Pseudothrombocytopenia with multiple anticoagulant sample collection tubes

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Abstract: The knowledge of pseudothrombocytopenia (PTCP) is important for the accuracy of a clinical assessment and for avoiding unnecessary treatment. An elderly patient was hospitalized with left lung pneumonia. Severe thrombocytopenia [platelet (PLT) number: $18 \times 10^9/L$] without any clinical bleeding was found in ethylenediaminetetraacetic acid blood collection tube. PLT measurement was repeated in various anticoagulant [sodium citrate, lithium heparin, disodium oxalate, hirudin, and magnesium sulfate (Mg-sulfate)] sample collection tubes and all of them showed thrombocytopenia except with Mg-sulfate. To the best of our knowledge, PTCP with five anticoagulant sample collection tubes has not been reported earlier.

Keywords: pseudothrombocytopenia, platelet aggregation, sample collection, blood smear, magnesium sulfate

Introduction

Platelets (PLTs) play an essential role in preserving vessel wall integrity. Decreased PLT number (PLT count below the 2.5th lower percentile of the normal PLT count distribution) may cause defect of primary hemostasis and bleeding. As a result of incorrect measurements pseudothrombocytopenia (PTCP), without clinical significance may cause diagnostic failure. In vitro PLT clumping leads to spuriously low PLT counts by automatic hematology analyzers. The mechanism is not clearly defined. It is supposed that an immunologically mediated phenomenon due to the presence of anticoagulant-dependent anti-PLT autoantibodies induces PLT clumping. The knowledge of PTCP is important for the accuracy of a clinical assessment and for avoiding unnecessary treatment [1]. We present a case with multianticoagulant-dependent PTCP and introduce a diagnostic measurement which may help to avoid this problem.

Case

An elderly patient with metabolic syndrome was hospitalized due to fever, shortness of breath, and chest pain. The patient was diagnosed with left bacterial pneumonia. Severe thrombocytopenia (PLT number: $18 \times 10^9/L$; reference range: $150–450 \times 10^9/L$) without any clinical bleeding was found in laboratory studies. Ethylenediaminetetraacetic acid (EDTA)-induced PTCP was assumed; therefore, laboratory investigation has been conducted. Samples were taken into blood collection tubes containing different anticoagulants. Sodium citrate 3.2% (Na-citrate), lithium heparin (Li-heparin), disodium oxalate 0.1 M (Na-oxalate), Vacuette (Greiner-Bio One GmbH, Frickenhausen, Germany), and hirudin Roche (Roche Diagnostics, Basel, Switzerland) tubes were used. The measurements were performed with Cell Dyn 3700 analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). In addition, blood smears were made from each anticoagulated samples to demonstrate the aggregation. Cold agglutinin and anti-PLT antibody tests were performed in the immunological laboratory of Medical University of Debrecen, Hungary. Results of the measurements with different anticoagulants are shown in Fig. 1 (straight lines). Values of Na-citrate and Na-oxalate blood collection tubes were corrected for the 10% dilution. PLT number decreased rapidly in three types of blood collection tubes containing EDTA, Na-citrate, and Na-oxalate after blood sampling. The decrease of PLT number was slower in two other types of blood collection tubes (Li-heparin, Mg-sulfate).
hirudin). Blood smears coming from five different blood collection tubes showed PLT aggregation (Fig. 2A1, B1, C1, D1, and E1). Antibiotics were administered and the patient got well. The measurements were repeated with the same anticoagulants 1 year later in order to see whether the phenomenon is persistent. The examinations were supplemented with magnesium sulfate (Mg-sulfate) anticoagulant containing blood collection tube S-Monovette ThromboExact (Sarstedt AG & Co., Nümbrecht, Germany). According to the literature data, magnesium sulfate is obviously suitable to effectively avoid spontaneous in vitro PLT aggregation – or in other terms – PTCP. The results of the repeated measurements are shown in Fig. 1 (dashed lines). Decreased PLT number was observed in collecting tubes aside from collecting tube containing Mg-sulfate. Blood smears come from six blood collection tubes showed PLT aggregation except collecting tube containing Mg-sulfate (Fig. 2A2, B2, C2, D2, E2, and F). Cold agglutinins were not found, but anti-PLT antibodies were detected in samples with qualitative test.

**Discussion**

Anticoagulant-dependent PTCP is an in vitro phenomenon characterized by spuriously low PLT counts caused by the presence of anti-PLT antibodies, which cause PLT clumping in blood samples collected into tubes containing anticoagulant. The combined action of the chelating effect of anticoagulant (EDTA, citrate, or oxalate) on calcium ions and the low temperature affects the PLT membrane glycoprotein complex IIb/IIIa, which reveals the epitope of glycoprotein IIb that is normally hidden in the glycoprotein complex IIb/IIIa. If anti-PLT autoantibodies are present against these epitopes, autoantibodies

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**Fig. 1.** Change in PLT counts according to time

**Fig. 2.** PLT agglutination in blood smears (A: EDTA, B: Na-citrate, C: Li-heparin, D: Na-oxalate, E: hirudin, F: Mg-sulfate; arrows: PLTs)
Binding to the epitopes can cause aggregation of PLTs [2, 3]. In case of heparin, PTCP is caused by PLT, endothelial, and monocyte-activating antibodies that target multimolecular complexes of PLT factor 4 and heparin. These anti-PLT autoantibodies belong to the IgG, IgM, or IgA class of immunoglobulins. These antibodies caused by several reasons may exist transiently or permanently [4, 5]. Cold agglutinins can also cause PTCP [6]. PTCP was described in connection with diseases other than of hematological origin (viral infections, neoplastic diseases, and autoimmune diseases) and drug abuse [4, 7–9]. It may appear in a newborn of mother with hirudin-dependent PTCP [10]. This phenomenon if unrecognized leads to incorrect diagnosis, inappropriate treatment, and debilitating iatrogenic disease [11, 12]. EDTA-dependent PTCP is the most common but two (EDTA and citrate), three (EDTA, citrate, and heparin), and even four (EDTA, citrate, heparin, and sodium fluoride) anticoagulant-dependent PTCPs were also described in the literature [2, 8, 9]. So far, only one study reported a hirudin-dependent PTCP [13]. Automated blood counting devices count PLTs more quickly and more accurately than the manual methods. Cell Dyn 3700 analyzer recognizes PLT based on its size. Therefore, the larger aggregates cannot be identified as PLT. Thus, it may lead to falsely low PLT number result. PLT clumping was observed with five different anticoagulants in our patient and falsely low PLT number was measured by analyzer. Mg-sulfate did not cause aggregation, so it is the best anticoagulant to use in these cases [14]. In the case of patient described in this paper, the phenomenon was proved to be permanent and probably was triggered by pneumonia. Multianticoagulant-dependent PTCP theoretically may cause problems in patients in three different circumstances: (1) if the patient gets heparin or recombinant hirudin analog treatment; (2) if hirudin gets into the patient’s blood by leech bite; and (3) if the patient needs PLT aggregometry but it is not feasible because citrate or hirudin anticoagulated blood is required for measuring devices. To the best of our knowledge, multianticoagulant-dependent PTCP caused by five anticoagulants has not been reported earlier.

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