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A Peripheral Smear Finding Beyond Cells: Cryoglobulins in a Case of Lymphoplasmacytic Lymphoma

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Abstract

A well prepared peripheral smear is an indispensable requisite for the diagnosis of hematological disorders and in some situations, non-hematological diseases. Here, we present the typical hemogram and peripheral smear findings of circulating cryoglobulins that led to the unraveling of the underlying lymphoproliferative disorder. A 51-year lady presented with symptoms of anemia since four months. Her hemogram showed anemia and leucopenia with flagging of platelet count. Peripheral smear at room temperature revealed marked artefactual changes in red blood cells including fragmentation. The morphology was better appreciated after incubating the sample at 37°C and showed normocytic normochromic RBCs, thrombocytopenia with a few lymphoplasmacytic cells and amorphous pinkish material in the background. Bone marrow examination showed near total replacement of marrow spaces by lymphoid cells positive for CD20 and CD138 and was diagnosed as lymphoplasmacytic lymphoma. Cryoglobulins can thus cause a range of laboratory artefacts which need to be recognized, warranting further search for possible underlying etiologies.

Keywords: Artifacts, Cryoglobulinemia, Peripheral-Smear, Pseudothrombocytosis

1. Introduction

Cryoglobulins are abnormal circulating immunoglobulins (monoclonal or polyclonal) that are precipitated at low temperatures (below 37°C) and re-dissolve at higher temperatures.[1] They are usually secondary to systemic causes ranging from infectious to neoplastic disorders. Their presence may result in a clinical syndrome of inflammation which can affect multiple organs due to deposition of immunoglobulins in the vessels of organs. The commonly involved organs are kidney, skin, small blood vessels etc. Depending upon the excess immunoglobulin, Cryoglobulinaemia is classified into three subgroups as described by Brouet et al.,[1].

1. Type I - composed of a single monoclonal immunoglobulin
2. Type III - characterized of polyclonal IgM with Rheumatoid factor activity along with polyclonal Ig
3. Type III- characterized by a mixture of polyclonal IgG and IgM.

The term Essential cryoglobulinemia refers to the very small percentage of patients with mixed cryoglobulinemic vasculitis in the absence of any identifiable underlying disease.[2] Monoclonal cryoglobulinemia i.e., type I cryoglobulinemia is often seen in patients with hematologic malignancies (Waldenstrom's macroglobulinemia, multiple myeloma etc) while mixed cryoglobulinemia (Types II and III) often presents as a systemic vasculitis due to immune complex deposition in the small vessels and is usually associated with infectious (especially Hepatitis C), connective tissue disorders etc.[2]

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Cryoglobulins are detected either in known conditions or their onset sometimes precedes the appearance of the primary disease making it essential to identify their presence. Circulating cryoglobulins are known to cause laboratory artefacts in automated cell counters and peripheral smear morphology. Thus, their recognition could be the first pointer to the diagnosis of cryoglobulinemias. The present case report highlights the characteristic hemogram and peripheral smear findings in a patient with cryoglobulinemia secondary to lymphoplasmacytic lymphoma.

2. Case Report

A 51-year-old lady presented to the hematology clinic with complaints of fever, vomiting, weakness and giddiness for the past one and half months. She also gave a history of one episode of bleeding and hematuria. She had received about four packed red cell transfusions in these 2 months. In the past, she had a history of menorrhagia for which hysterectomy was performed 2 years back. There was no jaundice, palpable lymphadenopathy or organomegaly. The first peripheral blood sample drawn for routine laboratory investigation was grossly hemolysed. A repeat sample was taken under warm conditions and incubated at 37°C for 30 minutes. Her hemogram (run with sample at 37°C) was Hb: 6.5g/dl, TLC: 2220/µl, MCV: 97.5fL, MCH: 31pg, MCHC: 31.8g/dl with flagging of platelet count. Peripheral smear showed a pinkish amorphous material in the background and marked rouleaux formation with normocytic normochromic RBCs, leucopenia, a differential leucocyte count of P3 L94 M3 with few lymphoplasmacytic cells and showed a manual platelet count of about 10,000/ µl (Figure 1). A direct Coomb's test was done to rule out in vivo hemolysis which was

