Increased mean platelet volume in rheumatic mitral stenosis: assessment of clinical and echocardiographic determinants

Mehmet İleri, Selçuk Kanat, Havva Tuğba Gürsoy, Deniz Şahin, Pınar Türker Bayır, Gökhan Şahin, Funda Başyigit, Özgül Uçar Elalıms, Ümit Güray
Ankara Numune Education and Research Hospital, Ankara, Turkey

Abstract

Background and aim: The aim of this study was to investigate mean platelet volume (MPV) in patients with rheumatic mitral stenosis (RMS) and to define the determinants of a possible platelet activation reflected as platelet volume enlargement.

Methods: Peripheral venous plasma value of MPV was measured in 84 consecutive patients (16 men, 68 women; mean age ± SD = 44 ± 13 years) with RMS who had no left atrial thrombus by transoesophageal echocardiography. The control group consisted of 32 healthy subjects (nine men, 23 women; mean age ± SD = 38 ± 7 years).

Results: The patients had significantly higher MPV values (mean ± SD = 10.07 ± 0.58 fL) compared to the healthy subjects (mean ± SD = 8.15 ± 0.60 fL, p < 0.001). Among many clinical and echocardiographic variables, left atrial spontaneous echo contrast-positivity (beta = 0.426, p < 0.001) and severe mitral regurgitation (beta = 0.577, p < 0.001) appeared as significant predictors of platelet enlargement in RMS in multiple linear regression analysis.

Conclusions: Patients with RMS have increased platelet activity reflected as elevated MPV; and the coexistence of severe mitral regurgitation and presence of left atrial spontaneous echo contrast are determinants of this increment.

Key words: mean platelet volume, rheumatic mitral stenosis

INTRODUCTION

Thromboembolism is a frequent complication in the course of rheumatic mitral stenosis (RMS). In addition to coagulation factors, platelets may play an additional role in thromboembolic complications of RMS. Using different in vivo haemostatic markers, some investigators have reported increased platelet activity in patients with RMS [1, 2]. Platelets are formed from the cytoplasm of megakaryocytes, their precursor cells, which are present in the bone marrow [3]. Megakaryocytes are the largest (50–100 µm) cells in the bone marrow. To arrange and release platelets, megakaryocytes become polyploid and then undergo a maturation process in which the bulk of their cytoplasm is packaged into multiple long processes called proplatelets. A megakaryocyte may extend to 10–20 proplatelets, each of which starts as a blunt protrusion that over time elongates, thins, and branches repeatedly. Platelets form selectively at the tips of proplatelets. During development, platelets receive their granule and organelle content as streams of individual particles transported from the megakaryocyte cell body. Human platelets survive 7–10 days once released into the bloodstream. Thrombopoietin functions as the major regulator that promotes the growth and development of megakaryocytes from their haematopoietic stem cell precursors.

Mean platelet volume (MPV) is a potentially useful marker of platelet activity and a quick and easy determinant of thrombotic risk. Larger platelets are metabolically more active and have greater prothrombotic potential [4–6]. The few studies in the literature that have measured platelet volume indices in RMS have reported enlarged platelets in these patients [7–11]. However, the precise determinants of increased MPV in RMS among many clinical and echocardiographic variables interacting with each other have not been clarified.
Therefore the aim of this study was to investigate MPV in patients with RMS and to define the determinants of a possible platelet activation reflected as platelet volume enlargement.

**METHODS**

**Study and control groups**

Eighty four consecutive patients (16 men, 68 women; mean age ± standard deviation [SD] = 44 ± 13 years) with RMS admitted to our department were included in this study. Twenty eight of them were in sinus rhythm and 56 were in atrial fibrillation (AF). Patients with left atrial thrombus, rheumatic activation, diabetes mellitus, systemic hypertension, coronary artery disease, renal or hepatic dysfunction or a history of thromboemboli, and those who had used any anti-platelet or anticoagulant drug within the two weeks prior to blood sampling were excluded from the study. The control group consisted of 32 healthy subjects (nine men, 23 women; mean age ± SD = 38 ± 7 years), who had also not used any drug that could interfere with the platelet and coagulation system within the previous two weeks.

No patient was stopped from taking a prescribed anticoagulant drug in order to make him/her fit the inclusion criteria. The study cases with AF were generally newly presenting patients. They were mostly sent from rural areas with poor socio-economic conditions and medical care, which was possibly why they were not on anti-platelet or anticoagulant prophylaxis. The study protocol was approved by our institutional ethical committee, and informed consent was given by all patients and control subjects.

