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Case Report | *Medicine*

Pseudothrombocytopenia due to platelet aggregates in an infant: A case report

Pseudotrombocitopenia por agregados plaquetarios en lactante menor: Un reporte de caso

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Abstract

This case report describes the finding of EDTA-dependent Pseudothrombocytopenia in an 8-month-old infant, an extremely rare condition not reported in the literature. The patient presented with a fever due to insect bite and was diagnosed with abscessed cellulitis. A complete blood count showed a thrombocytopenia of 47 mil/L, with no history of bleeding or hematologic disease. The following day a new test was performed, which showed a platelet count of 214 mil/L, which was not consistent with the expected evolution of a real thrombocytopenia. Pseudothrombocytopenia was suspected and confirmed by observing platelet aggregates in the blood smear and upon recounting in a tube with sodium citrate, showed a normal value of 298 mil/L. Treatment consists of avoiding the use of EDTA as an anticoagulant and using other anticoagulants such as citrate or heparin. Timely identification of this phenomenon is essential to avoid diagnostic confusion and unnecessary or harmful treatment. This case brings to the scientific literature an example of EDTA-dependent Pseudothrombocytopenia in a pediatric patient, which can be easily confused with other causes of thrombocytopenia and requires a high index of clinical suspicion.

Resumen

Este reporte de caso describe el hallazgo de Pseudotrombocitopenia dependiente de EDTA en un lactante menor de 8 meses, una condición extremadamente rara que no se encuentra reportada en la literatura. El paciente presentó un cuadro febril por picadura de insecto y se le diagnosticó celulitis abscedada. Al realizarle un hemograma se observó una trombocitopenia de 47 mil/L, sin antecedentes de sangrado o enfermedad hematológica. Al día siguiente se le realizó una nueva prueba, que mostró un conteo plaquetario de 214 mil/L, lo que no concordaba con la evolución esperada de una trombocitopenia real. Se sospechó de Pseudotrombocitopenia y se confirmó al observar agregados plaquetarios en el frotis sanguíneo y al realizar el recuento en un tubo con citrato de sodio, que mostró un valor normal de 298 mil/L. El tratamiento consiste en evitar el uso de EDTA como anticoagulante y utilizar otros como citrato o heparina. La identificación oportuna de este fenómeno es esencial para evitar confusiones diagnósticas y tratamientos innecesarios o perjudiciales. Este caso aporta a la literatura científica un ejemplo de Pseudotrombocitopenia dependiente de EDTA en un paciente pediátrico, que puede ser fácilmente confundida con otras causas de trombocitopenia y que requiere un alto índice de sospecha clínica.

Introduction

Thrombocytopenia is defined as a platelet count below 150,000/mL [1]. According to its mechanism of action, it can be caused by: *i*) defective production; *ii*) accelerated destruction; iii) abnormal distribution and *iii*) artificial thrombocytopenia or pseudothrombocytopenia[2].
Pseudothrombocytopenia (PTCP) is the finding of spuriously low platelet counts, first described in 1969 [3]; it is a well-known phenomenon regularly observed in the hematology laboratory, its prevalence ranges from 0.01 to 1% [4,5] and it is thought to be present in 0.1%-0.2% of the general population [6,7].

It is most commonly seen in women under 50 years of age, and there is a slight male predominance in age groups over 50 years [8]. The main causes of Pseudothrombocytopenia are: *i*) problems in sample collection and processing (inadequate shaking of tubes, dilution of samples, difficulties in peripheral blood collection); *ii*) giant platelet syndrome; *iii*) induction by anticoagulants, such as ethylenediaminetetraacetic acid (EDTA), citrate, oxalate and heparin; *iv*) autoimmune diseases; *v*) drugs; *vi*) solid tumors and *vii*) proliferative syndromes [9].

EDTA-dependent pseudothrombocytopenia, which is the anticoagulant recommended by the International Council for Standardization in Hematology (ICSH) for the performance of hematology [10], is a laboratory phenomenon due to the presence of antiplatelet antibody in the patient's serum. The antibody targets a cryptic antigen within the surface of glycoprotein IIb/IIIa that is revealed only when the glycoprotein undergoes a conformational change in the presence of EDTA. To suspect this condition, 5 criteria must be met: i) abnormal platelet count, typically < 100mil/mL; ii) thrombocytopenia with EDTA, but to a much lesser extent in samples collected with other anticoagulants; iii) decreasing platelet count over time in the EDTA sample; iv) platelet aggregates and clumping in EDTA samples; v) absence of signs or symptoms of platelet disorders. Reliable and timely identification of this phenomenon is essential, as there is a high probability that it can be confused with other disorders or lead to inappropriate decision making [11].

