Case report

Pseudothrombocytopenia Induced by Ethylenediaminetetraacetic Acid: A Case Report

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SUMMARY

Introduction: Pseudothrombocytopenia induced by ethylenediaminetetraacetic acid is a rare phenomenon caused by autoantibodies against platelet membrane with overall prevalence of 0.1% in the general population. This phenomenon is an *in vitro* artifact and with different anticoagulant used, the patient had the physiological platelet count.

Case report: This case is a report of a 2-year-old patient, whose initial complete blood count analysis revealed low platelet count (18×10^9/L) without any visible signs of hemorrhage. After detecting multiple platelet clumps in the peripheral blood smear, the complete blood count was repeated using 3.2% sodium-citrate. The analysis of the new specimen revealed the physiological platelet count. Thus, the patient was diagnosed with pseudothrombocytopenia induced by ethylenediaminetetraacetic acid.

Conclusion: Pseudothrombocytopenia induced by ethylenediaminetetraacetic acid should always be suspected whenever thrombocytopenia without bleeding tendency is presented. The peripheral blood smear examination is the most convenient and the cheapest diagnostic test for excluding this artifact.

Keywords: thrombocytopenia, pseudothrombocytopenia, platelet count, low platelet count

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INTRODUCTION

Pseudothrombocytopenia (PTCP) is defined as spuriously low platelet count without any signs or symptoms of haemorrhage. The most common causes are ethylenediamine-tetraacetic acid (EDTA)-dependent agglutination, platelet satellitosis, giant platelets, platelet cold agglutinins and partial clotting of blood samples due to improper blood withdrawal techniques or inadequate amount of anticoagulant (1). EDTA is an aminopolycarboxylic acid that chelates calcium ions in the blood specimen, and therefore, it is used extensively in clinical laboratories as an anticoagulant additive for a complete blood count test. In most European countries, the most common formulation used is K_2EDTA. EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) is caused by EDTA-depentent antiplatelet IgM and IgG antibodies which recognize cytoadhesive glycoprotein IIb/IIIa receptors on the platelet plasma membrane, and it occurs at room temparature (2). EDTA-PTCP is a rare phenomenon with overall prevalence of 0.1% in the general population and is not sex- or age-dependent. Patients with infection, autoimmune diseases and malignancy might have an increased risk of EDTA-PTCP. However, it was recorded in a disease-free population as well (2, 3). This phenomenon ocurrs only *in vitro* and is solely a laboratory artifact because with a different anticoagulant used, the patient presented the physiological platelet count (4). Its clinical significance lies in unnecessary and potentially harmful treatment decisions. These patients might undergo needless bone marrow aspiration or even splenectomy or be treated with adrenocorticosteroids. Patients are sometimes treated with thrombocyte concentrate as well (especially if they undergo surgical procedures) thus increasing the risk of thrombotic complications. Therefore, it is important that laboratory staff together with clinicians recognize this artifact so that such erroneous and misleading result do not cause further diagnostic and therapeutic mistakes (4, 5).

CASE REPORT

A 2-year-old female with a history of recurrent obstructive bronchitis was admitted to our hospital in the year 2020 due to frequent coughing and shortness of breath. She had no other complaints. There was no history of any bleeding tendency. Physical examination was unremarkable. An initial blood analysis revealed low platelet count (18 x 10^9/L) with normal white blood cell count and hemoglobin level. The analysis was performed using K_2EDTA (1 mg/mL) blood sample on automated hematological analyzer Advia 2120 (Siemens Healthineers, Germany). Microscopic examination of the blood smear revealed multiple platelet clumps in
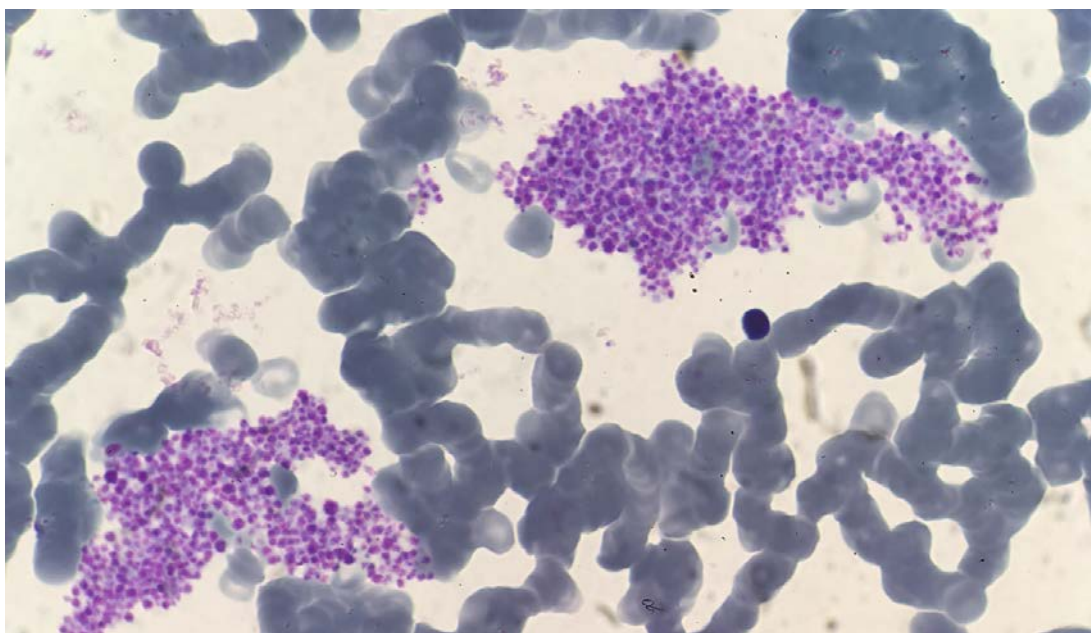

