DE GRUYTER OPEN

Case report

DOI: 10.2478/rrlm-2014-0017

Multi-anticoagulant and EDTA dependent pseudothrombocytopenia. Case reports on two pediatric patients

Pseudotrombocitopenia dependentă de multi-anticoagulant și de EDTA. Cazurile a doi pacienți pediatrici

Smaranda Arghirescu[1], Mihaela Bătăneanț[1], Cristian Jinca[1*], Andreea Pașcalău[2], Mihaela Lelik[2], Mihaela Preja[2], Ladislau Ritli[3], E.C. Ursu[1], Margit Șerban[2], Hortensia Ioniță[1]

1. "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania
2. "Louis Turcanu" Emergency Hospital for Children Timisoara, Romania
3. "Dr. Gavril Curteanu" Hospital Oradea, Romania

Abstract

Pseudothrombocytopenia is an in vitro sampling problem which may mislead the diagnosis towards the more critical condition of thrombocytopenia. The phenomenon occurs when the anticoagulant used while testing the blood sample causes clumping of platelets which mimics low platelet count without any clinical signs. This may determine unnecessary, expensive and invasive investigations and even treatment. In this article we report two cases of pseudothrombocytopenia diagnosed in pediatric patients.

Keywords: *pseudothrombocytopenia, multi-anticoagulant, EDTA*

Rezumat

Pseudotrombocitopenia este o anomalie in vitro legată de recoltarea probei de sânge, care poate conduce în mod eronat la diagnosticarea unei afecțiuni mai grave și anume trombocitopenia. Acest fenomen apare atunci când anticoagulantul folosit pentru recoltarea probelor de sânge în vederea testării acestora cauzează agregarea trombocitară, mimând astfel un număr scăzut de trombocite fără ca pacientul să prezinte semnele clinice ale unei trombocitopenii. Această anomalie poate determina efectuarea unor investigatii inutile, costisitoare și invazive, și chiar instituirea unui tratament nejustificat. În acest articol prezentăm două cazuri de pseudotrombocitopenie diagnosticată la pacienți pediatrici.

Cuvinte cheie*: pseudotrombocitopenie, anticoagulante, EDTA*

Received: 17th July 2013; Accepted: 15th March 2014; Published: 11th May 2014.

***Corresponding author**: Cristian Jinca, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania
e-mail: cristian_jinca@yahoo.com

Introduction

The preanalytic phase is an important component of laboratory medicine. It should always be considered, as a wide range of exogenous (methods of blood collection, handling, processing factors) or endogenous determinants (drugs, circulating antibodies) can spuriously affect the results of investigations (1-3).

Its importance is illustrated by multi-anticoagulant- and more frequently ethylenediaminetetraacetic acid (EDTA)–dependent pseudothrombocytopenia (PTCP), an *in vitro* phenomenon of a false low platelet count due to the presence of antibodies that cause platelet agglutination in anticoagulated blood (4). This event can occur in both healthy subjects and patients with various pathological conditions: autoimmune, neoplastic, atherosclerosis-related and liver diseases. The EDTA – dependent PTCP has an incidence reported in a range of 0.09 to 0.21 %. Although less frequently, we are confronted with the multi-anticoagulant dependent pseudothrombocytopenia, induced by EDTA and heparin (5,6). The responsible pathogenic mechanism is still not clearly defined. Antiplatelet antibodies, mostly IgM (58.3% - 60.9%), reacting optimally between 0-4 ºC, recognize the cytoadhesive receptor glycoprotein IIb/IIIa (GpIIb/IIIa) of platelets, stimulate the expression of antigens, trigger and induce activation of tyrosine kinase and determine agglutination and clumping *in vitro*. They lead to a spuriously, mostly time and temperature dependent, decreased platelet count, without clinical relevance (7).

Patients and methods

We present two illustrative clinical cases, admitted and investigated in the Clinic of Pediatrics III, Department of Oncohematology, Timisoara.