Studies have shown that this phenomenon is not necessarily associated with any disease, and patients do not show any clinical features of thrombocytopenia, but it may rarely be a chance finding in association with a disease or syndrome [8].

Case presentation

An 8-month-old male patient, with no significant personal or family history, was admitted to the emergency department in his mother's arms, who conducted the interview, and manifested a clinical picture of 2 days' evolution consisting of an insect bite in the areolar region of the left breast. Subsequently, the patient presented with febrile peaks of 38.5°C, associated with uncontrollable crying and hyporexia. He did not report any other associated symptoms. He was self-medicated by his mother with paracetamol syrup 150mg/5mL, 5cc orally every 6 hours.

Physical examination showed preserved vital functions, temperature with fever peak of 38.5°C, blood pressure (mmHg): 80/65, mean arterial pressure (mmHg): 70, heart rate (beats/min): 120, respiratory rate (resp/min): 30, oxygen saturation: 98%, with presence of febrile facies and dehydrated mucous membranes. On inspection of the chest in the left breast there was an insect bite in the areolar region, on superficial palpation with inflammatory signs: redness, heat, pain, induration, functional limitation and with the presence of purulent material, measuring approximately 5 x 6 cm, mobile, flexible, rounded shape with well-defined edges, with a smooth surface and purulent cystic consistency, without the presence of secretions, petechiae or rash. In lower limbs presence of multiple mosquito bites. Nervous system alert, without presence of neurological, motor or sensory focalization.

On admission, the patient underwent a complete blood count (CBC), C-reactive protein (CRP), sodium (Na), potassium (K). The paraclinical report showed a hemogram with leukocytosis 16,460, lymphocytes 58.1%, hemoglobin 11.3, hematocrit 34.6 and platelets 47,000, drawing attention to the presence of moderate thrombocytopenia. At that moment a viral condition was suspected due to the presence of febrile peaks plus thrombocytopenia and it was decided to send the patient for renal and hepatic function, coagulation time, IgM and IgG for dengue and arbovirus antigen. These last tests reported preserved renal function, liver function with slight elevation of AST, non-extended times, negative dengue IgG, negative IgM and negative arbovirus (see Table 1).

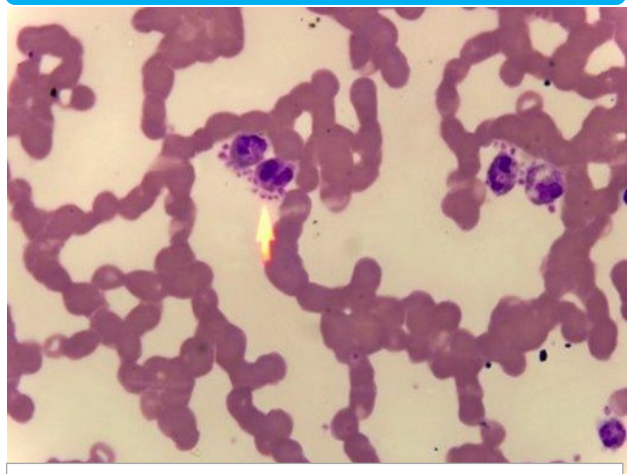
Table 1. Laboratories

Test name	Results	Reference range
Hemograma Automatizado III		
recuento de glóbulos blancos	16.46	5.00 - 10.00
recuento de glóbulos rojos	4.37	4.00 - 5.00
linfocitos (%)	58.1	17.0 - 49.0
linfocitos (#)	9.59	0.90 - 4.90
monocitos (%)	6.9	3.0 - 12.0
monocitos (#)	1.07	0.20 - 1.20
neutrófilos (%)	30.5	55.0 - 70.0
neutrófilos (#)	4.77	2.80 - 7.00
eosinófilos (%)	0.1	0.0 - 5.0
eosinófilos (#)	0.01	0.00 - 0.50
basófilos (%)	0.3	0.0 - 2.0
basófilos (#)	0.05	0.00 - 0.20
volumen corpuscular medio	72 fL	80 - 137
hemoglobina corpuscular media (mch)	23.8 pg	27.0 - 32.0
concentración de hemoglobina corpuscular media (mchc)	32 g/dl	31 - 34
volumen plaquetario medio (mpv)	-----	6.0 - 10.0
rdw cv	15.8	11.5 - 15.0
hematocrito	34.6	36.0 - 45.0
hemoglobina	11.3 g/dl	12.0 - 15.0
recuento de plaquetas	47	150 - 450
proteína c reactiva	25	
Electrolitos		
Sodio en suero	137 mmol/L	137.0-145.0
Potasio en suero	4.8 mmol/L	3.5- 5.1
Química		
deshidrogenasa láctica	392 U/L	120 - 246
.nitrógeno ureico	11.00 mg/dl	
.transaminasa glutámico-piruvica (alanino aminotransferasa)	20 U/L	0-50
.transaminasa glutamino oxalacetica (aspartato aminotransferasa)	70 U/L	14-36
.creatinina en suero u otros fluidos	0.30 mg/dl	
Coagulación		
tiempo de protrombina (pt)		
· plasma control pt	11.2 seg	
· inr	1.29	
· tiempo de protrombina (pt)	13.3 seg	9.9 - 11.8
tiempo de tromboplastina parcial (ttp)		
· plasma control ptt	27.0 seg	
· tiempo de tromboplastina parcial (ttp)	25.4 seg	24.5 - 32.8
Pruebas especiales		
Arbovirus antígeno semiautomatizado o automatizado	0.29	Negativo: <1.0 Positivo: ≥ 1.0
Inmunología		
Dengue anticuerpos ig g	Negativo	Negativo
Dengue anticuerpos ig m	Negativo	Negativo

