The association between mean platelet volume and chronic atrial fibrillation and the presence of thrombotic events

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Abstract. Mean platelet volume (MPV), a marker of platelet activation, is a surrogate marker of platelet function and a potential mediator of the association between inflammation and thrombosis. The present retrospective study sought to investigate the association between MPV and the presence of thrombotic events (TEs) in patients with chronic atrial fibrillation (AF). A total of 114 consecutive patients with chronic AF were enrolled from a Chinese hospital. Individuals were divided into three groups: The AF+TE group (n=57, 33.1%), which comprised patients in AF with concomitant TEs; the AF group (n=57, 33.1%), which comprised patients in AF with no identifiable TEs, as confirmed by brain computed tomography, transesophageal echocardiography, or a combination of the two; and a control group (n=58, 33.7%), which consisted of patients who were in sinus rhythm. MPV, high-sensitivity C-reactive protein (hsCRP), D-dimer and the left atrial diameter (LAD) were analyzed in the 172 participants. The MPV level of patients in the AF+TE group was significantly higher than that of patients in the AF and control groups (P<0.05). In the correlation analysis, MPV levels were found to be positively correlated with LAD, D-dimer concentrations and hsCRP levels in patients with AF (r=0.960, P<0.05; r=0.896, P<0.05; and r=0.924, P<0.01, respectively). In the receiver operating characteristic curve analysis, the value for MPV levels required to detect TEs with a sensitivity of 77.5% and specificity of 78% was 10.5 femtoliter (fl). A high MPV level (>10.5 fl) was significantly associated with the occurrence of TEs (odds ratio, 3:1; 95% confidence interval, 1.6-5.1; P=0.000). The results of the present study suggest that an additional biomarker, MPV, has a predictive value for the presence of TEs in patients with AF. MPV may be a potential mediator between inflammation and thrombosis.

Introduction

Atrial fibrillation (AF) is one of the most commonly observed arrhythmias in clinical practice. Epidemiological surveys have shown that its incidence is increasing annually (1,2). Patients with AF, in particular those with a disease duration >6 months (chronic AF), are at an increased risk of stroke and other thrombotic events (TEs), which are significant causes of mortality and morbidity in these patients (1,2). Although the treatment options for AF are continually improving, the prevention of TEs remains one the primary goals for the long-term treatment of patients with chronic AF. Therefore, improving the clinical risk stratification of TEs, early evaluation of the risk of developing TE among patients with AF, and the development of individualized antiplatelet or anticoagulant therapies are important strategies. However, the development of TEs involves a number of factors. Recent studies in this area have hypothesized that platelet activation is one of the most important factors. Activated platelets have larger volumes and contain larger quantities of vasoactive substances and prothrombotic factors. Therefore, mean platelet volume (MPV) is a marker of platelet activation and function, and may also be a response to inflammation and thrombosis (3,4,22). Recent studies (5,6,22) have shown that smoking, hypertension, diabetes mellitus, dyslipidemia and abdominal obesity are associated with a raised MPV. An increased MPV is associated with overall cerebrovascular and cardiovascular mortality rates, including those due to myocardial infarction, transient ischemic attack (TIA) and cerebral infarction. However, few studies have been conducted on the association between MPV and AF, or their effect on the presence of TEs. The present study aimed to evaluate the association between MPV and chronic AF, and their effect on the presence of TEs. To the best of our knowledge, the association between MPV and inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP)
or markers of thrombosis, such as D-dimer, in patients with chronic AF has not been studied to date.

Patients and methods

Study design and patients. Consecutive patients who were hospitalized at the Department of Cardiology and Neurology of the Third Xiangya Hospital at Central South University (Changsha, China) were referred to our center between November 2012 to July 2014. A total of 172 consecutive participants (males, 51.2%; females, 48.8%; mean age, 67.05±9.35) were enrolled. Study individuals were divided into three groups: The AF+TE group (n=57, 33.1%), which comprised patients in AF complicated by the presence of TEs; The AF group (n=57, 33.1%), which comprised patients in AF with no identifiable TEs, as confirmed by brain computed tomography (CT), transesophageal echocardiography (TEE), ultrasonic cardiogram, magnetic resonance imaging with diffusion weighted imaging (MR1+DWI), pulmonary vein imaging, or a combination of these techniques; and a control group (58, 33.7%), which comprised patients in sinus rhythm. The AF+TE group included 45 patients with cerebral infarctions, seven patients with left atrium or left atrial appendage thrombosis, three patients with both conditions, and two patients with cerebral infarction and a venous thrombosis in a lower extremity. These 114 patients were confirmed to be in chronic AF by electrocardiography on at least two separate occasions prior to recruitment and met the diagnostic criteria used in the 2011 ACCF/AHA/HRS Guidelines for the management of patients with AF (1).

