The Relationship Between Disease Activity and Platelet Indices in Pemphigus: An Observational Preliminary Study

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ABSTRACT Tests which have proven their efficacy and reliability in the follow-up of pemphigus patients are used only on a limited scale or take time to complete due to a lack of technical facilities in several centers. Therefore, more accessible methods are being considered for monitoring disease activity. We aimed to investigate the relationship between platelet function and disease activity based on the change in proinflammatory cytokine profile in pemphigus pathogenesis. The size of platelets correlates positively with their activity. Platelet sizes can be evaluated by the platelet volume index consisting of mean platelet volume (MPV), platelet-crit (PCT), and platelet distribution width (PDW). These indicators can be easily measured in complete blood count (CBC) with automatic blood counting devices, which do not require additional costs and are readily available. Patients diagnosed with pemphigus between April 2010 and February 2016 (n=18) in our center were retrospectively included in the study. Demographic data, follow-up period, clinical variants of the patients, platelet parameters (MPV, PDW, PCT), and platelet count (PLT) in CBC analysis with concurrent clinical activity, as well as indirect immunofluorescence (IIF) findings (positive highest titer) at the 6th and 12th month were recorded for each patient. MPV changes were consistent with the course of the disease. A statistically significant decrease in PCT levels was observed at the 12th month compared with the baseline levels ($P<0.05$). According to the baseline measurement, a statistically significant positive correlation (58.9%) was found between the 12-month difference measurements of IIF and PCT. Our data demonstrated that PCT decrease is correlated with IIF values. The significant correlation between PCT and IIF values in our study is important in showing the possible role of platelet index in the measurement of disease activity.

KEY WORDS: pemphigus, blood platelets, mean platelet volume

INTRODUCTION

At present, indirect immunofluorescence (IIF) and Desmoglein (Dsg) ELISA tests are recommended for serological monitoring of pemphigus disease activity and serum autoantibody levels correlated with clinical activity (1,2). IIF and Dsg ELISA tests, which have proven their efficacy and reliability in the follow-up of patients with pemphigus, are used only on a limited scale or take time to complete due to the lack of technical facilities in several centers. Therefore, more accessible methods are being considered for

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monitoring disease activity. The size of platelets correlates positively with their activity (aggregation, release of thromboxane A2 and beta thromboglobulin, expression of glycoprotein 1b and 2b/3a receptors). Platelet sizes can be evaluated using the platelet volume index consisting of mean platelet volume (MPV), platelet-crit (PCT), and platelet distribution width (PDW). Complete blood count (CBC) analysis is commonly requested from patients receiving systemic treatment in daily practice. These indicators can be easily measured in CBC with automatic blood counting devices (3), which do not require additional costs and are readily available. Hence, they are advantageous to both clinicians and patients. In the present study, we aimed to investigate the possible relationship between platelet function and disease activity based on the change in proinflammatory cytokine profile in pemphigus pathogenesis.

PATIENTS AND METHODS

Between April 2010 and February 2016, eighteen patients were diagnosed with pemphigus employing clinical, histopathological, and immunological methods at the Dermatology Clinic of Bakırköy Dr. Sadi Konuk Training and Research Hospital, and were included in the present study. The study was conducted retrospectively by scanning medical records in the electronic database. Demographic data, follow-up period, clinical variants of the patients, platelet parameters (MPV, PDW, PCT), and platelet count (PLT) in CBC analysis with concurrent clinical activity and IIF findings (positive highest titer) at the 6th and 12th month were recorded for each patient. Anti Dsg values were excluded because not every patient could have regular Dsg measurements during the follow-up period.

Cases of chronic inflammatory diseases (e.g., rheumatoid arthritis [RA] and inflammatory bowel disease [IBD]), hypertension (HT), diabetes mellitus (DM), cardiovascular diseases (CVDs), malignancy, sepsis, hematological diseases, chronic liver and kidney diseases, pregnancy, and chronic drug use that can affect platelet functions (e.g., aspirin, anticoagulants, anti-epileptic, metformin, oral contraceptives) were excluded from the study. Patients whose simultaneous CBC and IIF test results could not be obtained from medical records were not included in the study. Additionally, patients who did not attend regular outpatient follow-up for at least 1 year after their admission were excluded from the study (Figure 1).

