



Pseudothrombocytopenia

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SUMMARY

Introduction: Pseudo thrombocytopenia (PTP) is a phenomenon of falsely low platelet counts obtained on Hematology Analyzers (HA) due to in vitro platelet clumping in the presence of anticoagulants.

Methods: Papers on the subject of pseudothrombocytopenias effects were searched for in biomedical journals indexed in MEDLINE from 1969 to 2016. All other thrombocytopenia types were not analyzed.

Topic: Pseudothrombocytopenia occurs using the EDTA and other anticoagulants, in the process of determining the platelet count on the HA. Agglutination of platelets occurs at temperatures lower than 34°C and sample enhances if exposed to longer period of time. Agglutination of platelets is most expressed 4 hours after blood sampling. Agglutination occurs by binding of IgM, IgG and IgA immunoglobulin to antigen or crypto- antigen of platelets. Hematologic cell Analyzers, (HA) do not count platelets from large agglutinations, therefore, the number of platelets that provides HA represents the sum of the number of free non-agglutinating platelets and small agglutinations consisting of 3-5 platelets. Pseudothrombocytopenia shall be suspected in the case of the absence of clinical signs of hemorrhagic diathesis. Undiagnosed pseudothrombocytopenia may lead to unnecessary aggressive diagnostic procedures such as biopsy or puncture of the bone marrow, inadequate treatment and even transfusion of platelets. The following types of pseudothrombocytopenias are described herein:

- a) pseudothrombocytopenia occurred due to platelet agglutination,
- b) platelet satellitism and
- c) aggregation of platelets and leukocytes.

Conclusion: In order to obtain the actual count of platelets, peripheral blood smear shall be done for all samples with low values of thrombocytes ($<100 \times 10^9 / L$), and for samples of the results having flags on HA. In the case of finding agglutinated platelets, the following measures should be taken in order to obtain correct interpretation of laboratory results: to warm blood sample at 37°C and re-test, to test the blood sample on the another anticoagulant (citrate, heparin), to make blood smear, and to determine the platelets using ammonium oxalate by counting platelets from capillary blood in microscopic counting chamber. In case of all phenomena of agglutinations with all the previously mentioned anticoagulants is only possible to use magnesium sulphate and to make smears on the site of capillary blood sampling. Using this method is as well possible to detect agglutination of platelets without using anticoagulants, which indicates the presence of cold agglutinins.

Keywords: pseudothrombocytopenia, EDTA

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INTRODUCTION

Pseudothrombocytopenia (PTP) is a phenomenon of falsely low platelet counts obtained on Hematology Analyzers (HA) due to in vitro platelet clumping in the presence of anticoagulants.

Anticoagulant to which this phenomenon is usually described is ethylene-diamine-tetra-acetic acid (EDTA) and is thus called EDTA- dependent pseudothrombocytopenia.

EDTA in some patients and healthy persons may induce platelet agglutination/aggregation, resulting in a spuriously low automated platelet count.

Agglutination is caused by EDTA-dependent anti-platelet antibody. This antibody induces platelet agglutination in vitro, resulting in a decrease of platelet counts [1, 2].

Hematology Analyzers (HA) do not count platelets from large agglutinates. Therefore, the number of platelets provided on HA represents the sum of the number of free non-agglutinated platelets and platelets of small agglutinates (3-5), although the actual number of platelets is within normal values (Table 1) [3].

Hematological analyzers indicate pseudothrombocytopenia in routine analysis of blood in 10-15 cases per 10 000 samples. Apheresis procedure shows pseudothrombocytopenia in approximately 0.2% of voluntary platelet donors. Pseudothrombocytopenia occurs in hospitalized patients in 1-2% of cases. However, even 15-17% of patients diagnosed with thrombocytopenia and sent from primary health care centers to secondary and tertiary levels suffers from pseudothrombocytopenia [2, 4].

In addition to EDTA, phenomenon of thrombocyte agglutination is as well described in the case of using other anticoagulants such as citrate, oxalate and heparin. In 15 to - 20% of the EDTA-dependent pseudothrombocytopenia cases, platelets agglutinate in the presence of citrate [2].