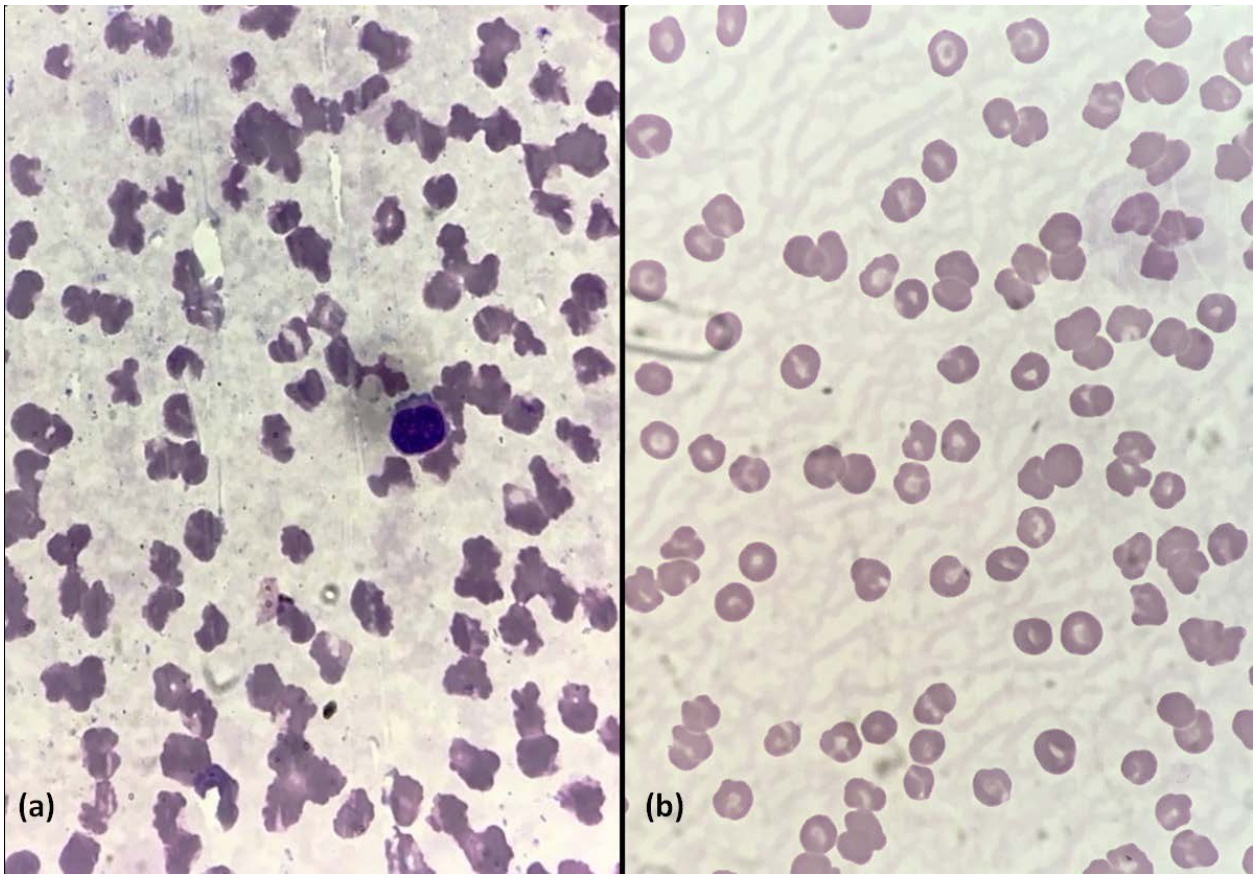

Figure 1. (a) Peripheral smear at room temperature showing marked artefactual changes in red cells with fragmentation and distortion, a lymphoplasmacytic cell and pink amorphous material in the background (b) Peripheral smear after incubation at 37°C showing normocytic normochromic RBCs.