All patients with cerebral infarction and TIA had symptoms of cerebral ischemia for the first time, occurring within 6 months. The exclusion criteria were i) the presence of other organic heart diseases, such as myocardial infarction, rheumatic valvular heart disease, valvular heart disease, dilated cardiomyopathy, chronic heart failure (grade III or IV according to the New York Heart Association heart failure classification) (7) and pulmonary heart disease; ii) AF due to hyperthyroidism or alcoholic cardiomyopathy; iii) hemorrhagic disease, such as cerebral hemorrhage and gastrointestinal hemorrhage; iv) hematological disease, such as severe anemia, thrombocytopenia or diseases of hematopoiesis; or v) others factors, such as hepatic insufficiency (alanine transaminase >3x normal upper limit), renal insufficiency (glomerular filtration rate <30 ml/min/1.73 m²), an acute or chronic systemic inflammatory state, connective tissue disease, autoimmune disease or malignant tumors. Our Ethics Committee approved the present study and all patients provided informed consent.

Collection of clinical information. Patient gender, age, smoking history, medication, as well as history of hypertension, diabetes mellitus, hematological disease and other related diseases, were recorded.

Blood collection and measurement of biomarkers. Fasting blood samples of the patients were collected within 2 h of admission. A total of 2-3 mL venous blood was collected in an EDTA-K2 tube (Sysmex Corporation, Kobe, Japan), and MPV and platelet counts were analyzed using a Sysmex XE5100 hematology analyzer (XE5100; Sysmex Corporation) within 2 h of sample collection. The instrument accompanied the reagents: Hemolytic agent, dilution solution and washing solution. The manipulation was performed according to the manufacturer's instructions. The normal values for MPV in our laboratory range from 7.6 to 13.2 femtoliter (fl).

A total of 2 mL non-coagulated fasting blood samples were collected, and low-density lipoprotein-cholesterol, high sensitivity C-reactive protein (hsCRP) and creatinine were measured using a Hitachi 7600-020 automatic biochemistry analyzer (Hitachi Ltd, Tokyo, Japan). Serum hsCRP was determined using immune-enhanced nephelometry. Hitachi provided all reagents. D-dimer and fibrinogen (Fbg) quantification was performed using the Sysmex CA-1500 via latex-enhanced nephelometry. Sysmex Corporation provided all reagents.

Iconography analysis. Specialist physicians determined left ventricular ejection fraction using standard methods with an HP-5500 color Doppler ultrasound scanner at a 25-MHz probe frequency. The left atrial diameter (LAD) and the presence of atrial thrombosis were examined using TEE. Cloudy shadows in the left atrium or left atrial appendage were assumed to represent thrombi.

TIA and cerebral infarction were determined according to the 2013 AHA/ASA guidelines for the early management of patients with acute ischemic stroke (8) and confirmed using brain CT (64-detector CT; Siemens, Munich, Germany), MR1+DWI (Siemens) and cerebral angiography at our hospital.

Calculation of CHADS2 score (9) and CHADS2 scoring (10). All 114 patients who met the inclusion criteria underwent CHADS2 scoring, where one point was assigned for congestive heart failure, hypertension, age >75 years or diabetes mellitus, and two points indicated cerebral infarction or a history of TIA. The CHA2DS2-VASc score was calculated based on a point system in which two points were assigned for a history of stroke or TIA, or age ≥75 years. One point was assigned for age between 65 and 74 years; a history of hypertension, diabetes, recent cardiac failure or vascular disease (myocardial infarction, complex aortic plaque or peripheral arterial disease); or female gender.

