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ORIGINAL RESEARCH

Left Atrial Appendage Morphology and Local Thrombogenesis-Related Blood Parameters in Patients With Atrial Fibrillation

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BACKGROUND: Left atrial appendage (LAA) morphology predicts stroke risk in patients with atrial fibrillation. However, it is not precisely understood how LAA morphology influences stroke risk. The present study aimed to investigate the relationship between LAA morphology and local thrombogenesis-related blood parameters in LAA.

METHODS AND RESULTS: We enrolled 205 patients undergoing catheter ablation of atrial fibrillation. The prevalence of chicken wing–, cactus-, windsock-, and cauliflower-type LAAs were 23.9%, 32.7%, 29.3%, and 14.1%, respectively. Blood samples were collected from the femoral vein, left atrium, and LAA in each patient. The levels of blood parameters were tested for each blood sample. The cauliflower-type LAA was associated with elevated platelet P-selectin expression, and interleukin-6 levels and with lower NO levels in LAA blood samples ($P<0.05$) independent of LAA flow velocity and LAA volume. LAA flow velocity, which was lowest in the cauliflower-type LAA, was the only independent predictor of von Willebrand factor antigen and plasminogen activator inhibitor-1 levels in LAA blood samples. In femoral vein blood samples, no significant difference was detected in the above blood parameters among the four LAA morphological types. In all blood samples, the levels of thrombin-antithrombin complex, D-dimer, fibrinogen, and tissue plasminogen activator were comparable among the four LAA morphological types.

CONCLUSIONS: In patients with atrial fibrillation, LAA morphological types might be associated with local platelet activity, fibrinolysis function, endothelial dysfunction, and inflammation.

Key Words: atrial fibrillation ■ coagulation ■ endothelial dysfunction ■ inflammation ■ left atrial appendage

Atrial fibrillation (AF) is a common arrhythmia in clinical practice that is associated with ischemic stroke. In patients with nonvalvular AF, the left atrial appendage (LAA) represents a major source of cardiac thrombus formation. The mechanism by which the LAA precipitates thrombogenesis has not been fully elucidated, but Virchow's triad (abnormal blood flow, abnormal blood constituents, and abnormal vessel walls) might apply.[1]

Given the important role of the LAA in AF-related thromboembolism, the anatomy and physiology of the LAA have been widely investigated. Di Biase et al classified the LAA into 4 morphological types (chicken wing, cactus, windsock, and cauliflower), which provided additional value in stratifying the risk of stroke or transient ischemic attack (TIA).[2] Nevertheless, it is not precisely understood how LAA morphology influences the risk of stroke/TIA. Previous studies have suggested that the size and flow velocity of the LAA vary among LAA morphologies, resulting in varying degrees of blood stasis, which might partially explain the relationship between LAA morphology and the risk of stroke/

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CLINICAL PERSPECTIVE

What Is New?

- Compared with the non–cauliflower-type left atrial appendage (LAA), the cauliflower-type LAA was associated with higher platelet activity, worse fibrinolysis, more severe endothelial dysfunction, and greater inflammation in the LAA.
- The results provided a new insight into understanding the relationship between LAA morphology and stroke risk.

What Are the Clinical Implications?

- The study supports individual anticoagulation based on LAA morphology.
- Evaluating LAA morphology might be indispensable in patients with atrial fibrillation.

Nonstandard Abbreviations and Acronyms

FV	femoral vein
LAA	left atrial appendage
LAAFV	left atrial appendage flow velocity
PAI-1	plasminogen activator inhibitor-1
TAT	thrombin-antithrombin
vWF	von Willebrand factor

TIA.[3–7] Besides abnormal blood flow, whether LAA morphology affects the other 2 factors of Virchow's triad, abnormal blood constituents and abnormal vessel walls, has not been well studied. This study aimed to investigate the relationship between LAA morphology and local blood parameters in LAA including those reflecting hemostasis, fibrinolysis, endothelial dysfunction, and inflammation.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

Between January 2018 and June 2020, patients with AF undergoing initial catheter ablation of AF under guideline recommendations were consecutively enrolled at Shaoxing People's Hospital in a prospective manner. The exclusion criteria were as follows: (1) reversible causes of AF; (2) previous ablation of atrial flutter or tachycardia originating from the left atrium; (3) valvular heart disease; (4) severe heart disease (ejection fraction <35%, dilated cardiomyopathy, hypertrophic cardiomyopathy, myocardial infarction within the past year); (5) hepatic or renal dysfunction; (6) known bleeding or platelet disorders; (7) known cancer; and (8) hormonal stimulation, including pregnancy. The study was conducted in accordance with the declaration of Helsinki, and the Ethics Committee of Shaoxing People's Hospital approved the study design. Written informed consent was obtained from all patients.

Echocardiographic Data Analysis

Enrolled patients received transthoracic echocardiography and transesophageal echocardiography within 2 days before the scheduled procedure. Transthoracic echocardiography and transesophageal echocardiography were performed with IE33 (Philips, Amsterdam, Netherlands) and Acuson SC2000 (Siemens USA, Washington, DC) ultrasound system using X5-1 and Z6Ms transducers, respectively, by an experienced sonographer following a standard protocol according to the recommendations of the American Society of Echocardiography.[8,9] LAA flow velocity (LAAFV) was measured with pulsed-wave Doppler at a site ≈1 cm from the LAA orifice in the longitudinal view of the LAA. During sinus rhythm, LAAFV was defined as the peak of the outflow velocity during diastole, and the average LAAFV over 3 consecutive cardiac cycles was used for analyses. During AF, LAAFV was defined as the mean velocity of peak outflow velocity over 10 consecutive cardiac cycles.

Cardiac Multidetector Computed Tomography Imaging Analysis

ECG-gated computed tomography was performed in all patients within 2 days before the scheduled procedure. Between January 2018 and December 2019, a 64-slice spiral scanner (Brilliance 64, Philips) was used. After December 2019, a 320-slice spiral scanner (Aquilion One; Canon Medical, Otawara, Japan) was used. The slice acquisition thicknesses were 0.625 and 0.3 mm, respectively. Three-dimensional left atrium and LAA structures were reconstructed using the postprocessing system. The LAA orifice plane was defined by a combination of the center of the pulmonary ridge and the centerline of the proximal left circumflex artery. Standard measurements of LAA volume, orifice area, and number of lobes were obtained from the volume-rendered 3-dimensional images.

