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CASE REPORT

EDTA-induced pseudothrombocytosis and citrate-induced platelet agglutination in a patient with Waldenstrom macroglobulinemia

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Key Clinical Message

Hematology analyzers sometimes generate spurious results. A patient had EDTA-induced pseudothrombocytosis and platelet agglutination in citrate blood samples. This case verifies that addition of 1% paraformaldehyde to the citrate tubes can prevent platelet clumping. Further, it illustrates the advantages of having access to more than one platelet count method.

Keywords

Agglutination, platelet, pseudothrombocytosis, Waldenstrom macroglobulinemia.

Introduction

Today hematology analyzers automatically perform platelet counts. These analyzers are advantageous as they deliver quick and accurate results in both normal and abnormal samples. However, in some cases the analyzers generate spurious results which can both be too low and too high [1]. Pseudothrombocytopenia is a transient or persistent in vitro phenomenon that can be due to tube anticoagulant induced platelet clumping. In blood anticoagulated with ethylene diamine tetracetic acid (EDTA) the prevalence is ~ 0.2% [2–5]. The prevalence is lower in the general population (0.1%) than in hospitalized patients. There is no known dependency of age or sex. The clinician should suspect EDTA-dependent pseudothrombocytopenia when the following five criteria are met: First and second, abnormal platelet count and thrombocytopenia in EDTA-anticoagulated samples at room temperature, but to a lesser extent when the samples are kept at 37°C and/or another anticoagulant is used. The third criterion is a time-dependent fall in platelet count in the EDTA sample, and the fourth: platelet aggregation and clumping in EDTA-anticoagulated samples. Last, but not less important – lack of signs or symptoms of platelet disorders [6].

In rare instances, other anticoagulants also cause agglutination. Falsely elevated platelet count – pseudothrombocytosis – is a much rarer event. Fragmented erythrocytes, cytoplasmic fragments of nucleated cells, microorganisms, lipid droplets, or protein aggregates are present in the blood in case of pseudothrombocytosis [1, 7]. To minimize the clinical impact of spurious platelet counts the modern hematology analyzer automatically flag samples in which platelet clumping is suspected based on certain rules in the instrument [1].

Waldenstrom macroglobulinemia is a B-cell neoplasm characterized by lymphoplasmacytic infiltration of the bone marrow and a monoclonal immunoglobulin type M (IgM) protein [8]. The IgM may vary from <1 g/L to very

Figure 1. Micrographs and scattergrams of platelets and the platelets counts. The upper section shows micrographs of platelets agglutinates (arrows with tail) induced by citrate (Left panel). Addition of 1% paraformaldehyde (PF) abolishes agglutination (Right panel). Single platelets that do not agglutinate are indicated (arrows). The middle section shows the platelet scattergrams obtained with the EDTA, citrate or citrate + 1% PF. The upper panels show representative Sysmex XE-2100 platelet scattergrams from blood samples with different anticoagulants drawn at day 3 (PLT-O). The corresponding optical platelet count is given for each of the samples. The lower panels shows the platelet volume distribution curves generate by the Sysmex XE-2100 when counting platelets in the impedance mode (PLT-I). The impedance platelet counts are given. After the addition of 1% paraformaldehyde (PF), there is massive auto-fluorescence (encircled area) in the citrate anticoagulated samples. Therefore, the XE-2100 flags the samples as it has problems identifying the platelets (marked with the green color and encircled with the dashed encircled area). Despite this, fairly accurate platelet counts can be obtained in the impedance mode on citrate anticoagulated blood. The lower section shows manual and automated platelet counts. Manual platelet counts were determined in blood sampled in citrate tubes containing 1% paraformaldehyde (PF) (Citrate-PF manual). Automated optical (EDTA-Optical, Citrate-Optical) and impedance platelet counts (EDTA-Impedance, Citrate-Impedance) were determined on the Sysmex XE-2100 automated analyzer.

high. Elevated concentration of IgM can cause clinical symptoms and increases the viscosity of the blood. The high concentration of IgM can interfere with both blood counts and other routine biochemical analyzes [9], but Waldenstrom macroglobulinemia is not commonly associated with pseudothrombocytopenia or pseudothrombocytosis. Here, we report a patient with Waldenstrom macroglobulinemia with citrate-induced platelet clumping as well as EDTA-induced pseudothrombocytosis in samples.

