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Research Article

Pseudothrombocytopenia: Automate-Blood Smear Confrontation

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Abstract: The platelet count is performed systematically as part of complete blood count and in the evaluation of patients with hemorrhagic syndrome or those at risk of bleeding. The automatic counting of platelets by the latest generation hematology analyzers is more precise and less expensive than conventional manual methods. However, this reliable and fast technique can lead to counting errors which must not be ignored. The aim of this work is to focus on the different situations leading to false thrombocytopenia, mainly EDTA dependent or not EDTA dependent, to help biologists detect this trap and thus avoid unnecessary and costly diagnostic and therapeutic procedures for patients.
Keywords: Pseudothrombocytopenia, EDTA, blood smear.

Introduction

The platelet count is a vital test, the results of which influence clinical or therapeutic decisions. The latest generation of hematology machines combine impedance techniques and optical methods (laser diffraction and fluorescence) to obtain a platelet figure that is closest to reality. However, in some situations, the platelet count may be wrong and there are two cases: a falsely high platelet count called pseudothrombocytosis and a falsely low platelet count called false thrombocytopenia, pseudothrombocytopenia or artefactual thrombocytopenia. Pseudothrombocytopenia can be caused by several factors such as, pre-analytical conditions, anticoagulant used, cold agglutinins, temperature, platelet size.

In the laboratory, the discovery of thrombocytopenia is always verified by examination of the blood smear. This always begins with the search for platelet aggregates, since their presence makes it possible to confirm artefactual thrombocytopenia. The problem in our study is to understand the different elements involved in this in-vitro phenomenon, in order to avoid any inappropriate clinical decision or unnecessary therapeutic interventions and to establish an etiological diagnostic approach in the face of the discovery of a false thrombocytopenia.

Materials and Methods

This is a prospective and descriptive study carried out over a period of 2 months from May 1, 2019 to July 31, 2019, at the Avicenne military hospital in Marrakech.

Inclusion criteria

This descriptive study concerned all samples sent to the laboratory, from patients hospitalized in the various departments or as an external consultant within the Avicenna military hospital.

The patients were selected according to the following criteria:

- ✓ With a platelet count <150 G/L, for which a blood smear stained with MGG was carried out.
- ✓ Lack of bleeding tendency
- ✓ Presence of an alarm on the machine indicating an abnormal distribution of platelets
- ✓ Normal hemostasis assessment

Exclusion criteria

The cases excluded from our study were all cases with:

- ✓ A platelet count> 150 G/L
- ✓ Bleeding
- ✓ Taking drugs inducing thrombocytopenia
- ✓ A coagulated sample
- ✓ A sample from a citrated tube
- ✓ Patients known to have true thrombocytopenia.
- ✓ All samples that do not comply with the pre-analytical phase (such as a difficult sample, a lack of anticoagulant by overfilling the tube, an absence of agitation).

Results

During this study 3682 complete blood counts were performed, of which 369 patients presented with thrombocytopenia, among whom a false thrombocytopenia was confirmed in 30 patients.

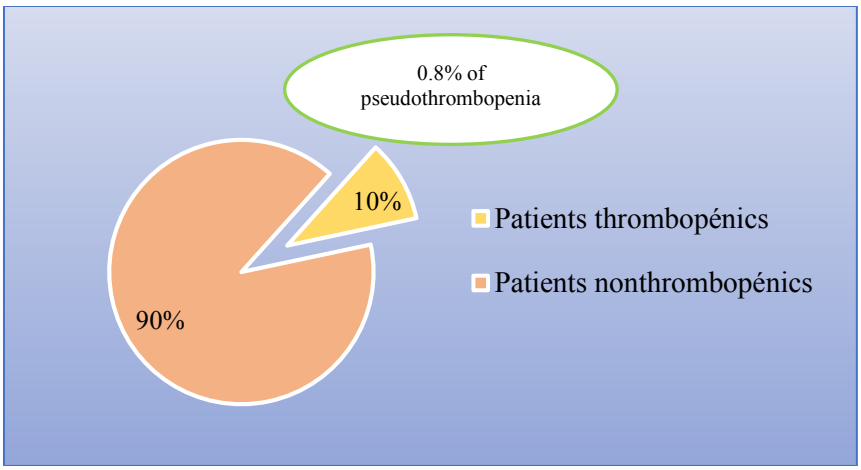

Figure 1. Overall frequency of thrombocytopenia

Average age of patients with pseudo-thrombocytopenia: 47 years with extremes ranging from 30 years to 65 years, there is a clear male predominance with a sex ratio of 1.5.