The initial diagnosis was abscessed cellulitis and thrombocytopenia under investigation. She was admitted to the paediatric ward with the following medical management: breastmilk diet plus complementary feeding, ringer lactate 500cc to pass at a rate of 42 cc/hour, paracetamol syrup 150 mg/5 cc give 5 cc orally every 6 hours if there is fever or pain, clindamycin amp 600 mg/4 cc apply 100 mg intravenous (IV) slowly diluted every 6 hours, Apply fusidic acid cream 2% every 8 hours to the lesion, monitor in the outpatient clinic the following day: CBC, AST, ALT, electrolytes, LDH, nursing care, report any changes, monitor vital signs.

Control blood tests showed a platelet count of 214,000, which was inconsistent with the patient's clinical symptoms due to an increase of 4 times the initial value. A manual platelet count was therefore requested and 205,000 directly observed platelet aggregates were reported as shown in Figure 1, suggesting a diagnosis of sticky platelets or Pseudothrombocytopenia (see Table 2).

Figure 1. Thrombocytopenia observed on peripheral blood smear



Wright's staining shows platelet adhesion around neutrophils, an in vitro phenomenon known as platelet satelitism. This means that CD16 (FCRIIIa) is a receptor present on the surface of neutrophils that is involved in the phenomenon of platelet satelitism observed in the peripheral blood smear when EDTA is used as an anticoagulant to analyse a haemogram.

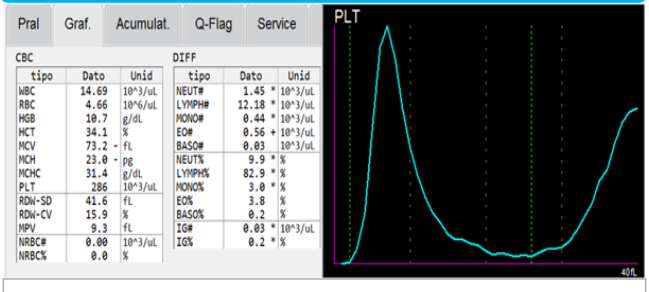
Given the variability of platelet concentrations on two consecutive days and the absence of changes in coagulation values, signs of bleeding or thrombotic events, a false thrombocytopenia was suspected. To confirm this diagnosis, laboratory samples were requested in different tubes with different reagents, sodium citrate and EDTA. Blood biometry in a sodium citrate tube showed a normal platelet count of 286,000/mm^3 and a manual platelet count of 298,000/mm^3 with a normal platelet curve. There were no platelet aggregates in the smear of this sample, the EDTA reactant reported a platelet count of 69,000/mm^3, with platelet aggregates, a small platelet

curve, both samples are compared as shown in Figures 2,3.

