Echocardiography WILEY

# Useful indices of thrombogenesis in the exclusion of intra-cardiac thrombus

Uzoma N. Ibebuogu MD[1,2]   |   Joseph H. Schafer PharmD[1] ⓘ   |   Mark J. Schwade BS[1] ⓘ   |   Jennifer L. Waller PhD[3]   |   Gyanendra K. Sharma MD[1]   |   Vincent J. B. Robinson MD[1] ⓘ

[1]Division of Cardiology, Department of Medicine, Augusta University Medical Center, Augusta, GA, USA

[2]Department of Medicine, University of Tennessee Health Sciences Center, Memphis, TN, USA

[3]Division of Biostatistics and Data Science, Department of Population Health Sciences, Augusta University Medical Center, Augusta, GA, USA

**Correspondence**
Vincent J. B. Robinson, Section of Cardiology, Augusta University Medical Center, 1120 15th Street (BBR6518), Augusta, GA 30912, USA.
Email:vrobinso@augusta.edu

## Abstract

**Background:** Cardioversion in patients with atrial fibrillation (AF) can cause cardioembolic stroke, and effective clinical management is necessary to reduce morbidity and mortality. Currently, transesophageal echocardiography (TEE) is the accepted standard to diagnose cardiogenic thromboemboli; however, a negative TEE does not eliminate the possibility of left atrial thrombus. The objective of this study was to evaluate the diagnostic value of supplementing the TEE with additional noninvasive markers to ensure thrombus absence.

**Methods:** A prospective study was conducted on 59 patients who underwent TEE for suspected intra-cardiac thrombi. The TEE indications included acute ischemic stroke (45.7%) and AF or flutter (59.3%). D-dimer level and white blood cell counts were assessed.

**Results:** A negative D-dimer level (<200 ng/mL) excluded the presence of intra-cardiac thrombi. Groups with either negative (n = 14) or positive (n = 45) D-dimer levels had comparable clinical characteristics. Comparing positive D-dimer–level patients with thrombus (n = 7) and without thrombus (n = 33), patients with thrombus had reduced left atrial appendage (LAA) velocity (*P* = .0024), reduced left ventricular ejection fraction (LVEF) (*P* = .0263), increased neutrophil percent (*P* = .0261), decreased lymphocyte percent (*P* = .0216), and increased monocyte counts (*P* = .0220). The area under the receiver operating characteristic (ROC) curve for thrombus diagnostics was larger for combinations of clinical and biochemical data than for each parameter individually.

**Conclusions:** Supplementing the gold standard TEE with the analysis of LAA velocity, noninvasive LVEF, D-dimer, and hemostatic markers provided additional useful diagnostic information. Larger studies are needed to further validate the efficacy of supplementing the TEE to better assess patients for intra-cardiac thrombi.

**KEYWORDS**
atrial fibrillation, cardiac thrombus, D-dimer assay, thrombosis, transesophageal echocardiography

# 1 | INTRODUCTION

Atrial fibrillation (AF) is associated with hemostatic hypercoagulability that may increase the susceptibility for formation of intra-cardiac thrombi, which may subsequently lead to systemic thromboembolic events and cerebral emboli.[1-3] In these patients, and especially those with left atrial enlargement, most of these thrombi originate in the left atrial appendage (LAA).[4] Among patients referred for evaluation of emboli from a potential cardiogenic source, spontaneous echo contrast within the left atrium is the most common transesophageal echocardiographic (TEE) finding in the setting of present thrombus.[5-10]

In the case of planned cardioversion for AF patients not on anti-coagulation, it is recommended that a TEE be obtained to confirm the absence of intra-cardiac thrombus prior to the procedure. Although a large randomized trial has shown that a negative TEE obviates the need for prolonged anticoagulation prior to cardioversion, it does not eliminate the presence of intra-cardiac thrombi, which may have embolized in entirety prior to TEE.[9,11] In one article, even after negative TEE results, an embolic rate of approximately 1% after electrical cardioversion was reported.[9] The complex structure of the LAA, which is often multilobed with unpredictable projections, makes the complete exclusion of small thrombi impossible.[12] For instance, in a quantitative autopsy study of 500 healthy subjects, the LAA had two or more lobes in 80% of hearts.[13] Moreover, the exclusion of LAA clots with the use of TEE still mandates close interrogation for left ventricular and proximal aortic thrombi.[14] Thus, despite being widely used in the diagnosis of atrial thrombi, TEE has a number of inherent limitations.[14-18]

Earlier studies proposed that hemostatic biomarkers of hypercoagulability, cardiac dysfunction, and pro-thrombotic systemic inflammation may be useful in identifying patients at highest thromboembolic risk.[19-25] In fact, thrombus formation in the cardiac chambers is mainly due to blood stasis, leading to a fibrin-rich clot very similar to venous thrombi.[26-28] Considering the mechanism of clot formation, it is clear that patients with AF/flutter may have increased levels of D-dimer[21,29] and up-regulated biomarkers of pro-thrombotic endothelial dysfunction.[30-32] However, the clinical role of hemostatic biomarker parameters as a supplemental approach in the exclusion/diagnostics of intra-cardiac thrombi remains to be determined. The aim of our study was to evaluate how the level of these biomarkers and D-dimer levels, a marker of cross-linked fibrin, may be useful in the risk assessment of cardiac thrombi in the context of current practice.

# 2 | PATIENTS AND METHODS

## 2.1 | Study population

This was a prospective, single-center study. We approached patients who underwent transthoracic echocardiography (TTE) and TEE at the Division of Cardiology at Augusta University Medical Center to evaluate for cardiogenic sources of emboli between May 2007 and July 2011. Our recruitment was based on patient willingness to participate, with the consent of their treating physicians. TEE was requested for indications including AF, atrial flutter, and acute stroke of cryptogenic etiology. Exclusion criteria were any history of malignancy, chronic or acute inflammatory disease, systemic disease, connective tissue disease, autoimmune disease, pregnancy, any coagulation disorders, or thromboembolic events 60 days prior to being enrolled in the study. Data on patient demographic characteristics, medical history, physical examination, clinical features, and routine hematologic blood tests were obtained from the patient chart, whereas TTE/TEE echo contrast and D-dimer data were collected prospectively. Anticoagulation therapy was started based on TEE results and CHADS$_2$ scores, as recommended in the AHA/ACC guidelines for the management of patients with AF. Cardioversion was successfully performed in 22 (76%) patients from 29 patients diagnosed with AF or atrial flutter without LAA thrombus. The study protocol was approved by the Institutional Review Board, and informed written consent was obtained from each patient prior to inclusion in the study.

