Evaluation of platelet parameters and neutrophil/lymphocyte ratio during omalizumab treatment in patients with severe chronic spontaneous urticaria

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1. Introduction

Urticaria is a frequent symptom defined by the appearance of wheals and angioedema (1). Chronic spontaneous urticaria (CSU), which was defined by at least six weeks of continuous or recurrent urticaria, is assumed to occur in approximately 1%–2% of the population (1,2). The current EAACI/GA²LEN/EDF/WAO guideline on urticaria recommends that second generation H1 antihistamines (sH2AH) should be used as the first line treatment of CSU and that doses are increased up to fourfold if needed to achieve disease control. When this fails, omalizumab is recommended to be used as a third line treatment (3).

Omalizumab, a humanized monoclonal antiimmunoglobulin E (IgE) antibody, is approved for treatment of moderate or severe allergic asthma (4). Recently, it has been approved for the patients with antihistamine-resistant, severe CSU (4). It shows its effect by binding IgE to the FcεRI receptor on the surface of target cells, including mast cells and basophils, thereby reducing receptor expression and the release of inflammatory mediators (5). In addition, omalizumab has been shown
to be effective in 70%–80% of patients with CSU (6). However, the cause of this nonresponse to omalizumab treatment in patients with CSU is still unclear as real-world data concerning omalizumab in CSU patients are still lacking (6–8). Clearly, new markers that can elucidate omalizumab’s mode of action and new predictive markers of anti-IgE treatment can improve our knowledge.

The mean platelet volume (MPV) is an index of platelet size, while the platelet distribution width (PDW) is an index of platelet anisocytosis, which results from pseudopodia formation; these are both important markers that significantly increase during platelet activation (9,10). During the inflammation process, an increase or decrease may be observed in the number and volume of platelets. MPV has been identified as an inflammatory marker in the literature; increased MPV has previously been demonstrated in many diseases, including CSU and other inflammatory diseases, such as rheumatoid arthritis (RA), familial Mediterranean fever, and cystic fibrosis (11,12). In the literature, increased MPV and PDW levels were found to be associated with an increased risk of cardiovascular (CV)/cerebrovascular (CBV) events (12,13). In addition, obtained by dividing the number of neutrophils by the number of lymphocytes, the ratio of neutrophils to lymphocytes (NLR) is considered an important indicator of inflammation in chronic diseases. At the same time, high NLRs are considered a bad prognostic factor in chronic diseases. The platelet–lymphocyte ratio (PLR) has recently emerged as a simple marker that can be conveniently calculated by dividing the platelet count by the lymphocyte count of the whole blood count parameters, similar to NLR (14,15).

We aimed to determine the changes in inflammation parameters, such as the NLR, PLR, and platelet parameters in patients with CSU compared with healthy controls. We also aimed to investigate the short-term effects of omalizumab therapy on the NLR, PLR, MPV, MPV/platelet count, and PDW in patients with CSU. In addition, we sought to reveal the predictive markers of omalizumab therapy in patients with CSU using real-world data.

2. Materials and methods
In this study, we retrospectively analyzed CSU patients who had presented to the outpatient clinic of Kayseri Education and Research Hospital between January 2014 and April 2017. This hospital-based study included 143 patients with CSU and 132 healthy controls, and it was approved by the local ethics committee.

CSU was defined by at least six weeks of continuous or recurrent urticaria as mentioned in the current guidelines (2). All the patients included in the study were resistant to antihistamines, and they were patients with severe CSU. We retrospectively analyzed the baseline neutrophils, lymphocytes, platelet counts, NLR, PLR, MPV, and PDW before the omalizumab treatment compared with healthy controls. We then observed the changes in these parameters during omalizumab treatment at week 4 and week 12.

Age- and sex-matched healthy adults who presented to the outpatient clinic for regular control visits were enrolled in the control group. The CSU patients who did not respond to high-dose antihistamine therapy were treated with a dose of 300 mg of omalizumab every 4 weeks. Seventeen of 143 CSU patients were excluded from the analyses at week 4, and 29 were excluded at week 12 due to missing test results. The neutrophils, lymphocytes, platelet counts, NLR, PLR, MPV, and PDW were analyzed before the omalizumab injection at weeks 4 (n = 126) and 12 (n = 114) after omalizumab treatment initiation.

