J Bras Patol Med Lab. 2020; 56: 1-4.

10.5935/1676-2444.20200005

Thrombogram: case report of pseudothrombocytopenia

Plaquetograma: relato de caso de pseudotrombocitopenia

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ABSTRACT

The thrombogram is one of the components of the blood count that includes platelet quantification and evaluation. The presence of laboratory artifacts, such as incorrect platelet counts by autoanalyzers, can lead to pseudothrombocytopenia, which is responsible for 15% to 30% of the cases of isolated thrombocytopenia observed in laboratory routine. Pseudothrombocytopenia induced by the anticoagulant ethylenediaminetetraacetic acid (EDTA) is one of the most frequent cases in which the presence of anticoagulant in blood samples can cause platelet aggregation or platelet satellitism. Careful observation of the data emitted by autoanalyzers, such as platelet and flag histograms, is crucial. Other procedures, such as checking for clotting in the sample, repeating the sample and viewing a peripheral blood smear, requesting a new sample taken with another type of anticoagulant, such as citrate, are imperative for the confirmation of cases of pseudothrombocytopenia.

Key words: thrombogram; pseudothrombocytopenia; anticoagulant.

RESUMO

O plaquetograma é um dos componentes do hemograma que inclui a quantificação e a avaliação plaquetária. A presença de artefatos laboratoriais, como as contagens incorretas do número de plaquetas pelos analizadores hematológicos, pode originar casos de pseudotrombocitopenia (15% a 30% de casos de trombocitopenias isoladas observados na rotina laboratorial). A pseudotrombocitopenia induzida pelo anticoagulante ácido etilenodiaminotetracético (EDTA) é um dos casos mais comuns em que a presença do anticoagulante em amostras de sangue pode provocar agregação plaquetária ou satelitismo plaquetário. A observação de forma criteriosa dos dados emitidos pelos analizadores (por exemplo, histograma de plaquetas e flags*) é crucial. Outros procedimentos, como a verificação da existência de coágulo na amostra, a repetição da amostra e a visualização de um esfregaço de sangue periférico, além do pedido de nova amostra colhida com outro tipo de anticoagulante, como o citrato, são importantes para confirmar casos de pseudotrombocitopenia.*

Unitermos: *plaquetograma; pseudotrombocitopenia; anticoagulantes.*

RESUMEN

El plaquetograma es uno de los componentes del hemograma que incluye la cuantificación y la evaluación de las plaquetas. La presencia de artefactos de laboratorio, como los recuentos incorrectos del número de plaquetas por los analizadores hematológicos puede originar casos de pseudotrombocitopenia (del 15% al 30% de los casos de trombocitopenias aisladas observados en la rutina del laboratorio). La pseudotrombocitopenia inducida por el anticoagulante ácido etilendiaminotetraacético (EDTA) es uno de los casos más comunes en los que la presencia de anticoagulante con muestras de sangre puede producir agregación plaquetaria

First submission on 04/29/19; last submission on 05/09/19; accepted for publication on 05/21/19; published on 01/14/19

o satelitismo plaquetario. La observación criteriosa de los datos emitidos por los autoanalizadores (por ejemplo, histogramas plaquetarios y alarmas) es crucial. Otros procedimientos, como la verificación de la existencia de un coágulo en la muestra, la repetición del frotis de sangre periférica, además de la solicitud de nueva muestra con otro tipo de anticoagulante, como el citrato, son importantes para confirmar casos de pseudotrombocitopenia.

Palabras clave: *plaquetograma; pseudotrombocitopenia; anticoagulantes.*

INTRODUCTION

The thrombogram is one of the analytical components of blood count that includes quantification and morphological evaluation of platelets. Laboratory parameters such as number of platelets (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) and platelet larger cell ratio (P-LCR) are available in the thrombogram[1, 2].

The presence of laboratory artifacts, such as incorrect platelet counts by autoanalyzers, can lead to pseudothrombocytopenia (a result of falsely decreased number of platelets)[3, 4].

Pseudothrombocytopenias correspond to 15% to 30% of the cases of isolated thrombocytopenia observed in the laboratory routine[5]. The main causes of pseudothrombocytopenia are: problems in collection and processing of samples (improper tube shaking, sample dilution, peripheral blood collection difficulties); giant platelet syndrome; induction by anticoagulants, such as ethylenediaminetetraacetic acid (EDTA), citrate, oxalate and heparin; autoimmune diseases; drugs (abciximab, valproic acid, mexiletine and olanzapine); solid tumors, myeloproliferative and lymphoproliferative syndrome[6].

