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## CASE REPORT

**EDTA-dependent pseudothrombocytopenia**

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Platelet clumping

**Summary** Pseudothrombocytopenia (PTCP) is an *in vitro* phenomenon of a spuriously reported low platelet count, typically caused by EDTA-dependent platelet aggregation. PTCP has no clinical significance, but misdiagnosis may lead to unnecessary diagnostic tests and treatment. In this study, two cases of EDTA-dependent PTCP are discussed to emphasize the importance of differentiating PTCP from true thrombocytopenia. The first case is of a 77-year-old woman with a femoral neck fracture, and the second case is of a 66-year-old woman with a frozen shoulder. Both cases showed low platelet counts in their routine blood tests and were ultimately proven to have PTCP. When identifying a patient with a low platelet count without any hematology disease, family history, and bleeding tendency manifestation, PTCP should also be considered. Re-examining blood samples with tubes containing other anticoagulants and a peripheral blood smear examination can help identify PTCP.

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**1. Introduction**

Pseudothrombocytopenia (PTCP) is an *in vitro* phenomenon of platelet aggregation that results in spurious reporting of a low platelet count by automatic cell counters, which are typically EDTA dependent.<sup>1</sup> In general, EDTA, a calcium chelator, is considered a safe and reliable anticoagulant for a complete blood count test because of its stability in blood cell counting and sizing. However, platelet clumping occurs occasionally. People with malignancy, chronic liver disease, infection, pregnancy, autoimmune diseases, and

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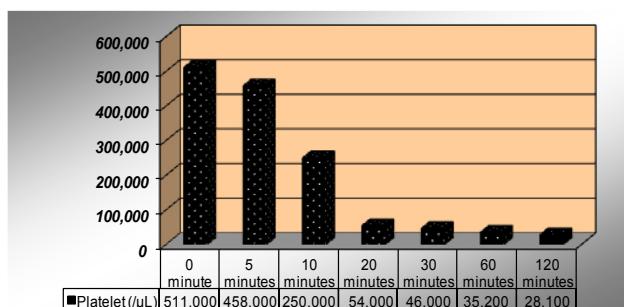
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cardiovascular diseases have an increased risk of EDTA-dependent PTCP. It has also been observed in disease-free patients.<sup>2,3</sup> Platelet clumping in the presence of EDTA is caused by an autoantibody against glycoprotein IIb/IIIa located on the cell membrane of platelets.<sup>4,5</sup> Although other anticoagulants such as heparin and sodium citrate rarely induce such a phenomena, it is possible.<sup>5,6</sup> Misdiagnosis of PTCP leads to unnecessary diagnostic tests and treatments, such as bone marrow biopsy, holding surgery, splenectomy, steroid therapy, and platelet transfusion.<sup>7</sup> Therefore, when a low platelet count is noted, PTCP must also be considered. In this study, we report two cases of EDTA-dependent PTCP.

## 2. Case reports

### 2.1. Case 1

A 77-year-old female patient with no personal or family history of bleeding was admitted with a left femoral neck fracture. A preoperative evaluation of complete blood count revealed thrombocytopenia (5700 platelets/ $\mu$ L). Other blood parameters were normal. The patient denied melena, hematuria, or easy bruising, and had no history of drug use or recent infection. On physical examination, no petechia, purpura, or lymphadenopathy was noted.

