Araştırma Makalesi
Research Article

Diagnostic role of platelet indices and leukocyte count in pseudothrombocytopenia

PSÖDOTROMBOSİTOPENİDE TROMBOSİT İNDEKSLERİ VE LÖKOSİT SAYISININ TANISAL ROLÜ

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ABSTRACT

Objective: This study aimed to evaluate whether platelet indices and leukocyte count predict the diagnosis of Ethylene Diamine Tetra Acetic Acid induced pseudothrombocytopenia (EDTA-PTCP) and differ this entity from immune thrombocytopenia (ITP).

Methods: One-hundred and sixty-nine individuals (52 patients diagnosed with EDTA-PTCP, 52 patients with newly diagnosed ITP, and 65 healthy individuals) were included. Demographic data and complete blood count (CBC) indices were compared between groups retrospectively.

Results: Age and sex distribution were similar in all groups. Between EDTA-PTCP and ITP groups, no difference was found for absolute leukocyte, neutrophil, lymphocyte, and monocyte counts as well as hemoglobin level, red-cell distribution width (RDW), and platelet distribution width (PDW). The median mean platelet volume (MPV) of the ITP group was found to be higher than the control and EDTA-PTCP groups ($p<0.001$ and $p=0.001$, respectively, 95% confidence interval; 2.52-4.45 and 0.56-2.6).

Conclusion: The results support that low platelet count in CBC may be a false result due to EDTA. Thrombocytopenic patients who have no bleeding symptoms and with normal MPV should be evaluated for EDTA-PTCP. This evaluation can be made by a peripheral blood smear or CBC in a blood tube containing other anticoagulants than EDTA (such as citrate or heparin). Therefore the increase in MPV may indicate a diagnosis of ITP rather than EDTA-PTCP. To make a more definite recommendation on this issue, studies involving more patients are needed.

Keywords: Pseudothrombocytopenia, immune thrombocytopenia, diagnosis, complete blood count indices

ÖZ

Amaç: Bu çalışmanın amacı, trombosit indekslerinin ve lökosit sayısının etilendiamintetraasetik asit ile indüklenen psödotrombositopeni (EDTA-PTSP) tanısını öngörüp öngörmediğini ve bu antiteyi immün trombositopeniden (İTP) ayırt edip etmediğini değerlendirmektir.

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Gereç ve Yöntem: Yüz altmış dokuz kişi (EDTA-PTSP tanısı alan 52 hasta, yeni İTP tanısı konmuş 52 hasta ve 65 sağlıklı birey) çalışmaya dahil edildi. Gruplar arasında demografik veriler ve tam kan sayımı (TKS) indeksleri retrospektif olarak karşılaştırıldı.

Bulgular: Yaş ve cinsiyet dağılımı tüm gruplarda benzerdi. EDTA-PTSP ve İTP grupları arasında mutlak lökosit, nötrofil, lenfosit ve monosit sayıları ile hemoglobin düzeyi, kırmızı hücre dağılım genişliği (RDW) ve trombosit dağılım genişliği (PDW) açısından fark bulunmadı. İTP grubunun medyan ortalama trombosit hacmi (MPV) kontrol ve EDTA-PTSP gruplarına göre daha yüksek bulundu (sırasıyla p<0,001 ve p=0,001, %95 güven aralığı; 2,52-4,45 ve 0,56-2,6).

Sonuç: Çalışmamızda elde edilen bulgular, TKS'deki düşük trombosit sayısının EDTA'ya bağlı yanlış bir sonuç olabileceğini desteklemektedir. Kanama semptomları olmayan ve MPV'si normal olan trombositopenik hastalar EDTA-PTSP açısından değerlendirilmelidir. Bu değerlendirme, EDTA'dan başka antikoagülan (sitrat veya heparin gibi) içeren kan tüpüne alınan örnekten TKS çalışılması veya periferik kan yaymasının değerlendirilmesi ile yapılabilir. Çalışmamızdaki bulgular MPV'deki artışın, EDTA-PTSP'den ziyade İTP tanısını gösterebileceğini düşündürmektedir. Bu konuda daha kesin önerilerde bulunabilmek için daha fazla hastayı içeren çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Psödotrombositopeni, immün trombositopeni, tanı, tam kan sayımı indeksleri

Thrombocytopenia is a common laboratory finding with a prevalence of up to 15% in particular settings and may indicate a significant pathology (1). Conditions that are mainly involved in the pathophysiology of thrombocytopenia are; increased platelet destruction, hemodilution, increased sequestration of platelets in the spleen (as occurring physiologically during pregnancy), and decreased platelet production, or use of drugs that attenuates thrombopoiesis (2). In case of a suspicion of thrombocytopenia, first of all, pseudothrombocytopenia (PTCP) -the in vitro situation which has no clinical significance- should be excluded to prevent further unnecessary diagnostic evaluation.