Although Di Biase et al originally categorized LAA into 4 morphological types,[2] a recent study has suggested that because of the lack of objective criteria and the presence of high interobserver and intraobserver variability, this classification might be unreliable.[10] Therefore, in our study, LAA morphology was classified using the Kimura et al[11] method, which

uses more objective measurements for distinguishing between LAA morphologies. Using this system, each LAA was classified as 1 of the following 4 types: (1) chicken wing: a main lobe measuring >4 cm in length, with a folded angle of <100°; (2) windsock: a main lobe measuring >4 cm in length, with a folded angle of >100°; (3) cactus: a main lobe measuring <4 cm in length, with >2 lobes over 1 cm; and (4) cauliflower: a main lobe of <4 cm in length, with no forked lobes. Patients were grouped according to the LAA morphology type.

Preprocedural Anticoagulation

Antiplatelet drugs were discontinued at least 1 week before the procedure. If patients had been receiving long-term non–vitamin K antagonist oral anticoagulants or warfarin, anticoagulants remained uninterrupted during the periprocedural period according to the guidelines.[12] In patients who had not been receiving oral anticoagulants, low-molecular-weight heparin was started before the procedure, with the last dose being administered at least 12 hours before the procedure.

Blood Sampling

The right femoral vein was punctured using the Seldinger technique, and a femoral cannula sheath was placed in the vein. All blood samples were collected using the slow withdrawal technique. Immediately after the vein was accessed, 20 mL of blood was drawn through the side arm of a short introducer sheath, from which the first 5 mL of blood was discarded to exclude intrasheath hemostasis activation (the femoral vein [FV] sample). Transseptal catheterization was then performed. A 5F pigtail catheter was inserted through the transseptal sheath to obtain blood samples from the LAA and left atrium in a randomized sequence (20 mL from each). Again, the first 5 mL of blood was discarded. Immediately after the collection of the left heart blood samples, 150 IU/kg body weight intravenous heparin was administered. From each blood sample, 5 mL was added to a sodium citrate tube, which was used to perform platelet function tests within 90 minutes. The remainder of each blood sample was anticoagulated with a mixture of buffered citrate, theophylline, adenosine, and dipyridamole and centrifuged twice at 1500*g* for 20 minutes at room temperature. Plasma samples were stored at –70°C until further analysis.

Laboratory Tests

All blood samples were tested for platelet function, hemostasis, fibrinolysis, endothelial dysfunction, and inflammation. Platelet activation status was evaluated according to platelet P-selectin expression density. Platelets were marked with the general platelet marker CD42b-PE and the activation-specific antibody CD62P-FITC. The fluorescence intensity of CD62P-FITC–positive platelets was measured by flow cytometer (FACSCanto II; BD Biosciences, San Jose, CA). D-dimer levels were assayed using routine laboratory techniques. The fibrinogen concentration was measured using the von Clauss method. Levels of the von Willebrand factor (vWF) antigen (Novus Biologicals, Centennial, CO), thrombin-antithrombin (TAT) complex (Novus Biologicals), tissue-type plasminogen activator (tPA) (Thermo Scientific, Waltham, MA), plasminogen activator inhibitor-1 (PAI-1) (R&D Systems, Minneapolis, MN), and interleukin-6 (R&D Systems) were measured using commercially available ELISA tests. The level of NO was identified by its stable metabolites, nitrite and nitrate, using the Griess reaction (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The inter- and intra-assay coefficients of variation for all tests were <8%. Technicians were blinded to sample origin and patient characteristics.

Statistical Analysis

For continuous variables, Kolmogorov-Smirnov tests were performed to check for a normal distribution. Normal distribution data were presented as mean±SD and were compared using 1-way ANOVA, and Tukey's or Dunnett's tests were used for multiple comparisons. Skewed data were presented as median (minimum-maximum) and compared using the Kruskal-Wallis tests. Categorical variables were expressed as counts (percentages) and were compared using Fisher's exact test or Pearson's chi-square tests, as appropriate. To ensure normal distribution and homoscedasticity, natural logarithm transformations were applied for the levels of TAT. To identify the independent relationship between LAA morphology and the laboratory parameters, multiple linear regression analyses were performed in a forward stepwise manner according to the F statistics (the significance level for entry of variables: $P<0.1$; for removal: $P>0.2$). In model 1, the following variables were included: age, sex, AF type, AF duration, hypertension, diabetes mellitus, heart failure, stroke/TIA history, CHA2DS2-VASc score, spontaneous echo contrast, anticoagulant administration (using dummy variable with low-molecular-weight heparin as a reference), antiplatelet drugs administration, left ventricular ejection fraction, left atrial dimension, and LAA morphology type (using dummy variable with the cauliflower type as a reference). In model 2, we added LAA volume and LAAFV to model 1. All data were analyzed using SPSS 20.0 (IBM, Armonk, NY). A $P<0.05$ was considered to be statistically significant.

Table 1. Characteristics of Patients With Different LAA Types

	Chicken Wing Type (n = 49)	Cactus Type (n = 67)	Windsock Type (n = 60)	Cauliflower Type (n = 29)	*P* Value	Groups With Pairwise Significant Difference
Age, y	67±8	65±9	64±10	65±8	0.328	/
Male, n (%)	28 (57)	45 (67)	37 (62)	17 (59)	0.708	/
BMI, kg/m^2	24.3±3.4	24.1±2.4	24.2±3.2	23±3.0	0.502	/
Persistent AF, n (%)	30 (61)	37 (55)	29 (48)	16 (55)	0.608	/
AF duration, mo	22±20	21±18	23±21	18±13	0.733	/
Hypertension, n (%)	27 (55)	45 (67)	32 (53)	18 (62)	0.384	/
Diabetes mellitus, n (%)	7 (14)	11 (16)	10 (17)	6 (21)	0.910	/
Heart failure, n (%)	4 (8)	5 (7)	3 (5)	1 (3)	0.785	/
Coronary heart disease, n (%)	4 (8)	5 (7)	3 (5)	2 (7)	0.915	/
Prior stroke/TIA, n (%)	5 (10)	5 (7)	6 (10)	7 (24)	0.114	/
CHA2DS2-VASc*	2.1±1.3	2.0±1.4	1.9±1.3	2.1±1.3	0.751	/
eGFR, mL/min	90±12	89±18	92±12	92±12	0.757	/
Antiplatelet drugs before blood drawing, n (%)†	3 (6)	3 (4)	2 (3)	1 (3)	0.931	/
Anticoagulant					0.990	/
LMWH, n (%)	7 (14)	10 (15)	9 (15)	4 (14)		/
Warfarin, n (%)	15 (31)	24 (36)	21 (35)	10 (35)		/
Rivaroxaban, n (%)	19 (39)	23 (34)	17 (28)	10 (34)		/
Dabigatran, n (%)	8 (16)	10 (15)	13 (21)	5 (17)		/
Rhythm during blood drawing					0.654	/
AF, n (%)	23 (47)	38 (57)	35 (58)	16 (55)		/
Sinus rhythm, n (%)	26 (53)	29 (43)	25 (42)	13 (45)		/
LVEF, n (%)	64±6	64±7	65±7	64±6	0.932	/
LAD, mm	39±7	39±6	39±6	38±7	0.710	/
SEC, n (%)	6 (12)	11 (16)	9 (15)	10 (34)	0.071	/
LAA flow velocity, cm/s	50±16	43±17	44±16	31±17	<0.001	1 vs 4; 1 vs 2; 2 vs 4; 3 vs 4
LAA orifice area‡, cm^2	3.24±0.81	3.24±0.88	3.55±0.81	3.63±0.76	0.037	2 vs 4
LAA volume‡, cm^3	11.7±3.9	12.2±4.5	14.6±3.8	15.2±3.3	<0.001	1 vs 3; 1 vs 4; 2 vs 3; 2 vs 4

