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Case Report

# Management of ethylenediaminetetraacetic acid and citrate-dependent pseudothrombocytopenia in the laboratory

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**Abstract**

This report describes the case of a patient with ethylenediaminetetraacetic acid- (EDTA) and citrate-dependent pseudothrombocytopenia (PTCP). An EDTA tube (BD Vacutainer K2 EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) platelet count indicated thrombocytopenia (15x109/L and 8x109/L), which was inconsistent with his clinical condition, and prompted further investigation. A repeat sample was drawn into both EDTA tubes and tubes containing 3.2% sodium citrate 9NC coagulation sodium citrate 3.2%, 3.5 mL, Vacuette®, (Greiner Bio-One International GmbH, Kremsmunster, Austria) and immediately measured in the laboratory. The platelet count was 157x109/L and 171x109/L in the EDTA and citrated samples, respectively. Simultaneous peripheral blood smear examinations were performed with capillary, EDTA, and citrated samples. Platelet clumps were observed only in the EDTA sample. The tubes were kept at 25°C and measurements were repeated at 10, 15, 60, 90, and 120 minutes. The platelet counts had decreased by 63% and 76% at the end of 120 minutes in the EDTA and citrated samples, respectively. After 20 minutes at 37°C, the number of platelets had increased by 76% and 87% in the EDTA and citrated samples, respectively. In cases of this kind of a contradiction between laboratory results and clinical status, laboratory specialists should suspect PTCP and be prepared to manage these findings. Close communication between the clinician and the laboratory helps to avoid unnecessary investigation and inappropriate treatment.

**Keywords:** Blood platelet count, EDTA-dependent pseudothrombocytopenia, platelet aggregation, pseudothrombocytopenia, thrombocytopenia

Pseudothrombocytopenia (PTCP) is a common laboratory artifact defined as a falsely low platelet count resulting from platelet aggregation. It is important to recognize these circumstances in the laboratory to prevent unnecessary investigation, false diagnosis, and unwarranted therapy [1]. Platelet satellitosis cold agglutinins, giant platelets, ethylenediaminetetraacetic acid (EDTA)-induced platelet clumping, or pre-analytical factors, like an incorrect amount of anticoagulant or the techniques used for the blood withdrawal, may cause PTCP [2]. EDTA-dependent PTCP is caused by the presence of EDTA-dependent antiplatelet antibodies, which react most between 0°C and 4°C, recognize the glycoprotein (GP) IIb-IIIa receptors, stimulate the expression of activation antigens, and generate activation of tyrosine kinase, platelet agglutination, and clumping *in vitro* [2]. Although these antibodies generally belong to the immunoglobulin (Ig) M class, IgG and IgA examples have also been observed. While most agglutinins are seen at room temperature or below, the cold agglutinins, some reactions occur independently of temperature and may be most strongly seen at 37°C [3]. The large size of platelet aggregates may mean that they not be recognized as platelets by automated hematology analyzers, leading to low platelet counts. In some cases, aggregates have even been counted as leukocytes by the analyzer and prompt results of pseudo-leukocytosis [4]. In the case of cold agglutinins, heating the blood samples to 37°C can help to achieve an accurate

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platelet count. However, in 20% of reported cases, aggregation continues despite heating [2]. This case report describes a patient with both EDTA- and citrate-dependent PTCP.