The activity/severity of CSU was assessed from the urticaria activity score (UAS). Briefly, the number of wheals and severity of pruritus were scored as follows: number of wheals: 0 = no wheals, 1 = mild (<20 wheals/24 h), 2 = moderate (21–50 wheals/24 h), and 3 = intense wheals (>50 wheals/24 h or large confluent areas of wheals); pruritus: 0 = none, 1 = mild, 2 = moderate, and 3 = intense (2). In this study, patients with score of UAS equal to or higher than three despite high dose antihistamine therapy were accepted as patients with severe CSU. The response statuses of the CSU patients were assessed at week 12. Patients who had lower UAS than baseline UAS or UAS = 0 were considered responders. Those with equal or higher UAS than baseline UAS at week 12 of omalizumab treatment were considered nonresponders (6,7).

Venous blood samples were collected in anticoagulant specimen tubes containing K2EDTA (Becton Dickinson Vacutainers). Complete blood count analyses were performed using an automated complete blood cell counter SysmexXN-9000 analyzer (Sysmex Corporation, Kobe, Japan) in two hours.

2.1. Statistical analysis
Analyses were conducted using SPSS version 20 (SPSS Inc., Chicago, IL, USA) and TURCOSA (Turcosa Analytics Ltd. Co., Turkey) statistical softwares. Parametric variables were presented as means and standard deviations, nonparametric variables were presented as medians and interquartile ranges (lower and upper quartiles). Kolmogorov–Smirnov test and histogram analyses were used to determine whether continuous variables were normally distributed. Levene's test was used for the evaluation of homogeneity of variances. Number of cases and percentages were used for categorical variables. Two independent groups of parametric variables were compared using Student's t-test. For nonparametric variables Mann–Whitney U test was administered. Categorical data were analyzed by Chi-square or Fisher's exact test, where applicable. The change in MPV and MPV/Platelet count by time was investigated.
using repeated measures analysis of variance with paired-t test as a post-test. Greenhouse–Geisser correction was used when sphericity assumption was violated. Friedman test with Wilcoxon signed rank test as a post-test were used to evaluate differences between variables across time (i.e. NLR, PLR, PDW, and platelet count). A P-value less than 0.05 was considered statistically significant.

3. Results
There was no significant difference in the baseline characteristics related to age and sex (Tables 1 and 2; P > 0.05). There were no statistically significant differences in the neutrophil, lymphocyte, platelet counts, NLR, or PLR values between CSU patients and healthy controls (P > 0.05).

3.1. MPV values were high and PDW values were low in CSU patients
CSU patients presented higher baseline MPV values (Mean (SD); 10.05 (1.001)) than healthy controls (Mean (SD); 9.78 (1.111); P = 0.035; Table 1; Figure 1). The baseline PDW values were low in patients with CSU (IQR; CSU patients; 15.7 [12.8–16.1], healthy controls 15.9 [15.7–16.2]), P < 0.001; Table 1; Figure 1).

3.2. NLR decreased and MPV increased during omalizumab treatment
The NLR values decreased during the omalizumab treatment (Table 3, P = 0.018). There were statistically significant increases in the MPV (P < 0.001), MPV/platelet

