Spurious Thrombocytopenia in Automated Platelet Count

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ABSTRACT

Spurious thrombocytopenia is a well-known phenomenon observed with the widespread use of hematology analyzers (HAs). In this study, 355 specimens with pseudo-thrombocytopenia (PTCP) were evaluated via epidemiology, identification, remedies, and platelet (PLT) count. Data showed that anticoagulants such as citrate and/or heparin-dependent PTCP (16.0%) became increasingly common, whereas ethylenediaminetetraacetic acid (EDTA)–induced PTCP (49.1%) remained the most frequent. We note that that nearly half of the patients with PTCP had veritable decreased PLT counts, even after PLT levels had been corrected. Our findings suggest that there were seasonal changes in patients with PTCP: PLT levels were higher in spring, compared with other seasons, with winter levels being the lowest. There were higher risks of PTCP for individuals with malignant neoplasms, liver diseases, infection, and hematologic disease, compared with other conditions. PTCP is related to diseases, rather than being a simple phenomenon, and thus demands careful attention.

Keywords: pseudo-thrombocytopenia, platelet aggregate, hematology analyzer, EDTA, cold agglutination, seasonal change

The use of hematology analyzers (HAs) in the clinical laboratory is now standard. The widespread use of those instruments has improved the accuracy and speed of the complete blood count (CBC) assay with differential tests. However, in a variety of conditions, automated parameters may be fictitious.[1,2]

Pseudo-thrombocytopenia (PTCP), or spurious thrombocytopenia, is an in vitro specimen-collection problem that occurs when the anticoagulant used while testing the blood specimen causes clumping of platelets (PLTs), which results in spuriously low PLT counts. It occurs most often in ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood; however, other anticoagulants (citrate, heparin, and oxalate) have also been implicated in several reports.[1-3]

Failure to recognize PTCP leads to undue patient anxiety and unnecessary diagnostic tests and treatments, such as bone marrow biopsy, surgical interventions, splenectomy, steroid therapy, and PLT transfusion because patients are considered to have thrombocytopenia.[4,5] Laboratory operators of these instruments and healthcare professionals must be aware of the possibility of spurious data from HAs.[6-8]

In this article, we describe the features of patients with PTCP and factors that contribute to the condition, such as demographics, seasonal changes, and PLT counts, for 355 specimens with spurious thrombocytopenia from 2013 to 2016.

Abbreviations

HAs, hematology analyzers; CBC, complete blood count; PTCP, pseudo-thrombocytopenia; PLTs, platelets; EDTA, ethylenediaminetetraacetic acid; EDTA-PTCP, EDTA-induced pseudo-thrombocytopenia; PTL1, aggregate specimens; PLT2, repeat specimens

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Materials and Methods

CBC, determined via differential tests anticoagulated with EDTA, were gathered using the XE-2100 (Sysmex Corporation) or LH750 (Beckman-Coulter Inc.) HA. Blood films were fixed and stained with Wright-Giemsa staining.

Blood was drawn again if PLT agglutination was discovered via peripheral blood smear. Methods included examining

a blood specimen containing EDTA as soon as possible, using different anticoagulants such as citrate and heparin, elevating the temperature of a blood specimen to 37°C, and using a counting chamber with immediate dilution without any anticoagulants.

All data from spurious decrease of PLTs were collected at the First Affiliated Hospital of Nanjing Medical University (Nanjing, China), from August 2013 through September 2016.

Statistical Analysis

Data were analyzed using Stata statistical software, release 14.0 (StataCorp LLC), with a statistical significance level set at $P < .05$. We used the paired *t*-test to compare the level of PLT counts in aggregate to repeat-specimen groups.

Table 1. Characteristics of Patients With PTCP

Sex[a]	No
Female	164
Male	191
Age, y[b]	
>60	142
≤60	166
Clinical context[c]	
Malignant neoplasms	69
Liver diseases	37
Infection	22
Hematology consultation	20
Autoimmune	12
Preoperative screening	11
Other	24
Season when diagnosed[d]	
Spring	58
Summer	38
Autumn	44
Winter	28

PTCP, pseudo-thrombocytopenia.
[a] *n = 355.*
[b] *n = 308.*
[c] *n = 195.*
[d] *n = 168.*

Results

Demographics of Patients With PTCP

A summary of the demographics of patients with PTCP is shown in **Table 1**. In total, 355 specimens from patients with PTCP were analyzed from August 2013 through September 2016. The incidence of PTCP was 0.04%, including outpatient, inpatient, and asymptomatic health examination results from individuals who came to the hospital only for health screening.