EDTA is the preferred anticoagulant used to perform blood counts, and its exposure to the blood samples from some patients may induce platelet aggregation or, less frequently, platelet aggregation around neutrophils (platelet satellitism), by an apparently immune-mediated process[7, 8]. This phenomenon, which is the most frequently associated with cases of pseudothrombocytopenia, requires that appropriate laboratory procedures be performed, since it represents a major laboratory and clinical problem[6-10].

CASE REPORT

A 3-year-old male patient was brought to the emergency room for a three-day episode of irritative cough and fever. Laboratory analyses were requested, and no significant changes were observed in the biochemical parameters. At the level of the blood count, there were no alterations in the red blood cell (RBC) count and in the white blood cell (WBC) count; they were observed just in the platelet count (thrombogram). Three peripheral blood samples were analyzed for the thrombogram study:

• sample 1 – peripheral venous blood collected in tube containing anticoagulant (EDTAK3, 3 ml, VACUETTE®, Premium, Greiner Bio-One, Austria). Blood volume collected: 3 ml;

• sample 2 – peripheral venous blood collected in tube containing anticoagulant (EDTAK3, 3 ml, VACUETTE®, Premium, Greiner Bio-One, Austria). Blood volume collected: 1.5 ml;

• sample 3 – peripheral venous blood, collected in tube containing anticoagulant (9NC coagulation sodium citrate 3.2%, 3.5 ml, VACUETTE®, Premium, Greiner Bio-One, Austria). Blood volume collected: 3.5 ml.

The three samples were processed in the XN-2000 autoanalyzer (Sysmex Corporation, Kobe, Japan), where the automatic platelet counting was carried out by an optical fluorescence method (PLT-O).

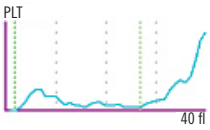
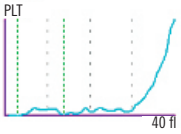
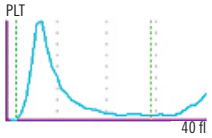
The results obtained for the three samples, referring to the different parameters that make up the thrombogram, are shown in **Table 1**.

The software associated with the autoanalyzer generated, for samples 1 and 2, messages for technical intervention: clot verification and peripheral blood smear. No clots were observed in samples 1 and 2 and, after repeated analysis, peripheral blood smears were studied. Sample 3 also underwent examination of peripheral blood smear. The final released platelet values and their respective platelet series observations are shown in **Table 2**.

DISCUSSION

About 0.1% of the world population has natural EDTA-dependent antiplatelet antibodies[5]. Most autoantibodies

TABLE 1 – Results obtained in the thrombogram for samples 1, 2 and 3

Thrombogram (units)	Sample 1	Sample 2	Sample 3
Platelets (10^3/µl)	26	5	153
PDW (fl)	11.8	n.d.	n.e.
MPV (fl)	11.7	n.d.	n.e.
P-LCR (%)	38.2	n.d.	n.e.
PCT (%)	0.02	n.d.	n.e.
Platelet histograms			
Flags	Thrombocytopenia	Thrombocytopenia abnormal distribution of PLT	Not flags

PDW: platelet distribution width; MPV: mean platelet volume; P-LCR: platelet larger cell ratio; PCT: plateletcrit; n.d.: not determined; n.e.: not effected; PLT: platelets.

TABLE 2 – Results of platelets released and platelet series observations

Sample	Platelets (10^3/µl)	Platelet series observations Hematology information
1	> 26	Platelet value highly underestimated by the presence of numerous platelet It is recommended to harvest in citrate tube
2	> 10	Numerous platelet aggregates It is recommended to harvest in citrate tube
3	> 168	Result of platelets in citrate Presence of some platelet aggregates

isolated from these individuals act as cold agglutinins at an ideal temperature range of 4°C-20°C, generally corresponding to immunoglobulin classes G (IgG) and M (IgM), and less frequently to immunoglobulin class A (IgA)[6, 11]. EDTA-induced pseudothrombocytopenia has a physiological mechanism not yet well known, but there are studies suggesting that autoantibodies present in plasma, in the presence of EDTA recognize and bind to the epitope of glycoprotein IIb (GPIIb), forming the GPIIb/IIIa complex in platelet aggregation, promoting platelet agglutination[1-3, 6].