Table 1. Comparisons of baseline characteristics of severe CSU patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>CSU patients (n = 143)</th>
<th>Controls (n = 132)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>40.0 (13.17)</td>
<td>42.0 (16.34)</td>
<td>NS¹</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>93 (65.5)</td>
<td>89 (67.4)</td>
<td></td>
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<tr>
<td>Male, n (%)</td>
<td>50 (35)</td>
<td>43 (32.6)</td>
<td></td>
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<tr>
<td>Neutrophil (/mm³) (IQR)</td>
<td>4.89 (3.95–6.30)</td>
<td>4.54 (3.65–5.96)</td>
<td>NS³</td>
</tr>
<tr>
<td>Lymphocyte (/mm³) (IQR)</td>
<td>2.29 (1.93–2.89)</td>
<td>2.19 (1.87–2.61)</td>
<td>NS³</td>
</tr>
<tr>
<td>NLR (IQR)</td>
<td>2.04 (1.64–2.74)</td>
<td>2.05 (1.56–2.57)</td>
<td>NS³</td>
</tr>
<tr>
<td>PLR (IQR)</td>
<td>121.84 (98.40–163.43)</td>
<td>128.05 (104.18–163.20)</td>
<td>NS³</td>
</tr>
<tr>
<td>Platelet count (10⁹ cells/L whole blood), (IQR)</td>
<td>292 (246–350)</td>
<td>284.5 (256.25–329.5)</td>
<td>NS³</td>
</tr>
<tr>
<td>MPV(fl), mean (SD)</td>
<td>10.05 (1.001)</td>
<td>9.78 (1.111)</td>
<td>0.035¹</td>
</tr>
<tr>
<td>PDW (%), (IQR)</td>
<td>15.7 (12.8–16.1)</td>
<td>15.9 (15.7–16.2)</td>
<td>&lt;0.001³</td>
</tr>
<tr>
<td>MPV(fl)/Platelet count (10⁹ cells/L whole blood), mean (SD)</td>
<td>0.036 (0.011)</td>
<td>0.036 (0.012)</td>
<td>NS¹</td>
</tr>
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</table>

Abbreviations: SD, standard deviation; CSU, chronic spontaneous urticarial; IQR, interquartile range (lower quartile–upper quartile; NLR, Neutrophil (mm³)/Lymphocyte (mm³); PLR, Platelet count / Lymphocyte (mm³); MPV, mean platelet volume; PDW, platelet distribution width.
¹t-test for parametric variables; ²Chi²-test; ³Mann–Whitney U test.

Table 2. Characteristics of CSU patients who are responsive or nonresponsive to the omalizumab treatment during 12 weeks.

<table>
<thead>
<tr>
<th></th>
<th>CSU Patients (n = 143)*</th>
<th>Responders (n=121)*</th>
<th>Non-responders (n=22)*</th>
<th>P  NR vs R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean (SD)</td>
<td>40.0 (13.17)</td>
<td>39.58 (13.01)</td>
<td>42.32 (14.11)</td>
<td>NS¹</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>93 (65.0)</td>
<td>78 (64.5)</td>
<td>15 (68.2)</td>
<td>NS²</td>
</tr>
<tr>
<td>Angioedema, n (%)</td>
<td>84 (58.7)</td>
<td>71 (58.7)</td>
<td>13 (59.1)</td>
<td>NS²</td>
</tr>
<tr>
<td>IgG-anti-TPO, n (%)</td>
<td>40 (26.5)</td>
<td>33 (27.3)</td>
<td>7 (31.8)</td>
<td>NS²</td>
</tr>
<tr>
<td>CSU duration, months, median (IQR)</td>
<td>36 (12–96)</td>
<td>36 (11.5–96.0)</td>
<td>41 (24–120)</td>
<td>NS¹</td>
</tr>
<tr>
<td>Atopy, n (%)</td>
<td>50 (35)</td>
<td>40 (33.1)</td>
<td>10 (45.5)</td>
<td>NS²</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation CSU; chronic spontaneous urticaria, IQR, interquartile range (lower quartile–upper quartile); NR, nonresponders; R, responders; NS, not significant; ¹t-test for parametric variables; ²Chi²-test; ³Mann–Whitney U test.

1257
count (P = 0.005), and PDW (P = 0.003) values at week 4 and week 12 of omalizumab treatment (Tables 3 and 4; Figure 2).

