Can mean platelet volume be used as a biomarker for asthma?

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Introduction: Platelets play important roles in airway inflammation and are activated in inflammatory lung diseases, including asthma.

Aim: We evaluated the mean platelet volume (MPV), used as a marker of platelet activation, in asthmatic patients during asymptomatic periods and exacerbations compared to healthy controls to determine whether MPV can be used as an indicator of inflammation.

Material and methods: Our patient group consisted of 95 children with exacerbation of asthma who were admitted to our allergy clinic. The control group consisted of 100 healthy children matched for age, gender, and ethnicity. Mean platelet volume values of the patient group obtained during exacerbation of asthma were compared to those of the same group during the asymptomatic period and with the control group. We investigated factors that can affect the MPV values of asthma patients, including infection, atopy, immunotherapy treatment, and severity of asthma exacerbation.

Results: The patient group consisted of 50 (52.6%) boys and 45 (47.4%) girls with a mean age of 125 ±38 months old. Mean MPV values in the exacerbation period, the healthy period, and in the control group were 8.1 ±0.8 fl, 8.1 ±0.9 fl, and 8.2 ±0.9 fl, respectively; there were no significant differences between groups (p > 0.05). The severity of asthma, severity of asthma exacerbation, immunotherapy, coinfection, eosinophil count, and IgE level also had no effect on MPV (p > 0.05).

Conclusions: Although platelets play a role in the pathophysiology of asthma, MPV measurement is insufficient to detect inflammation through platelets.

Key words: mean platelet volume, asthma, childhood, atopy, immunotherapy.
Material and methods

Patient population, study design, and hospital setting

All of the patients referred to the İzmir Dr Beçht Uz Children’s Hospital Allergy Department with exacerbation of asthma were included in our study. Complete blood counts (CBCs) were taken from all of the patients during both exacerbation and asymptomatic periods. Asthma was considered to be under control by the asthma control test at least 3 months after the last exacerbation. Asthma diagnosis, classification, and classification of exacerbation severity were all performed using the Global Strategy for Asthma Management and Prevention guidelines developed by the Global Initiative for Asthma (GINA) [1]. According to GINA guidelines, our cases have been classified as intermittent, mild persistent, moderate persistent and severe persistent asthma. If the patient had intermittent asthma, initial treatment was started from step 1, whereas if the patient had mild persistent asthma, initial treatment was started from step 2, and if the patient had moderate persistent asthma, initial treatment was started from step 3. Patients with severe persistent asthma commenced initial therapy from step 4.

Skin prick tests were applied in all of the cases with the same allergens. The study was approved by our local ethics committee.

One hundred healthy children without any chronic diseases, who were referred to our hospital for general medical examination, were chosen as the control group. The mean age and gender ratios were similar between the control group and the study group. Complete blood counts were taken and MPV values were noted. Children who were diagnosed with sepsis, iron deficiency anemia, obesity, hyperlipidemia, diabetes mellitus, hypertension, chronic renal failure, nephrotic syndrome, inflammatory bowel disease or connective tissue disease were excluded from our study as they were previously reported that these diseases affected MPV values.

Skin prick test

Skin prick tests were applied to the anterior surface of the forearm when the subjects were appropriate for testing (e.g. when not taking antihistamines). Skin prick tests for common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae), a mixture of grass pollens (Lolium perenne, Dactyliis glomerata, Phleum pratense, Anthoxanthum odoratum, Poa pratensis, Festuca elatior, Agrostis vulgaris, Holcus lanatus, Cydonia dactylon, Avena sativa, Avena fatua, Lotus Corniculatus), a mixture of grain pollens (oats, wheat, barley, corn), a mixture of tree pollens (Acer pseudoplatanus, Aesculus hippocastanum, Robinia pseudacacia, Tilia platyphyllos, Platanus vulgaris), weed-mix pollens (Medicago sativa, Trifolium pratense, Brassica nigra, Urtica dioica, Rumex acetosa), Alternaria alternaria, cockroaches (Blattella germanica), and cat and dog dander (Stallergenes SA, 92160 Antony, France) were performed using a Stallerpoint device. Histamine (10 mg/ml) and physiological saline were used as positive and negative references, respectively. Skin reactions were evaluated 20 min after the skin test. A positive reaction was characterized as wheal diameter ≥ 3 mm. Atopy was classified as at least one positive reaction to allergen sensitivity in the skin test.