**Echocardiography**

Transthoracic two-dimensional and colour flow Doppler echocardiography were performed in all patients using ultrasound equipment (Toshiba SSH160A) with commercially available 2.5-MHz transducers. Left atrial diameter was measured by M-mode echocardiography and mitral valve area was calculated by the Doppler pressure half-time method. Mean transmitral diastolic gradients were also measured by Doppler studies. Colour-flow Doppler was used to detect the presence of mitral regurgitation (MR) and its severity was assessed by a semi-quantitative method. The per cent ratio of the maximal flow disturbance produced by the MR jet to the left atrial area was measured and graded as previously described [12]: < 20% was considered as mild; 20–40% as moderate; and > 40% as severe. Pulmonary artery systolic pressure was calculated with the help of continuous-wave Doppler studies using the Bernoulli equation. Transoesophageal echocardiography was performed on all patients using a 5-MHz phased-array transducer just before the blood samples were taken to confirm the absence of any thrombus in the left atrial cavity or appendage and to assess the presence of left atrial spontaneous echo contrast (LASEC). All echocardiographic studies were recorded on super VHS videotape for subsequent independent review and analysed by two echocardiographers who were unaware of the clinical status of the patients. If there was disagreement, a third examiner was consulted.

**Blood sampling**

Blood samples at hospital admission were drawn from the antecubital vein by careful venipuncture using a 21-gauge needle attached to a sterile syringe without stasis between 8:00 and 10:00 am. Platelet volume indices were measured in blood samples collected in dipotassium ethylenediaminetetraacetic acid (EDTA) containing tubes by flow cytometry in an automated blood cell counter (Sysmex, XT-2000i) immediately within 60 min after sampling.

**Statistical analysis**

Student’s t-test was used to compare the MPV values between patients and control subjects. The effect of each echocardiographic or clinical variable on MPV values was analysed with Student’s t-test or Pearson’s bivariate correlation analysis, depending on whether categorical or continuous variables were compared, respectively. Clinical and echocardiographic variables which were correlated with MPV values in Student’s t-test and Pearson’s bivariate correlation analysis were included in multiple linear regression analysis to detect the determinants of possible platelet enlargement in the patient group. Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations. P values lower than 0.05 were considered to be statistically significant.

**RESULTS**

Baseline clinical characteristics and echocardiographic variables of patients and control subjects are shown in Table 1.

**Simple statistical calculations**

The patients had significantly higher MPV values (mean ± SD = 10.07 ± 0.58) compared to the control subjects (mean ± SD = 8.15 ± 0.60, p < 0.001) (Table 2).

Among categorical variables (rhythm, LASEC, degree of MR, New York Heart Association [NYHA] class), the MPV values were higher in every echocardiographic or clinical subgroup of patients compared to the control group (Table 2; Figs. 1–4). Significantly higher MPV values were observed in patients with severe MR, and in LASEC-positive-cases compared to those with mild to moderate MR and LASEC-negativity respectively (Table 2). Although mean ± SD values of MPV were higher in patients with AF and a higher NYHA class compared to the matched subgroups, this did not reach statistical significance. Among continuous variables (left atrial diameter, mitral valve area, pulmonary artery pressure and peak transmitral gradient), only left atrial diameter was significantly correlated with MPV in RMS patients.
Increased systemic and regional coagulation activity in patients with RMS and AF [13–15]. We have shown a hypercoagulable state in LASEC-positive RMS patients in sinus rhythm [16]. Previous studies have also demonstrated platelet activation, evaluated by measuring the secretory substances of platelets (i.e. platelet factor-4 and beta-thromboglobulin) that occur in the peripheral blood of patients with RMS [1, 2]. In one of these reports, left atrial platelet activity had a significant relationship with the severity of mitral stenosis [1]. Other authors have shown that the degree of platelet activation in RMS was positively correlated with the severity of MR [17].

Although a wide variety of different methods measuring platelet activity has been reported, most of them remain a research tool that are yet to be included in routine clinical decision-making. Among these, platelet volume indices are a group of parameters which are inexpensive, routinely measured by automated cell counters, and easy to interpret.

