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Case Report

Interpretation of pseudothrombocytopenia using platelet histograms and flags in a hematology autoanalyzer in a healthy child: a case report

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ABSTRACT

Background. Pseudothrombocytopenia is a spurious thrombocytopenia caused mostly by ethylenediaminetetraacetic acid (EDTA) use, and if detected early, unnecessary testing and treatment can be avoided. We present pseudothrombocytopenia caused by EDTA and citrate in an asymptomatic healthy child, as well as the value of using peripheral blood smear, platelet histogram, and flag data.

Case. A previously healthy 13-year-old girl with thrombocytopenia who developed tonsillitis 12 days previously was referred to our hematology department. Laboratory tests revealed severe thrombocytopenia ($17x10^3$/μL) in EDTA samples. A peripheral blood smear revealed numerous platelet clumping. We hypothesized EDTA-dependent pseudothrombocytopenia and ordered a platelet count by citrate tube. A citrate tube revealed thrombocytopenia with a platelet count of $55x10^3$/μL. The platelet count ($175x10^3$/μL) returned to normal with heparin tubing. All blood samples had a similar platelet histogram and flags in the autoanalyzer. The platelet histogram indicated a serrated/sawtooth curve containing the largest platelet aggregates. Platelet flags alert messages about platelet clumping.

Conclusions. Peripheral blood smear is the most reliable test for pseudothrombocytopenia. If the physician has no experience with smear examination, both laboratory technician and physician should be aware of abnormal platelet histograms and platelet clumping messages in platelet flags, which indicate pseudothrombocytopenia.

Key words: pseudothrombocytopenia, child, platelet histogram, platelet flags.

Pseudothrombocytopenia is a spurious thrombocytopenia caused by blood tube-dependent antibodies. The majority of antibodies target glycoprotein IIb/IIIa on platelet surfaces. Pseudothrombocytopenia should be considered in the differential diagnosis of thrombocytopenia. In the literature, pseudothrombocytopenia is often misinterpreted as immune thrombocytopenic purpura, resulting in overtreatment, splenectomy, and unnecessary laboratory tests.[1,2]

Although ethylenediaminetetraacetic acid (EDTA) is the most common cause of pseudothrombocytopenia, citrate-induced pseudothrombocytopenia has been documented on a few occasions.[2] When physicians see platelet clusters on a peripheral blood smear, they should consider pseudothrombocytopenia; the diagnosis is confirmed by a repeat blood count using citrate or heparin tubes other than EDTA. If the medical professional has no experience evaluating peripheral blood smears, the diagnosis is commonly missed. As a

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result, the technician must be able to accurately interpret platelet histograms and flags before reporting to the physician in the laboratory.[3-5] Furthermore, clinicians need to know how to evaluate platelet histograms and flags.

We present a healthy child who was diagnosed with both EDTA and citrate-induced pseudothrombocytopenia, as well as the value of platelet histogram and flags analysis.

Case Presentation

A 13-year-old girl was admitted for cancer screening due to a positive family and at the request of her parents, and her complete blood count revealed thrombocytopenia. She was referred to the hematology department for further examination into the cause of thrombocytopenia, such as immune thrombocytopenic purpura. She was asymptomatic upon admission to the hospital, however, she had tonsillitis at least 12 days before. She was administered antibiotics due to a suspected bacterial infection. Her personal history included no thrombocytopenia or bleeding symptoms, and she underwent a successful adenoidectomy when she was 10 years old. Her family history included her father's thyroid cancer diagnosis, her aunt's lung cancer diagnosis, and her sibling's Wilms tumor diagnosis. Upon admission, an automated hematology analyzer (DxH400, Beckman-Coulter Inc., Nyon, Switzerland) revealed isolated thrombocytopenia (platelet count: $17x10^3/\mu L$) with mean platelet volume (MPV): 8.4 fL, platelet distribution width (PDW): 16 fL and typical platelet histogram patterns (Table I and Figure 1). When we examined the peripheral blood smear, we observed numerous platelet clumping. We suspected pseudothrombocytopenia and performed a citrate tube platelet count. Citrate samples again indicated thrombocytopenia (platelet count: $55x10^3/\mu L$, MPV: 9.1 fL, PDW: 17.4 fL), as well as platelet clumping similar to EDTA. The platelet count in the heparin tube sample was normal ($175x10^3/\mu L$, MPV: 10.1 fL, PDW: 18.1 fL). However, platelet clumping was also seen in the peripheral blood smear, and abnormal platelet histogram and flags with heparin. We consulted the referring physician regarding the possibility of reviewing the peripheral blood smear. However, he indicated a lack of experience in interpreting peripheral blood smear assessments of this nature. Concurrently, we requested the laboratory technician to examine the platelet histogram and check for any indications of platelet clumping. He reported an inability to routinely assess the platelet