## 2.2 | Hemostatic blood tests

Routine hematologic and biochemical blood tests were performed at the Augusta University Medical Center Core Laboratory. Levels of D-dimer were measured by means of the HemosIL D-Dimer latex assay (IACLTOP 700 analyzer; Instrumentation Laboratory). The detection limit of this assay was 20 ng/mL. Measurements were carried out by personnel unaware of the patients' identities or clinical status.

## 2.3 | Transesophageal echocardiography

Transesophageal echocardiography was performed with patients in the left lateral decubitus position with a SONOS 7500 and iE33 echocardiographic imaging system (Philips) equipped with a 5-MHz multiplane transducer. The peak emptying velocity of the LAA was measured. Transesophageal parameters were reinterpreted off line by two investigators, without knowledge of patients' characteristics. The average values of five cardiac cycles were used for analysis.

## 2.4 | Statistics

The primary endpoint of the study was the presence or absence of left atrial thrombus as confirmed by TEE report. Any false negative TEE results would be recorded as a positive left atrial thrombus. All statistical analysis was performed using SAS 9.3, and statistical significance was assessed using an alpha level of 0.05. The results are given as mean ± standard deviation (SD) or frequencies and percentages where appropriate. Due to violations of assumptions of chi-square, t tests, and one-way ANOVA, nonparametric alternatives to these parametric tests were used. To examine differences in demographic and clinical variables between those with D-dimer levels <200 ng/mL and those with D-dimer levels ≥200 ng/mL, Fisher's exact tests and Wilcoxon rank sum tests were performed. The Pearson correlation was determined between age

and D-dimer levels. A Kruskal–Wallis test was used to examine differences in the median D-dimer and white blood cell (WBC) count between atrial fibrillation, atrial flutter, and neither groups. Differences in clinical measures between those with and without an intra-cardiac clot among those with D-dimer levels ≥200 ng/mL were determined using the Wilcoxon rank sum test. Logistic regression was used to estimate a receiver operating characteristic (ROC) curve and the area under the ROC (AROC) for D-dimer, LAA velocity, left ventricular ejection fraction (LVEF), and leukocyte counts depending on LAA clot status.

## 3 | RESULTS

### 3.1 | Clinical characteristics and usefulness of D-dimer in the exclusion of LAA thrombus

We enrolled 35 men and 25 women aged between 26 and 88 years (mean 63.2 ± 14 years). All patients were diagnosed with either AF or atrial flutter (59.3%) and acute stroke (45.7%), and referred to the TEE laboratory for a clinically indicated examination for intra-cardiac thrombi. Patients with acute stroke were initially diagnosed with a cryptogenic ischemic event by magnetic resonance imaging and/or CT of the brain. As TEE was the basis for thrombus presence or absence, one patient with acute stroke was excluded after enrollment due to thrombus detection by magnetic resonance angiography (MRA) and magnetic resonance imaging (MRI). After eligibility screening, all 59 of the remaining patients were involved in the study analysis. LAA thrombi were detected by TEE in seven patients (12%; five patients

with AF, one patient with atrial flutter, and one patient with acute stroke), which was consistent with the clot prevalence reported by others.[33] At baseline, there were no significant differences in the plasma D-dimer levels between LAA thrombus-positive (836 ± 917 ng/mL) and thrombus-negative (563 ± 991 ng/mL) groups (Figure 1A), due to the wide distribution of D-dimer levels in the thrombus-negative group (20-5779 ng/mL). In our population, the optimal D-dimer cutoff point was selected to be 200 ng/mL (Figure 1C,D), the D-dimer level that maximizes the sensitivity and specificity. Using a cutoff value of D-dimer ≥200 ng/mL, the D-dimer assay had a 100% sensitivity and 26.9% specificity (Figure 1B), 100% negative predictive value (NPV), and 15.6% positive predictive value (PPV), and a level ≥200 ng/mL will be referred to as a "positive D-dimer test result." Groups with either a positive or negative D-dimer test result had comparable clinical characteristics, except for sex (Table 1). Independent predictors of embolism, including diagnosis of ischemic stroke, hyperlipidemia or heart failure, LVEF, and LAA velocity, did not correlate with D-dimer elevation. The only demographic predictor that correlated with elevated D-dimer levels was decreased basophil count (P = .027; Table 2).

### 3.2 | Usefulness of cardiac morphological markers in the exclusion of LAA thrombus

Patients with both a positive D-dimer test and confirmed LAA thrombus had a significantly lower LAA velocity (22.4 ± 8.4 cm/s [95% CI, 14.7-30.2]) compared to patients with a positive D-dimer test and no LAA thrombus (44.4 ± 25.1 cm/s [95%CI, 36.3-55.6], P = .0024)
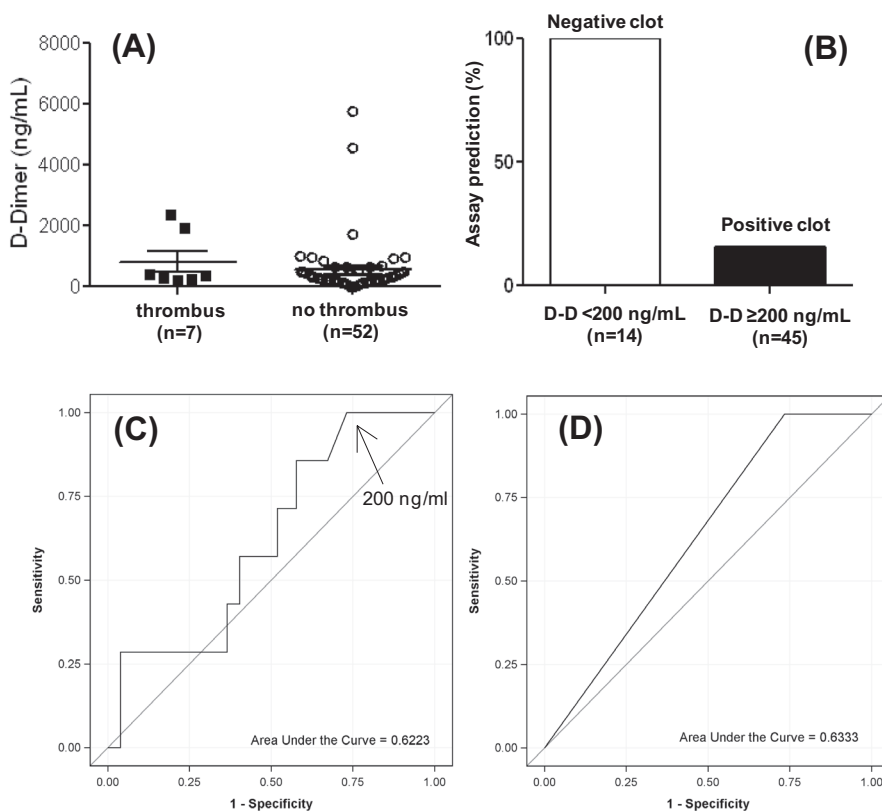


