Relationship between platelet indices and spontaneous echo contrast in patients with mitral stenosis


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Received 20 July 2011; accepted after revision 9 August 2011; online publish-ahead-of-print 5 September 2011

Aims
To determine the association of platelet indices with spontaneous echo contrast (SEC) in patients with mitral stenosis.

Methods and results
A total of 232 consecutive patients with mitral stenosis who undergoing mitral balloon valvuloplasty were enrolled to the study. Patients were divided into two groups according to the formation of SEC in the left atrium. Group 1: mitral stenosis complicated with SEC; Group 2: mitral stenosis without SEC. Transthoracic echocardiography and transoesophageal echocardiography were performed for each patient. Complete blood counting parameters were measured and all routine biochemical tests were performed. There were 133 patients (mean age 42 ± 11 and 74% female) in the SEC(−) group and 99 patients (mean age 45 ± 10 and 64% female) in the SEC(+) group. Plateletcrit (0.25 ± 0.06 vs. 0.27 ± 0.07, P = 0.043) and mean platelet volume (MPV) levels (9.4 ± 1.1 vs. 10.4 ± 1.2, P < 0.001) were significantly higher in the SEC(+) group. When we divided the SEC(+) patients into four subgroups according to previously reported criteria, MPV levels increased to correlate with the degree of SEC (P < 0.001). At multivariate analysis, MPV levels [odds ratio (OR) 2.365, 95% confidence interval (CI) 1.720–3.251; P < 0.001] and PCT levels (OR 2.699, 95% CI 1.584–4.598; P = 0.033) are independent risk factors of SEC in patients with mitral stenosis.

Conclusion
In patients with mitral stenosis, cheaply and easily measurable platelet indices including MPV and PCT levels are associated with the presence of SEC and are independent risk factors of SEC.

Keywords
Spontaneous echo contrast • Mitral stenosis • Mean platelet volume • Plateletcrit

Introduction
Spontaneous echo contrast (SEC) is a presence of smoke-like echoes with a characteristic swirling motion of blood in echocardiography. It occurs from aggregation in cellular component of blood in the situations with blood stasis and low velocity of bloodstream. SEC is commonly seen in the left atrium (LA) and particularly caused by mitral stenosis and non-valvular atrial fibrillation. Previous clinical studies have demonstrated that SEC is a risk factor for LA thrombus formation and an important indicator of potential systemic embolism originated from the heart. A hypercoagulable situation has been reported in patients with mitral stenosis who have both atrial fibrillation and sinus rhythm. Patients with rheumatic mitral stenosis may have thromboembolic events, although on oral anticoagulants treatment. So, in addition to the coagulation system, platelets may have an important role in the development of thromboembolic events in patients with mitral stenosis.

Platelet indices, including platelet count, platelet distributing width (PDW), mean platelet volume (MPV), and plateletcrit (PCT), are easily measurable parameters in complete blood count (CBC). To our knowledge, the relationship between platelet indices and SEC in patients with mitral stenosis has not been studied to date. In this
study, we aim to determine the association of platelet indices with SEC in patients with mitral stenosis.

Methods

Study population
A total of 232 consecutive patients (mean age 43 ± 11 and female 70%) with mitral stenosis who undergoing mitral valve valvuloplasty were enrolled to the study. Transthoracic echocardiography (TTE) and transoesophageal echocardiography (TEE) were performed for each patient to rule out the thrombus formation in the LA before mitral balloon valvuloplasty. The SEC was evaluated by TEE for all patients. The study population were divided into two groups according to the formation of SEC in the LA. Group 1: mitral stenosis complicated with SEC; Group 2: mitral stenosis without SEC.

Exclusion criteria for the study were LA thrombus formation, history of malignancy, connective tissue disease, thyroid disease, immune thrombocytopenic purpura, and other haematological disease. Previous medical history was recorded from patient’s anamnesis. Atrial fibrillation was determined by electrocardiogram. Blood pressure was measured after a 10 min resting period before TTE with a random-zero sphygmomanometer by trained observers. Heart rate was recorded at the same time with blood pressure measurement.

Informed consent was obtained from all patients and the protocol was approved by the Ethics Committee and the institutional review board of Erciyes University Medical School.