AF indicates atrial fibrillation; BMI, body mass index; eGFR, estimated glomerular filtration rate; LAA, left atrial appendage; LAD, left atrial dimension; LMWH, low-molecular-weight heparin; LVEF, left ventricular ejection fraction; SEC, spontaneous echo contrast; and TIA, transient ischemic attack.

"/": no significant difference was found among groups.

*The reported CHA2DS2-VASc was calculated before any thromboembolic event.

†The mean withdrawal time was 16 days.

‡Measured from computed tomographic image.

RESULTS

Patient Characteristics

A total of 205 patients (62.0% men) with a history of paroxysmal (54.6%) or persistent (45.4%) AF were enrolled at our center between January 2018 and June 2020. The mean age was 65±9 years and the average duration of AF was 22±19 months. A history of stroke/TIA was present in 11.2% of patients. The mean CHA2DS2-VASc score calculated before any thromboembolic event was 2.01±1.32. At the time of blood withdrawal, 93 patients (45.4%) were in sinus rhythm and 112 patients (54.6%) were in AF rhythm. Before the procedure, 51.2% of patients were being treated with non–vitamin K antagonist oral anticoagulants (33.7% rivaroxaban, 17.6% dabigatran), 34.1% were on vitamin K antagonists, and the remaining patients received low-molecular-weight heparin. Regarding echocardiography parameters, spontaneous echo contrast was detected in 17.6% of patients. The mean left ventricular ejection fraction and left atrial dimension values were 64±7% and 39±6 mm, respectively.

The prevalences of chicken wing–, cactus-, windsock-, and cauliflower-type LAAs were 49 patients (23.9%), 67 patients (32.7%), 60 patients (29.3%),

and 29 patients (14.1%), respectively. Table 1 shows the baseline demographic and clinical characteristics and LAA measurements of the patients with each LAA morphology type. No significant difference was found in baseline demographic or clinical characteristics between morphological types. LAA morphology and function characteristics were significantly different among the 4 LAA types. The LAAFV was highest in the chicken wing–type LAA and lowest in the cauliflower-type LAA. The LAA orifice area and LAA volume were greatest in the cauliflower-type LAA.

Blood Parameter Levels

In the FV, left atrial, and LAA blood samples, the levels of TAT complex, D-dimer, fibrinogen, and tPA were all comparable among the 4 LAA morphological types (Figures 1 and 2). Similarly, in FV blood samples, no significant difference was detected between the LAA morphological types in the levels of platelet P-selectin expression, vWF antigen, PAI-1, NO, or interleukin-6. However, in the left atrial and LAA blood samples, LAA morphology was associated with varying levels of platelet P-selectin expression, vWF antigen, PAI-1, NO, and interleukin-6 (Figures 1 through 3). The results of pairwise comparisons suggest that the cauliflower-type LAA was associated with elevated platelet P-selectin expression, vWF antigen, PAI-1, and interleukin-6 levels and with lower NO levels in left atrial/LAA blood samples (Figures 1 through 3). In addition to the relationship among the four LAA morphological types and thrombogenesis-related blood parameters, we also evaluated the effect of following factors on the blood parameters: age, sex, rhythm during blood drawing, AF type, AF duration, hypertension, diabetes mellitus, heart failure, stroke/TIA history, CHA2DS2-VASc score, left atrial dimension, left ventricular ejection fraction, spontaneous echo contrast, anticoagulant administration, and antiplatelet drug application before the procedure. The results are shown in Tables S1 and S2.

Moreover, we also compared blood parameters according to sample origins. The results suggested that parameters measured from left atrial and LAA blood samples were similar. However, the levels of platelet P-selectin expression, vWF antigen, PAI-1, and interleukin-6 were significantly higher in the left atrial/LAA blood samples than in the FV blood samples (Figures 1 through 3).

To identify the independent relationship between LAA morphology and LAA blood sample parameters, the associations between the LAA morphology and those local blood parameters identified as significant in univariate analysis (namely, platelet P-selectin expression, vWF antigen, PAI-1, NO, and interleukin-6), were adjusted for baseline demographic and clinical characteristics and the other LAA measurements. The results showed that LAA morphology was still independently associated with the above blood parameters, with the exception of vWF antigen and PAI-1. The level of vWF antigen was associated only with spontaneous echo contrast and stroke/TIA history in model 1 (adjusting for baseline demographic and clinical characteristics) and with LAAFV in model 2 (model 1+other LAA measurements). Although the