**FIGURE 1** Predictive values of D-dimer assay. A, Comparison of D-dimer levels between patients with and without LAA thrombus. B, Negative and positive predictive value using a D-dimer <200 ng/mL or ≥200 ng/mL. (C, D), Receiver operating characteristic curves of the continuous D-dimer level variable (C: AUC = 0.6223) and a D-dimer ≥200 ng/mL vs <200 ng/mL(D: AUC = 0.6333) to determine the presence of LAA thrombus by TEE. AUC is the area under the receiver operating characteristic curve

**TABLE 1** Demographic and clinical characteristics in relation to the D-dimer assay

| Variables | D-D < 200 ng/mL (n = 14) | D-D ≥ 200 ng/mL (n = 45) | Fisher's exact or Wilcoxon rank sum P-value |
|---|---|---|---|
| Sex, % male | 85.7 | 51.1 | .0292 |
| Race | | | |
| Black, % | 28.6 | 35.6 | .7529 |
| White, % | 71.4 | 64.4 | |
| BMI, mean (SD) | 30.7 (7.9) | 30.6 (11.0) | .7643 |
| Age, mean (SD), y | 61.3 (14.4) | 63.4 (13.6) | .7960 |
| <70, % | 71.4 | 64.4 | .7529 |
| ≥70, % | 28.6 | 35.6 | |
| History of hyperlipidemia, % | 35.7 | 44.4 | .7582 |
| History of DM, % | 42.9 | 22.2 | .1717 |
| History of stroke, % | 14.3 | 13.3 | 1.0000 |
| History of CAD, % | 50.0 | 28.9 | .1983 |
| History of HF, % | 14.3 | 33.3 | .3103 |
| History of CKD, % | 14.3 | 13.3 | 1.0000 |
| History of smoking, % | 42.9 | 31.1 | .5213 |
| Atrial fibrillation/atrial flutter | | | |
| Atrial fibrillation, % | 50.0 | 37.8 | .7831 |
| Atrial flutter, % | 14.3 | 20.0 | |
| None | 35.7 | 42.2 | |
| TIA or stroke, % | 35.7 | 48.9 | .5411 |
| Cardioversion, % | 57.1 | 31.1 | .1144 |
| CHADS2-Score, mean (SD) | 2.4 (1.4) | 2.6 (1.0) | .5783 |
| LVEF %, mean (SD) | 42.1 (15.5) | 31.6 (16.4) | .8574 |
| ≤35%, % | 33.3 | 35.7 | 1.0000 |
| >35%, % | 66.7 | 64.3 | |
| LAAV, mean (SD), cm/s | 46.4 (23.6) | 40.6 (24.5) | .3312 |
| ≤40 cm/s, % | 50.0 | 27.5 | .1733 |
| >40 cm/s,% | 50.0 | 72.5 | |

Abbreviations: BMI = body mass index calculated as weight (kg)/high (m$^2$); CAD = coronary artery disease; CKD = chronic kidney disease; CVA = cerebral vascular accident; D-D = D-dimer; DM = diabetes mellitus; HF = heart failure; LAA velocity = left atrial appendage velocity; LVEF = left ventricular ejection fraction; TIA = transient ischemic attack.

(Figure 2A). A ROC curve analysis showed that for a cutoff point of <40 cm/s, the LAA velocity had 100% sensitivity, 53.3% specificity, 25% PPV, and 100% NPV (Figure 2B). The large area under ROC curve of the continuous LAA velocity (AUC = 0.8603) suggests that the low peak emptying velocity of the LAA is a significant independent predictor of a thrombus and may be used alone to supplement the information provided by TEE echo contrast. In fact, the area under the ROC curve for the combination of LAA velocity <40 cm/s test with the positive D-dimer (Figure 2C) was increased compared with the single categorized LAA velocity (0.81 vs 0.77, P = .0383).

Furthermore, we determined that patients with both the positive D-dimer test and confirmed LAA thrombus had significant reduction in the LVEF (27.9 ± 14.7% [95% CI, 16.2-39.6]) compared with patients with a positive D-dimer test and no LAA thrombus (44.1 ± 15.5% [95%CI, 39.1-49.1], P = .0263) (Figure 2D). An ROC curve analysis

showed that for a cutoff point of <35%, the LVEF had 71% sensitivity, 73% specificity, 26% PPV, and 95% NPV (Figure 2E).To assess the usefulness of a noninvasive LVEF test for the diagnosis of cardiac thrombi, an ROC curve analysis was performed on LVEF values combined with a positive D-dimer test result (Figure 2F). The AUC curve for the combination of the LVEF test with a positive D-dimer was increased compared with the LVEF test alone (0.80 vs 0.72, P = .0264), suggesting that association of a low LVEF value and D-dimer ≥200 ng/mL may also serve as a noninvasive parameter for the risk of a thrombus.

## 3.3 | Association of LAA thrombus with WBC count

Among all patients with a positive D-dimer test, patients with LAA thrombus were more likely to have an elevation of pro-inflammatory

| Cell Types | Normal range | D-D < 200 ng/mL (n = 14) | D-D ≥ 200 ng/mL (n = 40) | Wilcoxon rank sum P-value |
|---|---|---|---|---|
| WBC (1000/mm$^3$) | 4.0–11.0 | 7.56 ± 2.10 | 8.02 ± 3.07 | .8056 |
| Neutrophil (1000/mm$^3$) (%) | 2.0–7.0 40–80 | 4.86 ± 1.98 62.39 ± 11.11 | 5.58 ± 2.96 66.67 ± 12.18 | .5804 .2324 |
| Lymphocyte (1000/mm$^3$) (%) | 1.0–3.0 20–40 | 1.89 ± 0.55 26.43 ± 9.54 | 1.55 ± 0.69 21.81 ± 10.39 | .0437 .1308 |
| Monocyte (1000/mm$^3$) (%) | 0.2–1.0 2–10 | 0.64 ± 0.19 8.64 ± 2.27 | 0.70 ± 0.36 8.94 ± 3.26 | .7961 .9130 |
| Eosinophil (1000/mm$^3$) (%) | 0.02–0.5 1–6 | 0.17 ± 0.16 2.36 ± 1.91 | 0.18 ± 0.16 2.41 ± 2.20 | .8713 .8889 |
| Basophil (1000/mm$^3$) (%) | 0.02–0.1 <1–2 | 0.06 ± 0.05 0.64 ± 0.50 | 0.02 ± 0.04 0.37 ± 0.45 | .0136 .0768 |
| Platelet (1000/mm$^3$) | 150–400 | 204.93 ± 66.15 | 215.14 ± 79.09 | .6633 |

