EDTA-Induced Pseudothrombocytopenia

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Physicians whose practices are affected by the Clinical Laboratory Improvement Act (CLIA) are decreasing in-house hematologic evaluations.[1] One unexpected consequence of this trend is the confusion caused by ethylenediaminetetraacetic acid (EDTA)-induced pseudothrombocytopenia (EDTA-PTCP). This in vitro clumping of platelets in the presence of EDTA results in a spuriously low platelet count,[2] with the platelet count inversely related to the length of time elapsed since the sample was drawn.[3] Immediate on-site manual or electronic platelet counts circumvent this problem,[4] but delayed off-site laboratory services can be plagued by EDTA-PTCP. The ensuing wild goose chase adds needless evaluations,[5-7] canceled surgical procedures,[8] avoidance of conduction anesthesia,[9] needless splenectomy, and undue patient expense and anxiety. One needs to differentiate between EDTA-PTCP and true thrombocytopenia. Practicing clinicians need to be aware of this EDTA-related anomaly when considering a differential diagnosis.

Case Report

The patient was a subject in a postmarketing study of finasteride (Proscar) conducted at the University of Wyoming Family Practice Residency at Casper. Samples for his initial hematologic and chemistry studies were collected and transported to the designated off-site study laboratory using the regular office collection protocol. On the following day the study laboratory telephoned a platelet count of 23,000/µL to our clinic. This report triggered a series of events that produced considerable anxiety for our patient and his family.

The patient's known medical history was re-viewed. His medical diagnosis included benign prostatic hypertrophy and ulcerative colitis. Sulfasalizine (Azulfidine) was his only current medication. His physical examination report was also reviewed. The patient had no sign or symptom of true thrombocytopenia.

The patient consented to follow-up studies. Complete blood counts and platelet counts were performed at the University of Wyoming Family Practice Clinic laboratory and at the study laboratory. The University of Wyoming Family Practice Clinic platelet count performed 7 minutes after collection was 190,000/µL. The study laboratory reported a follow-up platelet count of 20,000/µL. Suspecting pseudothrombocytopenia, the University of Wyoming Family Practice Clinic medical technologists performed serial platelet counts on the patient's blood that was collected in an EDTA tube. These determinations confirmed the diagnosis of EDTA-PTCP.[3] The patient's platelet counts declined serially with time (Figure 1).

Discussion

Vacuum tube specimens anticoagulated by EDTA produce the most common cause of pseudothrombocytopenia.[10] In a general population study including 33,623 subjects, the incidence of EDTA-PTCP was found to be approximately 1 in 1000.[11] In a retrospective study of patients
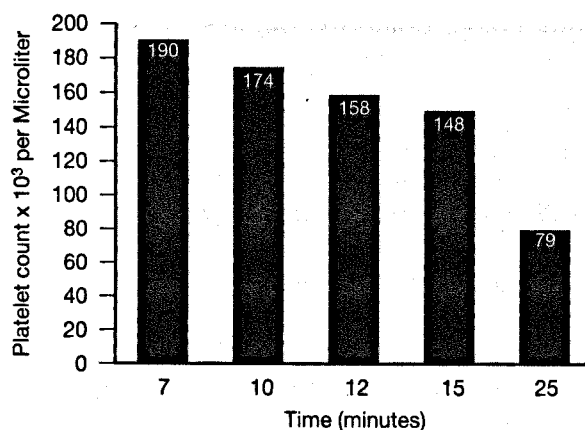
Figure 1. Plot of platelet count versus time in EDTA-induced pseudothrombocytopenia: time vs platelet count.

thought to have true thrombocytopenia, 15.3 percent of the patients were thought to have EDTA-PTCP. Cases of EDTA-PTCP have also been reported in the veterinary literature.[12,13]

When EDTA-PTCP is suspected, the diagnosis can be confirmed by finding normal platelet counts immediately after drawing the blood specimen. Serial platelet counts should then be performed on the EDTA-anticoagulated specimen. If the platelet count falls in a linear manner with time, the diagnosis is confirmed.[3]

Pseudothrombocytopenia usually has a normal mean platelet volume, whereas true thrombocytopenia has a decrease in mean platelet volume.[14] Although not a universal finding,[7] a shoulder on the white cell histogram generated by electronic cell counters is another clue that platelet agglutination is occurring.[15] Manual counts on a fresh blood smear will be normal.[16] EDTA can also induce platelet-granulocyte agglutination, which results in a complete blood count with apparent thrombocytopenia and leukopenia. The apparent low granulocyte count can be called a pseudoneutropenia, and the relative increase in lymphocytes results in a pseudolymphocytosis.[17]

The exact cause of pseudothrombocytopenia is probably a heterogeneous group of conditions.[18,19] There appears to be both a temperature-dependent and a temperature-independent mechanism.[7] Antibodies of the immunoglobulin (Ig) G, IgM, and IgA classes have all been implicated in causing EDTA-PTCP.[7,18,20] A variety of medical conditions can be associated with the development of EDTA-PTCP, including infections with human immunodeficiency virus,[21] rubella,[22] and cytomegalovirus;[23] autoimmune disorders; neoplastic diseases;[14] thrombotic disorders;[24] and perhaps trauma.[5] Pseudothrombocytopenia developing during the course of a hospitalization has been reported and can lead to errors in management.[5,8,14] The agglutinins that cause platelet clumping apparently cross the placenta. A mother with EDTA-PTCP gave birth to an infant who also transiently displayed the phenomenon.[25] The yield of platelets from plateletpheresis donors with EDTA-PTCP is not affected by this condition, and the platelet agglutination is not passively transferred to the recipients.[2]

Anticardiolipin antibodies have been found in 63.6 percent of 88 patients with EDTA-PTCP.[26] The suggestion that EDTA-PTCP can be used as a surrogate marker for thrombotic risks deserves more study.[24]

It is open to speculation how many drugs that have thrombocytopenia as a stated complication really represent EDTA-PTCP. Had the EDTA-PTCP not been recognized in our index case, this case would have been reported as a drug-induced thrombocytopenia.

The inverse of the course of EDTA-PTCP, which has a decreasing platelet count with time, has been found to occur when evacuated specimen tubes are overfilled. This latter condition has increasing platelet counts with time and is apparently due to inadequate mixing in the tube.[27] A bubble is needed in the tube to allow mixing on the oscillating mixer.

The principal intent of this report is to inform the practicing clinician of a potential laboratory collection problem that can lead to a factitious diagnosis of thrombocytopenia. Knowing about this possibility, the physician can save a patient from undue worry and expense. Pseudothrombocytopenia is a laboratory problem that can be simply evaluated in the average office laboratory.

In-office laboratories have the greatest potential for minimizing the incidence of pseudothrombocytopenia. The timely processing of the complete blood count from samples collected in EDTA containers is the simplest solution to this problem and can be complemented by a microscopic examination of a fresh blood smear. When the timely evaluation of hematologic laboratory tests is unavailable, anticoagulation with sodium citrate[28] or acid citrate dextrose[15] can be a valuable alternative. Kanamycin or colistin can also be added to blood anticoagulated with EDTA to reverse the EDTA-induced platelet agglutination, and the platelet count will revert to its true value.[29] Pretreatment of blood samples known to have EDTA-PTCP with the platelet inhibitors aspirin, prostaglandin E, and apyrase will also block the phenomenon.[18]

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