Laboratory measurements
In all patients, antecubital venous blood samples for the laboratory analysis were taken immediately after TEE. Tripotassium EDTA-based anticoagulated blood samples for CBC were measured by a Sysmex K-1000 auto analyzer within 5 min of sampling. The samples were studied immediately in order to avoid platelet swelling. All routine biochemical tests were carried out on an autoanalyser (Roche Diagnostic Modular Systems, Tokyo, Japan).

Echocardiography examination
Two-dimensional echocardiography was performed by using a commercially available machine (Vivid 7th GE Medical System, Horten, Norway) with a 3.5 MHz transducer for TTE and 5 MHz for TEE, during at least three (for sinus rhythm) or seven (for atrial fibrillation) consecutive cardiac cycles. TTE and TEE were performed within the same session. All patients were studied in the left lateral recumbent position after a 10 min resting period. Simpson’s method in a two-dimensional echocardiographic apical four-chamber view was used to assess the left ventricular ejection fraction (LVEF) as recommended by American Society of Echocardiography guidelines. Mitral valve area was measured with the planimetric method. The jet of tricuspid regurgitation was identified, its maximum velocity measured, and the pressure gradient calculated. This gradient was added to an assumed right atrial pressure (10 mmHg) to give an estimate of systolic pulmonary artery pressure (PAP). For TEE, after patient’s pharyngeal local anaesthesia with lidocaine spray, the probe was initially carried forward to a depth of 25–35 cm and then manipulated to optimum imaging. All images were archived and evaluated by two independent echocardiographer cardiologists. The degree of the LA SEC was defined as 0; 1+ (mild), minimal echogenicity in the LA appendage or sparsely distributed in the LA which was detected only transiently during the cardiac cycles; 2+ (mild to moderate), more dense than 1+ but similar distribution and detectable without increased gain settings; 3+ (moderate), more dense swirling pattern distributed to both LA appendage and LA which could change in intensity but was detectable throughout the cardiac cycle; 4+ (severe), very intense echo density and slow swirling motion distribution as for 3+.

The intra- and inter-observer variability were obtained from random 100 patients. The intra- and inter-observer variability for the presence of SEC were 1% and 2%, respectively. The intra- and inter-observer variability for 1(+) SEC were 4% and 3%, respectively; for 2(+) SEC, the corresponding values were 5% and 4%; respectively; for 3(+), 4(+) SEC, both intra- and inter-observer variability were 2%, and for 4(+). SEC both intra- and inter-observer variability were 1%.

Statistical analysis
Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. We report continuous data as mean and standard deviation or median. We compared continuous variables using Student’s t-test or the Mann–Whitney U-test between the groups. Categorical variables were summarized as percentages and compared with the χ² test. We compared the platelet indices in the SEC(+) group with one-way analysis of variance model. The effects of different variables on SEC were calculated in univariate analysis for each. The variables for which the unadjusted P-value was <0.10 in logistic regression analysis were identified as potential risk markers and included in the full model. We reduced the model by using backward elimination multivariate logistic regression analyses and we eliminated potential risk markers by using likelihood ratio tests. A P-value of <0.05 was considered significant and the confidence interval (CI) was 95%. All statistical analyses were performed with the SPSS version 15 (SPSS, Inc., Chicago, IL, USA).

Results
A total of 232 consecutive patients were enrolled to the study. Patients were divided into two groups according to the presence of SEC. There were 133 patients (mean age 42 ± 11 and 74% female) in the SEC(−) group and 99 patients (mean age 45 ± 10 and 64% female) in the SEC(+) group. Baseline characteristics are shown in Table 1. Mean age and sex of patients were similar between the groups (P = 0.091 and 0.138, respectively). There were no significant differences in the presence of hypertension, diabetes mellitus, smoking status, and prior coronary artery disease between the groups (Table 1). Previous cerebrovascular disease and peripheral embolism history were higher in the SEC(+) group, however did not reach the significance (P = 0.374 and 0.250, respectively).

With respect to the rhythm status of patients, 37% of patients in the SEC(−) group were in atrial fibrillation, while 48% of patients in the SEC(+) group were atrial fibrillation (P = 0.104). Aspirin, warfarin, and non-steroidal anti-inflammatory drug usage was also not significantly different between the groups (P = 0.248, 0.228, and 0.793 respectively).

