Laboratory Identification of Cryoglobulinemia From Automated Blood Cell Counts, Fresh Blood Samples, and Blood Films

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Abstract

Four cases showing different means to detect cryoglobulins are reported: effects on blood cell counts performed on 2 technologically different automated hematology instruments and microscopic features in fresh blood samples and on May-Grünwald-Giemsa–stained blood films. These cases were chosen for their instructive value in depicting all artifacts associated with cryoglobulins. Laboratory recognition of the cryoglobulins is important to correct factitious results with automated blood cell counters, mainly pseudoleukocytosis and pseudothrombocytosis. Moreover cryoglobulin-induced laboratory artifacts may be the first factor prompting the assessment for cryoglobulinemia and the diagnosis of the underlying cause.

Cryoglobulins are circulating immunoglobulins or immunoglobulin complexes characterized by reversible cold-induced precipitation occurring between 4°C and 37°C. Cryoglobulins have been reported in various infectious, renal, hepatic, autoimmune, hematologic (especially multiple myeloma and lymphoproliferative disorders), and neoplastic diseases, but they also can occur in the absence of any apparent relevant disease. Clinical manifestations, including purpura, Raynaud syndrome, arthralgia, peripheral neuritis, or renal disease, are inconstant, and this emphasizes the need for relevant laboratory screening tests.

The in vitro effects of cryoglobulins are less well documented, especially for blood cell counts ascertained by automated cell counters. Erroneous WBC counts have been reported as the major artifact, and abnormal platelet counts have been mentioned occasionally. Recognition of the hematologic abnormalities associated with cryoglobulins may be the first clue leading to the diagnosis of cryoglobulinemia and eventually to its underlying cause. We report anomalies in blood count histograms and scatterplots in 4 patients with cryoglobulinemia and the appearance of cryoglobulins in microscopy.

Materials and Methods

Cases were obtained from the hematology laboratory of the University Hospital, Nancy, France, and were selected for their instructive value in demonstrating different manifestations of cryoglobulinemia on blood cell analysis. Peripheral blood specimens were obtained in vacuum tubes containing tripotassium EDTA (8.4 mmol/L final concentration; Vacutainer, Becton Dickinson, San Jose, CA) and maintained at room
temperature until analysis. Blood counts were performed using 2 technologically different blood cell counters: an electrical impedance counter (STKS, Beckman Coulter, Hialeah, FL) and an optical-based instrument (Technicon H*2, Bayer, Tarrytown, NY). Blood analysis was performed at room temperature and after heating the sample for 30 minutes at 37°C. A drop of fresh blood was examined at room temperature with a phase-contrast microscope. This is readily accomplished by placing a small drop of blood beneath a coverslip on a glass slide.

Blood films were prepared from specimens obtained at room temperature and were stained according to the May-Grünwald-Giemsa technique. Cryoglobulinemia was confirmed by the detection of protein precipitates in the serum maintained at 4°C during at least 7 days, which dissolved when heated at 37°C.

Results

Cases 1 and 2 were associated with multiple myeloma and B-cell malignant lymphoma, respectively. Case 3 had a diagnosis of interstitial nephropathy. In case 4, cryoglobulinemia was transient, concomitant to a pulmonary infection. In all cases, the abnormalities on blood cell counts were observed before the clinical and biochemical diagnosis of cryoglobulinemia. Moreover, for case 2, investigation of the lymphocytosis-associated cryoglobulinemia (lymphocytes, 6,880/µL [6.9 × 10⁹ /L]) prompted the diagnosis of lymphoma. Cryoglobulinemia was confirmed by biochemical methods in serum samples for cases 1, 2, and 3. Interestingly, for case 2, a precipitate already was noted in the plasma of the sample used for blood cell counts after sedimentation at room temperature.