3.3. New predictor factors of the omalizumab response in patients with CSU

The increase of MPV was low in nonresponders during the 12 weeks of omalizumab treatment (P = 0.009; Table 4). At week 4 of treatment, the nonresponders had statistically significantly low PDW levels (P = 0.040; Table 4; Figure 3). When grouping the patients according to disease duration by the median of 36 months, the increase of PDW was low in patients with longer disease duration (<36 months, median, IQR; 1.27 (0 to 3.33); >36 months, median, IQR; 0 (−1.55 to 1.28), P = 0.012).

4. Discussion

The results of this study showed that most of the severe CSU patients whose disease was resistant to antihistamines exhibited higher MPV levels than the control group did as found in previous studies (16–19). As an easy, cost-effective, and less time-consuming marker, MPV is the most studied platelet activation marker (17).

Our results confirm and complement earlier studies showing that MPV is high in patients with severe CSU, which is a comparable result to those of previous studies (18,19). Although there is a previous publication that did not find a link between platelet markers and CSU (10), Chandrashekar et al. (17) found that high MPV and PDW levels are correlated with severe CSU and platelet aggregation. However, we observed low PDW values in our study, which may have been due to a difference in the current patient population, which consisted of all antihistamine-resistant and severe CSU patients. As in our study, Magen et al. (18) showed that MPV is higher in severe CSU patients than in both mild CSU patients and the control group. This may have been due to the high activity of inflammatory markers in severe CSU; the MPV values may indicate the intensity of the inflammatory process. As in the current study, prospective platelet activation markers, such as the PDW and MPV/platelet count, have been investigated in different diseases (17). In fact, we found higher NLR levels in patients with severe CSU than in healthy controls, although the difference was not statistically significant. In addition, Karabay et al. (20) found high NLR levels in CSU patients. This confirms a possible inflammatory process in patients with CSU.

Omalizumab exerts its effects quickly, and it has high treatment efficacy in patients with CSU who do not respond to high doses of antihistamines (4). One of the aims of this study was to investigate changes in the markers of platelet function, such as the MPV, PDW, and MPV/platelet count during omalizumab treatment in a large patient population with antihistamine-resistant CSU. This issue had not been previously addressed in CSU patients. However, the effect of omalizumab therapy on MPV was observed without finding any significant change in a small patient group with asthma (21). Our results indicate that, at week 12 of treatment with omalizumab, there was a significant effect on the MPV, PDW, and MPV/platelet count. In the current study, the size of platelets was observed to increase during omalizumab treatment. The mechanism of this effect of omalizumab had not been investigated in patients with CSU before. Gasparyan et al. (22) showed a similar effect on MPV during anti-TNF
They mentioned that excessive proinflammatory cytokines and high acute-phase reactants can suppress the size of platelets via the effects of megakaryopoiesis in patients with inflammatory diseases (22). Similarly, it is known that omalizumab shows its overall effects by stopping the degranulation of mast cells; reducing the secretion of mediators, cytokines, and chemokines; and showing additional antiinflammatory effects (23). The larger size of platelets could be due to lifting the suppression and modulation of megakaryopoiesis during the omalizumab therapy. We also found a statistically significant decrease in the NLR during omalizumab treatment. This can be accepted as a decrease of the inflammatory process in severe CSU patients during the treatment. Gökmen et al. (24) showed a similar decrease of NLR and CRP (C-reactive protein) levels during biological therapy in RA patients. These results also confirm our hypothesis concerning the suppression of inflammatory markers during omalizumab therapy. MPV can be considered a possible biomarker to predict omalizumab treatment response in patients with CSU.

Thrombocytopenia can be seen as a side effect of omalizumab, and this was previously shown in primates, albeit not in humans (25,26). Although a slight decrease was seen, there was no statistically significant change in the thrombocyte counts in the current study (Table 4). The mechanism of thrombocytopenia in primates remains unclear. Ledford (25) explained that it could be because the monoclonal antibodies (mAbs) with the
The highest binding affinity seem particularly prone to causing thrombocytopenia; however, this effect could stem from binding of complexes (mAb-IgE) to the surface of platelets via an IgG receptor (25). In addition, platelets are the source of inflammatory mediators implicated in histamine release from basophils and mast cells (17). It can be hypothesized that the large platelets seen during the therapy may be due to a direct or indirect stabilizing effect of omalizumab on platelets due to its possible antiinflammatory effects.