**TABLE 2** Hemostatic characteristics in relation to the D-dimer assay

Abbreviation: D-D = D-dimer.

leukocyte subpopulations (Table 3). Having a thrombus was significantly associated with increased neutrophil percent (P = .0261), increased monocyte counts (P = .0220), and decreased lymphocyte percent (P = .0216). In addition, patients with thrombus had nonsignificant moderate reduction of the eosinophil level. ROC curve analysis for prediction of LAA thrombus showed larger AUC for the combination of the positive D-dimer test and LVEF <35% with these surrogate hematologic parameters (0.84-0.88) as compared to the combination of positive D-dimer test and LVEF <35% (0.81) only (Figure 3A-D). Importantly, the levels of D-dimer (Kruskal–Wallis $\chi^2_{(2)}$ = 2.57, P = .2770), neutrophil count (Kruskal–Wallis $\chi^2_{(2)}$ = 0.50, P = .7778), monocyte count Kruskal–Wallis $\chi^2_{(2)}$ = 2.74, P = .2536), and peripheral lymphocyte count (Kruskal–Wallis $\chi^2_{(2)}$ = 3.98, P = .1370) had no significant association with clinical diagnosis of AF or atrial flutter.

## 4 | DISCUSSION

In this era of healthcare cost reduction combined with patient outcome improvement, the ability to harness the clinical utility of D-dimer, as well as other cost-effective and noninvasive blood tests, is paramount. Several authors have added information on D-dimer levels into clinical decision-making for patients with suspected deep venous thrombosis (DVT) and pulmonary embolism (PE).[34-36] Using this D-dimer information clinically has allowed emergency room physicians to discharge patients safely from the emergency department if they are a low clinical risk with a negative D-dimer level. One study has shown that in a low clinical risk group, the addition of a positive D-dimer level increases the likelihood of a positive CTA for PE to the same frequency as the high Wells score criteria group.[37] Separate from the clear diagnostic

value in DVT and PE, D-dimer levels have also been proposed as an etiologic diagnosis of cardioembolic stroke or prediction of subsequent thromboembolic cardiovascular events in AF patients during oral anticoagulant therapy.[22,38,39] D-dimer levels have not been well studied for acute cardioembolic management to exclude left atrial or LAA thrombi; however, Wan et al[40] have found that nine studies have looked at using D-dimer to exclude left atrial thrombus among 1667 patients, and found a pooled sensitivity of 0.75 (95% confidence interval: 0.65-0.83) and a pooled specificity of 0.81 (95% confidence interval: 0.59-0.93). Currently, TEE remains the gold standard for detecting intra-cardiac thrombi; however, the negative predictive value of the TEE for atrial thrombus detection may not be 100%. Ikegami et al found that among 107 patients scanned for atrial thrombus using TEE, there were 4 false negatives (found by intra-cardiac echocardiography), with a negative predictive value of 96%. Due to an atrial thrombus being a "can't miss" diagnosis, it is of interest whether noninvasive markers can help to catch false negative TEE test results.

Addressing this question, the aim of this study was to determine how the D-dimer test and its combination with other clinical parameters may assist TEE in detection of intra-cardiac thrombi. Our findings show that the low D-dimer level (<200 ng/mL), LAA velocity >40 cm/s, and LVEF >35% were associated with absence of a LAA thrombus. Interestingly, beyond a direct relationship between thrombus formation and reduction of LVEF and LAA velocity, cardiac thrombogenesis also appears to have an additional impact on circulating leukocyte count. Although the role of pro-thrombotic cellular markers in clot diagnosis is not clear, our study proposes that the presence of an intra-cardiac thrombus may mediate a spectrum of immunological/inflammatory reactions. These data support early studies, which proposed hemostasis biomarkers of
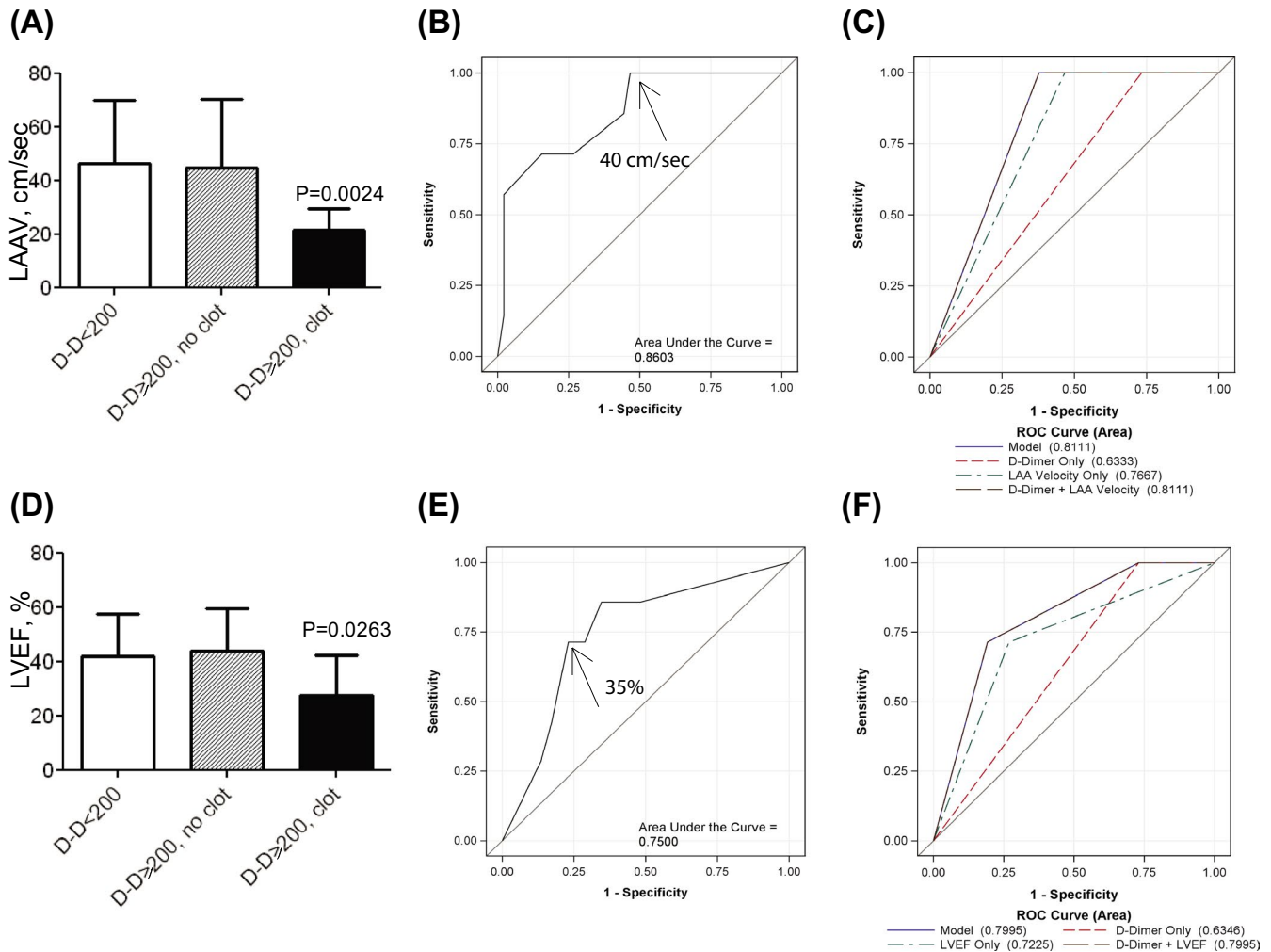
**(A)**