Table I. Simultaneous complete blood count in three distinct blood tube samples.

Variables	EDTA Samples	Citrate Samples	Heparin Samples
Erythroid series			
Red blood cell ($x10^6/\mu L$)	4.68	4.05	4.51
Hemoglobin (g/dL)	11	9.8	10.9
Hematoctrit (%)	34.1	30.1	32.9
MCV (fL)	74.3	72.9	73.1
RDW (%)	19.1	19.2	18.4
White Blood cell			
Leukocyte count ($x10^3/\mu L$)	12.1	5.5	7.4
Neutrophil count ($x10^3/\mu L$)	6.2	2.2	4.1
Lymphocyte count ($x10^3/\mu L$)	4.3	2.3	2.7
Platelet series			
Platelet count ($x10^3/\mu L$)	17	55	175
Mean platelet volume (fL)	8.4	9	10.1
PDW (fL)	16	17.4	18.1

MCV, mean corpuscular volume; PDW, platelet distribution width; RDW, red cell distribution width.

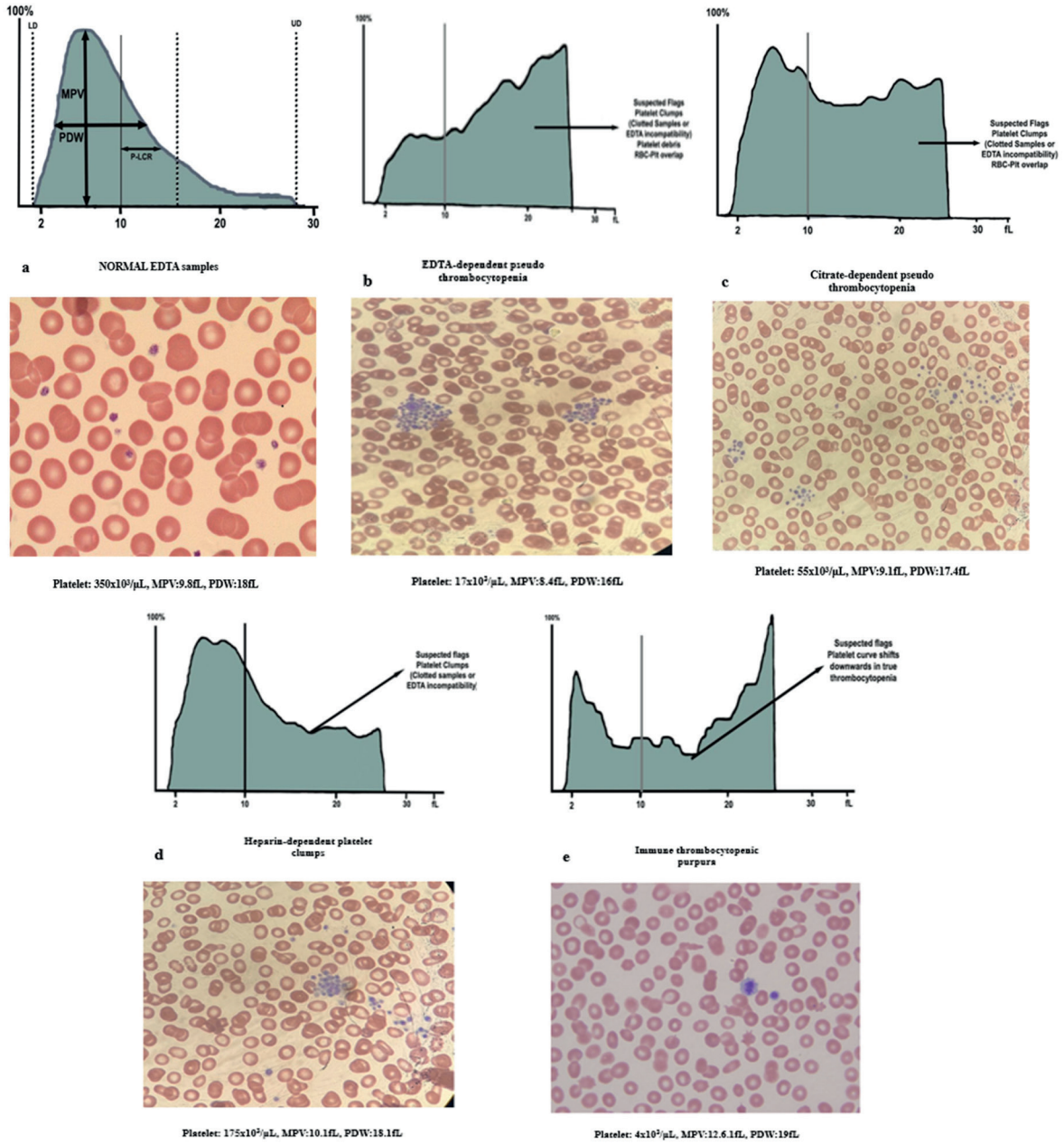

Fig. 1. Normal and abnormal platelet histogram, suspected flags, and peripheral blood smear of EDTA **(a,b)**, Citrate **(c)**, and Heparin **(d)** samples for the diagnosis of pseudothrombocytopenia in a child as well as EDTA sample **(e)** in a patient with immune thrombocytopenic purpura.

histogram and associated flags. The platelet histogram displayed a serrated/sawtooth curve, with the largest platelet aggregates appearing as a distinct peak on the platelet curve's rightward shift in EDTA and citrate samples. Furthermore, the platelet flag represents warning signs, such as platelet clumping or large platelets. Although the platelet count returned to normal with the heparin tube, a peripheral blood smear, platelet histogram, and flags suggested some platelet clumping message in the heparin tube, similar to the EDTA and citrate samples (Figure 1). Informed consent was obtained from the parents for the publication of this case report.