**DISCUSSION**

Systemic haemostatic abnormalities have been found in cross-sectional studies of patients with RMS. Using different *in vivo* haemostatic markers, many investigators have reported increased systemic and regional coagulation activity in patients with RMS and AF [13–15]. We have shown a hypercoagulable state in LASEC-positive RMS patients in sinus rhythm [16]. Previous studies have also demonstrated platelet activation, evaluated by measuring the secretory substances of platelets (i.e. platelet factor-4 and beta-thromboglobulin) that occur in the peripheral blood of patients with RMS [1, 2]. In one of these reports, left atrial platelet activity had a significant relationship with the severity of mitral stenosis [1]. Other authors have shown that the degree of platelet activation in RMS was positively correlated with the severity of MR [17].

**Multiple linear regression analysis**

Clinical and echocardiographic variables which were correlated with MPV values in Student’s t-test (LASEC, degree of MR) and Pearson’s bivariate correlation analysis (left atrial diameter) were included in multiple linear regression analysis to detect the determinants of possible platelet enlargement in the patient group. Beta and p values for each independent factor in multiple linear regression analysis are summarised in Table 3. In multiple linear regression analysis, both LASEC-positivity (beta = 0.426, p < 0.001) and severe MR (beta = 0.577, p < 0.001) appeared as independent predictors of platelet enlargement (Table 3).
Mean platelet volume, being a practical and prognostically important biomarker of platelet reactivity, is the most validated and prominent of these. Larger platelets are metabolically more active and have greater prothrombotic potential with increased thromboxane-A2 and B2 per unit volume and glycoprotein IIb/IIIa receptor expression [18, 19]. Larger platelets are denser and contain more alpha granules which can release prothrombotic substances including platelet factor-4, P selectin and platelet-derived growth factor [18, 19]. Evidence derived from both retrospective and prospective studies suggests that increased MPV is an important determinant of thrombotic risk in predominantly arterial disorders [20–23]. Prospective studies, to a certain extent, support the value of elevated MPV in predicting recurrent myocardial infarction and death in the post infarction period [24], impaired myo-

Table 2. Effect of various clinical and echocardiographic variables on MPV in patients with RMS

<table>
<thead>
<tr>
<th>Comparison of MPV in patient subgroups of categorical variables and control subjects (Student’s t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV [fL]</td>
</tr>
<tr>
<td>------------------------------------</td>
</tr>
<tr>
<td>Patients (n = 84) 10.07 ± 0.58*</td>
</tr>
<tr>
<td>Control group (n = 32) 8.15 ± 0.60</td>
</tr>
<tr>
<td>Rhythm:</td>
</tr>
<tr>
<td>Atrial fibrillation (n = 56) 10.19 ± 0.57* 0.08</td>
</tr>
<tr>
<td>Sinus rhythm (n = 28) 9.84 ± 0.53*</td>
</tr>
<tr>
<td>LASEC:</td>
</tr>
<tr>
<td>Positive (n = 39) 10.30 ± 0.57* 0.01</td>
</tr>
<tr>
<td>Negative (n = 45) 9.88 ± 0.52*</td>
</tr>
<tr>
<td>Degree of mitral regurgitation:</td>
</tr>
<tr>
<td>Mild + moderate (n = 70) 9.95 ± 0.52* &lt; 0.001</td>
</tr>
<tr>
<td>Severe (n = 14) 10.68 ± 0.50*</td>
</tr>
<tr>
<td>NYHA class:</td>
</tr>
<tr>
<td>1 + 2 (n = 45) 9.95 ± 0.50* 0.06</td>
</tr>
<tr>
<td>3 + 4 (n = 39) 10.21 ± 0.64*</td>
</tr>
</tbody>
</table>

Correlations between each continuous variable and MPV in RMS (Pearson’s bivariate correlation analysis)

<table>
<thead>
<tr>
<th>Correlation coefficient (r)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left atrial diameter [cm]</td>
<td>0.280 0.01</td>
</tr>
<tr>
<td>Mitral valve area [cm²]</td>
<td>–0.23 0.84</td>
</tr>
<tr>
<td>Pulmonary artery pressure [mm Hg]</td>
<td>0.051 0.62</td>
</tr>
<tr>
<td>Peak transmitral gradient [mm Hg]</td>
<td>0.149 0.17</td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>0.034 0.43</td>
</tr>
<tr>
<td>Total cholesterol [mg/dL]</td>
<td>0.012 0.72</td>
</tr>
</tbody>
</table>

*p < 0.001 vs. control group; *between patient subgroups; MPV — mean platelet volume; RMS — rheumatic mitral stenosis; LASEC — left atrial spontaneous echo contrast; NYHA — New York Heart Association; Results are expressed as mean ± standard deviation

Figure 1. Relation between mean platelet volume and rhythm (atrial fibrillation and sinus) in patients with rheumatic mitral stenosis and control subjects

Figure 2. Relation between mean platelet volume and presence of left atrial spontaneous echo contrast (LASEC) in patients with rheumatic mitral stenosis and control subjects

Figure 3. Relation between mean platelet volume and severity of mitral regurgitation (MR) in patients with rheumatic mitral stenosis and control subjects
cardiac reperfusion after thrombolysis [25], or percutaneous coronary interventions in ST-elevation myocardial infarction [26, 27] and restenosis after stenting in angina pectoris [28].