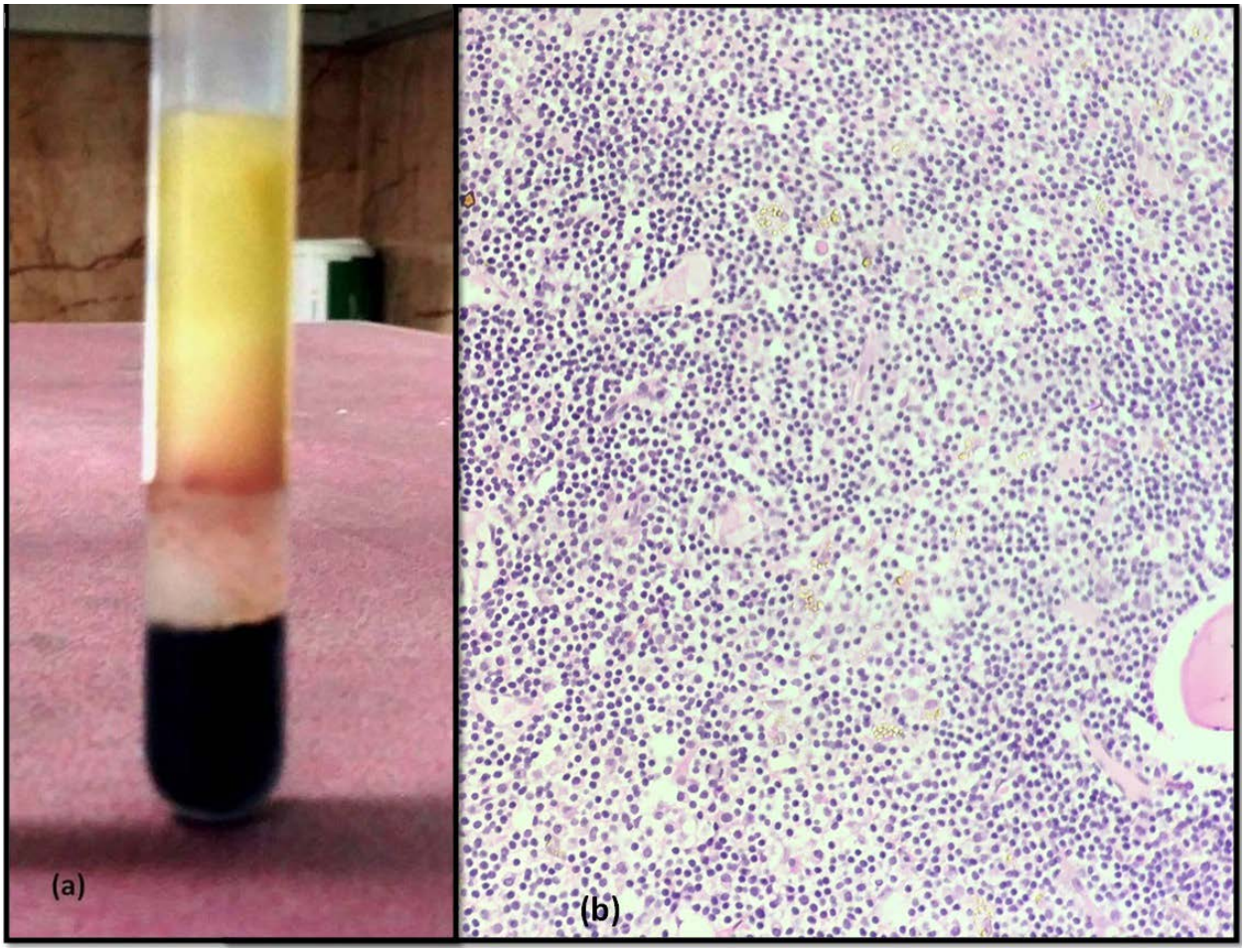

Figure 2. (a) A positive screening test for cryoglobulins seen in the patient's sample (b) Bone marrow biopsy showing diffuse infiltration of marrow spaces by lymphoplasmacytic cells.

negative. Screening test for cryoglobulins was positive (Figure 2a)

The bone marrow biopsy was hypercellular and showed near total replacement by monomorphic lymphoid cells with interstitial increase in plasma cells (Figure 2b). On immunocytochemistry, these cells were positive for CD20 and CD138 respectively, suggesting a lymphoplasmacytic lymphoma. Further investigations couldn't be done since the patient succumbed to the disease soon after.