CBC parameters were shown in Table 2. With respect to platelet indices, there was no significant difference in the platelet count and MPV between the groups (P = 0.377 and 0.395, respectively). However, PCT levels (0.27 ± 0.07 vs. 0.25 ± 0.06) and MPV levels (10.4 ± 1.2 vs. 9.4 ± 1.1) were significantly higher in the SEC(+) group.
When we divided the SEC(+) patients into four subgroups according to previously reported criteria,9 MPV levels have a positive correlation with the degree of SEC \( (P < 0.001) \) (Figure 1).

In the echocardiographic parameters, LVEF was higher in the SEC(−) group \((62.9 \pm 6.0 \text{ vs. } 60.3 \pm 5.8, P = 0.001)\). Mean gradient measured from mitral valve, systolic PAP, and right ventricle diameter was not significantly different between the groups (Table 3). However, LA volume was significantly higher in the SEC(+) group \((72.9 \pm 14.6 \text{ vs. } 68.3 \pm 13.2, P = 0.001)\) while
planymetric mitral valve area was higher in the SEC(−) group than in the SEC(+) group (1.22 ± 0.27 vs. 1.12 ± 0.19, P = 0.001).

In the groups, some of variables that can be effective on SEC were significantly different between the groups. So, the effects of multiple variables on the SEC were analysed with univariate and multivariate logistic regression analyses. The variables for which the unadjusted P-value was < 0.10 in univariate analysis were identified as the potential risk markers for SEC and included in the full model. At multivariate analysis, MPV [odds ratio (OR) 2.365, 95% CI 1.720–3.251; P < 0.001], PCT (OR 2.699, 95% CI 1.584–4.598; P = 0.033), mitral valve area (OR 0.161, 95% CI 0.011–0.546; P < 0.001), and LA volume (OR 3.012, 95% CI 1.543–5.579; P = 0.001) were independent risk factors of SEC in patients with mitral stenosis (Table 4).

Discussion

In this study, our results suggest that platelet indices including MPV and PCT levels were associated with SEC in patients with mitral stenosis. Also, in the subgroup of patients who complicated with SEC, MPV levels were correlated with the grade of SEC. Systemic thromboembolism is an important complication in patients with mitral stenosis. Stasis of blood that occurs in LA plays an important role in thromboembolism. Otherwise, a hypercoagulable situation has been reported in patients with mitral stenosis who have both atrial fibrillation and sinus rhythm.6,7 In addition to the coagulation system, platelets play an important role in the haemostatic system. Platelets have a critical role for the activation of intrinsic pathway factors.10 Also, patients with rheumatic mitral stenosis may have thromboembolic events.

### Table 3  Echocardiographic parameters of patients

<table>
<thead>
<tr>
<th></th>
<th>SEC(−)</th>
<th>SEC(+)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 133)</td>
<td>(n = 99)</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>62.9 ± 6.0</td>
<td>60.3 ± 5.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean gradient (mmHg)</td>
<td>16.5 ± 5.9</td>
<td>16.8 ± 6.2</td>
<td>0.732</td>
</tr>
<tr>
<td>Mitral valve area (cm²)</td>
<td>1.22 ± 0.27</td>
<td>1.12 ± 0.19</td>
<td>0.001</td>
</tr>
<tr>
<td>LA volume (mL)</td>
<td>68.3 ± 13.2</td>
<td>72.9 ± 14.6</td>
<td>0.001</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>44.1 ± 8.4</td>
<td>47.2 ± 9.7</td>
<td>0.121</td>
</tr>
<tr>
<td>RV diameter (cm)</td>
<td>4.3 ± 1.1</td>
<td>4.4 ± 1.2</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Degree of SEC

1(+) 31 (31%)

2(+) 37 (37%)

3(+) 18 (18%)

4(+) 13 (13%)

Data are expressed as mean ± standard deviation for normally distributed data. LVEF, left ventricular ejection fraction; LA, left atrium; PAP, pulmonary arterial pressure; RV, right ventricle; SEC, spontaneous echo contrast.