Discussion

In this case study, we present the platelet histogram, flags, and peripheral blood smear results for pseudothrombocytopenia in EDTA and citrate blood samples from a previously healthy child. Figure 1 also shows platelet histograms, flags, and smear results from individuals with normal and immune thrombocytopenic purpura samples collected at the hematology laboratory for comparison.

EDTA anticoagulants are widely used in hospitals for complete blood count (CBC) analysis.[3] The frequency of EDTA-dependent pseudothrombocytopenia ranges from 0.1-2% in hospitalized patients to 15-17% in outpatients tested for thrombocytopenia.[6] Furthermore, 83% of individuals have EDTA-induced pseudothrombocytopenia, and 17% have citrate-dependent pseudothrombocytopenia.[7-10] Multiple anticoagulant-dependent pseudothrombocytopenias have been reported in a variety of tubes, including heparin. Heating the whole blood specimen at 37 °C, in vitro amikacin supplementation or rapid sample analysis are further laboratory procedures for correcting low platelets on the laboratory bench.[10] These methods are time, temperature, and drug-dependent procedures. However, the use of alternate anticoagulant sample tubes is a quick and practical approach for clinicians.[10] In our case, we observed both EDTA and citrate-dependent pseudothrombocytopenia, but the platelet count was corrected using the heparin tube platelet count.