Platelet parameters of the cases were recorded with concurrent IIF findings at three different times: 1) new-onset disease (before treatment); 2) at the 6th month; and 3) at the 12th month of follow-up.

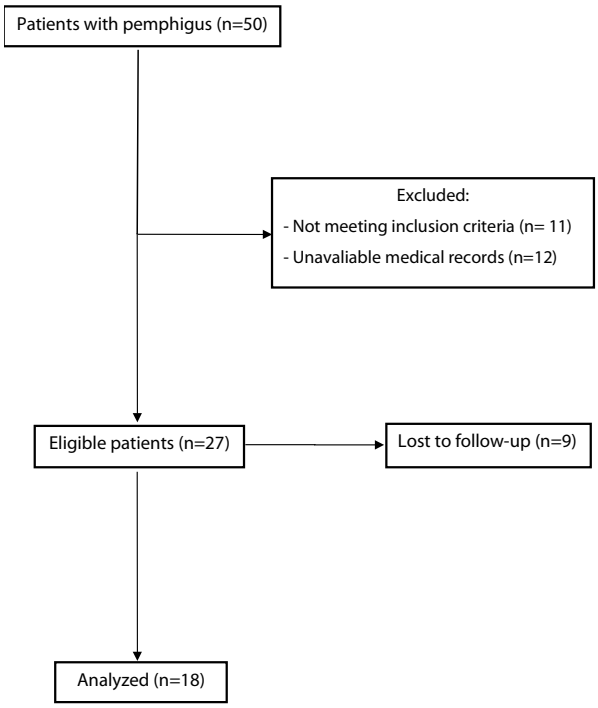

Figure 1. Flow chart of patient enrollment.

In-group comparisons of those parameters were compared with each other at all the time intervals. Moreover, we evaluated whether platelet parameters were correlated with concurrent IIF findings at all the time intervals.

Standard tubes with ethylenediaminetetraacetic acid (EDTA) are used in our hospital. The reference values of MPV, PDW, and PCT in our hematology laboratory are 6-10 fL, 8-18%, and 0.2-0.5%, respectively. Monkey esophagus was used for the IIF tests.

Statistical analyses were performed with the Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) software. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used in the evaluation of the study data. In addition, Friedman Test and Wilcoxon Signed Ranks were used for the in-group comparisons of non-distribution parameters and binary comparisons, respectively. Cochran's Q test was used to compare qualitative data, and the McNemar test was used to evaluate binary comparisons. Spearman correlation analysis was also used to evaluate the relationship between parameters. Significance was assessed at $P<0.01$ and $P<0.05$.

RESULTS

Eighteen patients (male/female: 9/9) aged between 29 and 81 were enrolled in the study. The median ages of men and women were 54.3±20.6 and

Table 1. Evaluation of PCT results

	PCT		
	Min-Max (Median)	**Mean±SD**	**[d]P**
Baseline	0.07-0.36 (0.2)	0.24±0.08	***0.108***
6th month	0.09-0.41 (0.2)	0.23±0.08	
12th month	0.12-0.32 (0.2)	0.21±0.06	
In-group comparisons;	**Mean±SD**	**[d]P**	
Baseline – 6th month	-0.01±0.08	***0.325***	
Baseline – 12th month	-0.02±0.04	***0.016****	
6th – 12th month	-0.01±0.06	***0.527***	

[c]Friedman Test; [d]Wilcoxon Signed Ranks Test; *P<0.05

45.5±12.9, respectively. All of the patients were diagnosed with pemphigus vulgaris (PV) and had mucocutaneous involvement. The patients had received a combined therapy with oral methylprednisolone and azathioprine.

Low doses of methylprednisolone (0.5 mg/kg/day) were started, and the doses were gradually tapered. At the baseline, the maximum corticosteroid dose was 80 mg, while the minimum dose was 24 mg and the mean dose was 46.8 mg/day. The mean corticosteroid doses at the 6th and 12th months were 17.7 and 7.2 mg/day, respectively. At 6 months of follow-up, 14 of 18 patients (80%) were lesion-free. Based on the endpoints defined by the international panel on pemphigus (1), all the patients showed complete remission during therapy at 12-month follow-up. Since the time of the end of the consolidation phase of the patients was variable, the consolidation phase was not included in the study.