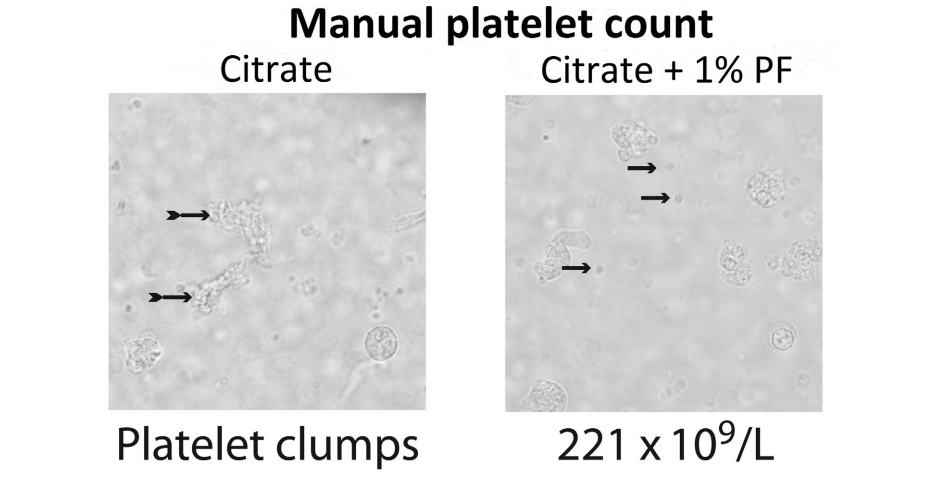
Case History

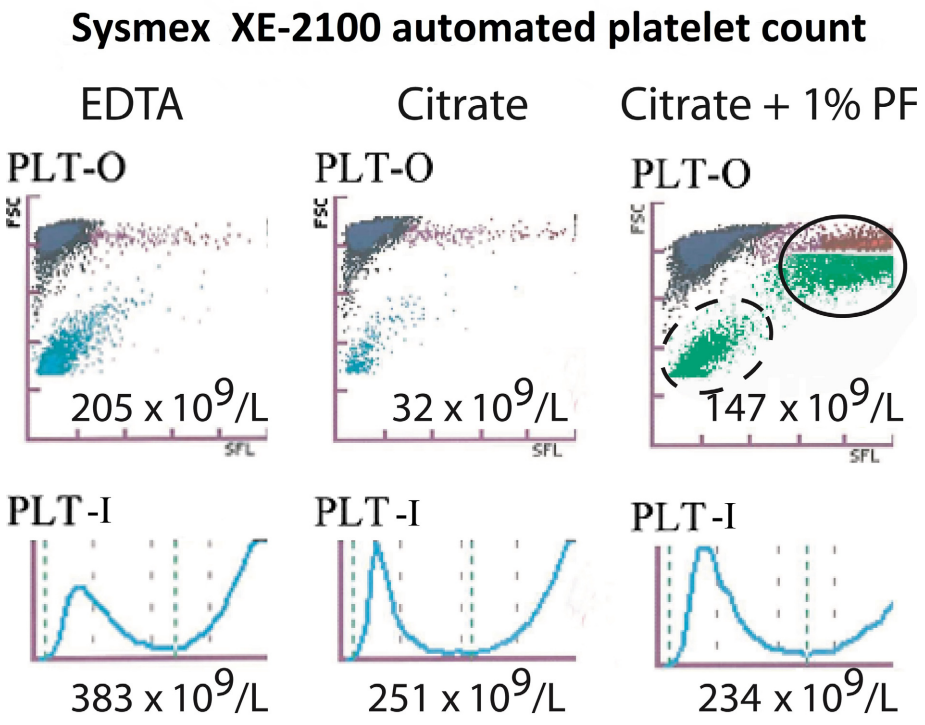
We noted persistent and severe discrepancy between platelet counts performed within the Sysmex XE-2100 (Sysmex, Denmark) optical and impedance methods on routine blood counts from a 49-year-old man during monitoring of his IgM myeloma. The patient had a history of intravenous (IV) drug abuse but was now clean. During his IV drug abuse, he became infected with hepatitis C virus (HCV). His virus titer was 5.5 million virions/mL at the time of diagnosis. He also had a history of chronic alcohol consumption but was currently only drinking intermittently. Previously he had been smoking 30 cigarettes per day, but had cut down smoking to 15 cigarettes per day before his admission.

