

Electronic Blood Cell Counters are by No Means Perfect

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Key words: electronic blood cell counters, artifacts, cold agglutinins, cryoglobulins, pseudothrombocytopenia, platelet satellitism, pseudothrombocytosis, pseudoleukocytosis, pseudoleukopenia, EDTA

IMAJ 2002;4:1082–1083

Measurement of complete blood cell count is one of the essential laboratory tests. Electronic blood cell counters simplify and speed up the performance of blood counts and the calculation of red cell indexes. Because of their high precision, physicians tend to accept their results as accurate. However, these counters can be “fooled” by changes in cell size with platelet clumping [1], agglutination of erythrocytes [2,3], or precipitation of abnormal proteins [4]. Failure of the physician to recognize these errors may lead to patients being subjected to unnecessary procedures and therapy.

In this issue of *IMAJ*, Breuer et al. [5] present four patients in whom the results of red blood cell indexes measured by an automated counter were incompatible and unreasonable. These patients had cold agglutinins: two of them had a respiratory infection, one had cytomegalovirus mononucleosis, and the fourth had a B cell lymphoproliferative disease. One of the patients also had a clinically overt autoimmune hemolytic anemia.

Cold agglutinins are polyclonal or monoclonal autoantibodies, usually of immunoglobulin M subtype, directed against I or i antigens and preferentially binding erythrocytes at cold temperatures [6]. These autoantibodies may be associated with malignant or benign disorders [6] (e.g., B cell neoplasm, post-infection, collagen vascular disease) and can be manifested by transient laboratory abnormalities up to severe autoimmune hemolytic anemia [3]. With automated analyzers, cold agglutinin laboratory abnormalities typically present as a discrepancy between the red blood cell indexes [2,3]. The agglutinated erythrocytes may be recognized as single cells or may be too large to be counted as RBC; therefore, measured mean corpuscular volume is falsely elevated and the red blood cell count is disproportionately low [2,3]. While the measured hemoglobin is correct [2,3], the calculated indexes are incorrect [2]: the hematocrit (red cell count x MCV) is low, while the mean corpuscular hemoglobin (hemoglobin/red cell count) and the mean corpuscular hemoglobin concentration (hemoglobin/hematocrit) are elevated. By rewarming the blood sample to 37°C, the erythrocyte agglutination is abolished and correct values will be read [3]. In the blood sample, hemagglutination may be visible to the unaided eye [6] and examination of the peripheral blood smear may reveal erythrocyte clumping [3].

Another problem might appear with the presence of cryoglobulins. Cryoglobulins are immunoglobulins that precipitate at temperatures below 37°C, producing high molecular weight aggregates [6]. The first clue to a diagnosis of cryoglobulinemia

could be laboratory artifacts detected in the automated blood cell counts [4]. The precipitated cryoglobulin particles of various sizes may falsely be recognized as leukocytes or platelets causing pseudoleukocytosis [4] and pseudothrombocytosis [4]. At the same time, the RBC indexes are generally unaffected [4]. Reliable automated counts can be obtained by warming the blood to 37°C or by keeping the blood at 37°C from the time of venipuncture to analysis [4]. May-Grunwald-Giemsa-stained blood films are usually normal, extracellular material is occasionally seen, and leukocyte cytoplasmic inclusion is rarely found [4].

