

EDTA-Dependent platelet clumping in a patient with hypothyroidism

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Abstract. The pseudothrombocytopenia by ethylenediaminetetraacetic acid is caused by natural antiplatelet antibodies and is responsible for thrombocytopenia when platelet count is performed by automatic devices. Although it is difficult to assess the rate of this phenomenon, it is believed that the incidence is higher in hospitalized patients, especially in those with liver and autoimmune diseases and cancer. In this article we describe a female patient with complaints of clinical hypothyroidism and repeated values of thrombocytopenia, which was diagnosed as pseudothrombocytopenia due to ethylenediaminetetraacetic acid after proper analysis.

Key words: Pseudothrombocytopenia; antibodies, platelet count, hypothyroidism

1. Introduction

Patterns of pseudothrombocytopenia (PTCP) detected by automatic instruments for the analysis and counting of blood cells have gained particular attention in recent years, as the use of these machines has become widespread in clinical laboratories. PTCP is characterized by a false reduction in the number of platelets in ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood, caused by platelet clumping at room temperature. The phenomenon is due to the presence of antiplatelet autoantibodies that recognize platelet antigens modified by or exposed (cryptoantigens) to the combined action of EDTA and low temperature on the platelet membrane glycoproteins (1,2). Failure to make this important distinction leads to unnecessary diagnostic tests, delay of surgery, and unwarranted exposure to transfusion-related complications (3).

2. Case report

A 51-year-old woman, resident in Rio de Janeiro, was admitted with signs of fatigue and thrombocytopenia for a long time. The patient complained about a progressive fatigue during the last six months and the presence of thrombocytopenia identified in her previous laboratory tests. The physical exam didn't reveal any abnormalities, except for overweight, because her body mass index was 28.3.

Laboratory analysis that included complete blood count, biochemistry, thyroid stimulating hormone (TSH) and free T4. Laboratory findings revealed TSH: 7.06 uIU/mL (normal range 1.15 to 3.5 uIU/mL), free T4: 0,82ng/mL (0,8 a 2,0 ng/100mL) and platelet count: 49.000/mm³ (normal range 150,000 to 400.000/mm³). Hypothyroidism was diagnosed, and treatment with levothyroxine was commenced. We request antibodies to Hashimoto thyroiditis were negative. For thrombocytopenia, the patient was consulted with the hematology department with the diagnosis of idiopathic thrombocytopenic purpura.

The first method proposed was the use of EDTA as an anticoagulant for complete blood count and resulted a platelet count of

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66.000/mm³ and then citrate was used as an anticoagulant and a platelet count of 157.000/mm³ was found then a diagnosis of PTCP by EDTA was made. (Figures 1 and 2)

The patient progressed well with levothyroxine treatment, she was consulted with endocrinology and the use of citrate for complete blood count was recommended.

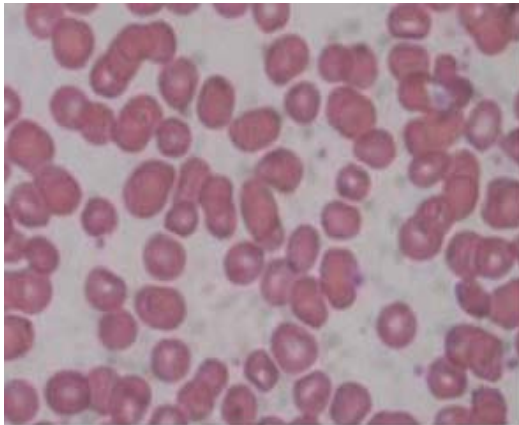


Fig. 1. Platelet clumps in EDTA-anticoagulated blood.