PTCP is a phenomenon caused by the aggregation of platelets in vitro and does not increase the risk of bleeding. The exposure of blood to the Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant can cause platelet clumping or the formation of platelet rosettes around leukocytes. Thus, automatic blood count analyzers – which's working principle is based on the size of the cell - can determine falsely low platelet counts, and this condition is so-called EDTA-induced false thrombocytopenia or pseudothrombocytopenia (EDTA-PTCP). A small percentage of healthy people can carry agglutinins in their serum against EDTA that can induce platelet aggregation in vitro, after the collection of the blood into the EDTA-containing blood tubes. This is thought to be due to a platelet autoantibody directed against a latent epitope on the platelet cell membrane glycoprotein revealed by EDTA-induced degradation of GPIIb/IIIa (3). The incidence of EDTA-PTCP has been reported to be up to 0.07-0.2% (4).

In plenty of diseases that cause thrombocytopenia other than immune thrombocytopenia (ITP), absolute erythrocyte and/or leukocyte counts can change. However, if EDTA-PTCP is not accompanied by any pathological condition, no abnormality is expected in absolute values of erythrocytes and/or leukocytes. Therefore, we can mention

for daily practice that in case of detection of isolated thrombocytopenia in routine complete blood cell count analysis (CBC), the cause may be EDTA-PTCP as well as ITP.

In routine daily practice, it is not possible to distinguish EDTA-PTCP from ITP according to the platelet counts measured by automatic blood count analyzers, and therefore the diagnosis of EDTA-PTCP requires another approach other than CBC; and is diagnosed by the examination of the peripheral blood smear. For this reason, we hypothesized to diagnose EDTA-PTCP according to the CBC indices other than platelet counts and whether if possible to eliminate the necessity of peripheral blood smear examination. In line with this hypothesis, we aimed to compare the CBC indices and leukocyte counts of patients with EDTA-PTCP, ITP, and healthy controls. We investigated whether there was a significant difference between the groups in terms of platelet indices and leukocyte counts and whether this difference could be predictive of the diagnosis of PTCP.

MATERIALS AND METHODS

This study was conducted between October 2016 and December 2021 in the hematology department of the Göztepe Prof. Dr. Süleyman Yalçın City Hospital, Istanbul Medeniyet University, including patients with EDTA-PTCP, newly diagnosed ITP patients, and a healthy control group.

The EDTA-PTCP group was selected from patients without any known diseases or a history of drug use and found to have thrombocytopenia in the CBC that was detected incidentally during routine health controls. EDTA-PTCP was defined as 1) Platelet counts below 100000/mm^3 in CBC for the blood sample drawn into the EDTA-containing tube and analyzed by the automatic analyzer, 2) The presence of platelet aggregates and/or satellites in the blood smear prepared from a blood sample collected into the EDTA containing tube as described before (4). Patients who were hospitalized at the time of CBC analyses, pregnant women, and those younger than 18 years of age were not included in the study.

ITP was diagnosed after the exclusion of all the possible secondary causes of thrombocytopenia (such as infections, rheumatic diseases, malignancies, drug use, viral hepatitis, microangiopathic hemolysis, autoimmune disorders, etc.). In the ITP patient group, patients who had concomitant diseases other than ITP or were on medication that would affect CBC parameters were excluded.

The control group was composed of healthy individuals who were admitted to the hematology outpatient clinic for routine control without any known diseases and drug use.

A total of 52 EDTA-PTCP patients, 52 newly diagnosed ITP patients, and 65 healthy controls were included in the study. Age, gender, leukocyte, and platelet counts, mean platelet volume (MPV), platelet distribution width (PDW), and percentage of platelets in the blood (plateletcrit = Pct) were analyzed from the patient files retrospectively.