Table 2. Laboratories

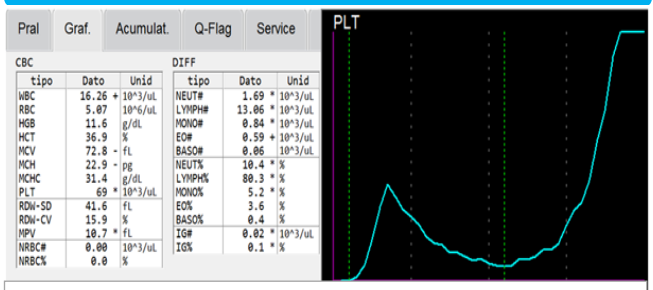
		range
Hemograma Automatizado III		
recuento de glóbulos blancos	15.34	5.00 - 10.00
recuento de glóbulos rojos	4.43	4.00 - 5.00
linfocitos (%)	64.1	17.0 - 49.0
linfocitos (#)	9.84	0.90 - 4.90
monocitos (%)	3.3	3.0 - 12.0
monocitos (#)	0.51	0.20 - 1.20
neutrófilos (%)	32.4	55.0 - 70.0
neutrófilos (#)	4.96	2.80 - 7.00
eosinófilos (%)	0.0	0.0 - 5.0
eosinófilos (#)	0.00	0.00 - 0.50
basófilos (%)	0.1	0.0 - 2.0
basófilos (#)	0.02	0.00 - 0.20
volumen corpuscular medio	71 fl	80 - 137
hemoglobina corpuscular media (mch)	23.5 pg	27.0 - 32.0
concentración de hemoglobina corpuscular media (mchc)	32 g/dl	31 - 34
volumen plaquetario medio (mpv)	9.4 fl	6.0 - 10.0
rdw cv	15.7	11.5 - 15.0
hematocrito	31.8	36.0 - 45.0
hemoglobina	10.4 g/dl	12.0 - 15.0
recuento de plaquetas	214	150 - 450
recuento de plaquetas	205 /mm3	150 - 450
se observan agregados plaquetarios		
Química		
cloro	101.00 mmol/l	
potasio en suero u otros fluidos	5.0 mmol/l	3.5 - 5.1
sodio en suero u otros fluidos	137.0 mmol/l	137.0-145.0
transaminasa glutámico-piruvica (alanino aminotransferasa)	24 u/l	0-50
transaminasa glutamino oxalacetica (aspartato aminotransferasa)	63 u/l	14-36

Figure 2. Automated blood count. A- (CITRATE)



Automated blood count is performed from a tube with sodium citrate anticoagulant, which reports a normal platelet count (286,000/mm^3). The histogram shows that there is an increase in curvature, with a subsequent decrease as the platelets increase in size. This indicates that the platelets are of adequate size (Mean platelet volume MPV normal).

Figure 3. Automated blood count. B (EDTA)



Automated blood count is performed from a tube with ethylenediaminetetraacetic acid (EDTA) anticoagulant. The histogram shows that its curvature grows to the right, indicating the presence of large platelet aggregates, suggesting an abnormal platelet count (69,000/mm^3), which is related to the increased mean platelet volume (Mean platelet volume MPV 10.7 Ft).

Subsequent biometrics were performed in sodium citrate tubes and showed platelet counts within normal ranges. Pseudothrombocytopenia induced by an immunological reaction to EDTA reagent was diagnosed and it was recommended that all samples be collected in sodium citrate tubes.

The patient received intravenous antibiotic treatment with clindamycin for 5 days with resolution of the abscessed cellulitis. Topical treatment with fusidic acid cream 2% was continued for 7 days and adequate wound healing was observed. Temperature, pain and fluid control were maintained with paracetamol and lactated Ringer's solution. The mother was educated about insect bite prevention and possible complications. Finally, the patient was discharged from paediatrics with wound care instructions and attended a haematology appointment where the diagnosis of EDTA-induced pseudothrombocytopenia (EDTA-PTCP) was confirmed.

Discussion

This is a case report of EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) in an 8-month-old infant, an extremely rare condition that has not been reported in the literature in younger infants. However, it is important to note that approximately 0.1% of the world's population has natural EDTA-dependent antiplatelet antibodies [12]. The patient was referred to the clinical laboratory because of a thrombocytopenia of 47 mil/L in a test requested after admission to the emergency department for febrile symptoms due to insect bites, with no history of bleeding, haematological disease or infection to explain the symptoms. A new test was performed the following day and showed a platelet count of 214 mil/L, which

was not consistent with the expected evolution of a true thrombocytopenia. There are no data on the incidence or prevalence of EDTA-dependent antibodies in this population. Most of the cases described in the literature are adults, especially women [13].

This case has some peculiarities that make it of interest to the scientific community. Firstly, the thrombocytopenia occurred abruptly in the initial platelet count, and no gradual decrease was observed when testing was performed. This may suggest that EDTA-induced platelet agglutination is a variable phenomenon, dependent on sample conditions, and that most cases have an unknown aetiology. It also contrasts with what was reported in the study by Morales et al. [13], where a progressive decrease in platelet count was observed in EDTA samples. In addition, despite advances in automated blood analysers, new methods still fail to detect platelet aggregation and satellitism, leading to a false decrease in platelet count [6,14,15]. It is recommended to suspect PTCP-EDTA in any discrepancy between automated platelet count and blood smear to avoid false positive thrombocytopenia and to guide appropriate treatment.