**(B)**



40 cm/sec

Area Under the Curve = 0.8603

**(C)**



ROC Curve (Area)
Model (0.8111)
D-Dimer Only (0.6333)
LAA Velocity Only (0.7667)
D-Dimer + LAA Velocity (0.8111)

**(D)**



P=0.0263

**(E)**



35%

Area Under the Curve = 0.7500

**(F)**



ROC Curve (Area)
Model (0.7995)     D-Dimer Only (0.6346)
LVEF Only (0.7225)     D-Dimer + LVEF (0.7995)

**FIGURE 2** Predictive values of cardiac morphological parameters. The left atrial appendage velocity (LAAV, top) and left ventricular ejection fraction (LVEF, bottom) were analyzed according to D-dimer levels and the presence of LAA thrombus detected by TEE. The significant reduction of LAAV (A) and LVEF (D) was associated with the presence of LAA thrombus. (B, E), ROC curves of continuous LAAV (B) and LVEF (E) variables to predict LAA thrombus. (C, F), The cut-point model of ROC curves of LAAV and LVEF and their combinations with D-dimer ≥200 ng/mL

hypercoagulability, indices of thrombogenesis, and systemic inflammation would be useful in identifying patients with high thromboembolic risk. There is substantial evidence that in addition to the elevated D-dimer, pro-thrombotic proteins and gene-expressed immune-inflammatory biomarkers (CRP, TNF-a, IL-1b, IL-6) are associated with duration of AF,[20,21] LAA dysfunction,[19] presence of atrial thrombus,[20,33,38] and diagnosis of cardioembolic stroke.[22-25] In this construct, it is clear that patients with AF have increased levels of D-dimer,[21,29] platelet activation, systemic inflammation,[30,31] and leukocyte adhesion, which is consistent with the amplified predisposition to thrombus formation and endothelial dysfunction.[32]

Based on our findings, we propose an algorithm to institute further investigations beyond TEE to incorporate the additional clinical information provided by the D-dimer level, LAA velocity, LVEF, and other cellular biomarkers of thrombosis (Figure 4). It is reasonable to consider that patients with true positive TEE and false negative TEE have similar parameters as both groups have a left atrial thrombus present. Per our results, no patient with a D-dimer level less than 200 ng/

mL had evidence of intra-cardiac thrombosis. This was regardless of whether or not other known imaging markers placed them in a high-risk group. Therefore, such a finding would confirm the absence of any possible thrombi following a negative TEE result. If patients have a D-dimer level greater than 200 ng/mL, the next step should be assessing whether the LAA velocity is <40 cm/s. This represents a very high-risk group where the likelihood of clot is so great that the clinical assumption is that the intra-cardiac clot has dislodged in entirety to the organ suspected of having suffered a thromboembolic event or the TEE may have been falsely negative. In this case, treatment with anticoagulation should be actively considered by the clinician even if the TEE is negative. Additionally, clinical judgment may warrant exploration of other etiologies outside of a cardioembolic source. In the situation where the LAA velocity is greater than 40 cm/s and no thrombus is observed in the heart, then collection of LVEF and other cellular biomarker data such as pro-thrombotic WBC count should be considered. If the LVEF is ≥35% and cellular markers of thrombosis are absent, then the patient can be considered to not have intra-cardiac

**TABLE 3** Hemostatic characteristics in the patient subgroup with the positive D-Dimer test in relation to detection of intra-cardiac thrombus

| Cell types | Normal range | D-D ≥ 200 ng/mL clot negative (n = 33) | D-D ≥ 200 ng/mL clot positive (n = 7) | Wilcoxon rank sum *P*-value |
|---|---|---|---|---|
| WBC (1000/mm³) | 4.0–11.0 | 7.55 ± 2.57 | 10.39 ± 4.37 | .1051 |
| Neutrophil (1000/mm³) (%) | 2.0–7.0 40–80 | 5.04 ± 2.39 65.00 ± 11.73 | 8.09 ± 4.17 74.54 ± 11.97 | .0545 .0261 |
| Lymphocyte (1000/mm³) (%) | 1.0–3.0 20–40 | 1.62 ± 0.71 23.46 ± 10.02 | 1.18 ± 0.38 14.00 ± 8.94 | .0718 .0216 |
| Monocyte (1000/mm³) (%) | 0.2–1.0 2–10 | 0.63 ± 0.28 8.60 ± 3.08 | 1.04 ± 0.52 10.56 ± 3.85 | .0220 .3434 |
| Eosinophil (1000/mm³) (%) | 0.02–0.5 1–6 | 0.20 ± 0.17 2.68 ± 2.27 | 0.07 ± 0.08 1.09 ± 1.26 | .0582 .0639 |
| Basophil (1000/mm³) (%) | 0.02–0.1 <1–2 | 0.02 ± 0.04 0.43 ± 0.47 | 0.005 ± 0.01 0.10 ± 0.26 | .4472 .0625 |
| Platelet (1000/mm³) | 150–400 | 221.66 ± 80.04 | 182.57 ± 70.42 | .0851 |