## Case Report

A 41-year-old male patient with nasopharyngeal cancer was referred to the laboratory by the hematology department with a low platelet count inconsistent with his clinical status. He had been treated with both radiotherapy and concomitant cisplatin chemotherapy for 3 months. He was hospitalized due to feeding difficulties and esophagitis and was provided with hydration and peripheral nutrition support. During hospitalization, his routine biochemical parameters, including a complete blood count (CBC) were evaluated. Although his platelet count had been within the reference range (150-450×$10^9$/L) in the previous days (≈210×$10^9$/L), on the 25$^{th}$ day, his platelet count was determined to be 15×$10^9$/L (BD Vacutainer K2 EDTA tube, no: 363048; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with a Beckman Coulter LH 780 analyzer (Beckman Coulter Inc., Brea, CA, USA). The platelets were measured using impedance technology. Laboratory specialists realized that the very low platelet count was inconsistent with that of the previous days and contacted the physician to obtain clinical information. The patient had no easy bruising, hematuria, melena, petechia, or purpura symptoms. He had been taking antifungal drugs for 8 days for esophagitis. The laboratory requested a new EDTA sample to exclude platelet clumping due to improper blood collection procedure. The reported platelet count of the new sample was 8×$10^9$/L and the analyzer flag noted platelet clumps. Since the patient didn't show any clinical symptoms of thrombocytopenia, PTCP was suspected. The laboratory requested 2 new samples from the patient using EDTA and citrated tubes (3.2% sodium citrate, Vacuette tube 3.5 mL 9NC; Greiner Bio-One International GmbH, Kremsmünster, Austria) and performed a CBC analysis. Peripheral blood smear (PBS) examinations were performed simultaneously with a capillary blood sample and the EDTA and citrated samples. The samples were kept at 25°C in the laboratory, and repeated measurements were performed at 10, 15, 60, 90, and 120 minutes. At the end of 120 minutes, both samples were stored at 37°C for 20 minutes and the platelet count was measured again. Repeat PBS examinations were performed at the third hour using samples kept at 25°C.

The platelet count was 157×$10^9$/L and 171×$10^9$/L in the EDTA and citrated samples, respectively, analyzed at the fifth minute. There was a decrease in the platelet count over time of 63% and 76% at 120 minutes in the EDTA and citrated samples, respectively, at 25°C (Fig. 1). When the samples were kept at 37°C for 20 minutes and measured again, the platelet count increased by 76% and 87% in the EDTA and citrated samples, respectively, but they never reached the initial level. The analyzer produced a flag of platelet clumps for all of the EDTA and citrated sample measurements except the citrated sample analyzed at the fifth minute. PBS analysis had initially revealed platelet aggregates in the EDTA sample but not in the capillary and citrated samples. After 3 hours, aggregates were observed in both the EDTA and citrated samples. PBS examinations performed at the fifth minute and the third hour are shown in Figure 2.

## Discussion

It is important to differentiate real thrombocytopenia from PTCP to avoid redundant diagnostic tests as well as therapeutic errors, such as a splenectomy, corticosteroid therapy, or platelet transfusion [1]. Platelet clumping due to EDTA is the most frequent reason for PTCP and has been observed in 0.1% to 2% of hospitalized patients [5], and in 15% to 17% of outpatients assessed for thrombocytopenia [6]. The following basic criteria should be used to diagnose EDTA-dependent PTCP: a low platelet count (<100×$10^9$/L), the existence of thrombocytopenia in only an EDTA-anticoagulated sample and at room temperature and less with alternative anticoagulated samples like citrated or calcium chloride/heparin and in samples maintained at 37°C, a time-dependent decrease in platelet count from 1 minute to 4 hours later, a PBS or automated cell counting device result of platelet clumps, and no clinical signs or symptoms of a platelet disorder [2]. Although EDTA-dependent PTCP is the most common, multi-anticoagulant-dependent PTCP has also been described in the literature [2]. About 20% of cases with EDTA-dependent PTCP show the phenomenon in citrate anticoagulant as well [7]. In a case reported by Kovacs et al., [8] 5 different anticoagulants caused PTCP; only magnesium sulfate didn't cause aggregation and permitted an accurate thrombocyte count measurement. According to the literature, magnesium sulfate is accepted as the most suitable anticoagulant to avoid spontaneous platelet clumping [8]. Laboratories should be able to recognize PTCP and be familiar with the methods to address it. When platelet clumping is observed, early blood collection methods should be checked and errors due to collection methods should be excluded [9]. Hematology analyzer histograms should always be examined for platelet clump flags and a peripheral smear should be examined for verification of the platelet count, shape, and morphology [9]. A PBS evaluation is accepted as the gold standard for the detection of PTCP [10]. If platelet clumping is observed, a new sample should be requested with different anticoagulants (citrate, heparin, etc.) and analyzed immediately. In case of delay, maintaining samples at 37°C during transfer and until analysis can prevent temperature-dependent aggregation. Platelet clumping may not entirely be avoided by these procedures in some cases and additional procedures may be required [11]. In our patient, the platelet counts of the second collection samples were within the reference range at the fifth minute (higher in the citrated tube sample). After storage at 25°C, the platelet counts decreased gradually until 120

