In this issue: Improving the preanalytical phase in laboratory medicine

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Pseudothrombocytopenia by ethylenediaminetetraacetic acid can jeopardize patient safety – a case report

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ABSTRACT

Pseudothrombocytopenia by ethylenediaminetetraacetic acid (EDTA) is an infrequent phenomenon of *in vitro* platelet agglutination due to the presence of antiplatelet autoantibodies. It has no clinical significance, but misdiagnosis may lead to clinical or therapeutic decision-making. In this study, we report a case of an 8-year-old boy with no history of platelet disorder presenting a low platelet count and a peripheral blood smear showing clumping of platelets by EDTA. The initial diagnosis hypothesis was of an idiopathic thrombocytopenic purpura, and an unnecessary bone marrow aspirate was made even though he did not have personal or family history of bleeding. A second sample collected in sodium citrate confirmed the pseudothrombocytopenia by EDTA. In conclusion, the laboratory should enhance a strong relationship with clinicians trying to avoid misunderstandings as that reflected in this case report. It should be reminded that, in those cases where a pseudothrombocytopenia by EDTA is suspected, a blood smear is mandatory

to confirm platelet clumps and blood must be tested anticoagulated with another anticoagulant (i.e., sodium citrate or heparin).

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INTRODUCTION

Several preanalytical factors could impact platelets evaluation and jeopardize patient safety (1). Therefore, laboratory professional should guarantee preanalytical procedures to avoid the following source of platelets variability:

- improperly fasting time (2,3)
- improperly tourniquet application time (4,5)
- unstandardized patient posture (6)
- improper order of evacuated tubes (7,8)
- improper blood venous sampling (9)

According to the World Health Organization (WHO), patient safety is the absence of preventable harm to a patient during the process of health care and reduction of risk of unnecessary harm associated with health care to an acceptable minimum (7). The work in healthcare services should focus in the culture of patient safety, understanding that the problem is often a succession of several oversights or errors, from which we can learn and implement solutions to prevent them in the future.

Ethylenediaminetetraacetic acid (EDTA) is a calcium chelator (10), widely used as an anticoagulant for a complete blood count test because it generally does not distort blood cells. The term pseudothrombocytopenia by EDTA define a low platelet count in patients without any bleeding tendency, and platelet distribution curve that indicates platelet aggregation (11). Moreover, the patient should present a normal platelet count if a different anticoagulant is used (12). The aim of this report is to show that a pseudothrombocytopenia by EDTA can jeopardize the patient safety.

CLINICAL-DIAGNOSTIC CASE

An 8-year-old boy was referred to paediatric onco-haematology for presenting a two-year evolution thrombocytopenia with a platelet count below $20x10^3/\mu L$ (Reference Interval: 130-$400x10^3/\mu L$) with normal values of white blood cells (WBC) and haemoglobin (Hb) (Table 1). He did not have a significant past medical history, besides his familial hypercholesterolemia, and his clinical examination was unremarkable.

The initial diagnosis hypothesis was of an idiopathic thrombocytopenic purpura even though he did not have personal or family history of bleeding. A bone marrow aspirate was made, with no significant anomalies in the sample obtained. It was described as a normocellular marrow, showing trilinear haematopoiesis, with a preserved myeloid/erythroid ratio and a megakaryocytic series without significative dysmorphias. At that moment the clinician realized that there was a commentary on the platelet count remarking the presence of plentiful platelet clumps in all the previous laboratory reports. The complete blood count was repeated in the emergency laboratory. The platelet count was of $16x10^3/\mu L$ together with a platelet clumps alarm in the automated cell counter (Advia 2120, Siemens Medical Solutions Diagnostics, Los Ángeles, CA, USA), without alterations in the other two haematological series (Table 1). Given this result, a peripheral blood smear was made, and multiple platelet clumps were observed (Figure 1). This was remarked in the laboratory report and a new sample was collected using an evacuated tube with 3.2% sodium citrate, as anticoagulant additive (BD Vacutainer). The platelet count in this sample was normal (Table 1) and this result was discussed with the clinician. Physicians decide to perform a new blood collection with both anticoagulants 14 days after these results to confirm the pseudothrombocytopenia by EDTA (Table 1).

Table 1 Laboratory results

Laboratory test (unit)		Anticoagulant	Platelet ($x10^3/\mu L$)	WBC ($x10^3/\mu L$)	Neutrofil ($x10^3/\mu L$)	Lympho-cyte ($x10^3/\mu L$)	Mono-cyte ($x10^3/\mu L$)	Eosinophil ($x10^3/\mu L$)	Basophil ($x10^3/\mu L$)	Hb (g/dL)	RBC ($X10^6/\mu L$)	Hct (%)	MCV (fL)
October 4th	1st	EDTA	20	8.70	4.69	3.00	0.62	0.34	0.05	13.9	4.61	38.1	82.6
November 6th	2nd	EDTA	16	6.15	2.25	2.00	1.49	0.20	0.04	13.5	4.54	38.3	84.4
November 6th	2nd	Na citrate	160	NA									
November 20th	3rd	EDTA	5	7.94	3.69	3.14	0.56	0.23	0.02	13.1	4.58	38.3	83.6
November 20th	3rd	Na citrate	198	NA									