Asthma Control Test

The control of asthma was assessed using the Asthma Control Test (ACT) questionnaire consisting of five questions regarding daytime and nighttime asthma symptoms, rescue medication use, and level of impairment in daily activities due to asthma. An ACT score of 25 points was considered full control, 20–24 points as partial control, and < 20 points as uncontrolled [1].

Counting blood samples

Whole blood count (WBC) was performed via Beckman Coulter LH 780 and blood samples which were anticoagulated with K3EDTA were used. The Coulter principle is volumetric analysis. The cells in suspension pass through a small aperture between two chambers between which there is an electrical current. As each cell passes, it creates an impulse which is considered to be proportional to the volume of the cell detected between the two electrodes. In the LH780 analyzers, particles between two and 20 fl are counted as platelets, with possible extrapolation up to 60.00 fl. A log-normal curve is fitted to these points. The curves have a range of 0–70 fl, and the platelet count and parameters are derived from this curve. The hemoglobin level, WBC, platelet count, and MPV values were recorded for each patient. The reference range for MPV was between 7.0 and 11 fl.

Statistical analysis

The data were primarily evaluated using descriptive statistical methods. For the numerical data, mean and median as measures of central tendency, and standard deviation (SD) and interquartile range (IQR) as measures of spread were used. The Kolmogorov-Smirnov test and the coefficient of variation were used to assess the distribution of the data and histograms, stem and leaf diagrams, and box plot graphs were also used. The numerical data were compared using the Mann-Whitney U test and t-test, and categorical data were compared using the χ2 and Fisher’s exact test between groups. SPSS 15.0 was used for statistical analyses, and p < 0.05 was taken to indicate statistical significance.

Results

In our retrospective cohort study, the study group consisted of 95 children with asthma and the control
group consisted of 100 healthy children with similar age and gender distribution. The study group was composed of 50 (52.6%) boys and 45 (47.4%) girls with a mean age of 125 ±38 months. The median age at hospital admission was 78.5 (IQR 60, min 10, max 168) months and the median of the starting age of the symptoms was 43 (IQR 62, min 1, max 144) months. Cases were classified according to the severity of asthma as mild intermittent (1.1%), mild persistent (37.9%), moderate persistent (58.9%), and severe persistent (2.1%). The median eosinophil count and IgE measurement values were 360 mm$^3$ (IQR 400, min 0, max 1800) and 340 (IQR 356, min 3, max 3949) IU/ml, respectively. According to skin test results, 64 (67.4%) and 31 (32.6%) patients were atopic and non-atopic, respectively. Sixteen (16.8%) patients were receiving immunotherapy. Asthma exacerbation was mild in 7 (7.4%) patients, moderate in 87 (91.6%), and severe in 1 (1.1%) patient. A diagnosis of coexisting infection was made in 34 (35.8%) cases (Table 1).

Mean MPV counts in the study group during asthma exacerbation and during the asymptomatic period were 8.1 ±0.8 fl and 8.1 ±1.06 fl, respectively, whereas that in the control group was 8.2 ±0.9 fl. There were no significant differences in mean MPV values between the exacerbation period and asymptomatic period ($p = 0.62$), the exacerbation period and the control group ($p = 0.64$), and the asymptomatic period and the control group ($p = 0.37$). However, the platelet counts in the patient group were significant higher during both the exacerbation and asymptomatic periods than that in the control group ($p = 0.001$) (Table 2).

Various factors, including immunotherapy, infection, atopy, severity of asthma, eosinophil count, and severity of asthma exacerbation had no effect on the MPV values of asthma patients both during periods of exacerbation and remission. Mean platelet volume values were only higher during the asymptomatic period in cases of severe persistent asthma compared to the other asthma groups ($p = 0.025$) (Table 3).

**Discussion**

Chronic airway inflammation is detected at the bronchial walls caused by eosinophils, mast cells, macrophages, lymphocytes, and mediators released from some other cells in asthma. Recent studies have shown that platelets play a role in the inflammation during asthma [5, 7–10]. Previous studies in adults have suggested roles of platelet activation in different inflammatory lung diseases, including asthma [11, 12].