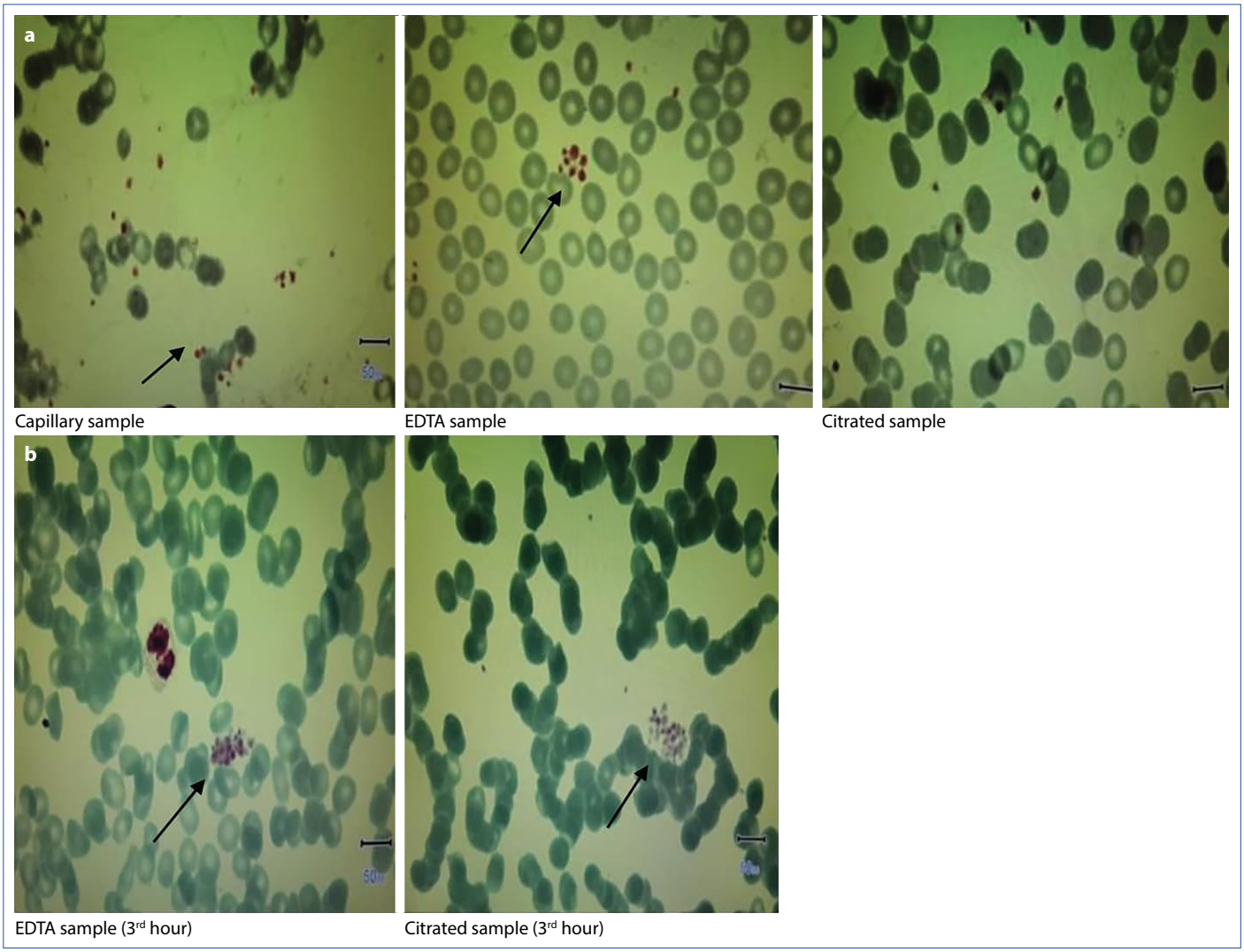


**Figure 2.** Peripheral blood smears performed at the fifth minute and at the third hour. (a) Peripheral blood smears performed at 5th minute with capillary, edta and citrated samples. (b) Peripheral blood smears performed at 3rd hour with EDTA and Citrated samples.