Another important laboratory artifact seen with the automated analyzers is pseudothrombocytopenia. This condition is caused by diverse mechanisms, including: anticoagulant-induced pseudothrombocytopenia [1], platelet satellitism [7,8], giant platelets [9], and cold agglutinin-induced platelet agglutination [10]. The anticoagulant-induced pseudothrombocytopenia is an *in vitro* platelet agglutination generally seen in specimens collected into EDTA [1]. It has been reported both in healthy subjects and in patients with various diseases [11] (e.g., collagen vascular disease, neoplasm, and in severely ill patients) and has an overall incidence of approximately 0.1% [9,12]. Although the agglutination is most pronounced with EDTA, it may occur with other anticoagulants as well, such as heparin, citrate or oxalate [1]. Because the generated platelet aggregates are large, the automated counters do not recognize them as platelets, leading to lower platelet counts [1]. In some cases the aggregates are large enough to be counted as leukocytes, causing a concomitant pseudoleukocytosis [1]. The aggregation in pseudothrombocytopenia is time-dependent and usually temperature-sensitive [1], with maximal activity at room temperature. The EDTA-induced pseudothrombocytopenia is mediated by autoantibodies of IgG, IgM and IgA subclasses [13] directed at an epitope on glycoprotein IIb [14]. This epitope is normally hidden in the membrane GP IIb/IIIa [14]. Ionized calcium has an important role in maintaining the heterodimeric structure of the GP IIIb/IIa complex [14]. The EDTA, through its chelating effect, dissociates the GP IIIb/IIa complex with epitope exposure [14]. In Glanzmann's thrombasthenia, a disorder characterized by the quantitative and/or qualitative abnormality of glycoprotein IIb/IIIa, pseudothrombocytopenia does not occur [14]. Interestingly, in recent years, Abciximab—a GP IIb/IIIa antagonist has been associated with pseudothrombocytopenia [15]. If anticoagulant-induced pseudothrombocytopenia is suspected a per-

RBC = red blood cells
MCV = mean corpuscular volume

Ig = immunoglobulin
GP = glycoprotein

ipheral blood smear should be examined for platelet clumping [1].

Platelet satellitism is similar to anticoagulant pseudothrombocytopenia. In the presence of EDTA, platelets bind to leukocytes and form rosettes [7,8]. The binding is usually to neutrophils [7] but binding to other white blood cells has been reported [8]. The automated analyzers do not correctly recognize platelet-neutrophil clumping, resulting in pseudothrombocytopenia [7,8]. Platelet satellitism is mediated by autoantibodies of IgG type [16]. These autoantibodies are directed at GP IIb/IIa on the platelet membrane and to an Fc gamma receptor III on the neutrophil membrane [16].

Pseudothrombocytopenia occurs with giant platelets [9]. Because of their size, the giant platelets are excluded from electronic platelet counting. Platelet cold agglutinin-induced pseudothrombocytopenia is a rare condition. The platelet agglutination is anticoagulant-independent, occurs at maximal activity at 4°C, and is mediated by IgM autoantibody directed against GP IIb/IIIa [10]. Because this autoantibody has little activity at temperatures above 30°C, no clinical complication occurs [10].

Other technical problems and less known situations may cause abnormal cell counts and indexes with automated analyzers. These include clots [17] or overfilling of tubes [18], hypertriglyceridemia [17], hyperbilirubinemia [17], and extreme high white blood count [17] – any of which may interfere with cell counting and cell indexes. Severe microcytosis [17], microorganisms [19], and cytoplasmic fragments of leukocytes [20] may cause spurious elevation of the platelet counts. These conditions are characterized by small particles that are wrongly counted as platelets. Larger particles may be recognized as leukocytes, e.g., circulating normoblasts [21], giant platelets [21], and erythrocytes with more resistance to lysis [22]. The latter occurs in automated analyzers when leukocyte counting is based on prior erythrocyte lysis. Erythrocyte resistance to lysis, causing interference with leukocyte counting, was reported in hemoglobinopathies [22] (e.g., hemoglobin C trait, CC, SC, and SS) and fetal (cord) red cells [22]. EDTA-dependent leukoagglutination [23] (similar to platelet satellitism) and cold-induced leukoagglutination [24] uncommonly cause pseudoleukopenia. Rarely does severe hyperglycemia cause spurious macrocytosis. The hyperosmolar glucose-“loaded” erythrocytes become swollen when they are diluted into a relatively hypotonic counting medium, but after hyperglycemia is corrected the MCV returns to normal [25].

To conclude, in our modern era, automated analyzers are able to increasingly recognize pathologic conditions and artifacts. First, the results are presented in numbers, histograms, and scatter plots with or without flags for internal laboratory review. The results are then transferred to the clinician, usually as numbers only. Still, undetected artifacts occur and go unnoticed. The clinician should be alert to those artifacts, thus avoiding unnecessary investigations and therapies.

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