There are a few studies in the literature investigating platelet volume indices in RMS [7–11]. Most have reported increased MPV in this situation. One of them suggested that larger platelets in RMS were associated with the presence of LASEC [9]. However, the precise determinants of increased MPV in RMS among many clinical and echocardiographic variables interacting with each other have not been clarified in the literature. Our patients with RMS have increased MPV compared to control subjects. This data was consistent with the results of the previous reports. Evidence from several studies has shown that shear stresses in turbulent flow as a result of stenotic mitral valve induce platelet activation. The second mechanism contributing to increased platelet activity might be the presence of endothelial dysfunction in RMS.

In multivariate analysis, LASEC positivity and severe MR appeared as independent predictors of platelet activation reflected as enlarged platelets in RMS. Associations of LASEC positivity and severe MR with increased MPV in these patients have not been documented previously. Three mechanisms may contribute to the increased systemic platelet activity associated with the presence of severe MR: (1) the washing effect of severe MR dislodges pre-formed left atrial microthrombi, and subsequent embolisation of them leads to systemic activation of platelets; (2) severe MR is probably a manifestation of a long-lasting disease process and, in this group of patients, multiple minute risk factors of platelet activation (e.g. AF; increased left atrial size, heart failure) accumulate; and (3) although MR decreases left atrial stasis, the regurgitant jet itself exhibits a turbulent blood flow in the left atrial cavity and thereby leads to platelet activation [29–32]. The activated platelets may then adhere and aggregate on the abnormal mitral valvular surface to form a platelet-fibrin thrombus on the leaflet, potentially resulting in the thromboembolic events.

Spontaneous echo contrast is the presence of smoke-like echoes with a characteristic swirling motion of blood detected by echocardiography and is most commonly seen in the left atrium, particularly in conditions of AF and/or mitral stenosis. Both the presence and increased severity of LASEC are risk factors for the development of left atrial thrombus formation and an important indicator of potential systemic embolism [33, 34]. According to our data, the presence of LASEC appeared as an independent predictor of enlarged platelets in RMS patients. In the pathophysiology of LASEC, several mechanisms have been reported. It occurs from aggregation in the cellular component of the blood in situations with blood stasis and low velocity of bloodstream [33]. Echogenicity of blood in LASEC occurred with erythrocyte aggregation in the plasma. A previous study found evidence of increased platelet aggregation in patients with LASEC and resolution after anti-platelet therapy [35]. These findings suggest that LASEC may reflect not only stasis of blood in left atrium, but may also be associated with blood characteristics including erythrocytes and platelets. In LASEC positive patients, at a low blood flow rate, platelet reactivity and adhesion increase as the haematocrit value is increased.

Therefore it is reasonable to observe that there is a further activation of the returned circulating platelets within red cell aggregates in the left atrium as a result of stasis. In a previous study, the authors found a profound increase in left atrial platelet and leukocyte activation in patients with LASEC, as indicated by increased surface antigen expression on platelets and leukocytes [36]. Furthermore, as a result of an increased activation status, they found a higher degree of platelet leukocyte aggregation in the left atrium of patients with LASEC. These results suggested the hypothesis that the origin of LASEC could be the platelet aggregates. Since larger platelets are more aggregable, one could argue that LASEC in RMS is a result of, rather than a cause of, MPV elevation in particular patients in our study group.