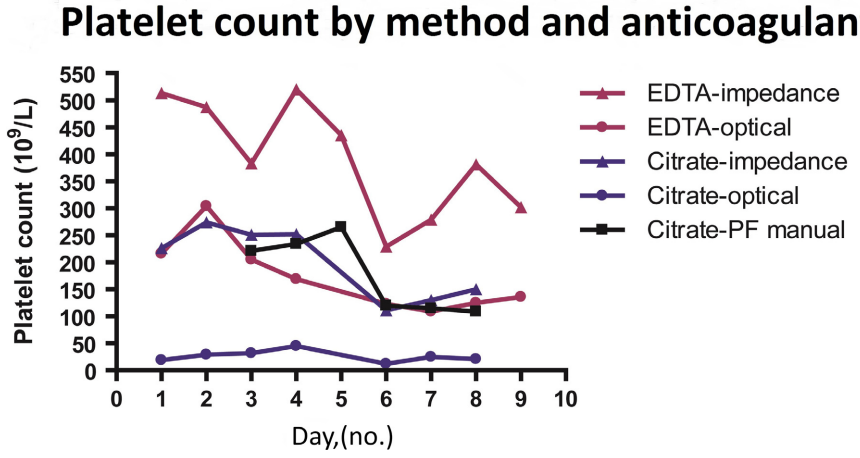
The Sysmex XE 2100 usually measures the platelet count using the impedance method, but can produce both an impedance platelet count and a fluorescence-based optical platelet count when the instrument runs in the reticulocyte mode [10, 11]. Routine platelet counts performed with the impedance method consistently gave a count of 300–500 $\times$ 10^9/L, without reporting any flags indicating clumps or other problems with the count. However, when trying to assess the patient's reticulocytes the machines switched mode, now measuring platelet count in both the optical and impedance mode. In the optical detection block, platelet count was around 200 $\times$ 10^9/L. The results were therefore flagged with either "abnormal platelet distribution" or "abnormal platelet scattergram," due to the great discrepancy between the two platelet measurements (Fig. 1, middle and lower section). In such cases, the routine procedure is to order retesting of the platelet count in citrate anticoagulated blood samples in addition to perform a manual platelet count [1]. However, the platelet agglutinated when sampled in citrate anticoagulated blood (Fig. 1, upper section).

Platelet agglutination was also observed in the Sysmex XE-2100 optical count (Fig. 1, middle and lower section). To avoid platelet clumping, we sampled the blood in citrate with paraformaldehyde (PF) (final concentration 1% vol/vol) as described by van der Meer et al. [12]. As expected, this prevented clumping of the platelets and enabled manual counting in a hemocytometer. We also analyzed the PF-treated samples in Sysmex XE-2100. In the optical channel, the PF gave a massive auto-fluorescence which invalidating the optical counts. The Sysmex XE-2100 instrument therefore flagged the samples due to problems identifying the platelets (Fig. 1, Citrate + 1% PF: marked with the green color and encircled). Despite this, accurate platelet counts were obtainable in the impedance mode. Furthermore, the citrate impedance measurement of platelets was in good agreement with the manual platelet count on citrate anticoagulated blood.