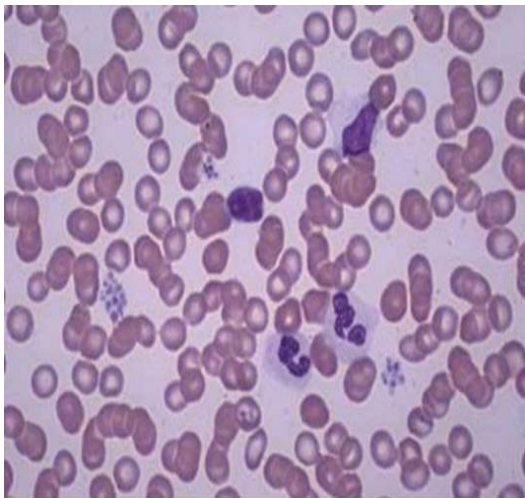


Fig. 2. Five small clumps of platelets. This phenomenon of platelet clumping in an EDTA anticoagulated blood sample is a rare occurrence but will be consistent in some patients. The platelet count will be erroneously low.

3. Discussion

The first description of EDTA-dependent PTP was carried out by Shreiner and Bell in 1973. In the following years, various papers on the same topic, consisting of either case reports or studies on the pathogenesis of this phenomenon, have been published. Moreover, two epidemiological surveys have appeared,

both analysing the North American population (4).

This case is particularly interesting because in vitro presence of platelet clumping due to EDTA-dependent autoantibodies of the IgM class. The platelet-clumping phenomenon is attributed to the presence of naturally occurring antiplatelet autoantibodies that recognize platelet antigens that are modified or exposed (cryptoantigens) by the combined action of EDTA and low temperature on the platelet membrane glycoproteins. Cold agglutinins can also be part of the natural autoantibody repertoire and may be present in small amounts in normal serum, but they mostly occur as the result of the either benign or malignant proliferation of a clone of antibody-producing cells or the immune system reaction to infection (5).

Thrombocytopenia is the most common cause of bleeding diathesis. When a patient has low platelet counts in the absence of a consistent clinical history and symptoms, one should suspect of PTCP. Although PTCP is the most common artifact, one should not forget other causes, such as the presence of giant platelets or platelet satellitism. PTCP is usually caused by the anti-coagulant EDTA (a calcium chelator). EDTA alters the conformation of platelet glycoproteins, and anti-coagulant dependent agglutinins (IgG, IgA, or IgM) bind several altered platelets, resulting in platelet aggregation (6,7).

PTCP occurs most frequently in hospitalized patients; however, there seems to be no correlation between age or sex. In addition, no consistent association has been observed between particular pathophysiologic conditions or medications. The annual incidence of moderate to severe idiosyncrasic thrombocytopenia is still unclear. European and American studies estimate an incidence of 10 cases per million patients a year, which is comparable to other cytopenias, including drug-induced neutropenias or agranulocytosis (8). EDTA-dependent PTCP occurs in approximately 0.2% of asymptomatic individuals, but the incidence may be as great as 1.9% in hospitalized patients (9). Given the widespread use of EDTA-containing vacutainers for blood collection for platelet counts, identification of PTCP requires an increased index of suspicion after the identification of thrombocytopenia in the absence of a consistent medical history and symptoms (3).

The elapsed time existing between blood withdrawal and the analysis is crucial: in some cases, clumping of the platelets occurs well after blood collection. This behaviour may affect a sure identification of EDTA-dependent platelet clumping and may compromise the intra- and especially interlaboratory reproducibility of the results. We would emphasize that in the present study the phenomenon of platelet clumping took place even many hours after blood collection: previous descriptions showed the evidence of clumping occurring 10, 15 or 30 min after collection (4).

One should use the citrate-containing tube to determine the number of platelets, and this is the easiest test to detect PTCP. As for correction of thrombocytopenia with EDTA, Sakurai et al. (10) stated that the addition of aminoglycosides (kanamycin) to the EDTA-containing tube before drawing the blood. Maintaining tubes with kanamycin is impractical and it is better to send the sample to be analyzed in a citrate containing tube when PTCP is suspected (10). Early identification of PTCP in a patient with thrombocytopenia decreases the risk of transmission of infectious diseases by preventing unnecessary transfusion of platelets.

In conclusion, unrecognized PTCP may result in unnecessary laboratory testing, bone marrow aspiration, and unwarranted transfusions. Examination of the peripheral blood smear provides definitive evidence of PTCP in the form of overt platelet clumping.

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