Statistical analysis. Statistical analysis was performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables with a normal distribution were expressed as the mean ± standard deviation, and group comparisons were performed by one-way analysis of variance (the F-test) and Kruskal-Wallis tests, depending on whether the data was normally or non-normally distributed, respectively. Non-normally distributed data are presented as the median (interquartile range) and discrete variables are presented as frequencies and percentages. Group comparisons were performed using the χ² test, Fisher's exact test or Wilcoxon rank-sum test. Correlation analyses of non-normally distributed data were performed using Spearman's rank correlation. To assess for significant differences between the three groups, Tukey's post hoc test was used to determine the intergroup differences, using log-transformed data where appropriate. The diagnostic performance of each indicator was evaluated.
using the receiver operating characteristic (ROC) curve. The correlation of clinical variables associated with TEs were analyzed using univariate and multivariate logistic regression analysis. The P-value for entry stepwise multivariate linear regression analysis was set at 0.05, while the P-value for removal was set 0.10. P<0.05 was considered to indicate a statistically significant difference.

**Results**

Baseline clinical characteristics, laboratory examination results and MPV levels are shown in Tables I and II, and Fig. 1. The levels of hsCRP, Fbg and D-dimer in the AF+TE group were significantly higher than those in the AF and the control groups. In addition, patients in the AF+TE group exhibited a
Figure 1. MPV values in the three groups. Analysis was conducted using analysis of variance, F=48.260, P<0.001. Intergroup differences were assessed using Tukey’s test. *P<0.05 between control and AF groups, †P<0.05 between control and AF+TE groups and ‡P<0.05 between AF and AF+TE groups. AF, atrial fibrillation; TE, thrombotic event; MPV, mean platelet volume.

Figure 2. (A) Scatter diagram of MPV levels and LAD concentration in patients with AF. Regression equation between MPV levels and LAD: ŷ=−0.044+3.589X (R²=0.858, P<0.05). (B) Scatter diagram of MPV levels and D-dimer concentration in patients with AF. Regression equation between MPV levels and D-dimer concentration: ŷ=−35.012+80.996X (R²=0.856, P<0.05). (C) Scatter diagram of MPV levels and hsCRP concentration in patients with AF. Regression equation between MPV levels and hsCRP: ŷ=−1.213+0.341X (R²=0.762, P<0.05). MPV, mean platelet volume; LAD, left atrial diameter; AF, atrial fibrillation; hsCRP, high-sensitivity C-reactive protein.
higher MPV and left atrial volume than patients in the other two groups (Fig. 1).

Spearman’s correlation coefficients between MPV levels, LAD, D-dimer levels and hsCRP levels, among patients with AF were r=0.960 (P<0.05), r=0.896 (P<0.05) and r=0.924 (P<0.01), respectively. MPV was positively correlated with LAD, D-dimer and hsCRP \[\text{the standardized partial regression coefficient } (\beta) \text{ was 0.926, 0.905 and 0.762, respectively; the correlation coefficient (b) was 3.589, 80.966 and 0.341, respectively}\]. Using MPV as the independent variable, the regression equations between MPV levels, and LAD, D-dimer and hsCRP are shown in Fig. 2.

The ROC analysis demonstrated a cut-off value of MPV>10.5 fl to predict the presence of TEs. At this level, sensitivity was 77.5\% [95\% confidence interval (CI), 58.7-91.2] and specificity was 78\% (95\% CI: 60.2-89.5). Area under the curve (AUC)=0.781; 95\% CI, 0.706-0.855; P=0.005. The value for LAD required to detect TEs with a sensitivity of 79\% (95\% CI, 52.6-91.7) and a specificity of 78.5\% (95\% CI, 54.7-90.1) was 36.5 mm. AUC=0.824; 95\% CI, 0.701-0.868; P=0.018 (Fig. 3A and B).

Univariate analysis demonstrated that age ≥75 years, MPV >10.5 fl, LAD >36.5 mm, hsCRP >2.5 mg/l and D-Dimer >306 µg/l, as well as CHA2DS2-VASc score ≥2 were significantly correlated with the presence of TEs (Table III). These variables were entered into a multivariate logistic regression model and MPV was found to be significantly associated with the presence of TEs (odds ratio 3.1; 95\% CI, 1.6-5.1; P=0.000; Table III).

Discussion

As an indicator measured in routine blood samples, MPV is a low-cost, rapid and low-risk test. Bath et al. (11) studied 3,134 patients who had experienced a stroke, with an average follow-up time of 3.9 years. The authors showed that MPV levels were positively correlated with the risk of stroke. When MPV levels increased by 1 fl, the risk of stroke increased by 11\%.