When compared with the baseline levels, mean PDW increase and also MPV, PCT, and PLT decrease were observed. However, no statistically significant difference was found between the platelet index measurements of the cases at the baseline and the 6th and 12th months (P>0.05).

In-group comparisons

A statistically significant decrease in PCT levels was observed at the 12th month, compared with the baseline levels (0.02±0.04 units) (P=0.016; P<0.05).

The differences obtained from the other time intervals of the PCT measurements were not statistically significant (P>0.05) (Table 1).

When compared with the baseline levels, there was no statistically significant correlation between the 6th month difference values of IIF with MPV, PDW, PCT, and PLT (P>0.05) (Table 2).

No statistically significant correlation was found in the 12th month difference values of IIF according to the baseline levels with MPV, PDW, PCT, and PLT difference values (P>0.05) (Table 2).

According to the baseline measurement, a statistically significant positive correlation (58.9%) was found between the 12-month difference measurements of IIF and PCT. It was observed that PCT decreased consistently with the IIF at the 12th month compared with the baseline levels.

DISCUSSION

Despite the low sample size, our data demonstrated that PCT decrease was correlated with IIF values, which is indicative of disease activity (Table 2). The difference in the values of platelet indices was not statistically significant at baseline, 6th, and 12th month. Increased mean PDW with decreased mean MPV and PCT values (Figure 2) were considered to be related to decreased inflammatory response during the remission period. Given that PLT is an indicator of the inflammatory response, decreased mean PLT levels during the remission period (Figure 2) may be an expected outcome. However, if other acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values were included in this study, it would have been possible to interpret this data more accurately. The fact that CRP was not analyzed may be a weakness in terms of the

Table 2. Comparison of variables according to IIF at the 6th and 12th month difference measurements

		Differences by initial measurement			
		IIF 6th and 12th difference			
		[b]r		**[a]P**	
MPV 6th	12th	-0.203	-0.045	**0.420**	**0.861**
PDW 6th	12th	0.348	-0.202	**0.156**	**0.421**
PCT 6th	12th	-0.245	0.589	**0.327**	**0.010***
PLT 6th	12th	-0.288	0.376	**0.247**	**0.124**

[b]r: Spearman's correlation coefficient; *P<0,05

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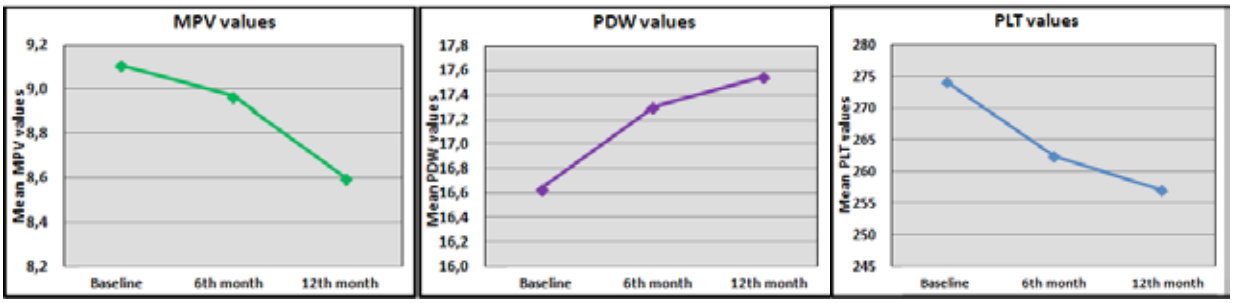

Figure 2. Mean platelet indices measurements.

evaluation of inflammatory response. We could not find any literature in which CRP and MPV were studied together, nor any literature about the course of CRP in pemphigus. In a cross-sectional study evaluating the acute phase reactants ESR and CRP in patients with PV, CRP levels were found to be positively correlated with the disease severity (4). Although Hayta *et al*. (5) reported that CRP levels were higher than the control group at the new-onset pemphigus, the finding was not reassessed during the course of the disease. Thus, there were no data that we could compare. A study integrating disease severity index, disease activity, CRP, and MPV could provide more accurate information.