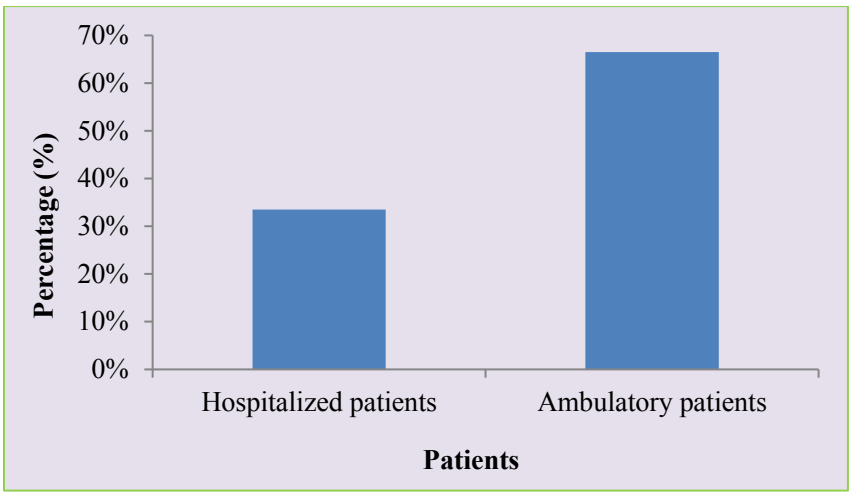

Figure 2. Distribution of patients

Average platelet count: 115G / L (90G-140G)