3. Discussion

The diagnosis of a cryoglobulinemia syndrome should be suspected in individuals presenting with skin ulcers, glomerulonephritis arthralgia, purpura and neuropathy. The suspicion should be raised further if these occur in the setting of a chronic viral hepatitis (especially hepatitis C virus [HCV]), a monoclonal gammopathy (eg, multiple myeloma, Waldenström macroglobulinemia, or connective tissue disease.[1,2]

The clinical symptoms of cryoglobulinemia depends on the level of globulins as well as the underlying condition. The prevalence of clinically significant cryoglobulinemia has been estimated at approximately 1 in 100,000. The in vitro effects of cryoglobulins are less well known, especially in automated cell counters. The most common hemogram abnormalities were false elevation of leucocyte count and platelet count (pseudoleucocytosis and pseudothrombocytosis respectively). The spuriously elevated cell counts are due to the presence of precipitated

cryoglobulin particles that are being counted as WBCs or platelets in relation to their physical properties such as size and shape.[3–5] Though RBC counts are not affected, overestimation of hemoglobin values may be seen in some instruments due to apparent decrease in light transmittance. For reliable counts, samples should be prewarmed at 37°C (for at least 30 minutes in a water bath) before analysis. If the results are still spurious, blood should be collected in pre-warmed syringes and tubes and maintained at 37°C until analysis.[6]

The presence of cryoglobulins can affect the peripheral smear morphology both on fresh wet smear as well as on fixed stained smears. Cryoglobulins are seen as large flakes of amorphous material on fresh smears or as fusiform crystals at room temperature. The precipitates may be mistaken for platelets on morphology and usually dissolve on incubating the sample at 37°C. However, cryoglobulin precipitates are thinner and rougher than platelet clumps.[6] These crystals distort the morphology of the blood cells thereby causing their fragmentation and they can be confused with schistocytes.[7–9] On leishman giemsa stained smears, they usually appear as extracellular pale pink amorphous material. They can even look like neutrophilic inclusions and need to be differentiated by cytochemical staining which will be negative for cryoglobulin thereby helping to distinguish it from other common inclusions.[6]

For detection of cryoglobulin, 10 to 20 mL of blood is drawn into prewarmed syringes without any anticoagulant. After clotting at 37°C for one hour, the serum is separated by centrifugation at 37°C, placed in a graduated (Wintrobe) tube, and then refrigerated at 4°C. In type I cryoglobulinemia, precipitates are often seen within 24 hours.[10] However, longer period is required (three to five days) for complete precipitation, especially in the mixed cryoglobulins, Cryocrit (analogus to hematocrit) is a measure of the packed volume of the precipitate as a percentage of the original serum volume at 4°C. The precipitate can be redissolved in saline at 37°C to confirm the warm solubility of the cryoglobulins. Further subcharacterization can be accomplished by immunofixation, Enzyme-Linked Immunosorbent Assay (ELISA), or another specific immunologic assay. To look for the underlying disorder is the next step in evaluation of the patient because treatment is directed towards the primary disorder.[10]

Similar case of secondary cryoglobulinemia have been recently reported by Tulio M et al., where in they studied an elderly woman with a non-cirrhotic hepatitis C virus infection presenting with weakness, arthralgias, purpuric rash with left leg ulcerative lesions, bilateral peripheral sensorimotor polyneuropathy, renal impairment and cardiac failure. The investigation was compatible with a severe type II mixed cryoglobulinemia with multisystemic involvement, including a low-grade B cell lymphoma and concomitant intestinal tuberculosis.[11]

4. Conclusion

This case is an illustration of the fact that meticulous peripheral smear screening can provide answers to many clinical scenarios. Cryoglobulinemia can cause a spectrum of hemogram and peripheral smear changes and awareness of these findings are essential for their early diagnosis and further work-up.

5. References

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