It is necessary for clinicians to consider the possible presence of PTP in cases of patients having low platelet counts without any hemorrhagic tendency.

Undiagnosed pseudothrombocytopenia may lead to unnecessary aggressive diagnostic procedures such as biopsy or puncture of the bone marrow, inadequate treatment and even transfusion of platelets [3, 5, 6].

METHODS

Papers about effects of pseudothrombocytopenias were searched for in biomedical journals indexed in MEDLINE from 1969 to 2016. All other thrombocytopenia types were not analyzed.

TOPIC

Pseudothrombocytopenia is in vitro laboratory phenomenon that usually occurs when EDTA is used as an anticoagulant while taking blood sample. It raises concerns of both patients and doctors and causes doctors to resort to extensive diagnostic procedures and often the patient undergoes treatment. This phenomenon occurs rarely in 1:1000 cases and is slightly more common in women. It is characterized by the in vitro formation of platelet agglutinates or aggregates. Agglutinates are formed by binding of immunoglobulins (mainly IgG) for platelet membrane antigens expressed only in the presence of EDTA. Based on many years of testing routine samples of patients with pseudothrombocytopenia is concluded that pseudothrombocytopenia or agglutinate formation depends on the ambient temperature and the elapsed time of blood collection until the time of testing. The temperature at which the aggregates are formed is lower than 34°C. By specifying the number of platelets every 30 minutes from the moment of sampling it was concluded that the most pronounced decline in the number of platelets appears 4 hours after blood sampling. This phenomenon is less frequent in heparinized blood or blood taken using the Na-citrate [7].

Rare cases of pseudothrombocytopenia are described at chromosomal aberrations such as 48, XXYY syndrome (47 XXY / 48 XXYY mosaicism) [8].

There are many types of laboratory pseudothrombocytopenias induced in the presence of EDTA such as:

- a) agglutination / aggregation of platelets;
- b) due to adhesion of platelets to polymorphonuclear leukocytes (platelet satellitism);
- c) aggregation of platelets and leukocytes.

Agglutinates of thrombocytes are typically immunoglobulins IgG, IgM and to a lesser extent IgA [2]. Thrombocyte agglutination examination has shown that they have reacted to the glycoprotein (GP) GPIb GPIIb/IIIa or other antigens on the surface of thrombo-

Table 1. Disorders which lead to the reduction of false platelet counts in HA *

*HA - Hematological Analyzers,
 PLT - Platelet,
 Er - erythrocyte,
 BC - Blood Count WBC -White
 Blood Cells (Leukocytes)

Disorder	Results on HA
PLT-agglutination (EDTA, other anticoagulants)	PLT- aggregates are counted as WBC
PLT-satellitism (mainly due to EDTA) <ul style="list-style-type: none"> • Around polymorphic nuclei • Around other WBC (normal and pathological) 	Low PLT
PLT-Neutrophil agglutination (mainly due to EDTA)	Number of WBC is falsely low
Large PLT (higher than the normal range of values)	PLT-counted as WBC and Er
Coagulation sample (micro)	Overall BC wrong
Too much blood related to the amount of anticoagulants	Improper blend

cytes or crypto-antigens expressed only in the presence of EDTA. One of the investigated antigens is GP78 (kD), which participates in the process of thrombocyte agglutination. Treating the thrombocyte with anti-GPIIb/IIIa antibody and anti GP78 blocks agglutination of thrombocytes under the effect of the EDTA. In contrary, addition of anti-GPIb does not prevent the agglutination of thrombocytes under the effect of EDTA [9]

Thrombocyte antigens GPIIb/IIIa, GP78 (kD), GPIb were blocked in order to prove which antigens were responsible for the pseudothrombocytopenia. Research has proven that thrombocyte agglutinins GPIIb/IIIa and GP78 (kD) bind to the target antigens (Ag). Agglutination specific for these antigens blocks them and causes lack of thrombocyte agglutination under the effect of EDTA. On the other hand, blocking GPIb does not lead to an inhibition of thrombocyte agglutination under the effect of EDTA [10].