Abbreviation: D-D = D-dimer.
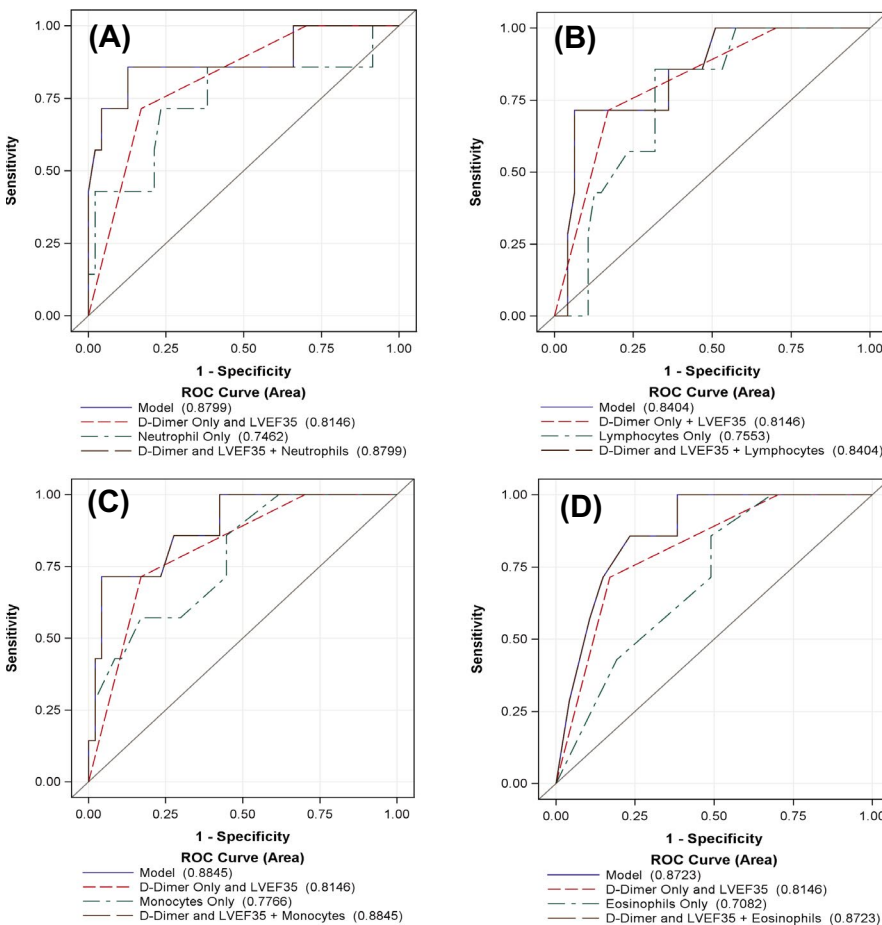


**FIGURE 3** Effectiveness of the model to predict cardiac thrombus, combining noninvasive morphological and biomarker data. The ROC curves for cut-point D-dimer ≥200 ng/mL and LVEF <35% and its combination with continuous WBC variables such as neutrophil (A), lymphocyte (B), monocyte (C), and eosinophil (D) counts. The area under the ROC curve for the multibiomarker model was significantly larger than that for isolated data
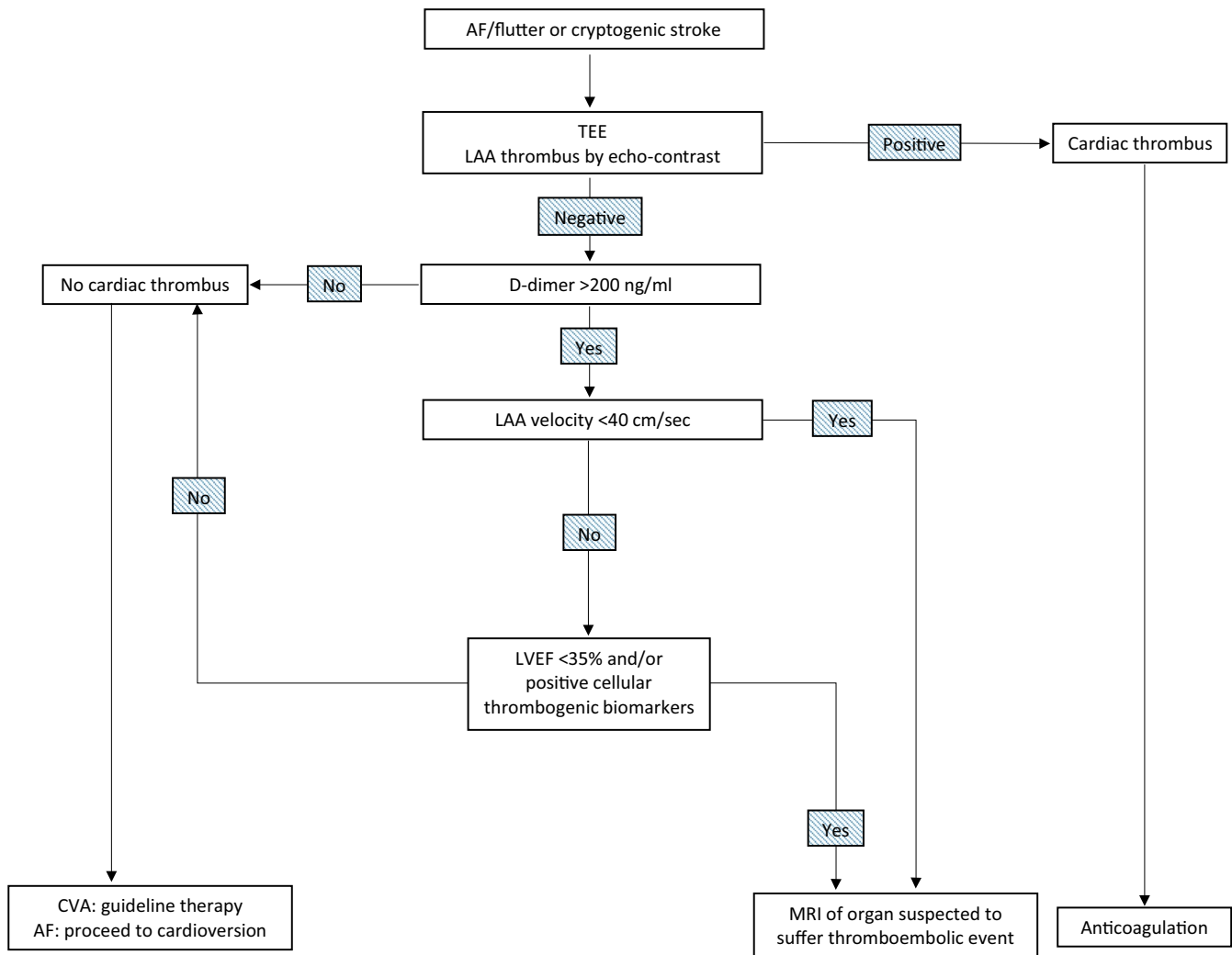
**FIGURE 4** Flow diagram that suggests a potential algorithm to use indices of thrombogenesis for management of patients with suspected cardiac thrombi. The cutoff points of morphological parameters and biochemical data still need to be carefully determined in larger prospective studies

thrombosis that has subsequently embolized. However, if the LVEF is <% and/or positive cellular biomarkers for thrombosis are present, then a search for thromboembolism in the suspected organ should be embarked upon.