Blood specimens were collected on EDTA and sodium citrate vacutainers and were analyzed immediately, at 60 minutes and at 120 minutes after sampling (7). Complete blood count (CBC) on the automated analyzer, blood smears, platelet (PLT) aggregation test, thrombelastography, global hemostasis tests, as well as serological tests, endocrine work-up and imaging investigations have been performed.

Complete blood count measurement was performed using the MYTHIC 22 CT automated cell counter as well as the SYSMEX XT-4000 I automated cell counter. The detection principle of the MYTHIC 22 CT device relies on the impedancemetry technique.

Concerning the SYSMEX XT-4000 I automated cell counter, red blood cells (RBC) and platelets (PLT) are counted in a dedicated channel using the Impedance or Direct Current (DC) detection method combined with hydrodynamic focusing technology.

Thrombelastography was performed using a TEG® 5000 Thrombelastograph® Hemostasis Analyzer system.

Platelet aggregation tests

These tests were performed using the Chrono-Log 570 blood aggregation system, based on light transmission aggregometry using adenosine diphosphate (ADP) and collagen agents.

Results

Case 1

A 16 year-old girl, without personal or family history of bleeding, was found with thrombocytopenia on routine blood work-up in November 2012 (46 x 10^9/ L - 66 x 10^9/L). She was referred to our hospital in January 2013. Her medical history was not significant and physical examination was normal without any signs of purpura; a modest enlargement of the thyroid gland was revealed.

Her CBC from an EDTA sample, collected by venopuncture, revealed, by automated counter, a white blood cell count of 12.4× 10^9/L, a hemoglobin of 137 g/L, and platelets of 56 × 10^9/L. A peripheral blood smear performed from

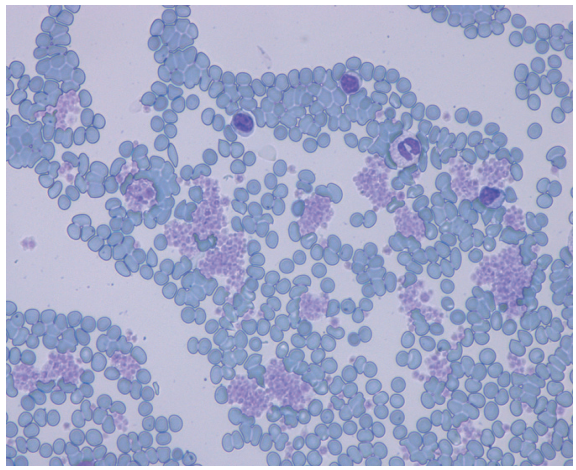

Figure 1. Platelet clumps on EDTA blood smear in patient 1 (May Grunwald Giemsa, x 200)

the EDTA-vacutainer revealed PLT clumps (Figure 1).

The antiplatelet antibody test was negative. Global hemostasis tests were normal: bleeding time, blood clot retraction.

Thrombelastography was normal: R =5.5 minutes (min) with normal range between 2-8 min, K = 2.6 min (normal range = 1-3 min), Angle=60.1 degrees (normal range = 55-78 degrees), MA=53.3 mm (normal range =1-69 mm) as presented in figure 2.

Aggregation with ADP showed an Amplitude of 17% *versus* a Control Amplitude of 78% (normal range = 71-88%) as shown in figures 3a, b.

Serologic tests were also performed revealing negativity for antiphospholipid antibodies type Ig G and Ig M . Serologic tests for hepatitis B, hepatitis C, human immunodeficieny virus (HIV) and toxoplasmosis were also negative.

Imaging investigation of thyroid gland revealed a mild thyroid hypertrophy. Endocrine workup comprised: thyroid-stimulating hormone (TSH) = 3.29 µIU/ml (normal values = 0.53-3.59 µIU/ml), free triiodothyronine (fT3) = 6.09 pmol/l (normal values = 3.5-7.7 pmol/l) and free thyroxine (fT4) =17.76 pmol/l (normal values = 12-20.6 pmol/l).