It was previously shown that omalizumab may be associated with arterial thrombotic events in asthmatic patients (27). Recently, Iribarren et al. (28) showed a higher incidence rate of CV/CBV events in omalizumab-treated patients. The cause of the high risk of CV/CBV is still unknown (8,9). Platelets play a pivotal role in arteriosclerosis and arterial thrombotic disorders (29); high MPV and PDW levels are associated with a variety of established risk factors, such as CV/CBV disorders. In addition, platelets are thought to be involved in the pathogenesis of CSU (17). The mechanisms of MPV changes have not been fully explored yet. However, NLR is known as a novel CV risk factor (30). The decrease of NLR during omalizumab therapy may be considered a good prognostic factor for CV/CBV.

We found that increasing levels of MPV and levels of PDW are linked to the CSU response at week 12 of omalizumab treatment; to our knowledge, this is the first time this has been shown. A low increase

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<th>Table 4. Changes in PDW and MPV values of CSU patients who are responsive or nonresponsive to the omalizumab treatment during 12 weeks.</th>
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<tr>
<td>CSU Patients (n = 143)*</td>
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<tr>
<td>Baseline PDW (%)</td>
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<tr>
<td>Week 4 PDW (%)</td>
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<tr>
<td>Week 12 PDW (%)</td>
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<td>△MPV Baseline-week 12 (%)</td>
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<td>△PDW Baseline-week 4 (%)</td>
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Abbreviations: SD, standard deviation; CSU, chronic spontaneous urticaria; IQR, interquartile range (lower quartile–upper quartile); NR, nonresponders; R, responders; NS, not significant; MPV, mean platelet volume, PDW, platelet distribution width. †Friedman test with Wilcoxon signed rank test, a: Baseline vs week 12 (P < 0.003), △: [(week 4 or 12 value–baseline value)/baseline value] x 100. *Seventeen of 143 CSU patients were excluded from the analyses at week 4, and 29 were excluded at week 12 due to missing test results.

Figure 2. A, MPV values; B, MPV/Platelet count values of patients during omalizumab treatment. Circles indicate the mean and error bars indicate the standard deviation.
in the percentage of MPV during 12 weeks of omalizumab treatment and a low value of PDW at week 12 were seen in nonresponders (Figure 3). Long disease duration in patients with CSU may be thought to be correlated with the severity of disease. So, lower increase of PDW during 12 weeks of omalizumab treatment in patients with long disease duration can be associated with the resistance to omalizumab treatment. These results were associated with the ineffectiveness of omalizumab treatment; however, the explanation for this finding is still unclear. A possible explanation related to nonresponder patients may be the inability to bind to the omalizumab receptors (7), faster clearance of omalizumab (7), or an unknown blocking mechanism. Consequently, in the nonresponder group, omalizumab did not show an effect either on clinical examination or laboratory tests. Possible usage of MPV and PDW values as inexpensive and easy biomarkers for predicting the response of omalizumab in patients with CSU needs to be emphasized as an important issue. On the other hand, even though the mean values of MPV (Baseline: 9.97, week 4: 10.30, and week 12: 10.49) and median values of PDW (Baseline: 15.7, week 4: 15.8, and week 12: 15.8) are close, the difference is statistically significant. However, it is still unclear whether these values may be useful parameters in clinical practice. More comprehensive prospective studies are needed to elucidate this issue.

In conclusion, MPV may be an important indicator of inflammation in CSU, as well as in other diseases. The subsequent increase of MPV, and other effects presented on PDW and NLR can be regarded as the effects of omalizumab on the platelets and peripheral blood cells in patients with CSU. This finding is also supported in that the MPV and PDW did not increase in the nonresponder group. Our findings need to be investigated with sophisticated in vivo and in vitro platelet activation tests and other inflammation markers in comprehensive prospective studies.

References

ERTAŞ et al. / Turk J Med Sci