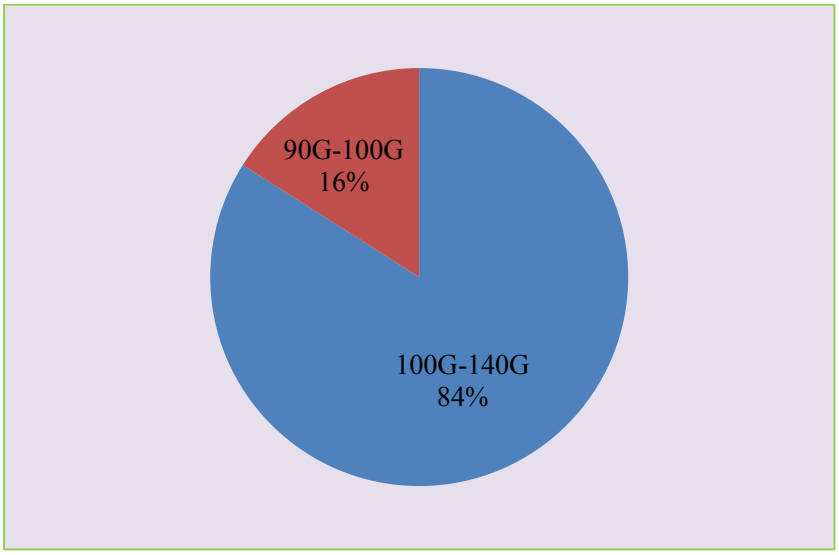

Figure 3. Distribution of patients by platelet count

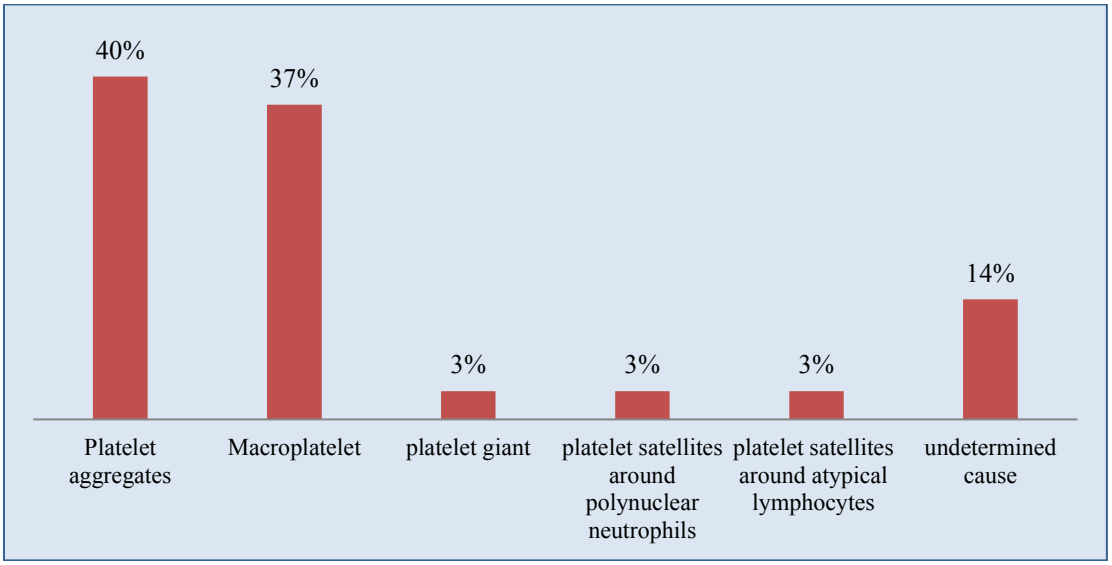

Figure 4. Distribution of pseudothrombocytopenia by etiology

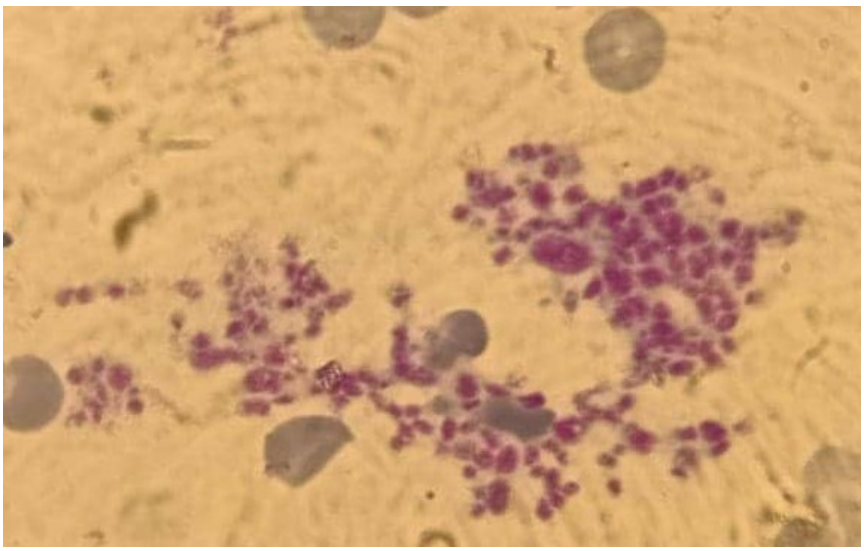

Image 1. Platelet aggregates

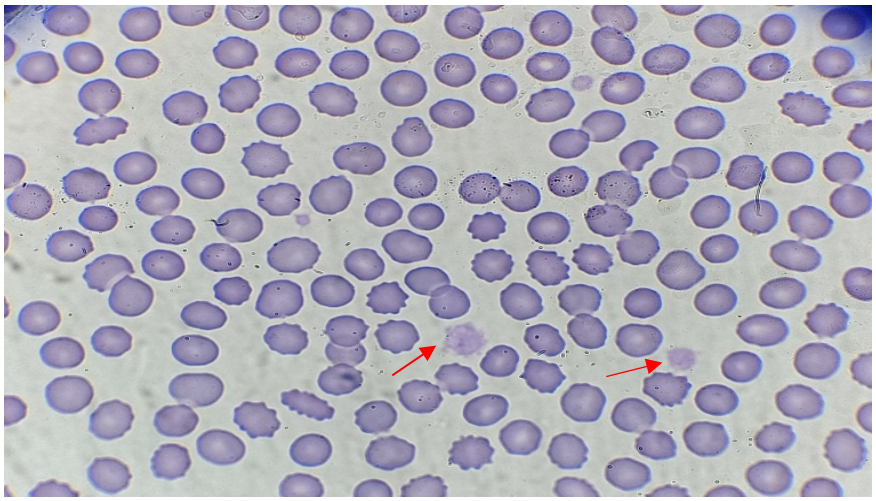

Image 2. Giant platelet + macroplatelet

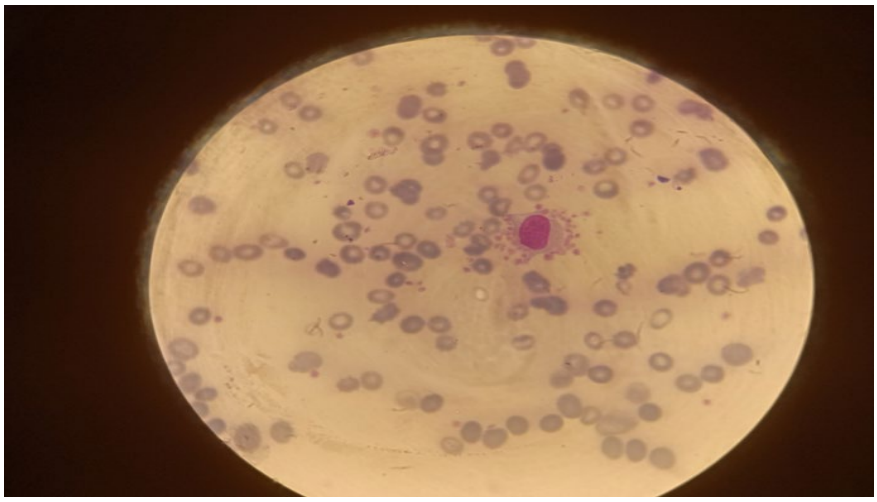

Image 3. Platelet satellites around an atypical lymphocyte

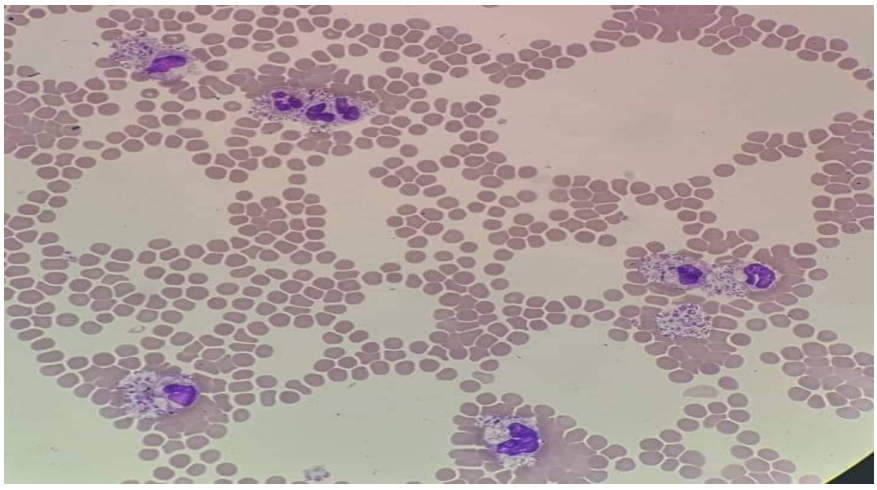

Image 4. Platelet satellites around polynuclear neutrophils

Discussion

False thrombocytopenia or pseudothrombocytopenia (PTP) is a phenomenon purely artefactual, relatively rare, often discovered by chance in which the number of PLT reported by the PLCs is much lower than their real number circulating in vivo. It is the result of the presence of PLT of size abnormal in large percentage or agglutination of PLT in vitro and therefore, the formation of platelet aggregates that automata are unable to differentiate individual cells, leading to a platelet count falsely diminished. It is responsible for 15-20% of all unexplained "thrombocytopenia" [1].

A study by Vicari et al. gave an incidence of pseudothrombocytopenia of 0 to 13% in 33,623 subjects referred from a general hospital.

PTP should be considered especially in asymptomatic individuals with unexpected or new-onset thrombocytopenia without petechiae or bruising or bleeding tendency even if no alarm indicative of an artifact is given by the automatic numbering machine.

According to an Italian study, PTP accounts for 0.13% of total platelet counts while in a hematology clinic in Italy, 15% of patients with isolated thrombocytopenia could be due to PTP and 7.5 to 15.3% in another report [1].

The incidence of PTP is slightly higher in sick or hospitalized than in ambulatory patients. Thus, Dutch studies, Japanese and Italian women reported that its incidence is 1.9% among hospitalized patients and 0.15% to 0.9% for outpatients.