In the situation of abnormally low platelet count and in the absence of a history and physical examination consistent with thrombocytopenia, pseudothrombocytopenia was suspected. In order to substantiate the suspected diagnosis in our patient, blood specimens were collected by venopuncture in different tubes containing EDTA or sodium citrate; the platelet count was then measured at different time points: 0, 60 and 120 minutes from each sample

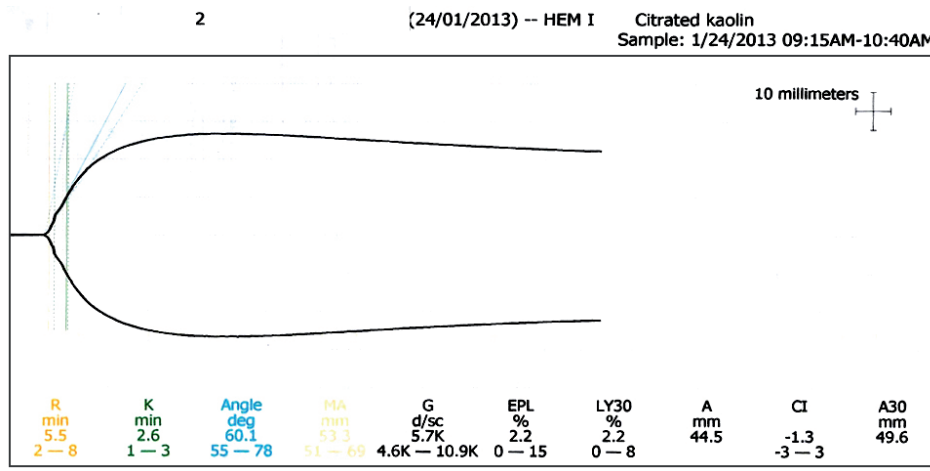

Figure 2. Thrombelastography trace in patient 1.