CBC was studied from blood samples anticoagulated with 5% sodium EDTA in an automatic Abbott Diagnostic CELL DYN Sapphire hematology analyzer. For the preparation of the blood smear, a venous blood sample was drawn into the EDTA-containing tube and scattered on the glass slide, dried at room temperature, and then stained by the May-Grünwald-Giemsa method by the same laboratory technician within 2 hours after the collection of the blood sample. All blood smears were examined by a hematology specialist with a 10x ocular and 100x magnification objective with an Olympus CH20 microscope. The study was approved by the local ethics committee of Istanbul Medeniyet University Göztepe Prof. Dr. Süleyman Yalçın City Hospital (Date: 12.01.2022, Decision number: 2021/0706) and conducted per the principles of the Helsinki Declaration.

Statistical Analysis

SPSS 22 (Statistical Package for the Social Sciences Inc. Chicago, IL, ABD) software was used for the statistical analysis of the data. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for normality analysis. Differences between groups were compared with the Chi-square test for categorical variables. Continuous variables were compared with an independent sample t-test and one-way ANOVA analysis. In the correlation analysis, the Pearson correlation coefficient was used for the comparison of

normally distributed continuous variables, and the Spearman correlation coefficient was used for non-normally distributed continuous variables. All analyses were two-tailed and the type 1 error rate was determined as 5%.

RESULTS

A total of 169 subjects were evaluated; 52 patients with EDTA-PTCP, 52 patients diagnosed with ITP, and 65 healthy controls. According to the age and sex, there was no difference between groups (Table 1). The patients' characteristics and CBC parameters according to the groups were summarized in Table 1.

Table 1: Patients charateresristic according to the groups

	ITP (n=52)	EDTA-PTCP (n=52)	Control (n=65)	*p*
Sex				
Female	34 (65,4%)	36 (69,2)	43 (66,2)	-
Male	18	16	22	
Age (min–max), years	20-91	19-81	27-79	0,59
Median	48,5	45	44	
Leukocyte, 10^6/µL				
Median (min–max)	7090 (3950-11300)	7045 (3210-12500)	6850 (3650-10470)	0,73
Neutrophil, 10^6/µL				
Median (min–max)	4340 (1860-8150)	4190 (1250-9200)	4000 (2200-8220)	0,477
Lymphocyte 10^6/µL				
Median (min–max)	1950 (738-4100)	1995 (270-4690)	2140 (1000-3940)	0,387
Monocyte 10^6/µL				
Median (min–max)	410 (70-930)	440 (100-1050)	460 (180-950)	0,643
Hgb, g/dl				
Median (min–max)	13,4 (10,4-17,5)	13,4 (8,2-16,5)	13,6 (8,6-16,5)	0,581
RDW, %				
Median (min–max)	13 (10,3-27,5)	13 (10,6-19,3)	13 (10,9-18,2)	0,994
Platelet, 10^3/µL				
Median (min–max)	31450 (1160-87700)	62700 (10000-98000)	234000 (152000-421000)	**<0,001**
MPV, fL				
Median (min–max)	12,3 (9,08-19)	11,3 (7,9-14,35)	9,5 (5,53-13,9)	**<0,001**
Pct, %				
Median (min–max)	0,04 (0-0,16)	0,072 (0-0,167)	0,23 (0,14-0,39)	**<0,001**
PDW, %				
Median (min–max)	15,3 (10-35,6)	16,7 (10,1-24,6)	16,3 (15,3-22,7)	0,116

Hgb: hemoglobin, RDW: red cell distribution width, MPV: mean platelet volüme, Pct: plateletcrit, PDW: platelet distribution width, fL: femtoliter. $p \leq 0.05$: statistically significant.

There was no statistically significant difference in the leukocyte, neutrophil, lymphocyte, monocyte, hemoglobin, RDW, and PDW values between EDTA-PTCP and ITP groups (Table 1). The median platelet count of the control group was found to be higher than the ITP and EDTA-PTCP groups (p<0.001). The median platelet count of the EDTA-PTCP group was higher than the ITP group (p<0.001). The median MPV value was higher in the ITP group than in the control and EDTA-PTCP groups (p<0.001 and p=0.001, respectively, 95% confidence interval: 2.52-4.45 and 0.56-2.6, respectively). There was no statistically significant difference between the groups in terms of median PDW value (p=0.116).