### Table 4  Effects of various variables on spontaneous echo contrast in univariate and multivariate logistic regression analyses

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted OR</th>
<th>95% CI</th>
<th>P-value</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.021</td>
<td>0.997–1.046</td>
<td>0.092</td>
<td>1.033</td>
<td>1.001–1.064</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.932</td>
<td>0.549–1.582</td>
<td>0.795</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.108</td>
<td>0.581–2.111</td>
<td>0.755</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>0.628</td>
<td>0.327–1.208</td>
<td>0.164</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td>1.562</td>
<td>0.580–4.206</td>
<td>0.377</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral embolic events</td>
<td>1.756</td>
<td>0.666–4.625</td>
<td>0.255</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1.549</td>
<td>0.913–2.630</td>
<td>0.105</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>1.371</td>
<td>0.802–2.345</td>
<td>0.248</td>
<td></td>
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</tr>
<tr>
<td>Warfarin</td>
<td>1.383</td>
<td>0.815–2.347</td>
<td>0.229</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAIDs</td>
<td>0.915</td>
<td>0.470–1.781</td>
<td>0.793</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>1.002</td>
<td>0.998–1.005</td>
<td>0.377</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV</td>
<td>2.209</td>
<td>1.680–2.904</td>
<td>&lt;0.001</td>
<td>2.365</td>
<td>1.720–3.251</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCT</td>
<td>1.082</td>
<td>1.001–1.136</td>
<td>0.044</td>
<td>2.699</td>
<td>1.584–4.598</td>
<td>0.033</td>
</tr>
<tr>
<td>PDW</td>
<td>0.998</td>
<td>0.983–1.012</td>
<td>0.740</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>LVEF</td>
<td>0.924</td>
<td>0.881–0.969</td>
<td>0.001</td>
<td>0.933</td>
<td>0.863–1.010</td>
<td>0.102</td>
</tr>
<tr>
<td>Mean gradient</td>
<td>1.008</td>
<td>0.965–1.052</td>
<td>0.731</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral valve area</td>
<td>0.150</td>
<td>0.045–0.507</td>
<td>0.002</td>
<td>0.161</td>
<td>0.011–0.546</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA volume</td>
<td>2.943</td>
<td>1.619–4.702</td>
<td>0.001</td>
<td>3.012</td>
<td>1.543–5.579</td>
<td>0.001</td>
</tr>
<tr>
<td>PAP</td>
<td>1.023</td>
<td>1.001–1.082</td>
<td>0.122</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV diameter</td>
<td>1.001</td>
<td>0.998–1.009</td>
<td>0.111</td>
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</tr>
</tbody>
</table>

*Adjusted for age, MPV, PCT, LVEF, mitral valve area, and LA volume.

CVD, cerebrovascular disease; NSAIDs, non-steroidal anti-inflammatory drugs; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; LVEF, left ventricular ejection fraction; LA, left atrium; PAP, pulmonary arterial pressure; RV, right ventricle.
although on oral anticoagulant treatment. Therefore, in addition to the coagulation system, platelets may have an important role in the development of thromboembolic events in patients with mitral stenosis.

SEC is a dynamic smoke-like echo with a characteristic swirling motion of blood detected by echocardiography. SEC is commonly seen in LA and particularly caused by mitral stenosis and non-valvular atrial fibrillation. Several epidemiological studies previously demonstrated that LA SEC is an independent predictor of thromboembolic events and a marker of thromboembolic risk in patients with mitral stenosis. In the physiopathology of SEC, there are several mechanisms that had been reported before. It occurs from aggregation in cellular component of blood in the situations with blood stasis and low velocity of blood-stream. Otherwise, Sigel et al. reported that echogenicity of blood in SEC occurred with erythrocytes aggregation in plasma. Erbel et al. found evidence of increased platelet aggregation in nine patients with SEC and resolution after antiplatelet therapy. These data suggest that SEC may reflect not only stasis of blood in LA, but also associated with blood characteristics including erythrocytes and platelets. Resolution of SEC after antiplatelet therapy suggests that patients with mitral stenosis complicated with SEC who have high MPV levels might gain advantage from intensive antiplatelet therapy and decrease the risk of thromboembolic events. However, importance of aspirin therapy in the treatment of SEC is still controversial. So, further prospective studies should be planned in order to know the effect of aspirin on SEC.