Table 1. Demographic characteristics of the study group and control group

| Parameter                  | Study group | Control group | P-value |
|----------------------------|-------------|---------------|---------|
| Age [months]               | 124 (60)*   | 144 (69)*     | 0.11    |
| Gender, female n (%)/male n (%) | 45 (47.4%)/50 (52.6%) | 52 (52%)/48 (48%) | 0.51 |
| Age at disease onset [months] | 78.5 (60)* |               |         |
| Eosinophil count [/mm$^3$] | 360 (400)*  |               |         |
| IgE level [IU/l]           | 340 (356)*  |               |         |
| Atopy, n (%)               | 64 (67.4)   |               |         |
| Immunotherapy, n (%)       | 16 (16.8)   |               |         |
| Infection, n (%)           | 34 (35.8)   |               |         |

*Data are shown as means ± standard deviation for normally distributed variables.

Table 2. Comparison of CBCs in the patient group obtained during exacerbation (group 1) and asymptomatic periods (group 2) with the control group (group 3)

| Parameters | n | Group 1 | n | Group 2 | n | Group 3 | Group 1–2 P-value | Group 2–3 P-value | Group 1–3 P-value |
|------------|---|---------|---|---------|---|---------|-------------------|-------------------|-------------------|
| MPV [fl]   | 95 | 8.1 ±0.8 | 95 | 8.1 ±1.06 | 100 | 8.2 ±0.9 | 0.62 | 0.64 | 0.37 |
| Plt [× 10$^9$/µl] | 95 | 321 ±81 | 95 | 344 ±89 | 100 | 285 ±60 | 0.015 | < 0.001 | 0.001 |
| Hb [g/dl]  | 95 | 12.6 ±1.1 | 95 | 12.6 ±1.06 | 100 | 12.9 ±0.8 | 0.832 | 0.013 | 0.026 |
| WBC [× 10$^9$/µl] | 95 | 9.1 (5.6)* | 95 | 8.7 (3.9)* | 100 | 7.4 (2)* | 0.02 | < 0.001 | < 0.001 |
| CRP [mg/l] | 95 | 1.3 (1.5)* | 95 | 0.33 (0)* | 100 | – | < 0.001 | – | – |

Plt – platelet, WBC – white blood cell, Hb – hemoglobin, CRP – C-reactive protein. *Data are shown as means ± standard deviation for normally distributed variables; variables without a normal distribution are shown as the median (interquartile range).
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Atopic individuals have higher levels of chemokines, β-thromboglobulin, and platelet factor 4 (PF4) compared to healthy subjects after allergen exposure, which is evidence of an increase in thrombopoiesis and a role of platelets in airway inflammation [10]. Kowal et al. [13] investigated activation of platelets after exposure to house dust mites in asthmatic patients, and they reported that prolonged airway inflammation after allergen exposure of asthmatic patients was related to intravascular platelet activation. Similarly, recent studies indicated that β-thromboglobulin and PF4 levels were increased in the plasma and bronchoalveolar lavage fluid of symptomatic asthma patients [12, 14]. Studies in animal models showed that platelet activation plays an important role in transmigration of circulating lymphocytes and eosinophils to the airways of patients with allergic asthma [15, 16].

Mean platelet volume was shown to be correlated with platelet function and activation [6, 17]. Thus, platelet activation during inflammation can be measured indirectly from the MPV. Mean platelet volume alone reflects both platelet stimulation and the speed of platelet production [17]. CD62, CD63, GP IIb/IIIa, PF4, and thromboglobulin can be used as markers of platelet activation [18]. On the other hand, these tests are not routinely performed due to their high costs and requirements for specialized equipment. However, MPV measurement is cheap, effective, easy, and was suggested be a useful method to assess platelet function and activation [19]. Considering all of these advantages, we used MPV and platelet number to assess platelet activation in cases of lower respiratory tract infection and inflammation.

To assess whether MPV value reflects platelet activation in asthmatic children with lower respiratory tract inflammation, we compared MPV values of patients during periods of exacerbation and asymptomatic periods with the control group. The results indicated no significant dif-

Table 3. Comparison of the exacerbation and remission MPV values in the patient group in terms of immunotherapy, infection, atopy, eosinophil count, severity of asthma, severity of exacerbation, and number of exacerbations