Bivariate correlation analysis was performed and a positive correlation was found between platelet count and PDW and PCT (p<0.05 and <0.01, respectively). A negative correlation was found between MPV and platelet count and PCT (p<0.01) (Table 2).

Table 2. Bivariate correlation analysis

	Mean	SD	Pdw	MPV	Platelet	Pct
PDW	17,0358	3,45951	1	0,054	***,198****	***,227****
MPV	11,1148	2,38901	0,054	1	**-,596****	**-,450****
Platelet count (10^3/µL)	124877,87	104589,077	***,198****	**-,596****	1	**,956****
Pct	0,12721	0,093648	**,227****	**-,450****	**,956****	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

SD: standard deviation, MPV: mean platelet volüme, Pct: plateletcrit, PDW: platelet distribution width.

DISCUSSION

For the management of isolated thrombocytopenia, pseudothrombocytopenia should be excluded first. Due to this circumstance being ignored, unnecessary diagnostic evaluation such as bone marrow biopsy and redundant treatments such as initiation of corticosteroids, platelet transfusions, or even splenectomy can be applied to the patients (5, 6). This situation both brings an unessential workload to health care providers, and unessential cost to the reimbursement system, and more importantly can harm the patient. Different approaches have been tested to prevent time and temperature-dependent in vitro aggregation of platelets in the presence of EDTA, but none of them have been proven to be optimal for routine use (7). Numerous anticoagulants such as citrate, heparin, and magnesium sulfate have been used in blood tubes to reduce the risk of the development of PTCP, but this issue remains unresolved (8). EDTA-PTCP can easily be recognized by specialists by blood smear examination. But there may be difficulties in recognizing this circumstance in such health centers that do not have a hematology department or cannot perform blood smears.

In the present study, we found that the median MPV of the EDTA-PTCP group was significantly lower than the ITP patient group. The most common underlying pathophysiologic mechanism in ITP is increased platelet destruction by peripheral macrophages and as a result of the increased production of megakaryocytes in the bone marrow, young and large volumed platelets cross into the peripheral circulation and this situation results in an increase in MPV (9). Our results support this mechanism as the mean MPV of our ITP patients was found to be significantly higher than in the other two groups. On the other hand, some reports suggest that the MPV is higher in patients with EDTA-PTCP than in ITP patients (10, 11). It has been also reported that EDTA may increase the MPV by causing deformation and swelling in platelets giving rise to an increase in the platelet volume (12). This can be a time dependent shape change due to EDTA and so on it is suggested to measure MPV not later than 120 minutes after

the collection of the blood sample (12). In our study, the assesment of all blood samples were performed in 2 hours after collection and MPV was higher in the EDTA-PTCP group than in the healthy control group, therefore this may be because of the aggregation of platelets rather than swelling. The large clusters of platelets may be unrecognizable for the automated CBC analyzers and this can result in PTCP. The working principle of these devices is based on bioimpedance and optical analysis. In the impedance method, cells are defined according to their size, and 2 to 35 fl volumed particules are recognized as platelets. With the optical method, cells are defined according to their granularity and nuclear structure. Therefore a large clump of platelets bigger than 35 fl (as seen in our patient shown in Figure 1 and 2) cannot be defined as platelet by the analyzer because of the unexpectedly large volume with an anucleated structure.

Figure1-A-B Two neutrophils are seen on the middle part of the slide in one of the patients in the EDTA-PTCP group. Note platelets are absent in this part of the slide. The edge of the same slide (note the erythrocytes featured as spherocytes) shows numerous large platelet aggregates, a neutrophil on the left and a monocyte.