MPV is an easily measurable and favourable index for platelet function. It has been shown that a higher MPV is correlated with a greater platelet activation. Larger platelets contain more prothrombotic materials including thromboxane A$_2$ and B$_2$ and have more glycoprotein IIB–IIa receptor expression on the surface of platelets. Jakubowski et al. reported that the presence of a greater platelet decreases the inhibitory effectiveness of PG12 on both platelet aggregation and the release reaction. Thus, larger platelets are more aggregable, metabolically and enzymatically more reactive than the smaller ones. PCT is the percentage of blood volume occupied by platelets. Although a few published data are available, further studies are needed in order to know the importance of PCT in platelet indices. According to the current literature, PCT is an indicator of platelet mass in the blood just as haematocrit is an indicator of total erythrocyte mass in the blood. PCT is physiologically the most pertinent parameter and is superior to the platelet count to estimate the platelet status.

In our study, PDW, platelet count, and other haematological parameters including haemoglobin, haematocrit, red cell distribution width, mean corpuscular volume, and white blood cell count were not associated with SEC in patients with mitral stenosis. However, MPV, a sign of platelet activation, was significantly higher in the SEC(+) group. In the subgroup of SEC, MPV levels also correlated with the degree of SEC. In addition, MPV levels were the independent risk factor for SEC in patients with mitral stenosis. Although PCT levels were statistically independently associated with SEC in patients with mitral stenosis, PCT did not increase in a graded fashion with SEC intensity as did MPV. Because PCT represents the percentage of whole blood occupied by platelets (analogous to the haematocrit for red blood cells), PCT and MPV are different descriptive parameters of the platelet fraction of blood. Analogously, in a previous study, Beyan et al. found no correlation between PCT and platelet aggregation in healthy volunteers. Thus, the exact role of PCT in platelet aggregation and SEC awaits further study.

The possible limitation of this study may be the method of CBC measurement. Tripotassium EDTA-based anticoagulated blood samples were used to measure the CBC parameters in our study. Most laboratories use EDTA for anticoagulation of whole blood prior to automated cell counting but due to platelet swelling, MPV values may increase with its use. Dastjerdi et al. found that MPV can be measured accurately by using both methods of anticoagulation, EDTA, and citrate if analysis be performed within 1 h of sampling. Macey et al. also showed that the changes in MPV, which reflect platelet sphering and swelling, were greatest between 30 and 60 min in blood stored at ambient temperature. Whereas in our study, blood samples were analysed within 5 min. So, our study had adequate confidence about results. In addition to these, we did not measure the platelet functions.

In conclusion, in patients with mitral stenosis, cheaply and easily measurable platelet indices including MPV and PCT levels are associated with the presence of SEC and are independent risk factors of SEC. According to our study, we suggest that SEC(+) patients with mitral stenosis with high MPV and PCT levels might gain advantage from intensive antiplatelet therapy and decrease the risk of thromboembolic events. However, further prospective studies should be planned in order to know the effects of antiplatelet therapy on SEC in patients with mitral stenosis.

Conflict of interest: none declared.

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The myocardial architecture of cor triloculare biatrium resembling reptiles

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The reptilian heart includes a three-chamber: right and left atria and one partially divided ventricle. This is made up the non-compacted myocardium which includes ventricular trabeculations. Reptiles, which are poikilothermic, lack an advanced coronary tree in contrary to humans and the trabecular structure of the myocardium provides mainly a direct route of blood supply from the ventricular cavity. Univentricular heart or cor triloculare biatrium represents a wide variety of rare and complex congenital cardiac malformations. Generally, a second rudimentary or hypoplastic accessory ventricle is present. We describe a 21-year-old patient who has been admitted for dyspnoea, cyanosis, and clubbing with univentricular heart. In the following cardiac magnetic resonance imaging (Panel A) and classical ventriculography (Panel B; see Supplementary data online, Video S1), the right and left ventricles appear heavily trabeculated myocardial architecture resembling reptiles.

In reality, during embryonic development, the human heart resembles a reptilian heart. The human heart, like that of a fish, possesses only two chambers in a series in its early embryonic stage. Later, as in reptiles, the atrium is divided into two and the ventricle is partly separated. Finally, with the complete development of interventricular septum, the human heart becomes four-chambered composed of compacted myocardium.

Supplementary data are available at European Journal of Echocardiography online.

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