Due to missing information in files or non-regular follow-ups, 32 patients were excluded from the study (see the Patients and Methods section). A total of 18 patients (9 men, 9 women) were enrolled. The number of patients in the study was small. In our study, it was assumed that the statistically insignificant change in platelet index may have been primarily due to the insufficient number of patients. Since pemphigus disease severity index scores were not assessed for all the patients, no further conclusions could be made on the relationship between disease severity and platelet parameters. In two previous reports regarding platelet indices in pemphigus (5,6), no relationship was found between disease severity and MPV.

It is known that various inflammatory cytokines increase during the immunopathogenesis of pemphigus (7,8). Inflammatory cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) play a critical role in the binding of autoantibodies to desmosomal target antigens (9). In the cultured skin models of pemphigus, while platelet derivate materials or pemphigus plasma alone could not induce acantholysis, various platelet fractions were shown to induce acantholysis in the presence of the pemphigus plasma (10). Additionally, TNF-alpha and IL-6 alone were unable to induce acantholysis, but were detected along with increased levels in the serum of patients with PV, which was correlated with the disease activity (11,12). Furthermore, IL-6 is considered to be the key factor responsible for the decreased MPV (6). Activated platelets cause the release of inflammatory mediators. Change in platelet function can be observed in inflammatory diseases of the skin such as urticaria, atopic dermatitis, and psoriasis (8).

There are various studies in the literature on platelet indices, including varied inflammatory diseases such as RA (13), IBD (14), Behcet's disease (15), chronic urticaria (16), and psoriasis (17). The majority of the studies demonstrated that an elevated MPV is associated with low-grade inflammatory disorders (15-17), whereas high-grade inflammatory disorders such as RA and familial Mediterranean fever are associated with decreased MPV (18). However, the relationship between platelet index and inflammatory disease activity is variable. In psoriasis and psoriatic arthritis, MPV was positively correlated with the disease severity and duration of the disease (17). It was found that MPV in Behcet's disease (15), as well as MPV and PDW in chronic spontaneous urticaria (16), were not reliable in assessing disease activity. In a case-control study, MPV and eosinophil levels of bullous pemphigoid patients were compared with healthy controls 6 months before and after treatment. MPV and eosinophils were found to have increased in bullous pemphigoid patients, which could be a useful indicator for vascular events. No correlation was observed with disease activity, recurrence, and disease severity (19). PDW decline and PCT increase have been associated with the active period of inflammatory diseases (13,14).

To the best of our knowledge, there are two previous reports regarding platelet indices in pemphigus. Hayta *et al*. (5) reported that MPV levels were significantly higher in patients with PV than in the control group. Mean MPV levels showed an increase in the active period of the disease and a decrease in remission; therefore, it has been suggested that there may be an association between the course of the disease

and MPV. Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and disease activity were not correlated. Compared with our study, the mean age of the patients was similar (51.2/49.9 years) and the number of male patients was higher. Since hematological parameters were evaluated based on the treatment response in that study, the follow-up periods of the patients were not similar. In contrast, follow-up periods in our study were standard for each patient. MPV was not found to be associated with disease severity, but it was suggested that MPV may be associated with disease activity. Similar to previous studies, MPV changes were consistent with the course of the disease in our study, but not statistically significant. This may be related to the low number of our patients. Although the number of patients was low, a correlation was found between IIF and PCT, suggesting that PCT could be a beneficial tool for the follow-up of disease activity. White blood cell count (WBC), ESR, mean corpuscular volume, NLR, and PLR were not assessed in our study.

Recently, Kridin *et al*. (6) pointed to a significant decrease in MPV levels in PV, compared with the control group. Additionally, MPV was lower, especially among patients with laryngeal involvement. WBC and ESR have been found to be increased compared with the control group. The level of PLT was found to be decreased during the remission period of the disease, compared with the baseline, and no significant changes were observed in MPV and WBC levels. In the same study, the hematological parameters of the patients were evaluated at baseline and about 4-5 weeks after the baseline (at the end of the consolidation phase). This is a short period of time to determine the relationship between MPV and disease activity. If the follow-up period was longer, a significant relationship with MPV would have been detected. Unlike this study, the follow-up period of our patients was longer (see Patients and Methods section). These two studies on pemphigus are summarized in Table 3.