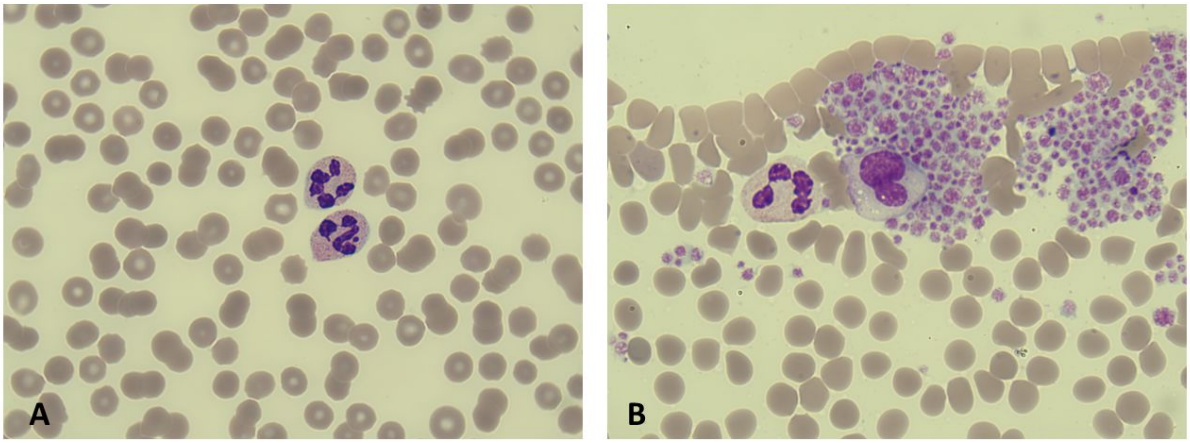
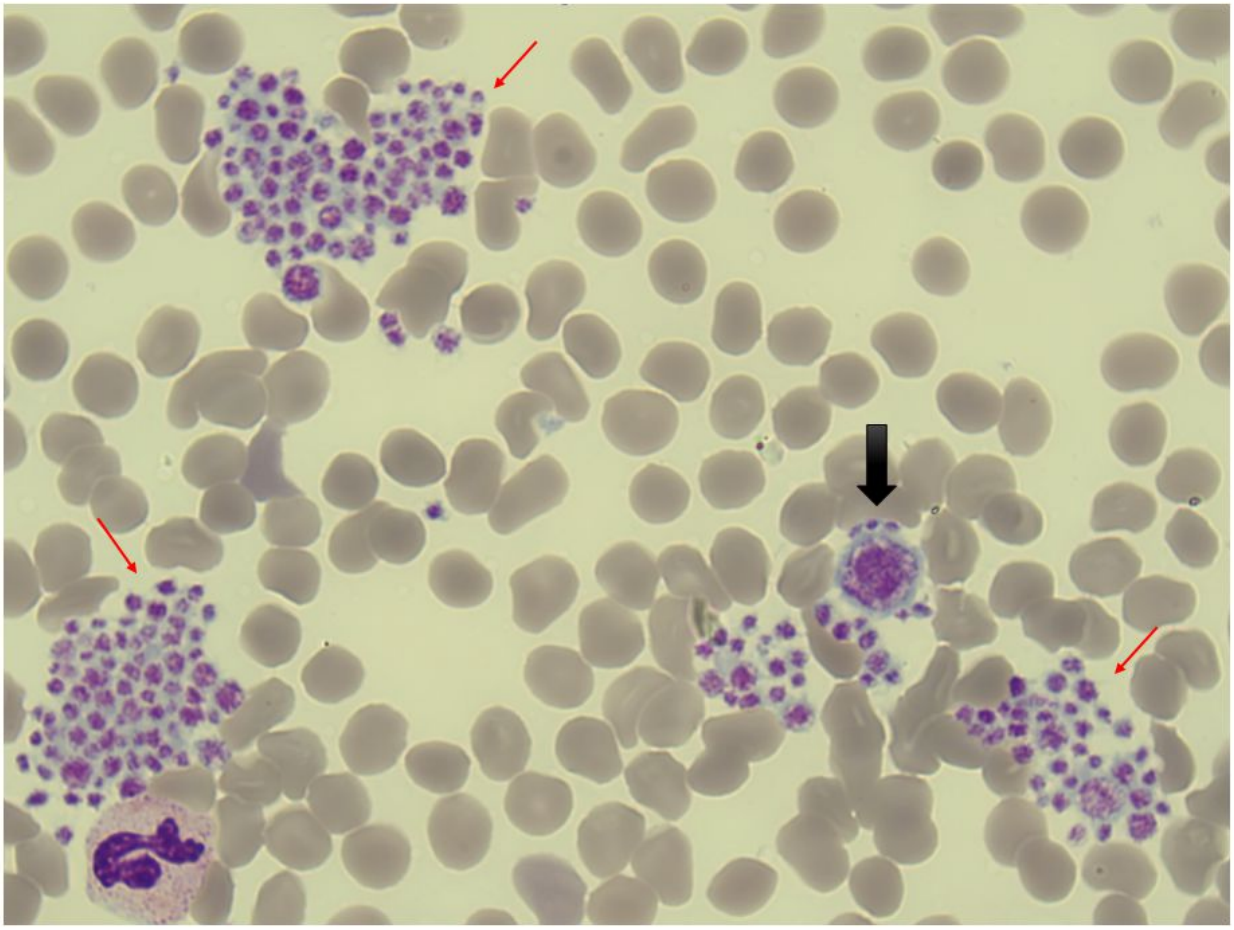