In PTP we distinguish [2]:

- ✓ EDTA-dependent PTPs which are due to either related platelet aggregation with EDTA, platelet orbiting, or mixed neutrophil platelet aggregation in the presence of EDTA.
- ✓ Non-EDTA-dependent PTPs also called EDTA-independent PTPs.

According to Italian studies, the frequency of EDTA-dependent PTP ranges from 0.03 to 1.9% of all counts performed. It represents up to 15% of the causes of "isolated thrombocytopenia" or even 17% for outpatients and between 75 to 90% of the aetiologies of PTP. It occurs in 0.2% of people asymptomatic, but the incidence is higher in hospitalized patients up to 2.0%. The prevalence of EDTA-dependent PTP in PLT donors is 0.2%.

The frequency of platelet orbiting is much lower than that of the agglutination of EDTA-dependent PLT (about 1/30,000 counts) but the real impact of the phenomenon can be underestimated.

EDTA-dependent PTP is transient (disappears in a few months) or permed. Some authors think it may be slightly more common in men and/or elderly patients. But generally, the incidence is quite superimposable between them. PTP has been documented in healthy subjects as well as in patients affected by a wide variety of disorders. In the literature there is a disagreement between the authors: some authors believe that it has a possible relationship between the occurrence of PTP and certain diseases such as autoimmune diseases, inflammatory, neoplastic, atherosclerotic diseases and diseases liver, Guillain-Barré syndrome, Sjögren syndrome as well as viral infections or sepsis as this phenomenon is more frequently observed in patients with these pathologies [3].

PTP is an often-unrecognized phenomenon and does not pose a hemorrhagic or thrombotic risk to the patient and the only clinical implication lies in its ignorance. Knowledge of this phenomenon is very important because PTP can lead to the incorrect diagnosis of thrombocytopenia and therefore inappropriate treatment which is sometimes disproportionate to the anomaly in question: hospitalization of patients, inappropriate transfusion of PLT and other costly and unnecessary additional laboratory tests such as bone marrow puncture and bone biopsy, long-term treatment with corticosteroids and even splenectomy, or on the contrary, delays in diagnostic or therapeutic procedures [4]. However, the most frequent consequence is an interruption of surgery for fear of the risk of bleeding preoperatively or a postponement of invasive examinations by discovery of thrombocytopenia in the preoperative workup. In addition, the presence of PTP may mask true thrombocytopenia.

A particular problem arises when subjects with PTP require surgery under hypothermia, as happens in surgery cardiac. However, it has been reported that surgery can be performed without complication in patients undergoing heart surgery for bypass grafting coronary heart disease or valve

replacement, even if the body temperature has been lowered to 28 or 30 ^{0}C. Pseudothrombopenia can be divided into two large groups: EDTA-dependent pseudothrombocytopenia and EDTA-independent or non-EDTA-dependent pseudothrombocytopenia.

A) EDTA-dependent pseudothrombopenia [5,6,7,8,9]: These are the most frequent. In these PTPs, the false decrease in platelet count is related to the presence of EDTA and does not occur in the presence of other anticoagulants such as citrate or heparin.

EDTA-dependent PTPs are due to either

1) EDTA dependent platelet aggregates

EDTA pseudo-thrombocytopenia is an in vitro phenomenon occurring in the collection tube. It is irreversible and is seen more often in patients with a known or hitherto asymptomatic inflammatory syndrome and in dysimmune states. It is an irreversible phenomenon. The formation of platelet aggregates is almost complete about 20 minutes after collection. At 90 minutes, the process is definitively completed and stabilized.

At the start of the aggregation process, the formation of microaggregates over time can increase the number of white blood cells by 10 to 100% (an "interference" type alarm is signaled by the automatons). If the platelet clumps are transformed into macro aggregates, the level of leukocytes at automatic counting returns to normal.