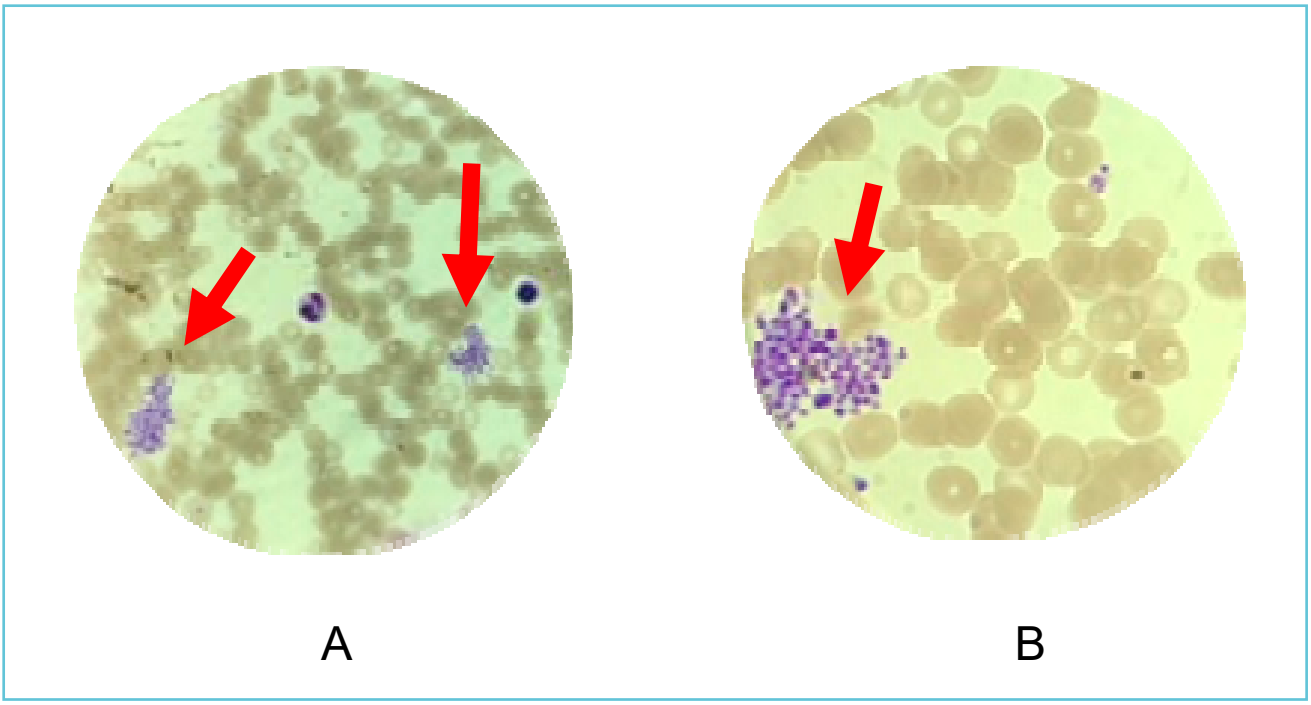
DISCUSSION

EDTA-dependent pseudothrombocytopenia is an infrequent phenomenon of *in vitro* platelet agglutination due to the presence of antiplatelet autoantibodies (13,14); its incidence has been reported to be between 0.10 and 0.29% (15). As described in our case the agglutination *in vitro* results in a decrease in platelet count, typically below $100x10^3/\mu L$ with the presence of a "platelet aggregation" alert flag from the analyser (16), the peripheral blood smear must be observed in order to confirm the presence of platelet clumps (17). In addition, a new blood sample from the same patient using sodium citrate as anticoagulant is the most suitable sample for the platelet count (18). The laboratory professional has the responsibility of detecting these false thrombocytopenias and notifying this information in the laboratory report (19). Likewise, the clinician should consider this condition in order to avoid unnecessary diagnostic tests and therapeutic interventions with the aim of preserving patient safety. Therefore, we would like to highlight the following learning points from this case report:

- The spurious low platelet count must be reported together with a commentary on the presence of platelet clumps that underestimate the real count.
- The clinical laboratory has an essential role over patient safety, but more efforts are required to prevent inappropriate clinical or therapeutic decision-making.

In conclusion, the laboratory should enhance a strong relationship with clinicians trying to avoid misunderstandings as that reflected in this case report. It should be reminded that, in those cases where a pseudothrombocytopenia by EDTA is suspected, a blood smear is mandatory to confirm platelet clumps and blood must be tested anticoagulated with other anticoagulant (i.e., sodium citrate or heparin).

Figure 1 Peripheral blood smear from blood sample collected with EDTA (May-Grünwald-Giemsa stained) observed in optical microscopy



Note: Arrows indicate platelet clumps at 400X (A) and at 1000X magnification (B).

Disclosures and contributions

Cristina Collazo Abal is the main responsible for the study conception design, intellectual content and drafting of this paper. All other authors confirmed they have contributed significantly to the analysis and interpretation of data, revising, and approva the article. Al authors dec are no conflicts of interest.

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