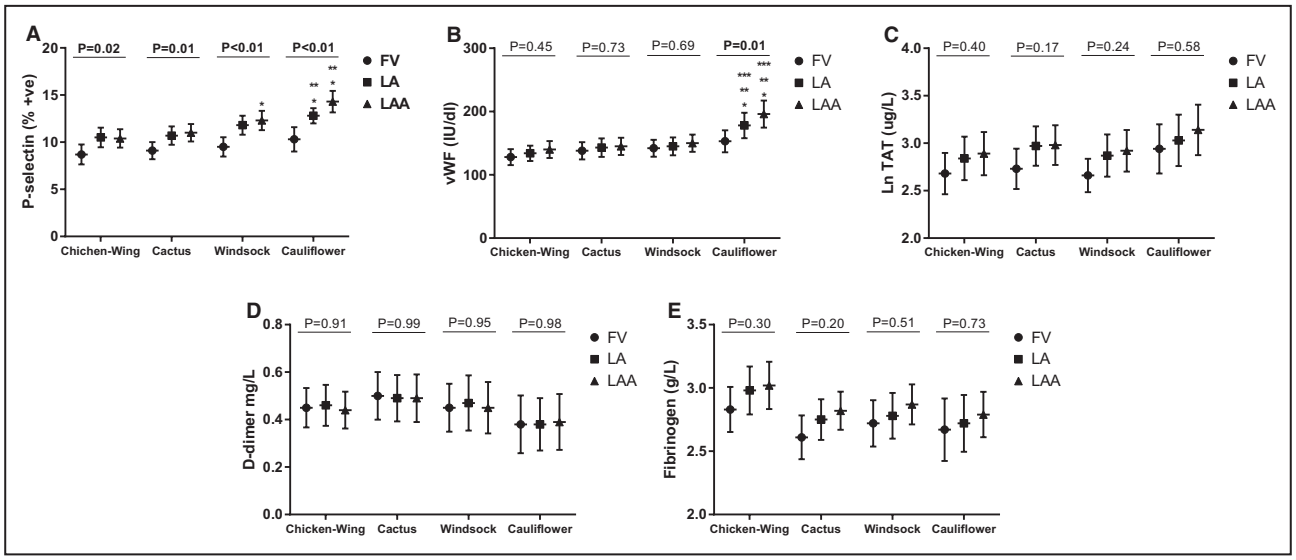

Figure 1. Levels of hemostasis parameters: P-selectin (A), vWF (B), TAT complex (C), D-dimer (D), and fibrinogen (E). Measures are presented graphically as mean and CIs. *: $P<0.05$ compared with the chicken wing–type LAA with the same blood sample source; **: $P<0.05$ compared with the cactus-type LAA with the same blood sample source; ***: $P<0.05$ compared with the windsock-type LAA with the same blood sample source. The horizontal line above indicates the comparison among different sample sources using 1-way ANOVA. FV indicates femoral vein; LA, left atrium; LAA, left atrial appendage; Ln, natural logarithm; TAT, thrombin-antithrombin complex; and vWF, von Willebrand factor.

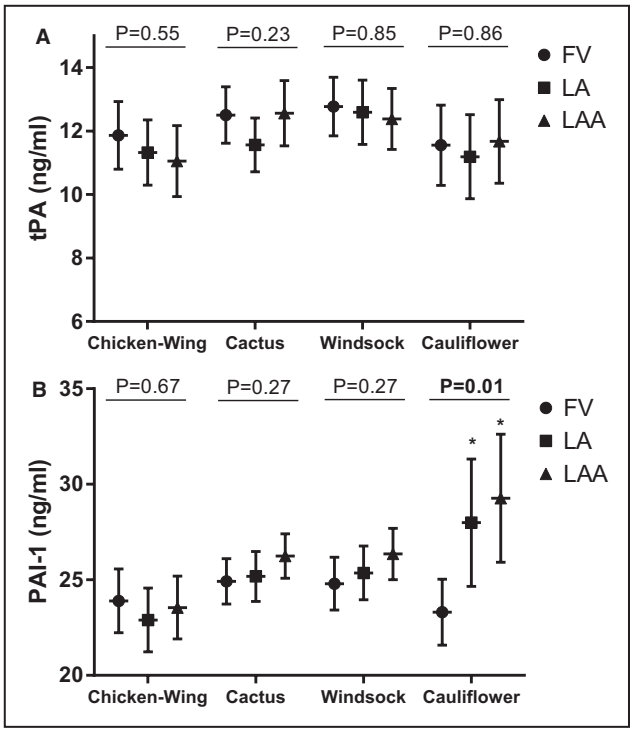

Figure 2. Levels of fibrinolysis parameters: tPA (A) and PAI-1 (B).
Measures are presented graphically as mean and confidence intervals. *: $P<0.05$ compared with the chicken wing–type LAA with the same blood sample source; the horizontal line above indicates the comparison among different sample sources using 1-way ANOVA. FV indicates femoral vein; LA, left atrium; LAA, left atrial appendage; PAI-1, plasminogen activator inhibitor-1; and tPA, tissue plasminogen activator.

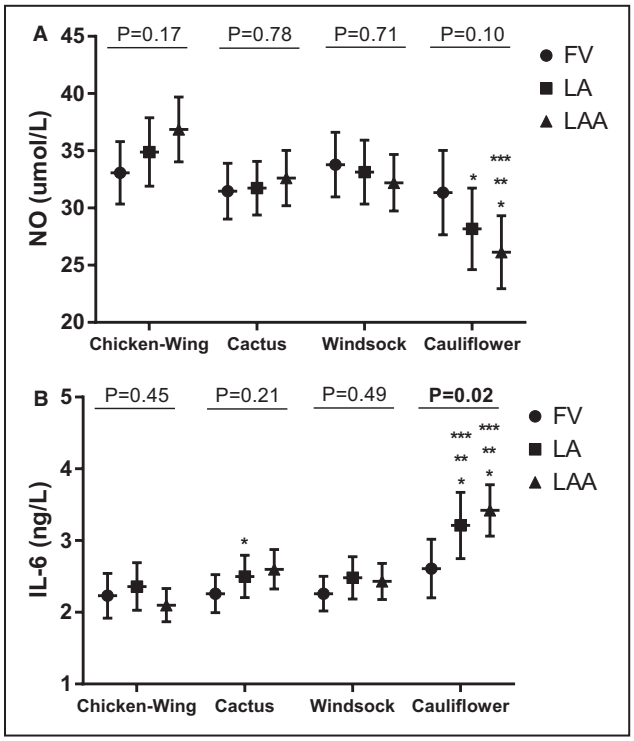

Figure 3. Levels of NO (A) and IL-6 (B).
Measures are presented graphically as mean and confidence intervals. *: $P<0.05$ compared with the chicken wing–type LAA with the same blood sample source; **: $P<0.05$ compared with the cactus-type LAA with the same blood sample source; ***: $P<0.05$ compared with the windsock-type LAA with the same blood sample source. The horizontal line above indicates the comparison among different sample sources using 1-way ANOVA. FV indicates femoral vein; IL-6, interleukin 6; LA, left atrium; and LAA, left atrial appendage.

level of PAI-1 was still associated with LAA types in model 1, LAAFV was the only independent predictor of PAI-1 level in model 2. The results of multiple linear regression analyses are summarized in Table 2.