EDTA can cause platelets to clump, either directly or by the antibody intermediary. The mechanism leading to the formation of platelet aggregates is complex and influenced by immunological factors (antiplatelet autoantibodies), chemical (anticoagulants), and physical (temperature) which together make this phenomenon possible only in vitro. The most likely mechanism of EDTA-related platelet aggregation is a normally hidden (cryptic) antigenic site of the αIIb /βIIIa complex platelet count is altered or exposed only in the presence of EDTA. After, we showed that this antigenic site is the GPIIb protein which is normally hidden but becoming accessible to antibodies after dissociation of the αIIb/βIIIa complex, under the effect EDTA chelator on calcium ions associated with alterations in protein conformation induced by low temperature. Thus, autoantibodies can recognize these glycoproteins and bind to them and other platelets thereby causing their agglutination.

2) Satellitism of platelets around leukocytes

Leuco-platelet rosettes is a phenomenon acquired in vitro particularly and not only in the presence of EDTA. It is due to the adhesion of platelets to the membrane of mature polynuclear neutrophils (PNNs) or sometimes to other cells. According to various Spanish and Italian studies, the mediators of this phenomenon are constantly naturally acquired autoantibodies like IgG inactive at 37 ^{0}C and /or IgM whose two thermal peaks of maximum activity are at 4 ^{0}C and 22 ^{0}C and which usually do not react in citrated blood. Alongside these autoantibodies, the Fc gamma receptors of PNNs could participate in this phenomenon. This phenomenon most often concerns PNNs, but it can also be observed, although rarely with other cells such as monocytes in blood with EDTA or/and heparin, basophils specifically in a case of chronic myeloid leukemia, eosinophils, lymphocytes or lymphoma cells with or without involving an immunoglobulin and CD16. The cause of the involvement of these cells in this phenomenon remains unknown.

B) Independent EDTA pseudo-thrombocytopenia [10,11,12]:

1) Causes related to errors in the preanalytical phase: In all biological analyzes, the quality of the analytical phase largely determines the quality of the results obtained and any preanalytical dysfunction can lead to abnormal results. Preanalytical errors are more frequent than analytical errors, which is why care must always be taken to ensure the quality of this important phase.

The main preanalytical errors that can be the cause of non-PTP EDTA-dependent and the measures to be taken to prevent their occurrence are described in Table 1.

Table 1. The main preanalytical errors causing non-PTP EDTA-dependent and how to avoid them

Preanalytical error	Corrective actions
Blood samples diluted by sampling at near an infusion or on a insufficiently purged infusion or catheter.	Avoid taking samples near an infusion or on an infusion line.
High concentration of anticoagulant (regardless either the latter) in the sample in case: ✓ difficult venipuncture (poor network venous, repeated samples, fragile veins); ✓ difficult (traumatic) harvest as in the newborn (risk of activation and therefore platelet aggregation or coagulation partial of the sample favored by hypercoagulability of newborn blood).	✓ Mention on the analysis prescription form this kind of levy and the difficulties encountered during the operation for take into account in the interpretation of results. ✓ Check samples of this kind, on tube and on smear, to detect any aggregates by PLT. ✓ Avoid traumatic samples and eliminate the first milliliters of blood taken because they often contain small traces of debris tissue that can promote activation platelet. ✓ The tubes should be 90% full at minimum. ✓ Use pediatric tubes suitable for this kind of sample.
Delay between sampling and analysis: may change the volume of PLTs and therefore a difficulty in the automaton to generate a smooth curve or to find precisely the usual criteria of definition of PLT.	✓ Perform blood count analysis on automaton and spreading the blood smear in within 3 hours of the sample stored at room temperature. ✓ Control tube and smear samples blood: search for any clusters platelets. ✓ Re-do the sample in case of detection platelet clusters with more precaution.

2) Pseudo-thrombocytopenia of an autoimmune nature

The mechanism is also immunological, but not attributable to EDTA. The formation of platelet aggregates is under the responsibility of a cold agglutinin-type autoantibody which appears in autoimmune diseases. It is a thrombocytoagglutinin because it is directed against platelets. It is a thermo-dependent and reversible phenomenon which disappears at 37 ^{0}C in a water bath. It develops in vitro at room temperature or 4 ^{0}C.

C-Pseudo-thrombocytopenia of a physical nature

Macrothrombocytes or giant platelets

By physical mechanism, we are talking about anomalies in the detection of platelets because of their size or volume. Counts by impedancemetry ignore mega or macrothrombocytes. The XE-2100™ from Sysmex uses dual optical measurement (fine diffraction angle providing information on the volume and fluorescence emission), allowing thus eliminate this type of trap in the majority of cases. Last resort, only counting in a counting cell under an optical microscope can check the platelet count.