These are the receptors normally affected by antithrombocyte drugs. Pseudothrombocytopenia is described when using these drugs, and discontinuation of therapy as well due to undiagnosed pseudothrombocytopenia that leads patient into the state of the risk of thromboembolic state [11]. Some investigators report about expression of platelet Integrins and activation antigens on platelets of persons with anticoagulant-dependent PTP and in healthy controls without PTP. In the presence of EDTA the expression of GpIIb/IIIa was significantly reduced in the PTP subjects compared to control. Activation antigens CD62, CD63 and thrombospondin-antigen

were upregulated in the presence of EDTA. These alterations in the expression of platelet antigens could also be induced on platelets of normal donors by incubation with sera of PTP subjects and EDTA [11].

EDTA-plasma of patients with pseudothrombocytopenia leads to platelet agglutination in healthy individuals. Using FITC method has proved that immunoglobulins IgG, IgM, IgA, IgM and kappa / lambda chains of healthy individuals bind to the plasma of patients with pseudothrombocytopenia. However, in some cases agglutination of thrombocytes may be induced by other plasma proteins different than immunoglobulins, or other mechanisms such as an interaction of circulating immune complexes with Fc receptors on the membrane of platelets in the presence of EDTA.

The immunofluorescence testing of platelet suspension (PSIFT) are determined by the details of an immunoglobulin subclasses. In IgG class, IgG1 is commonly found and occasionally may be found IgG2, I IgG3 and IgG4. Kappa light chains and lambda may as well be found in many cases [12].

Pseudothrombocytopenia is not only found in healthy individuals, but also in patients with malignancy, acute and chronic liver injury, in some autoimmune diseases, and chronic glomerulonephritis [13, 14, 15]. It is also described when antibiotics and other drugs are used, if the blood is sampled on EDTA. Although, very use of drugs does not lead to platelet aggregation [10, 16, 17, 18]

Aggregates observed in the chamber or in colored stained blood smears show a very wide range of sizes and may be found in groups of 3 to 5 platelets, and sometimes even over 100 platelets.

The consequences of undiagnosed pseudothrombocytopenia lead to incorrect treatment decisions. Thus, for example, following cases are described as:

- Termination of radiotherapy patients with carcinoma of the esophagus due to a false low platelet count on EDTA. In determining routine BC on EDTA number of platelets was $30 \times 10^9 / L$ while the number of platelets determined by other anticoagulant was $165 \times 10^9 / L$ [19].
- Patient diagnosed as Immune Thrombocytopenic Purpura (ITP) treated with corticosteroids and splenectomy after diagnosed pseudothrombocytopenia or BSS
- Platelet transfusions on patients with pseudo-

thrombocytopenia [20].

Most important goal in clinical treatment is to get the right count of platelets. For the proper interpretation of laboratory values or results following points need to be carefully addressed: familial and personal history of diagnosed diseases, pharmacological history and previous findings of laboratory tests [21].

In order to obtain the actual count of platelets, peripheral blood smear shall be done for all samples with low values of thrombocytes ($<100 \times 10^9 / L$), and samples for which the results have indicators of HA. In the case of finding agglutinated platelets, the following measures should be taken in order to obtain correct interpretation of laboratory results: to warm blood sample at 37° C and re-test, to test the blood sample on the second anticoagulant (citrate, heparin) and to determine the microscopic platelets from capillary blood using ammonium oxalate. In case of all phenomena of agglutination with all the above mentioned anticoagulants, it is only possible to use magnesium sulphate and to make smears on the site of capillary blood sampling.

It is rare but is still possible to detect agglutinates when making direct smears at the sampling capillary blood which indicates the presence of cold agglutinins [4, 23, 24].

To obtain the exact number of platelets, using aminoglycosides (kanamycin) as an anticoagulant or to adding to blood sample with EDTA is possible up to 30 min after sampling the blood [21].