DISCUSSION

The present study is, as far as we know, the first to evaluate the relationship between LAA morphology and hemostasis, fibrinolysis, endothelial dysfunction, and inflammation, as measured in blood samples from the LAA. Compared with non–cauliflower-type LAA, the cauliflower-type LAA was associated with higher platelet activity, worse fibrinolysis, more severe endothelial dysfunction and greater inflammation in the LAA. These findings might partially explain the higher risk of stroke in patients with AF with a cauliflower-type LAA.

Platelet P-selectin is an important adhesion molecule that mediates leukocyte-platelet-endothelium interactions and is expressed mainly in activated platelets. Although the role of platelet activation in the prothrombotic state in AF is controversial, multiple studies demonstrated that AF was associated with elevated P-selectin and that restoring the sinus rhythm contributed to a reduction in P-selectin.[1,13] The prognostic significance of P-selectin also remains in debate, but most previous studies focused on peripheral samples, which might attenuate the significance of P-selectin.[14] In our study, we found that the platelet P-selectin expression was significantly higher in the center samples than in peripheral samples, which might better predict outcomes. Previous studies suggested that P-selectin was not only a marker of platelet activation but also an indicator of endothelial dysfunction related to arrhythmia.[15] Therefore, in theory, local P-selectin levels might predict the risk of local thrombogenesis. The present study found that the level of P-selectin expression measured from LAA/left atrial samples varied among the 4 LAA morphologies, with the cauliflower-type LAA being associated with a higher P-selectin level than the chicken-wing and cactus

Table 2. Multiple Linear Regression Analyses for Blood Parameters Measured From LAA Samples

Model	Dependent Variables	Independent Variables	Unstandardized Coefficient (95% CI)	Standardized Coefficient*	*P* Value	R^2 for Overall Model
Model 1†	P-selectin	Chicken wing type	−3.81 (−5.46 to −2.17)	−0.424	<0.001	0.168
		Cactus type	−3.28 (−4.83 to −1.72)	−0.400	<0.001	
		Windsock type	−1.86 (−3.45 to −0.27)	−0.221	0.022	
		Cauliflower type	Reference	…	…	
		CHA2DS2-VASc score	0.52 (0.14 to 0.89)	0.177	0.007	
		Rhythm during blood drawing‡	1.09 (0.10 to 2.08)	0.141	0.031	
	vWF	SEC	31.35 (11.33 to 51.36)	0.215	0.002	0.079
		Stroke/TIA history	24.23 (0.10 to 48.36)	0.138	0.049	
	PAI-1	Chicken wing type	−2.74 (−4.98 to −0.50)	−0.203	0.017	0.077
		Cauliflower type	Reference	…	…	
		Stroke/TIA history	3.64 (0.041 to 7.233)	0.168	0.047	
	NO	Chicken wing type	5.47 (2.36 to 8.58)	0.233	0.001	0.091
		Cauliflower type	Reference	…	…	
		Diabetes mellitus	−4.94 (−8.51 to −1.37)	−0.183	0.007	
	Interleukin-6	Chicken wing type	−0.568 (−0.896 to −0.241)	−0.229	0.001	0.181
		Cauliflower type	Reference	…	…	
		Diabetes mellitus	0.581 (0.206 to −0.956)	0.204	0.003	
Model 2§	P-selectin	Chicken wing type	−3.17 (−4.84 to −1.51)	−0.352	<0.001	0.204
		Cactus type	−2.74 (−4.31 to −1.17)	−0.335	0.001	
		Windsock type	−1.79 (−3.35 to −0.23)	−0.226	0.025	
		Cauliflower type	Reference	…	…	
		Rhythm during blood drawing‡	1.14 (0.17 to 2.11)	0.145	0.021	
		LAA volume (per cm^3)	0.19 (0.07 to 0.31)	0.208	0.003	
	vWF	LAAFV (per cm/s)	−1.13 (−1.55 to −0.72)	−0.354	<0.001	0.125
	PAI-1	LAAFV (per cm/s)	−0.093 (−0.148 to −0.040)	−0.284	0.001	0.081
	NO	Chicken wing type	4.69 (1.51 to 7.87)	0.200	0.004	0.109
		Cauliflower type	Reference	…	…	
		Diabetes mellitus	−4.32 (−7.91 to −0.73)	−0.160	0.019	
		LAAFV (per cm/s)	0.082 (0.003 to 0.161)	0.142	0.043	
	Interleukin-6	Chicken wing type	−0.568 (−0.896 to −0.241)	−0.229	0.001	0.181
		Cauliflower type	Reference	…	…	
		Diabetes mellitus	0.581 (0.206 to −0.956)	0.204	0.003	

LAA indicates left atrial appendage; LAAFV, LAA flow velocity; PAI-1, plasminogen activator inhibitor-1; SEC, spontaneous echo contrast; TIA, transient ischemic attack; and vWF, von Willebrand factor.

*Effects are for per SD increase or vs reference category.

†Model 1 adjusted for age, sex, atrial fibrillation type, rhythm during blood drawing, atrial fibrillation duration, hypertension, diabetes mellitus, heart failure, stroke/TIA history, CHA2DS2-VASc score, SEC, anticoagulant application, antiplatelet drug administration, left ventricular ejection fraction, and left atrial dimension.

‡Atrial fibrillation vs sinus rhythm.

§Model 2 added LAA volume and LAAFV to model 1.

types. Recently, Kosiuk et al[15] found that LAA volume was a morphological predictor of P-selectin level. In fact, in the present study, the cauliflower-type LAA had the largest LAA volume among the 4 LAA morphologies. In multivariable linear regression analysis, LAA morphology, but not LAA volume, was independently associated with the platelet P-selectin expression level in the LAA blood samples.

vWF is mainly produced in endothelial cells and plays an important role in inflammatory and hemostatic processes. vWF is not only directly involved in clot formation but also reflects endothelial dysfunction in many cardiovascular diseases. Multiple studies demonstrated a close relationship between vWF and AF in both peripheral and central samples.[1,16,17] Moreover, the association between vWF and the risk of

thromboembolic events in patients with AF is supported by previous studies.[18] In the present study, compared with non–cauliflower-type LAAs, the cauliflower-type LAA was significantly associated with increasing vWF in LAA/left atrial blood samples rather than in FV blood samples, which might partially explain the local prothrombotic state in LAA and the higher risk of stroke in AF patients with a cauliflower-type LAA. However, after adjusting for LAAFV, the relationship between the LAA morphology and vWF was no longer significant, suggesting that the LAA morphology might influence the vWF level by affecting the LAAFV. Indeed, vWF is rapidly degraded by ADAMTS-13 (a disintegrin and metallopeptidase with thrombospondin type-1 motif, member 13), which is flow dependent. Therefore, reduced blood flow disrupts the regulatory process.[19] Consistent with the findings of previous studies, the present study also suggested that the LAAFV of the cauliflower-type LAA was the lowest among the 4 LAA morphologies.[4]