Table III. Univariate and multivariate analysis of risk factors for thrombotic events in chronic atrial fibrillation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>P-value</td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Age ≥75 years</td>
<td>2.9</td>
<td>1.7-4.7</td>
<td>0.000</td>
<td>2.1</td>
<td>1.2-3.7</td>
<td>0.011</td>
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<td>Male gender</td>
<td>1.4</td>
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<td>-</td>
<td>-</td>
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<td>Smoking</td>
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<td>DM</td>
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<td>0.74-6.81</td>
<td>0.106</td>
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<td>-</td>
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<td>Hypertension</td>
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<td>0.31-2.87</td>
<td>0.844</td>
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<td>-</td>
<td>-</td>
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<td>Using warfarin</td>
<td>1.55</td>
<td>1.02-2.35</td>
<td>0.039</td>
<td>1.04</td>
<td>0.96-1.13</td>
<td>0.281</td>
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<td>MPV &gt;10.5 fl</td>
<td>3.70</td>
<td>2.2-6.3</td>
<td>0.000</td>
<td>3.1</td>
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<td>0.000</td>
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<tr>
<td>LAD &gt;36.5 mm</td>
<td>3.3</td>
<td>1.8-5.7</td>
<td>0.000</td>
<td>3.2</td>
<td>1.8-5.4</td>
<td>0.000</td>
</tr>
<tr>
<td>hsCRP &gt;2.5 mg/l</td>
<td>2.9</td>
<td>1.7-4.8</td>
<td>0.000</td>
<td>2.1</td>
<td>1.2-3.7</td>
<td>0.011</td>
</tr>
<tr>
<td>D-dimer &gt;306 µg/l</td>
<td>2.7</td>
<td>1.5-4.5</td>
<td>0.000</td>
<td>1.9</td>
<td>1.1-3.4</td>
<td>0.039</td>
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<td>CHA2DS2–VASc score≥2</td>
<td>4.21</td>
<td>1.4-9.6</td>
<td>0.039</td>
<td>5.33</td>
<td>1.3-14.7</td>
<td>0.011</td>
</tr>
</tbody>
</table>

CI, confidence interval; DM, diabetes mellitus; MPV, mean platelet volume; LAD, left atrial diameter; hsCRP, high-sensitivity C-reactive protein; \(\beta\), standardized regression coefficients. The P-value for entry stepwise multivariate linear regression analysis was set at 0.05, and the P-value for removal was set 0.10. Adjusted R\(^2\)=0.578, P<0.001.

Figure 3. (A) ROC curve of MPV. (B) ROC curve of LAD. ROC, receiver operating characteristic; MPV, mean platelet volume; LAD, left atrial diameter.
(95% CI, 3.19%). The results of the current study showed that the difference among the three groups of patients was significant (P<0.05). Specifically, pairwise comparisons between these groups revealed a significant difference (P<0.05). These results are similar to those of Yuce et al (12), which indicates that the MPV levels of patients in AF are higher than those of individuals in sinus rhythm. Patients in the AF+TE group were in a hypercoagulable state. Thus, their platelet volumes were larger, and the platelet activity and aggregation were stronger. Recent studies (13) have shown that platelets with increased volume exhibit a significantly increased expression of platelet surface CD62P and CD63 molecules. As the platelet volume increases, the dense granules and α-granules within these cells also increase. The quantity of 5-hydroxytryptamine and β-thromboglobulin released by the dense granules, and the coagulation factors and von Willebrand (vW) factor released by the α-granules, are also increased, which may facilitate the formation of thrombi in blood vessels and continue to increase the volume of existing thrombi, thereby eventually occluding the affected blood vessels. In addition, platelets with large volumes have a relatively larger contact surface. Therefore, they are able to rapidly interact with adenosine diphosphate, the collagen receptor and the vW factor receptor on cell membranes. Furthermore, platelets with increased volumes express higher quantities of adhesion molecules, such as P-Selectin, which increases the aggregation and adhesion function of platelets. AF in combination with thrombosis, may consume platelets and cause a transient decrease in the numbers of these cells (14). Through α-granule proteins, this decrease in platelets may stimulate the proliferation of megakaryocytes in the bone marrow, increase the conversion rate of platelets and increase the number of platelets with large volumes in the peripheral blood via negative feedback (15). The current study also showed that the total platelet count of patients in the AF+TE group was higher than that in patients in the AF group, which may be due to the high turnover rate of platelets or the transient reduction in platelets among patients with AF complicated by TEs. These results are in accordance with those of Varol (16).