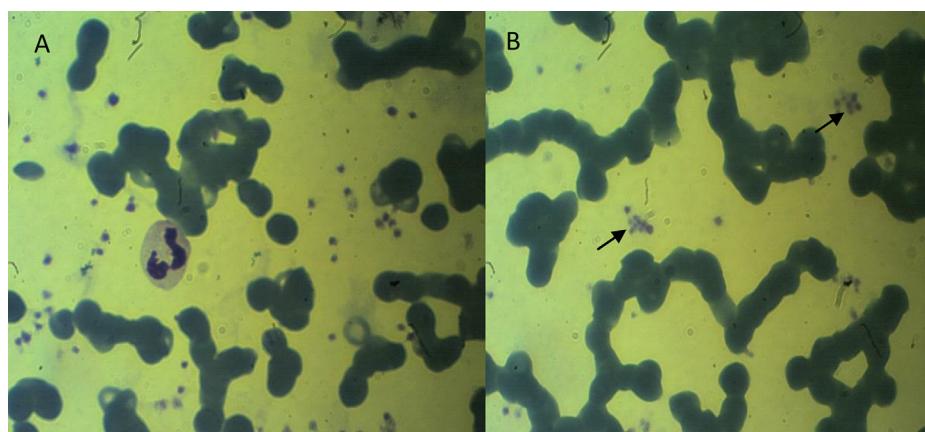


**Figure 1** Platelet count of the blood sample collected with EDTA decreased over time.

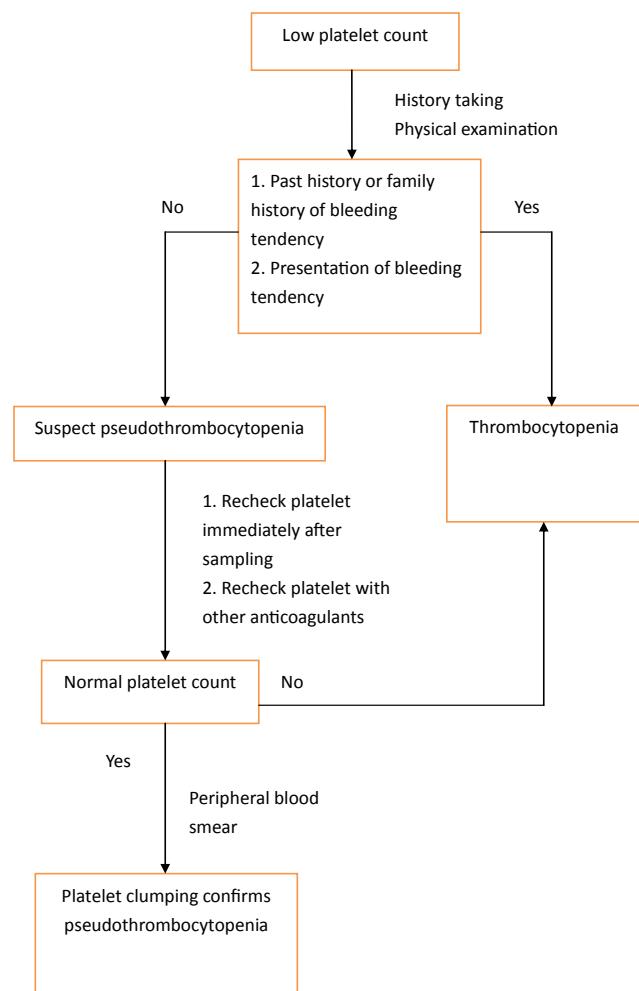
Because of the low platelet count, she received a blood transfusion with platelets (24 units). The platelet count was elevated to 126,000/ $\mu$ L. The surgery for dynamic hip screw went smoothly without any bleeding complications. Two months later, she was admitted again for a total hip replacement because of an implant failure. The platelet count varied during hospitalization as follows: 26,100/ $\mu$ L, 44,600/ $\mu$ L, 14,600/ $\mu$ L, 305,000/ $\mu$ L, and 69,100/ $\mu$ L. Presence of autoantibodies C3 and C4 were negative. Autoimmune diseases were excluded. No splenomegaly on abdominal sonography was noted. The dramatic change in the platelet count prompted suspicion of PTCP caused by EDTA or an error in the autoanalyzer. Two blood samples were collected again with two different anticoagulants, EDTA and heparin, respectively, for further study. The platelet count of the blood sample collected with EDTA decreased over time (Fig. 1). A peripheral blood smear examined immediately after blood collection showed no platelet clumping (Fig. 2A), while the platelet clumped 5 minutes after withdrawal (Fig. 2B). The platelet count of the blood sample collected with heparin was still within the normal range 120 minutes after blood collection. Based on these results, a final diagnosis of EDTA-dependent PTCP was made. The total hip replacement was completed with no bleeding complications.