Relevant hematologic findings are summarized in Table 1 and Table 2. RBC counts and hemoglobin measurements were slightly affected by the presence of the cryoglobulins. Case 2 was the only case associated with a flatness alarm on the H*2 analyzer, showing flow abnormality and leading to slightly decreased RBC count and hemoglobin measurement at room temperature compared with analysis of the sample after 30 minutes at 37°C (room temperature: RBC count, 4.77 × 10⁶ /µL [4.77 × 10¹² /L]; hemoglobin, 12.7 g/dL [127 g/L]; 37°C: RBC count, 5.00 × 10⁶ /µL [5.00 × 10¹² /L]; hemoglobin, 12.3 g/dL [123 g/L]).
Cases are illustrated in Figure 1, Figure 2, Figure 3, and Figure 4, respectively, depicting the principal manifestations of cryoglobulins on the blood count depending on the instrument used. For the STKS counter, the lymphocyte monocyte granulocyte (LMG) diagram showing the volume distribution of WBCs, with the anomaly of partially lysed leukocytes, is represented. Volume conductance scattering and platelet volume distribution histograms are not shown because they were not altered by the presence of cryoglobulins. The STKS counter, based on an impedance principle, mainly detects cryoglobulins as small particles (<35 fL) at the left
of the LMG diagram, leading to spuriously elevated WBC counts (R* flag). For the optical-based H*2 analyzer, the peroxidase activity (perox channel scatterplot) and the histogram of platelet volume distribution are represented. In scatterplots using the perox channel, forward light scatter, largely determined by cell volume (y-axis), is plotted against light absorbance largely determined by the intensity of the peroxidase reaction (x-axis). Cryoglobulins interfere mainly on the histogram of platelet volume distribution, generating a characteristic triangular figure. Platelet counts are spuriously elevated, and the mean platelet volume is usually absurd (too small for true platelets).
Cryoglobulins rarely manifest on the perox channel scatterplot, leading to falsely elevated WBC counts (Figures 1 and 4). The basophil/lobularity channel, analyzing WBCs according to their volume following differential cytoplasmic stripping, is not affected by cryoglobulins.

Examination of fresh blood samples at room temperature revealed that cryoglobulins might take different appearances depending on the patients. For example, a large flake of clear amorphous material is shown (case 1) as a thin refringent precipitates (cases 2 and 3) and fusiform crystals of different thicknesses (case 4).

(Figures 2 and 3).
May-Grünwald-Giemsa–stained blood films usually were normal (cases 2-4). But in case 1, the film revealed some pinkish amorphous deposits between RBCs and multiple basophilic inclusion bodies in the majority of neutrophils. These inclusion bodies sometimes were so abundant that they compressed and distorted the nucleus.

Table 3 proposes a guide to screen for and definitively diagnose the presence of cryoglobulins.

Discussion

Cryoglobulins may precipitate at temperatures less than 37°C; the precipitation temperature is highly variable from one cryoglobulin to another. An interference of the presence of cryoprecipitates on blood cell counts is thus likely to occur if cryoprecipitation develops rapidly and at room temperature (18°C-25°C). In the 4 cases reported, environmental cooling of the blood from venipuncture to analysis was sufficient to permit cryoprecipitates to form. Of clinical interest, and as in our case records, cryoglobulinemia may be diagnosed several years before the underlying cause, especially for hematologic diseases.

It has been reported that electronic cell counters may mistake the precipitated cryoglobulins for blood cells when the size of the precipitates falls within the range of the aperture through which cells in suspension are drawn. However, comparison of automated blood cell counts for patients with cryoglobulinemia using instruments with different technologies has seldom been reported in the medical literature. In almost all reported cases, blood cell counts were performed on a Coulter counter Model S8-14 or a Coulter counter Model S Plus, leading to falsely elevated WBC counts. We showed that cryoglobulins may interfere in WBC and/or platelet counts, even with current analyzers. The degree of interference in WBC or platelet counts seems to depend not only on the instrument used but also on the type of precipitate. Indeed, spuriously elevated cell counts are due to the presence of particles of cryoglobulin being counted as WBCs or platelets in relation to such physical properties as their size, structure, and shape.
Cryoglobulins manifested in the present study as erroneously elevated WBC counts on the STKS counter. Interference on the platelet count was much less frequent and of mild importance on this machine. On the H*2, the apparent largest cryoglobulin particles (cases 1 and 4) interfered in WBC counts, and the smaller ones interfered in platelet counts (cases 2 and 3). Of interest, we noted that the WBC count as assessed in the basophil/lobularity channel was never affected by cryoglobulins. The main differential diagnoses of cryoglobulins are platelet clumps, giant platelets, nucleated RBCs, and chylomicrons, which also interfere in the left part of the LMG diagram on the Coulter STKS counter. Interference on the perox channel plots on the H*2 also may correspond to platelet clumps or chylomicrons (the latter also are visible on the basophil/lobularity channel).