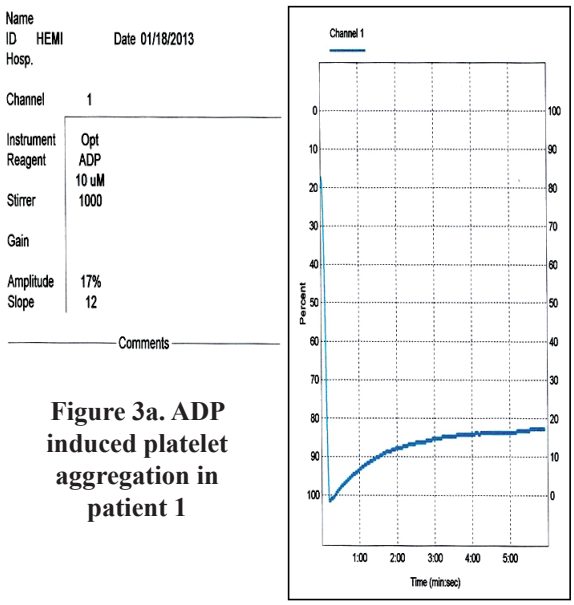

Figure 3a. ADP induced platelet aggregation in patient 1

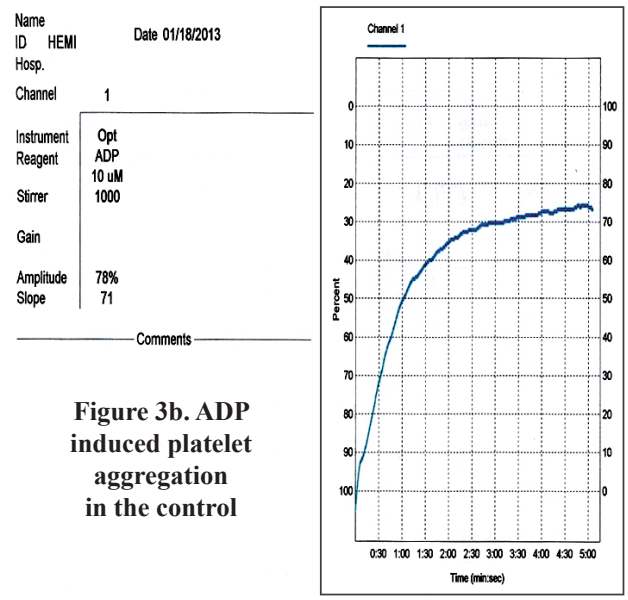

Figure 3b. ADP induced platelet aggregation in the control

collection at room temperature and heated to 37^0 C. The results are illustrated in figures 4 a, b, c.

We concluded that the patient had EDTA-dependent PTCP and no further treatment or blood work up was required.

Case report 2

A 13 year-old girl without personal or family history of bleeding was found to have thrombocytopenia in December 2012 (PLT=21 x 10^9/L) when she had an episode of faintness. She was admitted in a county hospital with the diagnosis of thrombocytopenia (ranging between: 19 x 10^9/L and 40 x 10^9/L). She was treated initially with intravenous immunoglobulin - 1 g/kg/day for 2 consecutive days. Because of the lack of response, she was referred to our hospital in February 2013. Her medical history was not significant and the physical examination was normal.

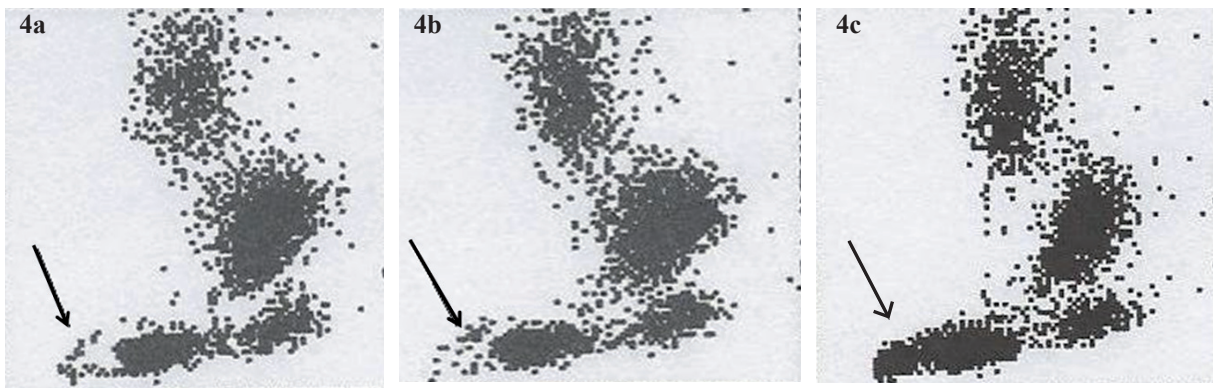

Figure 4a. Patient 1 sample on EDTA immediately after sampling, 4b. Patient 1 sample on citrate immediately after sampling, 4c. Patient 1 sample on EDTA 120 minutes after sampling

Her CBC on the automated cell counter revealed a white blood cell (WBC) count of 8.3× 10^9/L, hemoglobin of 116 g/L, and platelets of 44 × 10^9/L. A peripheral blood film from an EDTA tube showed platelets arranged in clumps as revealed in figure 5.

Antiplatelet antibody test was negative. Global hemostasis tests were normal: bleeding time, clot retraction and prothrombin consumption.