We believe that if larger multicenter trials replicate the results of our small prospective pilot study, then institution of our suggested algorithm will result in a significant reduction in the number of false negative TEEs. TEE is not 100% sensitive for detection of intra-cardiac thrombi, as a negative TEE was associated with 1% stroke rate after cardioversion.[9] In addition, Sun et al have found that the sensitivity of TEE for diagnosis of left atrial or LAA thrombus to be 93.3% (standard deviation: 90.5%-96.0%).[41] Cases may also occur where the cardiac thrombus has already migrated to the affected organ. We had such a case within the cohort of our recruited patients, and a diagnosis of cardiac thromboembolism with recanalization thrombosis in the left middle cerebral artery was made based on MRA/MRI. Additionally, this patient's D-dimer normalized only after three months, showing that the duration of elevation lags even after anticoagulation treatment is initiated.[42]

The addition of D-dimer and other risk factors will help to detect these patients. Identifying these patients is crucial, as it would alter their subsequent therapy from antiplatelet to anticoagulation, which is known to be much more effective in reducing subsequent thromboembolic events.[43]

There were several limitations to this study that merit discussion. This was a single-center, prospective cohort study conducted on a pilot observational basis. The study was performed in a relatively small number of patients, and further larger studies are needed. Second, TEE was accepted as the gold standard in the diagnosing of cardiac thrombi. However, TEE is not sensitive to atrial thrombi that may have already embolized. This weakness of the diagnostic standard was apparent in the exclusion of the patient with a negative TEE for intra-cardiac thrombus, but with confirmation of cardioembolic TIA by MRA/MRI modalities. Considering the clinical guidelines and the cost of health care, we cannot eliminate the possibility of the underestimation of the presence of thrombus in other patients with cryptogenic strokes. However, the prevalence of left atrial appendage thrombus in 12% of our study patients was

similar to that reported by other investigators. Third, though our data were essentially complete for our major values five patients with negative clot and the positive D-dimer level did not have a complete hematologic WBC profile, which could have obscured these results. Also, our cutoff value for the D-dimer assay may not apply to other individual laboratories that may have their own cut-off value based on the type of D-dimer analyzer used. Additionally, although intra-cardiac thrombus was correlated to levels of selected pro-inflammatory and pro-thrombotic subpopulation of circulating leukocytes, a relationship between thrombosis and systemic hemostasis is still unclear. Inflammation is not the only predictor of cardiac thrombus and cannot be used as a positive predictor for diagnosis. The data may be applicable only as exclusion criteria to supplement TEE findings or estimate potential risks for subjects who are not eligible for TEE.

## 5 | CONCLUSION

D-dimer levels <200 ng/mL and LAA velocities >40 cm/s were strongly associated with the absence of intra-cardiac clots. Individuals with plasma D-dimer greater than 200 ng/mL and LAA velocity <40 cm/s may be candidates for focused target organ imaging for evidence of prior thrombus. Pro-inflammatory and pro-thrombotic leukocyte indices were related to thrombus formation and may be used in combination with the positive D-dimer test and LVEF data as surrogate noninvasive parameters to help stratify risk of intra-cardiac thrombus. Further prospective studies of larger size are needed to validate our findings.

### CONFLICT OF INTERESTS

The authors have no conflicts of interest to disclose.

### ORCID

*Joseph H. Schafer* https://orcid.org/0000-0003-4513-8348
*Mark J. Schwade* https://orcid.org/0000-0002-6661-3438
*Vincent J. B. Robinson* https://orcid.org/0000-0003-3755-6515

### REFERENCES

1. Johnson W.D., Ganjoo A.K., Stone C.D., et al. The left atrial appendage: our most lethal human attachment! Surgical implications. *Eur J Cardiothorac Surg*. 2000;17:718–722.
2. Lloyd-Jones D., Adams R.J., Brown T.M., et al. Heart disease and stroke statistics–2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–e215.
3. Dangayach N.S., Kane K., Moonis M.. Paroxysmal atrial fibrillation in cryptogenic stroke. *Ther Clin Risk Manag*. 2011;7:33–37.
4. Malouf J.F., Ammash N.M., Chandrasekaran K., et al. Critical appraisal of transesophageal echocardiography in cardioversion of atrial fibrillation. *Am J Med*. 2002;113:587–595.
5. von der Recke G., Schmidt H., Illien S., et al. Transesophageal contrast echocardiography distinguishes a left atrial appendage thrombus from spontaneous echo contrast. *Echocardiography*. 2002;19:343–344.
6. Black I.W., Chesterman C.N., Hopkins A.P., et al. Hematologic correlates of left atrial spontaneous echo contrast and thromboembolism in nonvalvular atrial fibrillation. *J Am Coll Cardiol*. 1993;21:451–457.
7. Daniel W.G., Nellessen U., Schroder E., et al. Left atrial spontaneous echo contrast in mitral valve disease: an indicator for an increased thromboembolic risk. *J Am Coll Cardiol*. 1988;11:1204–1211.
8. Meenan R.T., Saha S., Chou R., et al. Cost-effectiveness of echocardiography to identify intracardiac thrombus among patients with first stroke or transient ischemic attack. *Med Decis Making*. 2007;27:161–177.
9. Klein A.L., Grimm R.A., Murray R.D., et al. Use of transesophageal echocardiography to guide cardioversion in patients with atrial fibrillation. *N Engl J Med*. 2001;344:1411–1420.
10. von der Recke G., Schmidt H., Illien S., et al. Use of transesophageal contrast echocardiography for excluding left atrial appendage thrombi in patients with atrial fibrillation before cardioversion. *J Am Soc Echocardiogr*. 2002;15:1256–1261.
11. Fatkin D., Kelly R.P., Feneley M.P.. Relations between left atrial appendage blood flow velocity, spontaneous echocardiographic contrast and thromboembolic risk in vivo. *J Am Coll Cardiol*. 1994;23:961–969.
12. Zabalgoitia M., Halperin J.L., Pearce L.A., et al. Transesophageal echocardiographic correlates of clinical risk of thromboembolism in nonvalvular atrial fibrillation. Stroke Prevention in Atrial Fibrillation III Investigators. *J Am Coll Cardiol*. 1998;31:1622–1626.
13. Veinot J.P., Harrity P.J., Gentile F., et al. Anatomy of the normal left atrial appendage: a quantitative study of age-related changes in 500 autopsy hearts: implications for echocardiographic examination. *Circulation*. 1997;96:3112–3115.
14. Black I.W., Fatkin D., Sagar K.B., et al. Exclusion of atrial thrombus by transesophageal echocardiography does not preclude embolism after cardioversion of atrial fibrillation. A multicenter study. *Circulation*. 1994;89:2509–2513.
15. Seward J.B., Khandheria B.K., Oh J.K., et al. Critical appraisal of transesophageal echocardiography: limitations, pitfalls, and complications. *J Am Soc Echocardiogr*. 1992;5:288–305.
16. Almeida J., Sepulveda F., Bastos P.T., et al. Transesophageal echocardiography. A critical appraisal. How to avoid false diagnoses. *Rev Port Cardiol*. 1996;15(775–83):1.
17. Klein A.L., Murray R.D., Grimm R.A., et al. Bleeding complications in patients with atrial fibrillation undergoing cardioversion randomized to transesophageal echocardiographically guided and conventional anticoagulation therapies. *Am J Cardiol*. 2003;92:161–165.
18. Khandheria B.K., Seward J.B., Oh J.K., et al. Value and limitations of transesophageal echocardiography in assessment of mitral valve prostheses. *Circulation*. 1991;83:1956–1968.
19. Igarashi Y., Kashimura K., Makiyama Y., et al. Left atrial appendage dysfunction in chronic nonvalvular atrial fibrillation is significantly associated with an elevated level of brain natriuretic peptide and a prothrombotic state. *Jpn Circ J*. 2001;65:788–792.
20. Lip G.Y., Lowe G.D., Rumley A., et al. Fibrinogen and fibrin D-dimer levels in paroxysmal atrial fibrillation: evidence for intermediate elevated levels of intravascular thrombogenesis. *Am Heart J*. 1996;131:724–730.
21. Wang T.L., Hung C.R., Chang H.. Evolution of plasma D-dimer and fibrinogen in witnessed onset of paroxysmal atrial fibrillation. *Cardiology*. 2004;102:115–118.