In addition to hemostasis parameters, the present study also evaluated the level of PAI-1, the endogenous inhibitor of the fibrinolytic system. An increased level of PAI-1 is associated with many cardiovascular conditions, but the relationship between PAI-1 and AF is controversial. Early studies suggested that hypofibrinolysis is associated with a high level of PAI-1 in patients with AF.[16] However, a recent study reported no difference in the level of PAI-1 between patients with AF and healthy participants.[17] Nevertheless, the prognostic significance of PAI-1 was supported by a meta-analysis, which demonstrated that the level of PAI-1 was associated with the risk of stroke in patients with AF.[14] In the present study, we found that the level of PAI-1 in LAA/left atrial samples was higher in the cauliflower-type LAA group than in the chicken wing–type LAA group, which suggests that LAA morphology might have an effect on the fibrinolytic system. PAI-1 is synthesized and secreted by endothelial cells that are mediated by a number of factors, such as NO and inflammatory cytokines. The increased intracardiac level of PAI-1 in patients with a cauliflower-type LAA might result from a reduction in endocardial NO synthase and stronger local inflammation, as discussed below.

NO is a key factor released by endothelial cells and plays an important role in thrombosis, platelet aggregation, oxidative stress, vascular tone, and inflammation. As a marker of endothelial dysfunction, NO was found to be closely associated with AF, and the relationship was independent of comorbidities such as hypertension and diabetes mellitus.[20] NO reduction has also been regarded as an important contributor to AF-related thrombogenesis.[21] In our study, the cauliflower-type LAA was associated with a lower level of NO in LAA blood samples, which indicated more severe endothelial dysfunction in the cauliflower-type LAA than in non–cauliflower-type LAAs. The mechanism underlying the phenomenon might be explained by a more impaired rheology in the cauliflower-type LAA. Lower blood flow velocity and turbulent flow resulting from AF have been recognized as potential mechanisms underlying NO reduction in patients with AF. In fact, the LAA morphology might play an important role in rheology in the LAA. As mentioned above, the cauliflower-type LAA was associated with lower LAAFV. Besides LAAFV, in a recent computational fluid dynamics study, LAA morphology also influenced vorticity independent of LAAFV. Lower flow velocity and vorticity in the cauliflower-type LAA contribute to lower wall shear stress, which is an important modulator of NO production, explaining why, in our study, NO level was lower in the cauliflower-type LAA than in the other LAAs.[7,22]

The close relationship between inflammation and AF is widely accepted. Among various inflammation factors, interleukin-6 has been widely studied and is closely associated with AF. Interleukin-6 enhances platelet activity, accelerates fibrinogen production, and reflects endothelial dysfunction. Therefore, an increased interleukin-6 level promotes the prothrombotic state in AF.[16] In the present study, a higher interleukin-6 level in LAA/left atrial blood samples was detected in patients with the cauliflower-type LAA than in those with non–cauliflower-type LAAs. As with NO, higher interleukin-6 levels in the cauliflower-type LAA might result from a more impaired rheology because interleukin-6 production is also regulated by wall shear stress.[23]

Although LAA morphology was associated with the levels of platelet P-selectin expression, vWF antigen, PAI-1, NO, and interleukin-6, which have roles upstream of thrombogenesis, the parameters involved in the end stage of thrombogenesis did not differ significantly different among the 4 LAA morphologies. The TAT complex is a marker of the rate of thrombin generation, D-dimer is a product of fibrin turnover, and tPA production results from secondary fibrinolysis. Therefore, levels of the TAT complex, D-dimer, and tPA levels all reflect the degree of thrombogenicity. All these indices increase in patients with AF and are associated with thromboembolic events.[1] Interestingly, our study failed to demonstrate an association between LAA morphology and these parameters. However, the results should be interpreted with caution, since >85% of the patients had been receiving oral anticoagulant before the procedure, which might greatly affect these parameters.

Overall, the present study demonstrated that the LAA morphology was associated with platelet activity, endothelial dysfunction, fibrinolysis function, and inflammation in the left atrium/LAA. However, the degree of thrombogenicity, as reflected by TAT complex, D-dimer, and tPA levels, did not differ among the 4 LAA

morphologies, which might be a consequence of the high proportion of patients in our study receiving long-term oral anticoagulants. Previous studies have largely attributed varying stroke risk to varying LAAFV among the different LAA morphologies. Our results provided a new insight into understanding the relationship between the LAA morphology and stroke risk, which might contribute to the delivery of more appropriate individual therapy in the future.

Limitations

There are several limitations to the present study. First, the present study was conducted in a single center with a small sample size in which type II error must be considered. Therefore, further large multicenter studies are warranted to confirm the results. Second, as mentioned above, most patients had been receiving oral anticoagulants before the procedure, which might affect the results, particularly in the levels of TAT complex, D-dimer, and tPA levels. However, according to the current guidelines, an uninterrupted anticoagulation strategy is strongly recommended, so ethical requirements prevented anticoagulant treatments from being stopped before the procedure.[12] Third, the present study enrolled only those patients receiving AF ablation, which might have introduced a selection bias. Moreover, extensive left atrial ablation, especially LAA isolation, might change the hemodynamics and thrombogenesis-related blood parameters in the left atrium and LAA. In the present study, we enrolled only patients without previous extensive left atrial ablation. Therefore, the results should be extrapolated with caution. Finally, the classification of left atrial morphology was subjective, and interobserver and intraobserver variation reduced the reliability of the LAA morphology classification.[8] In our study, the LAA morphology was classified by the method reported by Kimura et al, which uses a more objective approach for classification.[11]

CONCLUSIONS

In patients with AF, LAA morphological types were associated with the local prothrombotic state in the LAA. Compared with non–cauliflower-type LAAs, the cauliflower-type LAA might be associated with higher platelet activity, worse fibrinolysis function, more severe endothelial dysfunction, and greater inflammation. The results partially explain why the cauliflower-type LAA has a greater risk of thromboembolic events.

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Disclosures

None.