Figure 1. *Platelet clumps in peripheral blood smear from blood sample collected with EDTA (May-Grünwald-Giemsa staining; 100x oil microscope)*

blood samples with EDTA (Figure 1). We considered that thrombocytopenia was due to EDTA-induced platelet aggregation. Therefore, the complete blood count was repeated using a light blue-topped evacuated tube with 3.2% sodium-citrate (blood: anticoagulant = 1:9). The analysis of the new specimen revealed platelet count of 306 x 10^9/L. On the examination of blood samples with sodium-citrate, aggregation of platelets was not observed. Therefore, the patient was diagnosed with EDTA-PTCP. A written informed consent was obtained from the patient's parent. The study was approved by the Ethics Committee of the Institute for Child and Youth Health Care of Vojvodina (No. 725-2; 19 Feb, 2021).

DISCUSSION

The work in clinical laboratories, as in any other medical field, should focus on the patient safety. Patient safety is defined by the World Health Organization as the absence of preventable harm to a patient during the whole health care process and reduction of the risk of unnecessary harm associated with health care to an acceptable minimum. Understanding possible problems and putting efforts into preventing them is the key to every medical branch being successful (6). EDTA-PTCP is a sporadic, laboratory phenomenon of platelet agglutination caused by EDTA-dependent antibodies against platelet cell membrane glycoproteins. The prevalence of EDTA-PTCP in the general population is 0.1%. As described in our report, antiplatelet antibodies caused *in vitro* agglutination and subsequently, a decrease in platelet count, typically below 100 x 10^9/L (7). The presence of alert flag on hematological analyzer, warning us about a possible platelet aggregation, is not always the case. Namely, the size of platelet clumps may vary to a significant extent and they can be labeled as e.g. lymphocytes by hematological counters. Hence, the peripheral blood smear is an imperative when low platelet count is not correlating with the clinical picture of the patient. Peripheral blood smear should always be observed from samples with EDTA in order to detect the platelet clumps (7, 8). Additionally, a new blood sample from the same patient must be evaluated but with different anticoagulant, such as so-

dium-citrate, heparin or ammonium-oxalate. Evaluation of the new sample should be performed on the same hematological analyzer in order to have a comparable result. Platelet indices may be very helpful in distinguishing true thrombocytopenia from PTCP (8). One of the most common derived platelet parameters is mean platelet volume (MPV), which is calculated by dividing the plateletcrit (PCT) by the total number of platelets. It represents the mean size of platelets and is an indicator of their activity (9). Platelet distribution width (PDW) is another commonly used platelet index and it reflects equability in platelet size. Falsely elevated MPV and PDW are expected in PTCP due to platelet aggregation. After the evaluation of hematological results from the sample with different anticoagulant, in PTCP the MPV and the PDW tend to decrease, while in true thrombocytopenia and in several other inflammatory conditions they remain increased due to platelet consumption or sequestering (9, 10). In the testing process, an important number of errors in laboratory medicine occurs in postanalytical phase. An important postanalytical step is to specify in the laboratory report which anticoagulant has been used. It must contain a comment that specific blood sample was withdrawn with sodium-citrate, heparin or ammonium-oxalate, so clinician can be aware that the change in platelet count was not caused by any kind of treatment (8, 9).

CONCLUSION

EDTA-PTCP should always be suspected whenever thrombocytopenia without bleeding tendency is presented. Periferal blood smear analysis is the most convenient and the cheapest way to determine the difference between the EDTA-PTCP and the true thrombocytopenia. With our study, we emphasize the importance of interface between laboratory staff and clinicians in order to preserve patient safety.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Pseudotrombocitopenija uzrokovana etilendiaminotetraacetatnom kiselinom: prikaz slučaja

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S A Ž E T A K

Uvod. Pseudotrombocitopenija uzrokovana etilendiaminotetraacetatnom kiselinom je redak fenomen uzrokovan autoantitelima protiv membrane trombocita sa prevalencijom od 0,1% u opštoj populaciji. Ovaj fenomen prepoznat je u *in vitro* uslovima, a uz upotrebu drugog antikoagulansa kod bolesnika se postiže fiziološki broj trombocita.

Prikaz slučaja. Ovaj rad predstavlja prikaz slučaja bolesnika uzasta od dve godine, u čijoj je inicijalnoj kompletnoj krvnoj slici detektovan nizak broj trombocita (18 x 10^9/L), bez vidljivih znakova krvarenja. Nakon detektovanja multiplih trombocitnih agregata u razmazu periferne krvi, kompletna krvna slika ponovljenja je uz upotrebu 3,2% natrijum-citrata. Analiza novog uzorka pokazala je fiziološki broj trombocita. Bolesniku je postavljena dijagnoza pseudotrombocitopenije uzrokovane etilendiaminotetraacetatnom kiselinom.

Zaključak. Sumnja na pseudotrombocitopeniju uzrokovanu etilendiaminotetraacetatnom kiselinom treba biti postavljenja kad god postoji nizak broj trombocita bez znakova krvarenja. Razmaz periferne krvi najbolji je i jeftiniji dijagnostički test za isključivanje ovog artefakta.

Ključne reči: trombocitopenija, pseudotrombocitopenija, broj trombocita, nizak broj trombocita