**Limitations of the study**

The present study has some limitations. Firstly, physiologic assays such as platelet aggregation, adherence or secretion were
not performed in the study group, and therefore increases in platelet volume indices may not necessarily reflect activation of platelet physiologic function. However, in our study, we aimed to investigate particularly the MPV values. Secondly, it was a single centre experience with a modest sample size. Thirdly, this was a cross-sectional study, and we did not follow up patients; therefore we were not able to determine whether increased MPV in any subgroup of RMS patients predicts future thromboembolic events. Fourthly, the method of platelet volume measurement may be a possible limitation. The variability in the timing and methods of blood sample preparation and the type and calibration of particle counters may have had a significant impact in MPV measurements. A previous study revealed that platelets swelled until 120 min in EDTA and until 60 min in citrate. There was a plateau of swelling after 120 min in EDTA and after 60 min in citrate. It was crucial to wait until stability of swelling had been reached. Therefore they recommended that the optimal measuring time should be 120 min after venipuncture if a tube containing EDTA was used [37]. In another report, there was an increase in MPV in EDTA within 30 min with a mean increase of 0.7 fL, corresponding to a 7.9% increase over baseline. MPV however continued to increase over the 24-h period (overall 13.4% increase) with the majority of this increase by 6 h (11.2%) [38]. Recently, 844 articles on platelet volume indices published between 1966 and 2011 were reviewed [39]. In the vast majority of patients reviewed in this paper, blood samples were analysed within 2 h of collection. All haematology analysers had manufacturer-assigned ranges for platelet volume indices, which differed significantly between different analysers depending on the technology used, but no external quality assessment schemes are currently available. Until such schemes are in place, the authors of this review recommend that blood samples for MPV should be analysed within 2 h of venipuncture, in keeping with the methods used in most of the mentioned studies. For reliable MPV measurements, the potential influence of the anticoagulant must be carefully controlled by standardising the time delay between sampling and analysis (less than 2 h). In our study, in order to have a standardised time window for the analysis, blood samples of the subjects were collected in the same standard tubes containing EDTA and analysed immediately within 60 min after venipuncture. Platelet activity has seemed to have a circadian variation in some reports [40], and therefore all blood samples were drawn between 8.00 and 10.00 am. Lack of laboratory standardisation and cut-off values along with technical problems in platelet volume measurements presently limit the widespread usefulness of platelet volume indices. Future prospective studies with standardised measurements may clarify the clinical role of platelet volume indices.

CONCLUSIONS

Patients with RMS have increased platelet activity reflected as elevated MPV, and the presence of LASEC and severe MR are the determinants of this increment. Distinct pathogenesis and clinical significance of this platelet volume enlargement in RMS are not clear.

Conflict of interest: none declared

References

Zwiększenie średniej objętości płytek krwi w zwężeniu zastawki mitralnej o podłożu reumatycznym: ocena determinantów klinicznych i echokardiograficznych

Mehmet İleri, Selçuk Kanat, Havva Tuğba Gürsoy, Deniz Şahin, Pınar Türker Bayır, Gökhan Şahin, Funda Başyigit, Özgül Uçar Elalmış, Ümit Güray

Ankara Numune Education and Research Hospital, Ankara, Turcja

Streszczenie

Wstęp i cel: Celem badania było określenie średniej objętości płytek krwi (MPV) u chorych ze zwężeniem zastawki mitralnej spowodowanym chorobą reumatyczną (RMS) i zdefiniowanie determinantów potencjalnej aktywacji płytek krwi przejawiającej się zwiększeniem ich objętości.

Metody: Średnią objętość płytek w osoczu krwi obwodowej zmierzyono u 84 kolejnych pacjentów (16 mężczyzn, 68 kobiet; średnia wieku ± SD = 44 ± 13 lat) z RMS, u których nie stwierdzono skrzepin w lewym przedsiękoniu w echokardiografii przezprzężkowej. Grupa kontrolna składała się z 32 zdrowych osób (9 mężczyzn, 23 kobiety; średnia wieku ± SD = 38 ± 7 lat).

 Wyniki: U chorych z RMS stwierdzono istotnie większe wartości MPV (średnia ± SD = 10,07 ± 0,58 fl) niż u osób zdrowych (średnia ± SD = 8,15 ± 0,60 fl, p < 0,001). W analizie regresji liniowej wykazano, że spośród wielu zmiennych klinicznych i echokardiograficznych istotnymi czynnikami predykcyjnymi zwiększenia objętości płytek krwi w RMS jest samoistne kontrastowanie się krwi w lewym przedsiękoniu (beta = 0,426; p < 0,001) i ciężka niedomykalność zastawki mitralnej (beta = 0,577; p < 0,001).

Wnioski: U chorych z RMS aktywność płytek krwi jest nasilona, co przejawia się w zwiększonej MPV, a współistnienie ciężkiej niedomykalności zastawki mitralnej i samoistnego kontrastowania się krwi w lewym przedsiękoniu stanowi czynnik determinujący tę zwiększoną aktywność płytek.

Słowa kluczowe: średnia objętość płytki krwi, zwężenie zastawki mitralnej, choroba reumatyczna

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