Supplementary Material

Tables S1–S2

REFERENCES

1. Ding WY, Gupta D, Lip GYH. Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020. *Heart*. 2020;106:1463–1468. DOI: 10.1136/heartjnl-2020-316977.
2. Di Biase L, Santangeli P, Anselmino M, Mohanty P, Salvetti I, Gili S, Horton R, Sanchez JE, Bai R, Mohanty S, et al. Does the left atrial appendage morphology correlate with the risk of stroke in patients with atrial fibrillation? Results from a multicenter study. *J Am Coll Cardiol*. 2012;60:531–538. DOI: 10.1016/j.jacc.2012.04.032.
3. Lee JM, Seo J, Uhm JS, Kim YJ, Lee HJ, Kim JY, Sung JH, Pak HN, Lee MH, Joung B. Why is left atrial appendage morphology related to strokes? An analysis of the flow velocity and orifice size of the left atrial appendage. *J Cardiovasc Electrophysiol*. 2015;26:922–927. DOI: 10.1111/jce.12710.
4. Fukushima K, Fukushima N, Kato K, Ejima K, Sato H, Fukushima K, Saito C, Hayashi K, Arai K, Manaka T, et al. Correlation between left atrial appendage morphology and flow velocity in patients with paroxysmal atrial fibrillation. *Eur Heart J Cardiovasc Imaging*. 2015;17:59–66. DOI: 10.1093/ehjci/jev117.
5. Petersen M, Roehrich A, Balzer J, Shin DI, Meyer C, Kelm M, Kehmeier ES. Left atrial appendage morphology is closely associated with specific echocardiographic flow pattern in patients with atrial fibrillation. *Europace*. 2015;17:539–545. DOI: 10.1093/europace/euu347.
6. Yaghi S, Song C, Gray WA, Furie KL, Elkind MS, Kamel H. Left atrial appendage function and stroke risk. *Stroke*. 2015;46:3554–3559. DOI: 10.1161/STROKEAHA.115.011273.
7. Masci A, Barone L, Dedè L, Fedele M, Tomasi C, Quarteroni A, Corsi C. The impact of left atrium appendage morphology on stroke risk assessment in atrial fibrillation: a computational fluid dynamics study. *Front Physiol*. 2019;9:1938. DOI: 10.3389/fphys.2018.01938.
8. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, Horton K, Ogunyankin KO, Palma RA, Velazquez EJ. Guidelines for performing a comprehensive transthoracic echocardiographic examination in adults: recommendations from the American Society of Echocardiography. *J Am Soc Echocardiogr*. 2019;32:1–64. DOI: 10.1016/j.echo.2018.06.004.
9. Hahn RT, Abraham T, Adams MS, Bruce CJ, Glas KE, Lang RM, Reeves ST, Shanewise JS, Siu SC, Stewart W, et al. Guidelines for performing a comprehensive transesophageal echocardiographic examination: recommendations from the American Society of Echocardiography and the Society of Cardiovascular Anesthesiologists. *J Am Soc Echocardiogr*. 2013;26:921–964. DOI: 10.1016/j.echo.2013.07.009.
10. Wu L, Liang E, Fan S, Zheng L, Du Z, Liu S, Hu F, Fan X, Chen G, Ding L, et al. Relation of left atrial appendage morphology determined by computed tomography to prior stroke or to increased risk of stroke in patients with atrial fibrillation. *Am J Cardiol*. 2019;123:1283–1286. DOI: 10.1016/j.amjcard.2019.01.024.
11. Kimura T, Takatsuki S, Inagawa K, Katsumata Y, Nishiyama T, Nishiyama N, Fukumoto K, Aizawa Y, Tanimoto Y, Tanimoto K, et al. Anatomical characteristics of the left atrial appendage in cardiogenic stroke with low CHADS2 scores. *Heart Rhythm*. 2013;10:921–925. DOI: 10.1016/j.hrthm.2013.01.036.

12. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, Boriani G, Castella M, Dan GA, Dilaveris PE, et al.; ESC Scientific Document Group. 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association of Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2021;42:373–498. DOI: 10.1093/eurheartj/ehaa612.
13. Lim HS, Willoughby SR, Schultz C, Chakrabarty A, Alasady M, Lau DH, Roberts-Thomson KC, Worthley MI, Young GD, Sanders P. Successful catheter ablation decreases platelet activation and improves endothelial function in patients with atrial fibrillation. *Heart Rhythm*. 2014;11:1912–1918. DOI: 10.1016/j.hrthm.2014.07.030.
14. Wu N, Chen X, Cai T, Wu L, Xiang Y, Zhang M, Li Y, Song Z, Zhong L. Association of inflammatory and hemostatic markers with stroke and thromboembolic events in atrial fibrillation: a systematic review and meta-analysis. *Can J Cardiol*. 2015;31:278–286. DOI: 10.1016/j.cjca.2014.12.002.
15. Kosiuk J, Uhe T, Stegmann C, Ueberham L, Bertagnolli L, Dagres N, Dinov B, Müssigbrodt A, Richter S, Paetsch I, et al. Morphological determinators of platelet activation status in patients with atrial fibrillation. *Int J Cardiol*. 2019;279:90–95. DOI: 10.1016/j.ijcard.2018.11.096.
16. Khan AA, Lip GYH. The prothrombotic state in atrial fibrillation: pathophysiological and management implications. *Cardiovasc Res*. 2018;115:31–45.
17. Tóth NK, Csanádi Z, Hajas O, Kiss A, Nagy-Baló E, Kovács KB, Sarkady F, Muszbek L, Bereczky Z, Csiba L, et al. Intracardiac hemostasis and fibrinolysis parameters in patients with atrial fibrillation. *Biomed Res Int*. 2017;2017:1–10. DOI: 10.1155/2017/3678017.
18. Ye YZ, Chang YF, Wang BZ, Ma YT, Ma X. Prognostic value of von Willebrand factor for patients with atrial fibrillation: a meta-analysis of prospective cohort studies. *Postgrad Med J*. 2020;96:267–276.
19. Bortot M, Ashworth K, Sharifi A, Walker F, Crawford NC, Neeves KB, Bark D, Paola JD. Turbulent flow promotes cleavage of VWF (von Willebrand factor) by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type-1 motif, member 13). *Arterioscler Thromb Vasc Biol*. 2019;39:1831–1842. DOI: 10.1161/ATVBAHA.119.312814.
20. Guazzi M, Arena R. Endothelial dysfunction and pathophysiological correlates in atrial fibrillation. *Heart*. 2008;95:102–106.
21. Glikson M, Wolff R, Hindricks G, Mandrola J, Camm AJ, Lip GYH, Fauchier L, Betts TR, Lewalter T, Saw J, et al. EHRA/EAPCI expert consensus statement on catheter-based left atrial appendage occlusion—an update. *EuroIntervention*. 2020;15:1133–1180. DOI: 10.4244/EIJY19M08_01.
22. Grigoriadis GI, Sakellarios AI, Kosmidou I, Naka KK, Ellis C, Michalis LK, Fotiadis DI. Wall shear stress alterations at left atrium and left atrial appendage employing abnormal blood velocity profiles. *Annu Int Conf IEEE Eng Med Biol Soc*. 2020;2020:2565–2568.
23. Cheng C, Tempel D, van Haperen R, van der Baan A, Grosveld F, Daemen MJ, Krams R, de Crom R. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. *Circulation*. 2006;113:2744–2753. DOI: 10.1161/CIRCULATIONAHA.105.590018.