Alhaji et al (17) calculated the left atrial volume index by measuring LAD, and found that it was an independent risk factor for stroke. Beinart et al (18) performed studies investigating the prevention of stroke in patients with AF, and demonstrated that the left atrial appendage dimension predicted the development of thromboembolic complications. Atrial wall movement disorders, induced by the expansion of the left atrium, cause derangement of the direction of atrial blood flow, decreased flow speed and blood stasis, and frequent collision between platelets, thereby producing abnormal hemodynamic characteristics, injuring the myocardium and vascular endothelial cells, activating the exogenous coagulation system via the exposure of subendothelial collagen, and activating platelets. Therefore, Providencia et al (19) hypothesized that the increase in MPV is associated with the expansion of the left atrium and blood stasis. The above factors may result in the overactivation of platelets and enhance the function of coagulation substances. The coagulation factor 1, Fbg, is directly involved in the coagulation process, and is associated with platelet aggregation. An increased level of this molecule in the plasma is therefore an important risk factor for the development of thromboembolism. D-dimer is produced by the fibrinolysis of cross-linked fibrin proteins. An increase in D-dimer concentration also indicates an enhancement of coagulation. Both molecules are important indicators of a prothrombotic state (PTS) (20).

In addition to platelet activation and the hemodynamic response, the development of TEs is also associated with inflammation. Although the mechanism of inflammation in AF remains unclear, researchers have found that CRP and interleukin-6 (IL-6) appear to be involved in PTS (21). The results of a study by Gasparyan et al (22) suggested a correlation between an increase in MPV and the risk of thrombosis. Specifically, thrombopoietin and high levels of inflammatory factors, such as IL-1, IL-6 and tumor necrosis factor-α, may be involved in thrombosis or a PTS. Conway et al (23) found increased plasma levels of IL-6 and CRP, and raised plasma viscosity in patients with AF compared with healthy controls, and showed that plasma IL-6 levels were significantly higher among patients with AF at high risk of stroke. Further research (24,25) has demonstrated that an increased CRP, and an activated platelet and coagulation/fibrinolytic system, are involved in the pathogenesis of cerebral infarction and are positively correlated with known risk factors for the development of emboli in patients with AF. Furthermore, CRP levels were increased in patients with AF who had a history of embolism. A number of recent studies have confirmed that statins regulate lipids and stabilize plaques, and also exhibit anti-inflammatory and anti-oxidative functions (26). The measurement of LAD, D-dimer and hsCRP requires additional time in the assessment of patients with AF. Furthermore, the procedure is complicated, it incurs a relatively high cost and the controllability of the results is undesirable. However, the development of TEs appears to be correlated with MPV levels. Therefore, MPV may be a useful auxiliary indicator of the risk of developing TEs in patients with AF.

A number of studies have investigated MPV levels in stroke patients, such as that conducted by Turfan et al (27), and have found that high MPV values are associated with prognosis in patients who have had a stroke. Numerous studies (17,18) have shown that an increase of LAD is associated with the development of TEs. Similar results were found in the present study: Patient in the AF+TE group exhibited higher MPV values than those in the control group and the AF group. In patients with elevated MPV values (>10.5 fl), the risk of TEs was increased by ~three-fold. The risk of TEs is also increased in patients with a high MPV value, in particular in those ≥75 years, in patients with left atrial enlargement (LAD >36.5 mm) and in those with a CHA2DS2-VASc score ≥2. However, the current guidelines for primary risk assessment to prevent stroke in patients with non-valvular AF, that is, CHADS2 or CHA2DS2-VASc scoring, do not include LAD or MPV as risk factors. The results of the current study suggest that additional markers, such as MPV and LAD, have a predictive value for assessing the risk of TEs in patients with AF.

In conclusion, MPV levels were associated with AF and TEs. The measurement of MPV is simple, convenient and inexpensive. Therefore, MPV may be useful for indicating the risk of TE in patients with AF, and for improving the stratification of risk factors. MPV detection may also be used to guide the prescription of anticoagulation treatments in patients with AF.
The present study had a small sample size and was also a retrospective study. In addition, the effects of antiplatelet and anticoagulant drugs on MPV remain uncertain. Thus, it may be that MPV levels increased following the occurrence of TE events, possibly as a result of the initiation of different medications. Furthermore, the primary limitation was the long period (2012-2014) over which patients were recruited, which diminishes the significance of conclusions. Therefore, additional clinical studies are required in order to assess the usefulness of MPV as a predictive factor for the development of TEs in patients with AF.

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References