Agglutination of platelets may be inhibited by the addition of the bivalent ions (Ca^{2+} and Mg^{2+}), or by addition of an increased EDTA [22]. The mechanism of inhibition of platelet agglutination in increased EDTA is not yet clear [23, 24].

Literature data demonstrate that PTP is not restricted to EDTA, but is also present with other anticoagulants. In contrast, pseudo-leukocytosis was observed only in EDTA-anticoagulated blood. In the presence of EDTA, the expression of GpIIb/IIIa is significantly reduced in the PTP subjects compared to control. Activation antigens CD62, CD63 and thrombospondin-antigen are upregulated in the presence of EDTA. These alterations in the expression of platelet antigens could also be induced on platelets of normal donors by incubation with sera of PTP subjects and EDTA [10, 11, 26, 27].

As clinicians we underline transplacental trans-

mission of EDTA dependent pseudothrombocytopenia. The occurrence of pseudothrombocytopenia is also described in babies born to mothers with thrombocytopenia if blood sample is taken from the umbilical cord using EDTA. In this case, the analyzation of platelets need to be repeated from the sample taken out of the heel of the newborn preferably with another anticoagulant (ammonium oxalate, citrate) and tested immediately after sampling [25]. This phenomenon in a newborn disappears after a month of birth indicating transplacental transmission of plasma components (most likely IgG) that led to pseudohrombocytopenia of the newborn. Due to this phenomenon, concerning neonate with asymptomatic thrombocytopenia shall be proceeded with patterns as adults in order to avoid inappropriate and potentially harmful treatment decisions [28, 29].

CONCLUSION

The occurrence of thrombocytopenia in a laboratory result of BC obtained with hematology analyzers have to be alert for checking and shall indicate the doctor in the laboratory to check the number of platelets using another anticoagulant (citrate, oxalate), another method such as Neubauer chamber cell counting and analyzing peripheral blood smear according to ISLH. In case of all agglutinations with all the above anticoagulants, using magnesium sulphate and making smears at the sampling capillary blood remains as only possible method. Using this method is possible to detect platelet agglutinates without the use of anticoagulants, which indicates the presence of cold agglutinins.

REFERENCES

1. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. *Int. Jnl. Lab. Hem.* 2007, 29, 21-41
2. 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.
3. Cohen AM, Cycowitz Z, Mittelman M, Lewinski UH, Gardyn J. The incidence of pseudothrombocytopenia in automatic blood analyzers. *Haematologia (Budap).* 2000; 30(2):117-21.
4. García Suárez J, Merino JL, Rodríguez M, Ve-