Figure 2 Large clumps (aggregates) of platelets (red arrows) and a giant plaletet (black arrow, may be because of swelling due to EDTA) in a patient in the EDTA-PTCP group.



Yavaşoğlu et al. (13) reported the MPV to be lower in the EDTA-PTCP group than in the healthy controls. These unconvincing results bring the necessity of more studies involving more patients. However, following our findings, we can guess that unelevated MPV in a thrombocytopenic patient without bleeding symptoms should raise the suspicion of EDTA-PTCP rather than ITP.

The incidence of EDTA-PTCP has been shown to increase in some clinical situations. Işık et al. (11) reported that hospitalization, infection, low molecular weight heparin use, and pregnancy increase the incidence of EDTA-PTCP. Yıldız et al. (10) suggested in their study including 164 patients with EDTA-PTCP, 43 patients with ITP, and 45 healthy controls that the EDTA-PTCP patients had more comorbid diseases. In addition, some publications claim that EDTA-PTCP is more common in patients with autoimmune or neoplastic diseases, severe liver diseases, or atherosclerosis (3). We cannot comment on this issue because the presence of comorbid diseases was an exclusion criterion in our study group.

In the present study, we did not find a statistically significant difference between the groups in terms of PDW. Yıldız et al. (10) reported that the PDW was higher in the EDTA-PTCP group than in the ITP and control groups. On the other hand, Yavaşoğlu et al. found the PDW to behigher in the control group than in the EDTA-PTCP group. Therefore, there is no consensus about this issue.

Falsely high leukocyte counts (pseudoleukocytosis) may be present in patients with PTCP due to the misidentification of platelet aggregates as leukocytes in automatic CBC analyzers (11, 14). In addition, automatic CBC analyzers may miss the large platelet-leukocyte structures that occur due to platelet satellites around leukocytes, and pseudoleukopenia may occur (14). We did not find a significant difference between our study groups in terms of WBC. While all of our EDTA-PTCP patients had platelet aggregates in the blood smear (Figure 1 and Figure 2), we observed platelet rosettes around leukocytes in only particular cases.

Several limitations of our study deserve to be mentioned. The main limitation can be discussed as the small number of patients in each group. The main reason for this is that we strictly excluded patients with comorbidities and on medication to avoid bias. Another limitation is not being able to perform the CBC with anticoagulants (citrate, heparin, magnesium sulfate) other than EDTA.

The results we obtained in our study support that low platelet count may be a false result in a patient without bleeding symptoms and whose MPV has not increased. A blood smear examination remains an important diagnostic tool for EDTA-PTCP. To make a more definite recommendation on this issue, studies involving more patients are needed but currently, the most reliable method is the evaluation of these cases with blood smears as well as CBC. Remarkably, we would like to point out that the blood smear should be examined carefully in thrombocytopenic patients, and especially the edge of the slide should be carefully evaluated. Because, as an example just like in one of our patients represented in Figures 1 and 2, in EDTA-PCTP, the middle part of the slide can predict real thrombocytopenia (Figure 1-A) while large aggregates can be seen only on the edges of the slide. This is because of the scattering of the large volumed aggregates of thrombocytes moving towards the slide during the preparation of the smear and can be mistakenly regarded as real thrombocytopenia if the edges of the slide are not inspected.

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