EDTA: Ethylenediaminetetraacetic acid.

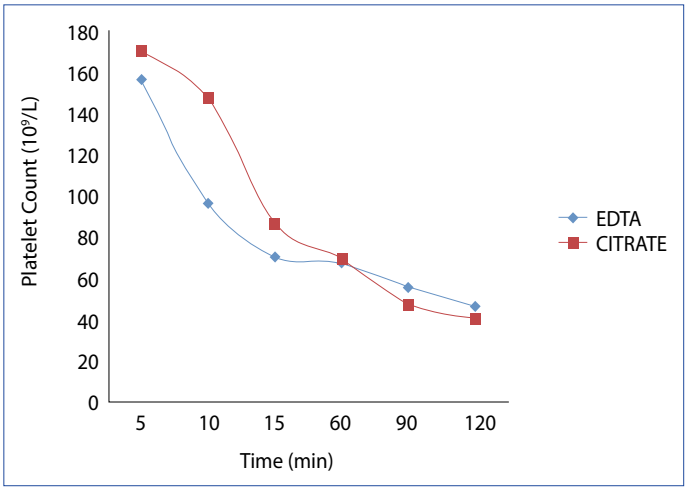


**Figure 1.** Area Platelet count of ethylenediaminetetraacetic acid (EDTA) and citrated samples over time.

minutes. At the 15th minute, which is generally a minimum length of time for a sample to be delivered to the laboratory, the platelet count was less than $100\times10^9$/L in both samples. Similar low platelet counts were obtained in follow-up for 3 days, and the patient was then discharged from the hospital. This case was not defined as solely EDTA-dependent PTCP as citrate also led to clumping over time and according to temperature beginning at 10 minutes. Thus, requesting another sample with a citrated tube would not be of help if the blood draw is not analyzed immediately. In our study, keeping the samples at 37°C for 20 minutes revealed a partial increase in the platelet count of both samples, but they never reached the initial level. Therefore, heating the blood does not necessarily yield an accurate platelet count. A marked increase in platelet count can be observed by heating the serum in cases of PTCP due to platelet cold agglutinins. It is a rare condition with anticoagulant-independent platelet agglutination that

usually occurs at 4°C and is mediated by IgM autoantibodies directed against GP IIb/IIIa [12]. Cold agglutinins usually interfere with other laboratory parameters as well, especially in a CBC [13]. For a definitive diagnosis, cold agglutinin titers or flow cytometric analysis of the Ig class should be measured [12]. In our patient, there was no pathology observed in the white blood or red blood cell counts, but cold agglutinin titers were not measured, nor was flow cytometric analysis performed. Nonetheless, this report is noteworthy, since time- and temperature-dependent aggregation was observed not only in the EDTA sample, but the citrated sample as well. PTCP can be seen with both types of anticoagulants. A platelet count measured in the first several minutes after blood is drawn may not be accurate since there may not have been enough time for anticoagulant-dependent platelet aggregation to occur. Also, it is important to notice that heating the blood does not help to determine an accurate platelet count. The principal limitation of this study was that we didn't draw a new sample with other anticoagulants, such as lithium heparin, disodium oxalate, or hirudin, to see whether the phenomenon was present there as well.

## Conclusion

In the event that laboratory results and clinical status are not consistent, laboratory specialists should suspect PTCP and be prepared to manage such findings. Close communication between the clinician and the laboratory helps to avoid unnecessary investigations and inappropriate treatments.

**Informed Consent:** Informed consent was obtained from the patient.

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