- lasco A, Moreno MC. Pseudothrombocytopenia: incidence, causes and methods of detection. *Sangre (Barc)*. 1991 Jun; 36(3):197-200.
5. Althaus K, Greinacher A. MYH9-related platelet disorders. *Semin Thromb Hemost*. 2009 Mar; 35(2):189-203. doi: 10.1055/s-0029-1220327. Epub 2009 Apr 30.
6. Kakkar N, Garg G. Inappropriate platelet transfusion in a patient with ethylenediamine tetra-acetic acid (EDTA)-induced pseudothrombocytopenia. *Indian J Pathol Microbiol*. 2006; 49(1):29-31. Erratum in: *Indian J Pathol Microbiol*. 2006; 49(3):476.
7. Oza N, Ingole N, Gangane N. Study of Ethylenediaminetetraacetic acid (EDTA) - Dependent Pseudothrombocytopenia. *Acta Medica Scientia 01 [01]* (2014). www.asdpub.com/index.php/ams
8. Kodama T, Imai H, Nakamoto Y, Sugawara T, Miura AB. 48, XXYY syndrome associated with ethylenediaminetetraacetic acid (EDTA)-dependent pseudothrombocytopenia. *Intern Med*. 1992 Jan; 31(1):143-6. PMID: 1568036
9. De Caterina M, Fratellanza G, Grimaldi E, Variante V, Scopacasa F, Di Maro G, Formisano S. Evidence of a cold immunoglobulin M autoantibody against 78-kD platelet glycoprotein in a case of EDTA-dependent pseudothrombocytopenia. *Am J Clin Pathol*. 1993 Feb; 99(2):163-7.
10. Stiegler H, Fischer Y, Steiner S, Strauer BE, Reinauer H. Sudden onset of EDTA-dependent pseudothrombocytopenia after therapy with the glycoprotein IIb/IIIa antagonist c7E3 Fab. *Ann Hematol*. 2000 Mar; 79(3):161-4. Erratum in: *Ann Hematol* 2000 Apr; 79(4):226.
11. Schrezenmeier H, Müller H, Gunsilius E, Heimpele H, Seifried E. Anticoagulant-induced pseudothrombocytopenia and pseudoleucocytosis. *Thromb Haemost*. 1995 Mar; 73(3):506-13.
12. Pegels JG, Bruynes EC, Engelfriet CP, von dem Borne AE. Pseudothrombocytopenia: an immunologic study on platelet antibodies dependent on ethylene diamine tetra-acetate. *Blood*. 1982 Jan; 59(1):157-61.
13. Ohashi-Fukuda N, Inokuchi R, Sato H, Nakamura K, Iwagami M, Wada T, Jona M, Hisasue T, Nakajima S, Yahagi N. Poorer prognosis with ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia: a single-center case-control study. *Medicine (Baltimore)*. 2015 Apr; 94(15):e674. doi: 10.1097/MD.0000000000000674
14. Reed BW, Go RS. Pseudothrombocytopenia associated with multiple myeloma. *Mayo Clin Proc*. 2006 Jul; 81(7):869.
15. Imai H, Nakamoto Y, Miki K, Miyakuni T, Miura AB. Pseudothrombocytopenia and IgA-related platelet agglutinin in a patient with IgA nephritis. *Nephron*. 1983; 34(3):154-8.
16. Kinoshita Y, Yamane T, Kamimoto A, Oku H, Iwata Y, Kobayashi T, Hino M. A case of pseudothrombocytopenia during antibiotic administration. *Rinsho Byori*. 2004 Feb; 52(2):120-3.
17. Fozza C, Pardini S, Marras T, Longu F, Isoni A, Contini S, Longinotti M. Pseudothrombocytopenia in a patient receiving romiplostim for immune thrombocytopenic purpura. *Ann Hematol* 2014; 93: 899-900.
18. Albersen A, Porcelijn L, Schilders J, Zuetenhorst H, Njo T, Hamberg P: Sunitinib-associated pseudothrombocytopenia induced by IgM antibody. *Platelets* 2013; 24: 566-570.
19. Shreiner DP, Bell WR. Pseudothrombocytopenia: manifestation of a new type of platelet agglutinin. *Blood*. 1973 Oct; 42(4):541-9.
20. Yin XL, Shen WD, Chen YS, Zhou Y, Zhang XH: Refractory platelet transfusion in a patient with CD36 deficiency due to pseudothrombocytopenia. *Platelets* 2011; 22: 237-240.
21. Ahn HL, Jo YI, Choi YS, Lee JY, Lee HW, Kim SR, Sim J, Lee W, Jin CJ. EDTA-dependent pseudothrombocytopenia confirmed by supplementation of kanamycin; a case report. *Korean J Intern Med*. 2002 Mar; 17(1):65-8.
22. Sakurai S, Shiojima I, Tanigawa T, Nakahara K. Aminoglycosides prevent and dissociate the aggregation of platelets in patients with EDTA-dependent pseudothrombocytopenia. *Br J Haematol*. 1997 Dec; 99(4):817-23.
23. Kurata Y, Hayashi S, Jouzaki K, Konishi I, Kashiwagi H, Tomiyama Y. Four cases of pseudothrombocytopenia due to platelet cold agglutinins. *Rinsho Ketsueki*. 2006 Aug; 47(8):781-6.
24. Saigo K, Sakota Y, Masuda Y. EDTA-dependent pseudothrombocytopenia: clinical aspects and laboratory tests. *Rinsho Byori*. 2005 Jul; 53(7):646-53].
25. Solanki DL, Blackburn BC. Spurious thrombocytopenia during pregnancy. *Obstet Gynecol*. 1985 Mar; 65(3 Suppl):145-175.
26. Shabnam I, Chuphal DS, Joshi BC. Ethylenediaminetetraacetic Acid (EDTA) - dependent pseudothrombocytopenia: a case report. *J Clin Diagn Res*. 2014 Oct; 8(10):FL03-4. doi: 10.7860/JCDR/2014/9603.5019. Epub 2014
27. Salama A. Autoimmune Thrombocytopenia Complicated by EDTA- and/or Citrate-Dependent Pseudothrombocytopenia. *Transfus Med Hemother*. 2015 Sep; 42(5):345-8.
28. Korterink JJ, Boersma B, Schoorl M, Porcelijn L, Bartels PC. Pseudothrombocytopenia in a neonate due to mother? *Eur J Pediatr*. 2013 Jul; 172(7):987-9.
29. Chiurazzi F, Villa MR, Rotoli B. Transplacental transmission of EDTA-dependent pseudothrombocytopenia. *Haematologica*. 1999 Jul; 84(7):664