Supplemental Material

Table S1. The impact of potential confounding factors on blood parameters (Categorical variables) [*]

PCF	Blood Parameters	site	Mean±SD	P-value
Rhythm during blood drawing [†]	P-selectin (%+ve)	LAA	12.29 ±3.87 vs 11.00 ±3.72	<0.05
	vWF (IU/dl)	LAA	159.6±55.2 vs 144.1 ± 55.3	<0.05
Heart failure [‡]	tPA (ng/ml)	LA	11.86±3.66 vs 10.08±3.11	0.09
Hypertension [‡]	P-selectin (%+ve)	FV	9.87±3.63 vs 8.40±3.78	<0.01
		LA	11.72±3.59 vs 10.56±4.06	<0.05
	PAI-1 (ng/ml)	FV	25.26±4.95 vs 23.16±5.33	<0.01
		LAA	26.75±6.21 vs 25.04±5.67	<0.05
Diabetes [‡]	IL-6 (ng/L)	LAA	3.05±0.92 vs 2.45±1.06	<0.01
	NO (umol/L)	FV	28.75±9.12 vs 33.26±10.14	<0.01
		LAA	28.29±9.24 vs 33.45±10.00	<0.01
	PAI-1 (ng/ml)	FV	25.80±5.75 vs 24.14±5.06	0.09
		LA	28.30±8.30 vs 24.44±5.38	<0.05
		LAA	29.07±8.80 vs 25.46±5.16	<0.05
Stroke/TIA history [‡]	LnTAT (ug/L)	LAA	3.22±0.76 vs 2.93±0.82	0.10
	IL-6 (ng/L)	FV	2.70±1.05 vs 2.26±1.03	0.06
		LAA	2.97±1.02 vs 2.49±1.06	<0.05
	PAI-1 (ng/ml)	LA	28.21±8.53 vs 24.96±5.64	<0.05
		LAA	29.29±9.01 vs 25.65±5.46	0.07
SEC [‡]	LnTAT (ug/L)	FV	3.20±0.61 vs 2.63±0.84	<0.01
		LA	3.43±0.52 vs 2.81±0.83	<0.01
		LAA	3.48±0.50 vs 2.85±0.83	<0.01
	vWF (IU/dl)	FV	157.03±35.04 vs 134.98±53.04	<0.01
		LA	171.69±42.95 vs 140.70±55.97	<0.01
		LAA	182.42±44.49 vs 146.21±55.82	<0.01

	IL-6 (ng/L)	LAA	2.94±1.10 vs 2.46±1.04	<0.05
	NO (umol/L)	FV	29.21±9.66 vs 33.21±10.08	<0.05
		LA	29.13±8.89 vs 33.09±10.43	<0.05
		LAA	28.83±9.28 vs 33.40±10.04	<0.05
	D-dimer (mg/L)	FV	0.60±0.56 vs 0.42±0.31	0.07
		LA	0.61±0.57 vs 0.43±0.32	0.07
		LAA	0.59±0.53 vs 0.42±0.31	0.07
	Fibrinogen (g/L)	LA	2.54±0.59 vs 2.87±0.67	<0.01
		LAA	2.65±0.55 vs 2.93±0.61	<0.05
Anticoagulant [§]	tPA (ng/ml)	FV	11.76±3.05 vs 13.08±3.26 vs 12.27±3.82 vs 11.25±3.97	0.07
		LAA	11.51±3.23 vs 13.14±3.75 vs 11.67±3.74 vs 10.94±4.66	0.02

FV, femoral vein; IL-6, interleukin 6; LA, left atrium; LAA, left atrial appendage; NO, nitric oxide, PAI-1, plasminogen activator inhibitor-1; PCF, potential confounding factors; SEC, SEC: spontaneous echo contrast; TAT, Thrombin-antithrombin complex; TIA, transient ischemic attack; tPA, tissue plasminogen activator; vWF, von Willebrand factor.

*, evaluate the following potential confounding factors and only show the factors with P-value less than 0.10 in univariate analyses: sex, rhythm during blood drawing, AF type, hypertension, diabetes, heart failure, stroke/TIA history, spontaneous echo contrast, anticoagulant administration and antiplatelet drugs before the procedure;

[†], atrial fibrillation vs. sinus rhythm;

[‡], yes vs. no;

[§], low-molecular-weight-heparin vs. warfarin vs. rivaroxaban vs. dabigatran.

Table S2. The impact of potential confounding factors on blood parameters (continuous variables) [*]

PCF	Blood Parameters	site	Correlation Coefficient	P-value
Left atrial dimension (mm)	IL-6 (ng/L)	LA	0.17	0.01
	NO (umol/L)	FV	-0.21	<0.01
		LA	-0.28	<0.01
	PAI-1 (ng/ml)	FV	0.83	<0.01
		LA	0.58	<0.01
AF duration (months)	P-selectin (%+ve)	FV	0.183	0.01
		LA	0.137	0.05
CHA_2DS_2-Vasc	P-selectin (%+ve)	FV	0.12	0.09
		LA	0.13	0.07
		LAA	0.18	0.01
	NO (umol/L)	FV	-0.18	0.01
		LAA	-0.13	0.06
	IL-6 (ng/L)	LAA	0.142	0.04
	PAI-1 (ng/ml)	LAA	0.12	0.09

FV, femoral vein; IL-6, interleukin 6; LA, left atrium; LAA, left atrial appendage; NO, nitric oxide, PAI-1, plasminogen activator inhibitor-1; PCF, potential confounding factors.

*, evaluate the following potential confounding factors and only show the factors with P-value less than 0.10 in univariate analyses: left atrial dimension, age, AF duration, CHA2DS2-VASc score, and left ventricular ejection fraction.