Consistent with the study by Kridin *et al*. (5), MPV and disease activity were found to be unrelated in our study, and PLT levels decreased during the remission period, compared with the baseline. Unlike these two studies in pemphigus (5,6), in addition to MPV and PLT, other platelet indices (PCT, PDW) were also assessed in the present study. In the literature, PCT has been investigated in very few studies, compared with other platelet indices in inflammatory diseases. In a case-control study, elevated PCT levels were detected in psoriasis compared with the healthy control group, and they positively correlated with disease severity (20). It has been also reported that PCT is higher than in the control group in preeclampsia, with HT (21), RA (13), and IBD (14). In the two latter studies, PCT levels were analyzed in both the active and remission period of the diseases and found to be higher among patients in the active period. Moreover, it was described as a useful marker in disease follow-up (14,20). According to our results, PCT changes were similar to these two studies, demonstrating higher levels in active phase than in remission, although the difference of the 6th and 12th month values was not statistically significant compared with the baseline. However, IIF-related change of PCT was found to be significant during the disease activity. The disease activity was evaluated with the treatment response in the former study (5); in our study, the disease

Table 3. Studies in the literature on platelet indices in pemphigus

	Hayta et al. (n=43)	Kridin et al. (n=160)	Our study (n=18)
Study design	Retrospective case-control		Retrospective case
Age, mean, years	51.2	54.9	49.9
Sex (male/female)	27/16	63/97	9/9
Hematological parameters compared with controls	Increased MPV and NLR	Decreased MPV	-
Platelet indices at the time of clinical response (compared with baseline)	Decreased MPV	Decreased PLT	Insignificant
PI at the time of remission (compared with baseline)	Decreased MPV	Not evaluated	Decreased MPV, PCT and PLT Increased PDW
Monitoring disease activity	Treatment response	Treatment response	IIF
Correlation with disease activity	MPV	None	PCT
Correlation with IIF	Not evaluated	Not evaluated	PCT
Correlation with pemphigus severity index	None	None	Not evaluated

activity was evaluated using alteration of the IIF titrations, while the correlations of platelet indices with IIF measurements were analyzed. In this respect, to the best of our knowledge, our study is the only study comparing IIF, which is one of the objective tests measuring disease activity and platelet indices. Since PCT and PDW were not evaluated in previous studies, this is the first study in which all platelet indices were included. Furthermore, this article is the first to describe the relationship between PCT and pemphigus disease activity.

Platelet indices reference ranges have been known to vary according to age, race, physical activity, the technology of the measurement device, its model, and its calibration (22). In several studies in Turkey, reference values were reported as 0.09-0.2% for PCT and 7.2-11.7 fL for MPV (23,24). However, the values obtained in our hospital laboratory vary (see Patients and Methods section). Storage of the blood sample in CBC tubes containing EDTA anticoagulants can cause an increase of platelet size of up to 13.4% during the first 6 hours. The optimum measurement time to ensure that MPV is unaffected is 2 hours (3). In the studies on platelet index in the literature, sufficient information about the above-mentioned variables, sample waiting time, or anticoagulant use (such as EDTA, citrate), etc., was not provided. This may be due to the small number of patients and retrospective design. Additionally, these parameters need to be standardized, and cut-off values for clinical use must be determined. The disadvantage is that the platelet index is particularly affected by common diseases such as DM, HT, and KVH and the use of certain drugs. The number of new studies showing the relationship between platelet index and various diseases is also increasing on a daily basis.

The low number of patients in our study, the lack of a control group, and the unpredictable waiting time of the blood samples in the laboratory were among the major limitations of our study. Due to the retrospective design, the control group could not be included in the study because the reasons that could affect the platelet index for the control group were not predicted.

CONCLUSION

In summary, platelet index in pemphigus may vary during disease follow-up. The significant correlation between PCT and IIF values in our study is important in showing the possible role of platelet index in the measurement of disease activity. Decrease in MPV, PCT, and PLT and an increase in PDW were observed during the non-lesion period. The correlation between MPV and PLT decline and IIF measurements, which were not statistically significant, may be related to the low number of patients in our study. The platelet index is affected by several factors such as demographics, technical conditions, comorbidities, and drug use. Furthermore, its place in clinical follow-up is unclear due to the lack of standardized reference ranges. However, it can be a guiding tool for physicians when methods such as Dsg ELISA and IIF, which are used to measure disease activity, are not available. In order to determine the role of platelet parameters in clinical follow-up, prospective controlled studies involving more patients and with longer follow-up periods are needed.

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