The patients with PTCP were aged 1 to 94 years; PTCP occurred more often in those older than 60 years of age, compared with those aged 60 years or younger. Patients with PTCP are comprised of a proportion of patients diagnosed with malignant neoplasms, liver diseases, and infection, who have sought hematology consultation, autoimmune, and preoperative screening, in that order. From 2014 through 2015, the PLT counts in spring were higher than those in other seasons, with winter values being the lowest.

Situations Leading to PTCP

As shown in **Table 2**, spurious decrease of PLT count is related to several situations, including PLT agglutination caused by anticoagulation (EDTA and other anticoagulants such as citrate and heparin, 65.1%), difficult venipuncture (26.4%), cold agglutination (6.6%), and PLT satellitism (0.94%). Also, there was a unique case of spuriously low PLTs counts caused by warming EDTA tubes at 37°C.[9]

Identification and Remedies

All decreased PLT counts detected by HAs should be confirmed by peripheral blood smear. For the PLT clumps, in many instances, drawing and testing another blood specimen will be helpful. Several remedies have been proposed to prevent PTCP, include examining blood specimens containing EDTA as soon as possible[10]; using different anticoagulants such as citrate, heparin, or oxalate[10]; elevating the temperature of a blood specimen to 37°C, and using a counting chamber with immediate dilution and without any anticoagulants (**Figure 1**). We believe that the most suitable and practical approach is rechecking the specimen for PTCP-induced anticoagulation immediately after it is drawn.

Also, before their next test, patients with PTCP should be given a warning about the possibility of spurious results from HA testing, to avoid misdiagnosis and blood redrawing. A comment about this warning should be added to the patient results, and the ordering physician should be notified.

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Table 2. Situations Leading to Spuriously Decreased Platelet Counts

Situation	No. (%)[a]
PLT agglutination (EDTA)	52 (49.1%)
PLT agglutination (EDTA and citrate or heparin)	10 (9.4%)
PLT agglutination (EDTA, citrate, and heparin)	7 (6.6%)
Difficult venipuncture	28 (26.4%)
Cold agglutination	7 (6.6%)
Being warmed at 37°C	1 (0.94%)
PLT satellitism	1 (0.94%)

PLT, platelet; EDTA, ethylenediaminetetraacetic acid.
[a]*n = 106.*

PLT Count of Aggregate and Repeat Specimens

The PLT count of aggregate and repeat specimens is shown in **Figure 2**. The mean level of repeat specimens significantly increased to 133.2 (27~328 × 10^9/L), and the PLT counts with EDTA (aggregate) are 45.4 (2~175 × 10^9/L) (p <.001). However, the PLT counts were still lower than 125 × 10^9 per L with 158 specimens (49.5%), although these counts had increased on reevaluation. Also, they are mostly (69.8%) diagnosed as indicating hematologic disease.

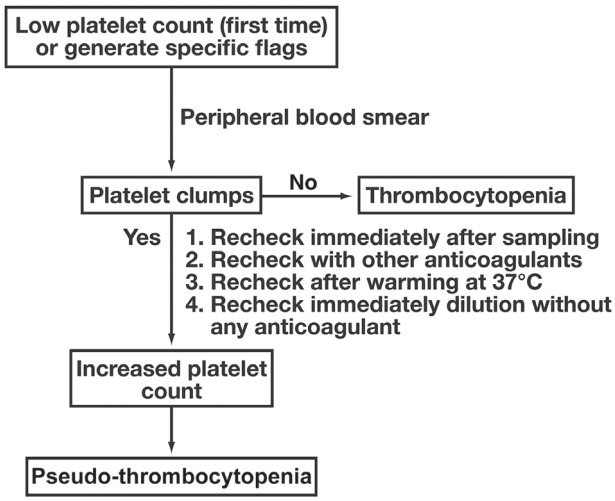

Figure 1

Flowchart of how to distinguish thrombocytopenia from pseudo-thrombocytopenia.