| Parameter                      | N (%) | Exacerbation MPV | Remission MPV | P-value |
|--------------------------------|-------|------------------|---------------|---------|
| Immunotherapy                  |       |                  |               |         |
| Yes                            | 16 (16.8) | 8.3 ±0.6 | 8.2 ±0.9 | 0.71 |
| No                             | 79 (83.2) | 8.09 ±0.89 | 8.1 ±0.89 | 0.49 |
| P                              | 0.38  | 0.85            |              |         |
| Asthma severity                |       |                  |               |         |
| Mild intermittent              | 1 (1.1)   | 7.7             | 7.2          | –      |
| Mild persistent                | 36 (37.9) | 8.1 ±0.8 | 8.0 ±0.9 | 0.38 |
| Moderate persistent            | 56 (58.9) | 8.1 ±0.89 | 8.2 ±1.07 | 0.41 |
| Severe persistent              | 2 (2.1)   | 8.8 ±0.2 | 10.05 ±1.2 | 0.33 |
| P                              | 0.49  | 0.255          |              |         |
| Asthma exacerbation severity   |       |                  |               |         |
| Mild                           | 7 (7.4)   | 8.4 ±0.3 | 8.01 ±0.5 | 0.15 |
| Moderate                       | 87 (91.6) | 8.1 ±0.8 | 8.1 ±1.1 | 0.44 |
| Severe                         | 1 (1.1)   | 7.8             | 7.9          | –      |
| P                              | 0.26  | 0.92           |              |         |
| Infection                      |       |                  |               |         |
| Yes                            | 34 (35.8) | 8.1 ±0.6 | 8.1 ±0.89 | 0.6  |
| No                             | 61 (64.2) | 8 ±0.9   | 8.2 ±1.1 | 0.37 |
| P                              | 0.51  | 0.76           |              |         |
| Skin test                      |       |                  |               |         |
| Atopic                         | 64 (64.7) | 8.2 ±0.9 | 8.1 ±1.1 | 0.62 |
| Non-atopic                     | 31 (32.6) | 7.9 ±0.6 | 8.2 ±0.9 | 0.08 |
| P                              | 0.18  | 0.64           |              |         |
| Number of asthma exacerbations |       |                  |               |         |
| < 2                            | 36 (36.7) | 8.1 ±1.05 | 7.9 ±0.9 | 0.09 |
| ≥ 2                            | 59 (61.2) | 8 ±0.7    | 8.3 ±1.1 | 0.102 |
| P                              | 0.63  | 0.1            |              |         |
| Eosinophil count               |       |                  |               |         |
| < 500                          | 72 (75.8) | 8 ±0.8   | 8.1 ±1.1 | 0.76 |
| 500–1500                       | 20 (21.1) | 8.1 ±0.79 | 8.2 ±0.98 | 0.59 |
| 1500–5000                      | 3 (3.2)   | 8.7 ±0.1 | 8.7 ±0.32 | 0.89 |
| P                              | 0.46  | 0.57           |              |         |

*Data are shown as means ± standard deviation for normally distributed variables.
ferences between the groups. Similar to our study, Tuncel et al. [20] also found no significant differences in MPV between asthmatic and control/symptomatic patients and in asymptomatic periods in asthma patients. In contrast, however, Sun et al. [21] reported that MPV values were lower during periods of asthma exacerbation compared to asymptomatic periods.

In the present study, platelet count was significantly higher in the patient group in both exacerbation and asymptomatic periods compared to the control group (p = 0.001). Kemona-Chetnik et al. [9] reported that platelet count and percentage of reticulated platelets were significantly higher in asthmatic children than in controls; they also reported that thrombopoietin concentration was higher in asthmatic patients, but the difference was not statistically significant. Compatible with the findings of Kemona-Chetnik et al. [9] platelet counts were significantly higher in the patient group in our study during both periods of exacerbation and remission. Taken together, these results suggest that an increased platelet count in asthmatic children may play a role in inflammation in asthma, but that MPV measurement is not sufficient to detect the inflammation via platelets.

Asthma, severity of asthma exacerbation, immunotherapy, infection, atopy, and eosinophil count were shown to have no effect on MPV. We only found that MPV values were significantly higher in severe persistent asthma during the asymptomatic period compared to the other asthma groups. The main reason for this difference may be the higher airway inflammation in severe persistent asthma. However, the number of patients with severe asthma in our cohort was small, which represented a limitation of our study. Studies in larger numbers of patients with severe asthma are required to confirm whether these patients tend to have higher MPV measurements. There have been no previous reports regarding the factors that affect the MPV of the asthmatic patients, so we could not perform comparisons with our results.

Conclusions
This study focused on the MPV values of asthmatic patients during periods of exacerbation and remission of asthma. We also examined whether the MPV values were affected by several factors, including immunotherapy, atopy, infection, asthma, and the severity of asthma. Our study was important due to the conflicting results reported previously in the literature. There were no differences in MPV values of asthmatic patients and controls both during periods of asthma exacerbation and asymptomatic periods. In addition, the severity of asthma, the severity of exacerbation, atopy, immunotherapy, and co-existing infection did not affect the MPV values. On the other hand, platelet counts were significantly higher in the patient group during both periods of exacerbation and remission. Taken together, these results suggest that an increased platelet count in asthmatic children may play a role in the inflammation in asthma, but MPV measurement is not sufficient to detect the inflammation via platelets.