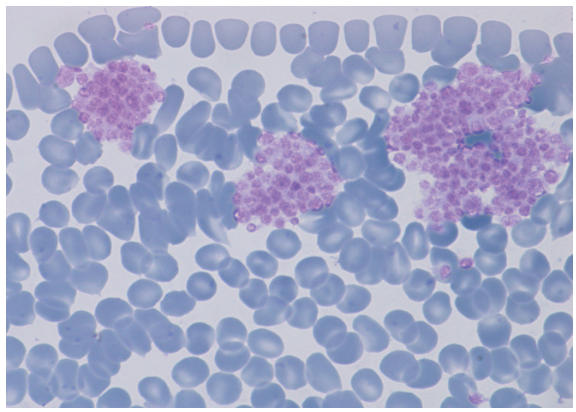

Figure 5. Platelet clumps on EDTA blood smear in patient 2 (May Grunwald Giemsa, x 400)

The TEG trace was not significantly modified: R =8.5 min (normal range = 2-8 min) , K=2.8 min (normal range = 1-3 min), Angle=53 degrees (normal range=55-78 degrees), MA=55 mm (normal range=51-69 mm) as shown in figure 6.

Antiphospholipid antibodies type Ig G and Ig M, antinuclear antibodies, rheumatoid factor, complement fractions C3 and C4 , enzyme-linked immunosorbent assay (ELISA) tests for hepatitis B, hepatitis C, HIV, toxoplasmosis, cytomegalovirus, Epstein-Barr virus and Helicobacter pylori were all negative. Abdominal and thyroid ultrasound were normal. Based on Holter EKG an extrasystolic ventricular arrhythmia Lown 1 was diagnosed.

Work-up prompted for collection of blood separately on EDTA, sodium citrate and heparin containing tubes, with CBC measurements at time-points: 0, 60 and 120 minutes, at room temperature and heated at 37ºC respectively (figures 7 a, b, c). No PLT aggregates could be identified immediately after sampling neither on EDTA nor on citrate tubes (figures 7a, b). One hundred

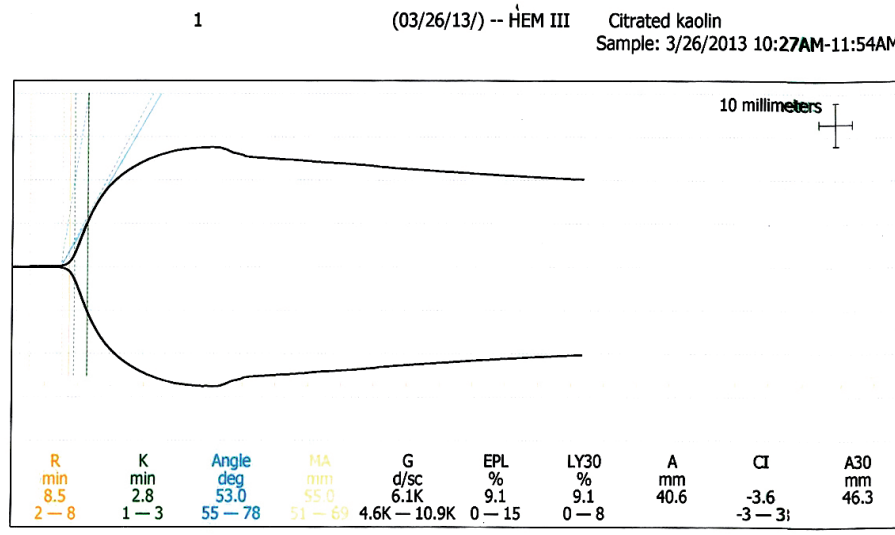

Figure 6. Thrombelastography trace patient 2

twenty minutes after sampling, in the sample tubes collected on EDTA tubes, PLT aggregates could be identified on the scattergram, as shown in figure 7c. Control CBC scattergram revealed clumps in the 120 minutes sample .

We concluded that the patient had EDTA-dependent PTCP and no further treatment or blood work-up was required.