22. Ageno W., Finazzi S., Steidl L., et al. Plasma measurement of D-dimer levels for the early diagnosis of ischemic stroke subtypes. *Arch Intern Med.* 2002;162:2589–2593.

23. Montaner J., Perea-Gainza M., Delgado P., et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. *Stroke.* 2008;39:2280–2287.

24. Licata G., Tuttolomondo A., Di Raimondo D., et al. Immuno-inflammatory activation in acute cardio-embolic strokes in comparison with other subtypes of ischaemic stroke. *Thromb Haemost.* 2009;101:929–937.

25. Squizzato A., Ageno W.. D-dimer testing in ischemic stroke and cerebral sinus and venous thrombosis. *Semin Vasc Med.* 2005;5:379–386.

26. Lee A.J., Fowkes G.R., Lowe G.D., et al. Determinants of fibrin D-dimer in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol.* 1995;15:1094–1097.

27. Woodburn K.R., Lowe G.D., Rumley A., et al. Relation of haemostatic, fibrinolytic, and rheological variables to the angiographic extent of peripheral arterial occlusive disease. *Int Angiol.* 1995;14:346–352.

28. Robinson V.J., Pineda G.E., Salah A.K., et al. Latex D-dimer signal in in situ femoral vein thrombus in swine and effect of minidose exogenous tissue plasminogen activator bolus. *Chest.* 2005;127:622–629.

29. Nozawa T., Inoue H., Hirai T., et al. D-dimer level influences thromboembolic events in patients with atrial fibrillation. *Int J Cardiol.* 2006;109:59–65.

30. Maehama T., Okura H., Imai K., et al. Usefulness of CHADS2 score to predict C-reactive protein, left atrial blood stasis, and prognosis in patients with nonrheumatic atrial fibrillation. *Am J Cardiol.* 2010;106:535–538.

31. Lip G.Y., Patel J.V., Hughes E., et al. High-sensitivity C-reactive protein and soluble CD40 ligand as indices of inflammation and platelet activation in 880 patients with nonvalvular atrial fibrillation: relationship to stroke risk factors, stroke risk stratification schema, and prognosis. *Stroke.* 2007;38:1229–1237.

32. Blann A.D., Choudhury A., Freestone B., et al. Soluble CD40 ligand and atrial fibrillation: relationship to platelet activation, and endothelial damage/dysfunction. *Int J Cardiol.* 2008;127:135–137.

33. Maehama T., Okura H., Imai K., et al. Systemic inflammation and left atrial thrombus in patients with non-rheumatic atrial fibrillation. *J Cardiol.* 2010;56:118–124.

34. Stein P.D., Hull R.D., Patel K.C., et al. D-dimer for the exclusion of acute venous thrombosis and pulmonary embolism: a systematic review. *Ann Intern Med.* 2004;140:589–602.

35. Kruip M.J., Leclercq M.G., van der Heul C., et al. Diagnostic strategies for excluding pulmonary embolism in clinical outcome studies. A systematic review. *Ann Intern Med.* 2003;138:941–951.

36. Bates S.M., Kearon C., Crowther M., et al. A diagnostic strategy involving a quantitative latex D-dimer assay reliably excludes deep venous thrombosis. *Ann Intern Med.* 2003;138:787–794.

37. Mohamad A., Tanna S., Sharma G., et al. Utility and cost analysis of the D-dimer assay in diagnosis of pulmonary embolism using computerized tomographic angiography in the emergency department. *J Am Coll Cardiol.* 2011;57:E1540.

38. Sadanaga T., Kohsaka S., Ogawa S.. D-dimer levels in combination with clinical risk factors can effectively predict subsequent thromboembolic events in patients with atrial fibrillation during oral anticoagulant therapy. *Cardiology.* 2010;117:31–36.

39. Sadanaga T., Sadanaga M., Ogawa S.. Evidence that D-dimer levels predict subsequent thromboembolic and cardiovascular events in patients with atrial fibrillation during oral anticoagulant therapy. *J Am Coll Cardiol.* 2010;55:2225–2231.

40. Wan H., Wu S., Yang Y., et al. Plasma fibrin D-dimer and the risk of left atrial thrombus: a systematic review and meta-analysis. *PLoS ONE.* 2017;12(2):e0172272.

41. Ikegami Y., Tanimoto K., Inagawa K., et al. Identification of left atrial appendage thrombi in patients with persistent and long-standing persistent atrial fibrillation using intra-cardiac echocardiography and cardiac computed tomography. *Circ J.* 2017;82(1):46–52.

42. Sazonova I.Y., Pondicherry-Harish R., Kadle N., et al. Embolic stroke diagnosed by elevated D-dimer in a patient with negative TEE for cardioembolic source. *J Investig Med High Impact Case Rep.* 2014;2(4):2324709614560907.

43. Sun L., Li Y., Zhang Y.T., et al. A computer-aided diagnostic algorithm improves the accuracy of transesophageal echocardiography for left atrial thrombi: a single-center prospective study. *J Ultrasound Med.* 2014;33(1):83–91.