Pseudotrombocitopenija

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KRATAK SADRŽAJ

Uvod: Pseudotrombocitopenija je fenomen lažno niskog broja trombocita dobijenog na hematološkim brojačima (HA) zbog formiranja trombocitnih aglutinata u prisustvu antikoagulansa i antitela.

Metodologija: Pretraživanje radova o pseudotrombocitopenijama u časopisima indeksiranim na MEDLINE-u od 1969. do 2016.

Tema: Pseudotrombocitopenija se javlja upotrebom EDTA i drugih antikoagulansa pri određivanju trombocita na HA. Aglutinacija trombocita nastaje na temperaturama nižim od 34 °C i pojačava se dužim stajanjem uzorka. Najizraženija je 4 časa nakon uzorkovanja krvi. Nastaje vezivanjem IgM, IgG i IgA imunoglobulina za antigene ili kriptoantigene trombocita u prisustvu antikoagulansa. HA ne broje trombocite iz velikih aglutinata tako da broj trombocita koji daje HA predstavlja zbir broja slobodnih neaglutinisanih trombocita i sitnih aglutinata koji se sastoje od 3-5 trombocita. U odsustvu kliničkih znakova za hemoragijsku dijatezu treba posumnjati na pseudotrombocitopeniju. Nепреpoznata pseudotrombocitopenija može dovesti do agresivnih dijagnostičkih procedura kao što je biopsija ili punkcija kostne srži, neadekvatnog lečenja i čak primene transfuzije trombocita. Opisane su sledeće pseudotrombocitopenije:

- a) usled aglutinacije trombocita,
- b) trombocitni satelitizam i
- c) agregacija trombocita i leukocita.

Zaključci: Da bi se dobio realan broj trombocita treba za sve uzorke sa niskim vrednostima trombocita ($<100 \times 10^9/L$) i za uzorke čiji rezultati imaju flagove na HA napraviti razmaz periferne krvi. U slučaju nalaza aglutinata trombocita, a radi pravilne laboratorijske interpretacije rezultata treba preduzeti sledeće mere: zagrejati uzorak krvi na 37 °C i ponovo testirati, uzorkovati krv na drugom antikoagulansu (citrat, heparin) i napraviti razmaz, upotrebiti drugu metodu-odrediti trombocite mikroskopski brojanjem u komori iz kapilarne krvi sa amonijum oksalatom. U slučaju pojave anglutinata sa svim navedenim antikoagulansima, kao mogućnost ostaje upotreba magnezijum-sulfata i pravljenje razmaza na mestu uzorkovanja kapilarne krvi. Moguće je da se, i na ovaj način bez upotrebe antikoagulansa detektuju anglutinati Tr, što upućuje na prisutvo hladnih aglutinina.

Ključne reči: pseudotrombocitopenija, EDTA

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