D-Pseudo-thrombocytopenia of a chemical nature

In this type of phenomenon, platelets in vitro in a given patient are also constitute in aggregates. This is a non-immunological phenomenon, independent of EDTA or conventional citrate used in coagulation and irreversible. The formation of aggregates in this case is due to an increase in pH in the tube over time corresponding to a phenomenon alkalization of the medium. To have an exact count, the blood must be taken on a tube with a special citrate called "acid citrate"

Conclusion

At the end of our work, we can conclude that the numeration automatic platelets although it is more precise than the methods manual, can lead to default errors such as pseudo thrombocytopenia. This is an in vitro phenomenon occurring in the collection tube which poses no risk to the patient, it is a

laboratory artefact which is never accompanied by a hemorrhagic phenomenon. Their ignorance can lead to unnecessary clinical decisions and harmful. In fact, unrecognized pseudo-thrombocytopenia with EDTA has carried out additional investigations including spinal cord puncture, iterative autoimmunity tests, ineffective long-term treatments by corticosteroids, and even extreme cases of splenectomy. However, the the most frequent consequence is an interruption of surgery or a postponement of invasive examinations by discovery of thrombocytopenia in the assessment preoperative.

Faced with the first discovery of thrombocytopenia, it is necessary to systematically search the tube for a clot whose presence corresponds to a partially or totally coagulated sample, in the absence of this, a manual control of the thrombocytopenia on a slide of MGG stained blood should be performed. The morphological study will possibly allow the detection of thrombopathies with dystrophic platelets.

The biologist must therefore always remain vigilant to this phenomenon of false thrombocytopenia. This is a diagnostic trap to be aware of, To be able to guard against these pitfalls, strict procedures can be proposed.

It is in this process that our work is inscribed, which after having explained the mechanisms of this phenomenon, proposed a decision tree. So in presence of platelet aggregates whether EDTA dependent or not EDTA dependent, the multi-step procedure allows us to obtain the actual rate platelets.

Conflicts of interest: Authors declare no conflict of interest.

References

1. James C. Zimring MD, PhD. Chapter 114–Platelet Count. Transfusion Medicine and Hemostasis 2009; 611-613.
2. Dalal BI, Brigden ML. Artifacts that may be present on a blood film. Clin Lab Med. 2002;22(1):81-100.
3. Silvestri F, Virgolini L, Savignano C, Zaja F, Velisig M, Baccarani M. Incidence and diagnosis of EDTA-dependent pseudothrombocytopenia in a consecutive outpatient population referred for isolated thrombocytopenia. Vox Sang. 1995;68(1):35-9.
4. Berkman N, Michaeli Y, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and a review of the literature. Am J Hematol. 1991;36(3):195-201.
5. Bragagni G, Bianconcini G, Brogna R, Zoli G. Pseudothrombocytopenia: clinical comment on 37 cases. Miner Med. 2001;92(1):13-7.
6. Lau LG, Chng WJ. Transfusion medicine illustrated. Unnecessary transfusions due to pseudothrombocytopenia. Transfus. 2004;44(6):801.
7. Satoh M, Hirose Y, Gamo M, Ohtsuka T, Yonezawa K, Tomioka T, Sakai K. Sudden onset of EDTA-dependent pseudothrombocytopenia in a patient scheduled for open heart surgery. Masui Japan J Anesthesiol. 2003;52(4):402-5.
8. Maslanka K, Marciniak-Bielak D, Szczepinski A. Pseudothrombocytopenia in blood donors. Vox Sang. 2008;95(4):349.
9. Mayan HA, Salomon OP, Pauzner RA, Farfel Z. EDTA-induced pseudothrombocytopenia. Sout Med J. 1992;85(2):213-4.
10. Schimmer A, Mody M, Sager M, Garvey MB, Hogarth M, Freedman J. Platelet cold agglutinins: a flow cytometric analysis. Transfus Sci. 1998;19(3):217-24.

11. Zupańska B, Maślanka K. Analysis of 15 cases with platelet EDTA-dependent antibodies. Acta Haematol Polon. 1995;26(4):361-5.

12. Berning H, Stilbo I. Pseudothrombocytopenia and the haematology laboratory. Lancet. 1982;2(8313):1469-70.

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