When a patient presents with isolated thrombocytopenia, with no family history of thrombocytopenia, hematological diseases or manifestation of bleeding episodes, a case of pseudothrombocytopenia should be suspected, which should not be confused with other serious clinical conditions, such as disseminated intravascular coagulation, idiopathic thrombocytopenic purpura or heparin-induced thrombocytopenia[12].

In the case reported, the presence of isolated thrombocytopenia in a child alerted for the possibility of pseudothrombocytopenia. With a low platelet count, clotting was assessed in the sample, but its presence was not observed (sample 1). After this procedure, a new hemogram was performed, in which the methodology used for platelet counting was not electrical impedance (PLT-I) but PLT-O. According to studies, PLT-O is more reliable in platelet counts below 100 × 10^3/µl[13]. The displayed histogram does not present a normal distribution of platelets, its trajectory being suggestive of the presence of platelet aggregates. After the analysis was repeated, a peripheral blood smear was performed, where numerous platelet aggregates were observed. The final result of the number of platelets released was > 26 × 10^3/µl, due to the presence of platelet aggregates. Given the suspicion of pseudothrombocytopenia, clinicians were recommended to send a new sample for platelet count in a tube of citrate.

The use of alternative anticoagulants to EDTA, such as sodium citrate, oxalate or heparin, is a good option to rule out the phenomenon of EDTA-induced pseudothrombocytopenia, but there are some rare cases of pseudothrombocytopenia associated with these anticoagulants[6-8]. Other procedures less used in laboratory practice to confirm cases of pseudothrombocytopenia are to reanalyze the sample taken in EDTA after incubation at 37°C for about 30 minutes (the objective of this incubation is the dissociation of the platelet aggregates), or to add certain aminoglycosides such as kanamycin and amikacin (these do not interfere with cell counts and prevent the formation of platelet aggregates, although their mechanism of action is still not well known)[11] to the sample harvested with EDTA.

By mistake, the second sample sent for analysis was placed in a tube with EDTA; besides, the sample volume was not in the proportion recommended for the amount of anticoagulant. About 1.5 ml of peripheral blood was collected, when the indicated was 3 ml. The results obtained in the second sample reflect this dilution with EDTA, resulting in a PLT-O count lower than in sample 1. The platelet histogram of sample 2 is also suggestive of the presence of

platelet aggregates. The same procedures were followed, as with sample 1, when the presence of numerous platelet aggregates was documented and a new sample was requested in citrate. Regardless of the platelet counting method in the autoanalyzers, platelet counts of less than 10×10^3/µl are imprecise, with this minimum platelet count being released as the result if the autoanalyzer has a lower count[14]. The released result was of platelets $> 10 \times 10^3$/µl, given the presence of platelet aggregates.

A third sample collected in citrate confirmed the existence of pseudothrombocytopenia, since the platelet count in the autoanalyzer was 153×10^3/µl. Although the platelet histogram exhibited normal distribution and did not raise the possible existence of platelet aggregates, some platelet aggregates were observed in the peripheral blood smear. The citrate tube sample was diluted with citrate (nine parts blood for a total of ten parts) and it was necessary to correct the platelet value of the autoanalyzer by multiplying the results by the dilution factor 1.1 (10%)[1]. In sample 3, a platelet count $> 168 \times 10^3$/µl was released, with the application of the respective dilution factor and the presence of platelet aggregates.

There are documented cases in which some autoanalyzers may erroneously count platelet aggregates as leukocytes, leading to a falsely elevated leucocyte count, called pseudoleukocytosis[4, 11].

CONCLUSION

An incorrect interpretation of platelet count results obtained by autoanalyzers may lead to false diagnosis. It is imperative to carefully observe the data emitted by autoanalyzers (such as platelet histogram, flags suggestive of pseudothrombocytopenia) to verify the existence of a clot in the sample, to repeat the sample and to visualize a peripheral blood smear. The collection of a new citrate sample may be sufficient to confirm the finding of pseudothrombocytopenia.

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