EDTA-induced pseudothrombocytopenia has a physiological mechanism that is not yet well understood, but there are studies suggesting that the autoantibodies present in plasma in the presence of EDTA recognise and bind to the epitope of glycoprotein IIb (GPIIb), forming the GPIIb/IIIa complex in platelet aggregation and promoting platelet agglutination [6,16,17]. The important thing about this problem is that these patients do not show clinical data of thrombocytopenia and no alterations in coagulation tests are found, so it is important to consider this disorder in order to avoid unnecessary therapeutic measures that could have serious adverse effects that would only complicate the patient's situation. A study has recently been published comparing patients with pseudothrombocytopenia and healthy individuals (n = 49 patients vs. n = 69 controls). Most of the patients were hospitalised (61%) and there were no significant differences between the groups in terms of the characteristics studied, except that the group of patients with pseudothrombocytopenia was more likely to use low-molecular-weight heparin [2].

The importance of microscopic examination of the blood smear is evident in any suspected PTCP-EDTA, as the presence of platelet clusters is the hallmark of this phenomenon. The diagnosis is based on the microscopic observation of platelet clusters, which may have different shapes and sizes, and which dissociate when the anticoagulant is changed or when antiaggregant agents are added [13,18,19,20].

Thirdly, treatment consists of avoiding the use of EDTA as an anticoagulant and using other anticoagulants such as citrate or heparin. Kanamycin, which has been shown to be effective in preventing IgG-mediated platelet agglutination, may also be used. The use of alternative anticoagulants to EDTA, such as sodium citrate, oxalate or heparin, is a good option to exclude EDTA-induced pseudothrombocytopenia, but there are some rare cases of pseudothrombocytopenia associated with these anticoagulants. Other procedures less commonly used in laboratory practice to confirm cases of pseudothrombocytopenia include reanalysis of the sample collected in EDTA after incubation at 37°C for about 30 minutes (the purpose of this incubation is to dissociate platelet aggregates), or addition of certain aminoglycosides such as kanamycin and amikacin to the EDTA-collected sample (these do not affect the cell count and prevent the formation of platelet aggregates, although their mechanism of action is not yet well understood) [9].

Conclusion

In the case report, the patient showed a favourable evolution of his initial symptoms without complications or sequelae. The prognosis is good, considering that PTCP-EDTA is a benign and transient condition that does not require specific treatment and does not pose a risk of bleeding or thrombosis. It has been recommended that blood samples requiring platelet count be taken using a sodium citrate tube. The patient is expected to have a normal quality of life.

EDTA-dependent pseudothrombocytopenia is a rare phenomenon but should be considered in the differential diagnosis of thrombocytopenia, especially in patients without clinical signs of bleeding or haematological disease. This condition is caused by the formation of platelet aggregates in the presence of EDTA, which interfere with automated platelet counting. To avoid this error, it is recommended to use other anticoagulants such as citrate, to add amikacin to the sample to reverse platelet agglutination, or to perform a manual count on the smear.

Early recognition of this phenomenon is essential to avoid unnecessary interventions, such as platelet transfusions or invasive tests, which can put the

patient's health at risk. It can also reduce the length of hospital stay and costs associated with the management of thrombocytopenia. This case provides an example of EDTA-dependent pseudothrombocytopenia or pseudothrombocytopenia in a young infant, which has not been previously reported in the literature, and illustrates the importance of clinical correlation and medical judgement for correct diagnosis and exclusion of true thrombocytopenia.

Abbreviations

ALT: alanine aminotransferase
AST: aspartate transaminase
CBC: complete blood count
CRP: C-reactive protein
EDTA: ethylenediaminetetraacetic acid
GPIIb: glycoprotein IIb
ICSH: International Council for Standardization in Hematology
IV: intravenous
LDH: Lactate dehydrogenase
PTCP: Pseudothrombocytopenia
MPV Mean platelet volume.

Consent for publication

The authors read and approved the final manuscript.

Competing interest

The authors declare no conflict of interest. This document only reflects their point of views and not that of the institution to which they belong.

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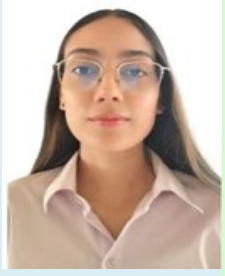

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