Over the next 6 days (day 3–8), we compared manual platelet counts obtained from PF-treated citrate anticoagulated blood to impedance and optical counts generated by the Sysmex XE-2100s when analyzing blood anticoagulated with either EDTA or citrate (Fig. 1, lower section). This showed that the optical platelet counts obtained in EDTA blood and the impedance platelet counts in citrate anticoagulated blood were close to the manual counts from the PF-treated citrate anticoagulated blood. The impedance platelet count in citrate anticoagulated blood was slightly closer to the manual counts and the Sysmex XE-2100 flagged the EDTA samples with either "abnormal platelet distribution" or "abnormal platelet scattergram."







Discussion

Platelet agglutination in citrate anticoagulated blood is rare phenomenon, but has previously been reported [13]. Oxalate and heparin are too occasionally reported to induce platelet clumping. Pseudothrombocytopenia is an in vitro phenomenon that can be transient or persistent, and platelet clumping is most often due to antibodies directed against epitopes on the platelets. The antibodies are IgG (33–50% of the cases), IgM (10–63%), or IgA (4–40%) [12–15]. The pathophysiology of the autoantibody production is unknown, but pseudothrombocytopenia can be found in both otherwise healthy individuals as well as in association with a variety of disease states, various states of immune stimulation, including autoimmune conditions, acute and chronic liver disease, malignancies, and different drugs [6].

Falsely elevated platelet count – pseudothrombocytosis – is a much rarer event but can occur when fragmented erythrocytes, cytoplasmic fragments of nucleated cells, microorganisms, lipid droplets, or protein aggregates are present in the blood [1, 7]. There are reports of spurious high platelet counts in patients with cryoglobulinemia [16, 17]. This patient was tested negative for cryoglobulin, but had highly elevated IgM levels. We presume that protein aggregates were the cause of the pseudothrombocytosis in this patient. We demonstrated that in the presence of agglutinins a correct platelet count can be achieved by adding PF to the citrate tubes and counting the cells manually.

The impedance principle, developed by Coulter, was the first automated method for platelet counting, and has for many years been the backbone methodology for platelet counting [18]. Computerized algorithms such as "Curve fitting" and "Moving threshold" have improved the impedance method as they correct for interference and increase the accuracy. However, the impedance method is still limited by its inability to distinguish platelets from other particles that overlap the size range of platelets. On the higher end of the size spectrum, microcytic and fragmented erythrocytes can interfere, whereas noncellular particulate interference and "electronic noise" may interfere at the lower end of the size spectrum. Spurious high platelet count may be a problem in patients with Waldenstrom macroglobulinemia and may lead to misclassification.

To overcome these limitations some instrument manufacturers developed optical platelet-counting methods, which use laser light scatter technique to identify platelets [19–22]. The Sysmex XE-2100 uses fluorescence light scatter technique [10]. All optical platelet-counting methods take advantage of the characteristic optical properties of platelets to distinguish them from nonplatelet and cellular elements or particles. Some have suggested that optical platelet-counting methods may be more accurate than impedance methods [10, 19–22]. However, other studies suggest that this may not apply in general [11, 23, 24]. In summary, which method is more accurate depends on the given situation.

This case story demonstrates some of the rare challenges associated with determining the correct platelet count in patients with elevated IgM and emphasize the advantage of having access to platelet counts based on impedance as well as optical methods.

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Conflicts of Interest

The authors have no conflicts of interest to report.

Authorship

JV, LFH, and PBL: study concept and design. LFH and JV: acquisition of data. JV, PBL, and LFH: analysis and interpretation of data. PBL and JV: drafting of the manuscript. LFH and PBL: critical revision of the manuscript for important intellectual content. LFH: administrative, technical, or material support LFH: study supervision.

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