Peripheral blood smears remain the gold standard test for diagnosing pseudothrombocytopenia. However, not every physician has experience assessing peripheral blood smears.[11,12] As a result, most individuals are misdiagnosed as immune thrombocytopenic purpura.[1,2] According to the literature, the majority of individuals with pseudothrombocytopenia are treated as immune thrombocytopenic purpura with steroids, immunoglobulins, or splenectomy, which is unnecessary. In this case, he was referred to our hematology department due to immune thrombocytopenic purpura suspicions. We were aware that the referral physician had not examined the peripheral blood smear. We believe that digital imaging technology will be able to detect pseudothrombocytopenia in routine peripheral blood smears, minimizing the need for physicians to evaluate smear testing.

A modern technology-based automated hematological analyzer, as well as suitable blood sample tubes, are required for reliable complete blood count results.[11-13] Most laboratories continue to report hemogram results obtained using impedance-based automated hematological analyzers. An advanced automated hematology analyzer that uses a fluorescent or optic-based platelet count, is effective for the correction of pseudothrombocytopenia. However, pseudothrombocytopenia is difficult to identify with an impedance platelet count since platelet histograms and flag warning messages are frequently ignored by technicians or physicians. As a result, if the physician has no experience assessing peripheral blood smears, it may be critical for the technician to notice pseudothrombocytopenia on the laboratory bench before reporting it to the physician. Furthermore, physicians should be qualified to interpret platelet histograms and flags in clinical settings.

The platelet histogram measures platelet size, MPV, PDW, and platelet large cell ratio (P-LCR).[10-13] MPV, PDW, and P-LCR values are artificially increased in the presence of platelet clumping or large platelets. However, MPV and PDW values in our patient were similar to those of a healthy sample (Figure 1). The platelet histograms have two flexible discriminators that help distinguish platelet curves from others: the lower and upper discriminators range from 2 to 20 fL. The platelet discriminator has an optimum value of 12 fL. The normal platelet histogram begins with a sharp climb to a peak and then progressively

declines as platelet size increases (Figure 1). This suggests that the majority of platelets are small, with fewer larger ones. The red blood cell peak can be seen starting to the right of the upper discriminator bar. When platelet size approaches 20 fL, it interacts with red blood cells, causing an inaccurate platelet count due to large platelets and platelet aggregates. Additionally, typical normal platelet curves are left-skewed. In cases where the platelet curve exhibits a rightward shift extending beyond the upper platelet discriminator, as observed in our patient, combined with an upper discriminator height of 40% and a serrated or sawtooth pattern, the resulting platelet count may yield an inaccurately low value. However, the platelet curve shifts downward in true thrombocytopenia, such as immune thrombocytopenic purpura (Fig. 1). In addition to the platelet histogram, platelet clumping and large platelet signals obtained from platelet flags should also be considered. Our platelet histogram and flag analysis results revealed platelet clumping in the EDTA and citrate samples. The heparin-based platelet histogram and peripheral blood smear show rare platelet clumping but appear to have a nearly normal platelet count. Our findings show that if EDTA and citrate cause pseudothrombocytopenia, the physician should consider using the heparin tube.

The main limitation of this report is that it is based on a single case. Additional research with a large cohort is required to confirm pseudothrombocytopenia in such individuals.

Finally, if the platelet histogram and flags suggest a platelet cluster warning, the laboratory technician and physician should be aware of pseudothrombocytopenia, one on the laboratory bench and the other in the clinical setting.

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Ethical approval

Informed consent was obtained from parents.

Author contribution

The authors confirm their contribution to the paper as follows: Study conception and design: ED; data collection: ZK; analysis and interpretation of results: ED, ZK; draft manuscript preparation: ED, ZK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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