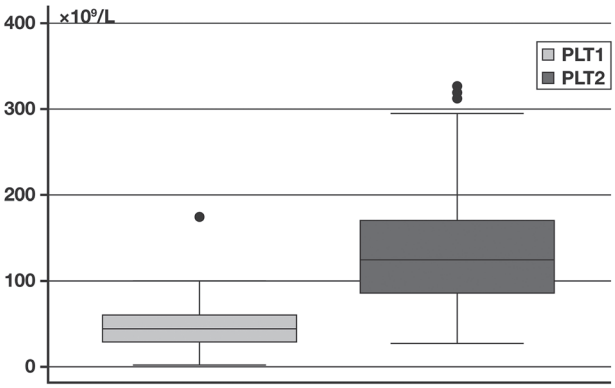

Figure 2

Platelet (PLT) count of aggregate (PLT1) and repeat (PLT2) specimens.

Discussion

When a patient has a low PLT count without any hematologic disease, family history, and/or bleeding-tendency manifestation identified, PTCP should be considered, and health care professionals treating that patient should request blood film testing. A repeat CBC assay should be performed before the PLTs agglutinate because agglutination will cause false counts.

PTCP is complicated and occurs in several circumstances. It not only occurs in PLT agglutination related to anticoagulants and/or temperature but also in PLT satellitism, coagulation within the specimen, large PLTs, etc. We did not evaluate large PLTs in this study. EDTA-induced pseudo-thrombocytopenia (EDTA-PTCP) is the form most frequently observed in clinical practice.[1] As one might expect based on that finding, in our study, EDTA-PTCP comprised nearly half of the incidences of PTCP. However, we believe it is notable that anticoagulants such as citrate and/or heparin-dependent PTCP became increasingly common, with an incidence of 16% incidence recorded in our study.

EDTA-PTCP incidence is approximately 0.07% to 0.2% in hospitalized patients;[11,12] however, it was approximately 0.04% in our study. A possible explanation might be that the population included not only outpatient and inpatient but also healthy examined individuals.

Our study demonstrated that PTCP occurs more frequently in people aged 65 years or older. Also, seasonal changes

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exist in PTCP: specifically, PTCP occurred more commonly in the spring than in the winter. The reason for this seasonal difference is unclear; we assume that it may be due to the protopathic disease of patients with PTCP; this theory requires further study for confirmation.

The results of a previous study[13] showed that people with malignant neoplasms, chronic liver disease, infection, pregnancy, autoimmune diseases, and cardiovascular diseases have increased risk of EDTA-PTCP. EDTA-PTCP has also been observed in patients who are disease free. Our study findings are consistent with these findings. Further, in our data, malignant neoplasms rank first among risk factors.

The EDTA-PTCP mechanism involves antibodies that may cause in vitro PLT aggregation by binding to Gp IIb/IIIa receptors, which reassociate and translocate in the presence of EDTA on the PLT surface. However, the mechanism of how other anticoagulants such as citrate and heparin cause thrombocytopenia is still unclear. von Willebrand disease, type 2B, may be the possible cause of spurious thrombocytopenia when agglutination occurs in all anticoagulants.

Most of the agglutinins reacted strongly at room temperature or low temperatures; however, some agglutinins are temperature independent or reacted most strongly at 37°C. Also, we observed several cold aggregations. In contrast, Zhang et al[9] report a unique case of spuriously low PLT counts caused by warming EDTA tubes at 37°C. The PLT aggregation is not only anticoagulant and temperature dependent but also time dependent. Thus counting PLT immediately after specimen drawing is an effective remedy.

It is worth noting that the reevaluated PLT levels were not all normal. Moreover, nearly half of the patients with PTCP had decreased PLT counts. Salama[14] recommended considering PTCP not only in the list of ITP differential diagnoses but also in the list of diseases associated with PTCP. Based on our data, PTCP seems not to be only spurious but also "veritable" thrombocytopenia.

In conclusion, laboratory operators and healthcare professionals must be aware of the possibility of spurious data from automated PLT counts. PTCP is related to diseases, rather than being a simple phenomenon; it demands careful attention and further research about its mechanism, seasonal changes, and other such factors. **LM**

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