### 2.2. Case 2

A 66-year-old female patient with a frozen shoulder was admitted to hospital for a manipulation. Her platelet count was 49,500/ $\mu$ L and her prothrombin time, activated partial thromboplastin time, and bleeding time were all within normal ranges. She had no hematologic personal or family history. A physical examination revealed no signs of bleeding tendency. According to her medical record, a complete blood count test taken 3 months previously did not show thrombocytopenia. Based on prior experience of such a case, EDTA-dependent PTCP was considered, and was confirmed when a peripheral blood smear examination showed platelet clumping. Hence, an unnecessary blood transfusion was avoided in this case.



**Figure 2** (A) A peripheral blood smear examined immediately after blood collection showed no platelet clumping. The platelet count was 511,000/ $\mu$ L; (B) 5 minutes later, the platelets had clumped together (arrows). The platelet count reported by the automatic cell counter was 458,000/ $\mu$ L.



**Figure 3** The algorithm for identifying pseudothrombocytopenia.

### 3. Discussion

Thrombocytopenia is caused by increased destruction of platelets (hemolytic uremic syndrome, immune thrombocytopenia, and disseminated intravascular coagulation), decreased production of platelets (leukemia, sepsis, human immunodeficiency virus, and decreased production of thrombopoietin), hemodilution, or the use of certain drugs (valproic acid, methotrexate, pantoprazole, and heparin).<sup>8</sup> PTCP caused by platelet aggregation in blood containing EDTA should also be considered when a low platelet count is noted. Since 1973, EDTA-dependent PTCP has been widely reported.<sup>1</sup> The prevalence rate of EDTA-dependent PTCP is approximately 0.1–2% in hospitalized patients.<sup>2,8</sup> The mechanism involves the binding of an antiplatelet autoantibody to the glycoprotein located on the cell membrane of platelets. A combined action of the chelating effect of EDTA on calcium ions and low temperature affects the platelet membrane glycoprotein complex IIb/IIIa and reveals the epitope of glycoprotein IIb, which normally remains hidden in the glycoprotein complex IIb/IIIa.<sup>4</sup> When the autoantibody binds to the epitope of glycoprotein IIb, platelet aggregation occurs.<sup>4,5</sup> The primary antibodies

involved in this mechanism are immunoglobulin (Ig)G, IgM, and IgA, and combinations of IgG and IgM, or IgA and IgG are also observed.<sup>7</sup> Most of the agglutinins react strongly at room temperature or low temperatures, but some agglutinins are temperature-independent or react most strongly at 37°C.<sup>5</sup>

Methods employed for distinguishing true thrombocytopenia from PTCP include using other anticoagulants (sodium citrate, oxalate, and heparin), elevating the temperature of a blood sample to 37°C, examining a blood sample containing EDTA as soon as possible, examining a blood smear under a microscope, and adding kanamycin or amikacin to a blood sample collected with EDTA.<sup>6</sup> Automated platelet clump count is also available for screening EDTA-dependent PTCP.<sup>9</sup> Misclassification of PTCP leads to unnecessary diagnostic tests and treatment. In Case 1, we conducted an unnecessary platelet transfusion and delayed surgery on the patient, which could have led to complications after the transfusion, making the patient anxious. Additional aggressive treatments, such as splenectomy, steroid therapy, and bone marrow biopsy have also been reported.<sup>7</sup>

When a patient with a low platelet count but without any hematologic disease, family history, and bleeding tendency manifestation is identified, PTCP should also be considered. When EDTA-dependent PTCP is suspected, a blood sample of the patient with tubes containing other anticoagulants can be re-evaluated. Later on, the simple, inexpensive, and diagnostic method of peripheral blood smear examination can be employed to confirm platelet clumping (Fig. 3).

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