Discussion and conclusions

In the case of thrombocytopenia without clinical history of bleeding one should consider to perform a peripheral blood smear. The microscopic examination can identify platelet clumping and repeated CBC tests, using a different anticoagulant, can clarify the diagnosis (8). When PLT aggregation occurs with EDTA, not only PLT counts are affected, but also WBC counts (in terms of increase) because most automated analyzers count cells by dimension, falsely recognizing the PLT clumps as being leukocytes (9-11). A reliable and timely identification of this artifact is essential since there is a high risk to confuse this abnormality with other life-threatening disorders, leading to inappropriate exploration and therapy. Some investigators have studied mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) in order to find additional parameters which could be considered for suspecting this disorder. These findings revealed that MPV, as well as PDW, were increased in PCTP as well as in true forms of thrombocytopenia, making these parameters less useful for differential diagnosis. In contrast, the same investigators showed that the automated platelet clump count (APCC) performed by some automated cell counters could provide accurate information regarding relevant anticoagulant – PTCP (12). Aggregation tests were also relevant when performed in the first presented patient with an amplitude of only 17% as compared to normal controls. Other investigators confirmed the role of this investigation in PTCP. They concluded that platelet aggregometry can evaluate platelet functions, but it has some limitations since standardization of this procedure is difficult and there is a demand for reproducibility of these tests in order to avoid the erroneous results (13). The basic criteria suggesting pseudotrombocytopenia are low platelet number in EDTA +/- anticoagulated samples at room temperature, the contribution of time and temperature to the decrease of platelet counts, evidence of aggregates and clumps on automated cell counting, microscopic analysis of blood

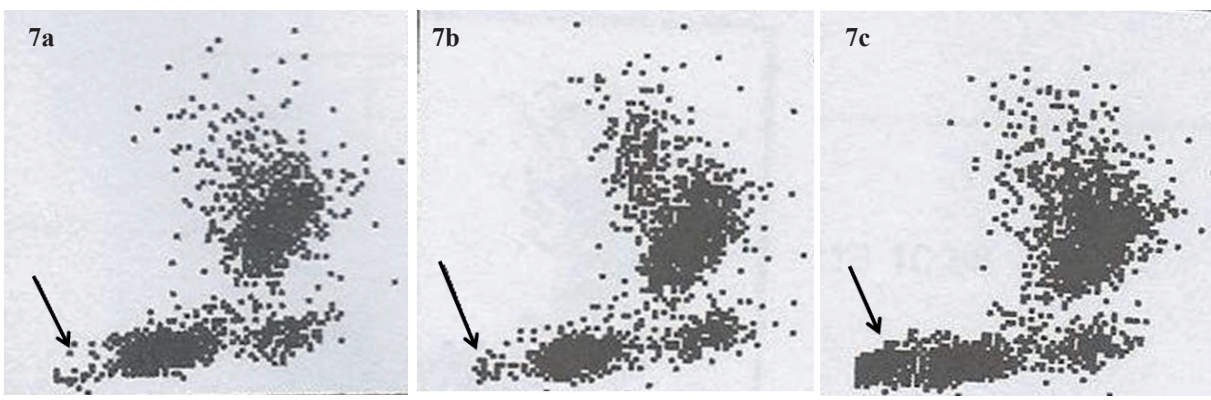

Figure 7a Patient 2 sample on EDTA immediately after sampling, 7b Patient 2 sample on citrate immediately after sampling, 7c Patient 2 sample on EDTA 120 minutes after sampling

smears and lack of signs and symptoms relevant for a bleeding disorder (7).

In order to prevent unwarranted time, resource consuming investigations and unnecessary treatment, it is meaningful to verify the platelet count. Repeated collection with another anticoagulant, microscopic examination of the blood smear, keeping the blood samples at 37^0 C, using additives like kanamycin, amikacin or other aminoglycosides are simple, accessible modalities (14). In case the peripheral blood count exhibits platelets clumps, pseudothrombocytopenia should always be a presumption. If in doubt, blood smear examination remains the gold standard for diagnosis (1,7,12).