Conflict of interest
The authors declare no conflict of interest.

References
1. Global strategy for asthma management and prevention. Global Initiative for Asthma (GINA); 2014 (revised), available from: http://www.ginasthma.org.
2. Tavacol R, Rahimi Z, Cheraghi M, et al. A cross-sectional study of prevalence and risk factors for childhood asthma in Ahvaz city, Iran. Postep Derm Alergol 2015; 32: 268-73.
3. Rogala B, Bozek A, Gluck J, Jarzab J. Prevalence of IgE-mediated allergy and evaluation of Th1/Th2 cytokine profiles in patients with severe bronchial asthma. Postep Derm Alergol 2015; 32: 274-80.
4. Brzozowska A, Majak P, Jerzyńska J, et al. Exhaled nitric oxide correlates with IL-2, MCP-1, PDGF-BB and TIMP-2 in exhaled breath condensate of children with refractory asthma. Postep Derm Alergol 2015; 32: 107-13.
5. Sullivan PJ, Jafar ZH, Harbinson PL, et al. Platelet dynamics following allergen challenge in allergic asthmatics. Respiration 2000; 67: 514-7.
6. Wijanwitkitt V. Plateletcrit, mean platelet volume, platelet distribution width: its expected values and correlation with parallel red blood cell parameters. Clin Appl Thromb Hemost 2004; 10: 175-8
7. Tutuoglu B, Gurel CB, Ozdas SB, et al. Platelet function and fibrinolytic activity in patients with bronchial asthma. Clin Appl Thromb Hemost 2005; 11: 77-81.
8. Pitchford SC. Defining a role for platelets in allergic inflammation. Biochem Soc Trans 2007; 35: 1104-8.
9. Kemona-Chetnik I, Bodzenta-Lukaszyk A, Butkiewicz A, et al. Thrombocytopenia in allergic asthma. Pol Arch Med Wewn 2007; 117: 9-13.
10. Pitchford SC, Riffo-Vasquez Y, Sousa A, et al. Platelets are necessary for airway wall remodeling in a murine model of chronic allergic inflammation. Blood 2004; 103: 639-47.
11. Kowal-Bielecka O, Kowal K, Lewszuk A, et al. B-thromboglobulin and platelet factor in bronchoalveolar lavage fluid of patients with systemic sclerosis. Ann Rheum Dis 2005; 64: 484-6.
12. Yamamoto H, Nagata M, Tabe K, et al. The evidence of platelet activation in bronchial asthma. J Allergy Clin Immunol 1993; 91: 79-87.
13. Kowal K, Pampuch A, Kowal-Bielecka O, et al. Platelet activation in allergic asthma patients during allergen challenge with Dermatophagoides pteronyssinus. Clin Exp Allergy 2006; 36: 426-32.
14. Yasuba H, Chihara J, Kino T, et al. Increased releasability of platelet products and reduced heparin-induced platelet factor 4 release from endothelial cells in bronchial asthma. J Lipid Mediat 1991; 4: 5-21.
15. Pitchford SC, Momi S, Giannini S, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. Blood 2005; 105: 2074-81.
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16. Pitchford SC, Yano H, Lever R, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. J Allergy Clin Immunol 2003; 112: 109-18.

17. Khandekar MM, Khurana AS, Deshmukh SD, et al. Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: an Indian scenario. J Clin Pathol 2006; 59: 146-9.

18. Tsiara S, Elisaf M, Jagroop IA, Mikhailidis DP. Trombosits as predictors of vascular risk: is there a practical index of trombosit activity? Clin Appl Thromb Hemostasis 2003; 9: 177-90.

19. Bath PM, Butterworth RJ. Platelet size: measurement, physiology and vascular disease. Blood Coagul Fibrinolysis 1996; 7: 157-61.

20. Tuncel T, Uysal P, Hocaoglu AB, et al. Change of mean platelet volume values in asthmatic children as an inflammatory marker. Allergol Immunopathol (Madr) 2012; 40: 104-7.

21. Sun WX, Zhang JR, Cao ZG, et al. A decreased mean platelet volume is associated with stable and exacerbated asthma. Respiration 2014; 88: 31-7.