RBC values generally are unaffected in cryoglobulinemias. Nevertheless, an apparent increment in hemoglobin because of decreased light transmittance in the measurement cuvette has been reported. According to our experience, cryoglobulin did not interfere in the hemoglobin measurement. We noted only 1 case of slightly reduced hemoglobin owing to a slightly decreased RBC count because of a flow...
disorder in relation to the presence of numerous thin precipitates in blood. Of practical interest, reliable automated counts can be obtained by warming the blood specimen at 37°C (for at least 30 minutes) before analysis. If this method is not sufficient (partial redissolution occurs), collection of blood in warmed tubes and maintenance at 37°C until analysis should be performed.

We also report on the variable morphologic appearance of cryoglobulins in a fresh blood drop and on a blood film. The morphologic appearance of cryoglobulins in fresh blood samples has been described rarely. According to our experience, cryoglobulins mostly appear as thin, bright deposits (cases 2 and 3). Care must be taken to not mistake them for platelet clumps, particularly since EDTA-associated platelet clumps may sometimes disappear at 37°C. In practice, cryoglobulin precipitates are thinner and rougher than platelet clumps. We also observed 2 cases of crystal formation (cases 1 and 4). Crystallization has seldom been reported. In such cases, the morphologic appearance of the crystal was varied from needle-like, boat-shaped, cubic, flake-like, rhomboidal, or hexagonal to rectangular.11,13-14 The coincidence of the appearance of long (50-150 µm), thin crystals on blood films with the detection of a falsely elevated leukocytosis has been suggested.11,13

Only a few reports on the manifestations of cryoglobulins on blood films have been published.7,14,15,18 Indeed, according to our experience, May-Grünwald-Giemsa–stained blood films usually are normal. In addition, the blood film examinations were negative in 3 of 4 cases (cases 2-4). Yet, cryoglobulins may sometimes become visible as extracellular or intracellular material. Extracellular materials reported are small grayish precipitates,14 fusiform or needle-shaped crystals,11,13 faintly basophilic or pinkish globules, droplets, flakes, or shapeless aggregates.9,15 Only 7 cases of leukocyte cytoplasmic inclusions in cryoglobulinemia have been reported in the literature15,18 (plus 2 cases, personal communication, J.M. Jackson, reported in Lucas et al8). It has been demonstrated that cryoglobulin-containing plasma might induce the generation of neutrophil inclusions in neutrophils from a healthy donor.18 Cytochemical staining has been reported on blood films from 2 cases: the inclusions were negative for Sudan black B, oil red O, Gram stain, myeloperoxidase, and periodic acid–Schiff. Ultrastructural and immunofluorescence studies have shown that the cytoplasmic inclusions in neutrophils corresponded to the cryoglobulin, likely phagocytosed by these cells.15,18

Morphologically, cryoglobulin inclusions should be distinguished from other neutrophil inclusions, such as Döhle bodies, abnormal mucopolysaccharides, and fragments of RBCs ingested in hemolytic anemias. Usual neutrophil cytoplasmic inclusions such as Döhle bodies, 

![Image 5](Case 1) Blood smear showing multiple basophilic inclusion bodies in neutrophils (May-Grünwald-Giemsa, original magnification ×1,000).

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Alder-Reilly anomaly, and Chédiak-Higashi syndrome are morphologically distinct. The principal cause of neutrophil vacuolization is bacterial septicemia, which may induce clear cytoplasmic neutrophil vacuoles, toxic granulations, and Döhle bodies. The presence of a distorted neutrophil nucleus should not be mistaken for nuclear pyknosis or karyorrhexis. Of note, inclusions in monocytes from patients with cryoglobulinemia also have been found.

The present study indicates that significant anomalies in automated blood cell counts may be related to the presence of cryoglobulin precipitates. The observation of such abnormal histograms must prompt microscopic examination of fresh blood samples and May-Grünwald-Giemsa–stained blood films, which may permit the visualization of specific features of these precipitates. In such cases, the clinician should be informed promptly, as such anomalies may be the first indication of the presence of a cryoglobulinemia.

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References