Abbreviations

ADP - Adenosine diphosphate
APCC - Automated platelet clump count
CBC - Complete blood count
DC - Impedance or Direct Current
EDTA - Ethylenediaminetetraacetic acid
ELISA – enzyme-linked immunosorbent assay
fT3 – free triiodothyronine
fT4 – free thyroxine
Gp IIb/IIIa - Glycoprotein IIb/IIIa
HIV – human immunodeficiency virus
MA-Maximum Amplitude
MPV- Mean platelet volume PCT - Plateletcrit
PDW - Platelet distribution width
PLT - Platelets
PTCP -Pseudothrombocytopenia
RBC - Red blood cells
TEG -Thrombelastography
TSH – thyroid stimulating hormone
WBC - White blood cells

Acknowledgements

No conflicts of interest to disclose.

References

1. Yoneyama A, Nakahara K. EDTA-dependent pseudothrombocytopenia-differentiation from true thrombocytopenia. Nihon Rinsho. 2003;61(4):569-74.
2. Schrezenmeier H, Müller H, Heimpel H, Seifried E. Anticoagulant-induced pseudothrombocytopenia and pseudoleucocytosis. Thromb Haemost. 1995;73(3):506-13.
3. Bizzaro N. EDTA-Dependent Pseudothrombocytopenia: A Clinical and Epidemiological Study of 112 Cases, With 10-Year Follow-Up. Am J Hematol. 1995 Oct;50(2):103-9. DOI: 10.1002/ajh.2830500206
4. Van der Meer W, Allebes W, Simon A, van Berkel Y, de Keijzer MH. Pseudotrombocytopenie: A report of a New Method to Count Platelets in a Person With EDTA-and Temperature-Independent Antibodies of the IgM Type. Eur J Haematol. 2002 Oct;69(4):243-7. DOI: 10.1034/j.1600-0609.2002.02680.x
5. Kabutomori O, Iwatani Y. „Correct" Platelet Count in EDTA-Dependent Pseudothombocytopenia. Eur J Haematol 1995;55(1):67-8. DOI: 10.1111/j.1600-0609.1995.tb00240.x
6. Bartels P, Schoorl M, Lombarts A. Screening for EDTA-Dependent Deviations in Platelet Counts and Abnormalities in Platelet Distribution Histograms in Pseudothrombocytopenia. Scand J Clin Lab Invest. 1997 Nov;57(7):629-36. DOI: 10.3109/00365519709055287
7. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. Clin Chem Lab Med. 2012;50(8):1281-5. DOI: 10.1515/cclm-2012-0081
8. Podda GM, Pugliano M, Femia EA, Mezzasoma AM, Gresele P, Carpani G, et al. The platelet count in EDTA-anticoagulated blood from patients with thrombocytopenia may be underestimated when measured in routine laboratories. Am J Hematol. 2012 Jul;87(7):727-8. DOI: 10.1002/ajh.23216
9. Chae H, Kim J, Oh EJ, Kim Y, Han K. Novel method to dissociate platelet clumps in ETDA–dependent pseudothrombocytopenia based on the pathophysiological mechanism. Clin Chem Lab Med. 2012 Mar 3;50(8):1387-91. DOI: 10.1515/cclm-2011-0892
10. Hagerman RS. Ethylenediamenetetraacetic Acid (EDTA)-Dependent Pseudothrombocytopenia: A case report of an Incidental but Important Finding. 2009. Available from: http://priory.com/med/Case%20Presentation.htm
11. Saigo K, Sakota Y, Masuda Y. EDTA-dependent pseudothrombocytopenia: clinical aspects and labora-

tory tests. Rinsho Byori. 2005 Jul;53(7):646-53.
12. Onder O, Weinstein A, Hoyer LW. Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. Blood. 1980 Aug;56(2):177-82.
13. Wu W, Guo Y, Zhang L, Cui W, Li W, Zhang S. Clinical utility of automated platelet clump count in the screening for ethylene diamine tetraacetic acid-dependent pseudothrombocytopenia. Chin Med J (Engl). 2011 Oct;124(20):3353-7.
14. Yavasoglu I, Acar B, Kadikoylu G, Bolaman Z. Platelet Aggregation Tests Are Affected in Pseudothrombocytopenia. Lab Med. 2010;41(8):483-5. DOI: 10.1309/LM9UXAORTFONZ6U5