Scholars Journal of Medical Case Reports
Abbreviated Key Title: Sch J Med Case Rep
ISSN 2347-9507 (Print) | ISSN 2347-6559 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Haematology

Pseudoleukocytosis, WBC Histogram and Peripheral Blood Smear Examination: The Clue to the Diagnosis of Rare Disorder Mixed Cryoglobulinemia - An Interesting Case Report

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DOI: 10.36347/sjmcr.2022.v10i11.019 | **Received:** 13.10.2022 | **Accepted:** 22.11.2022 | **Published:** 27.11.2022

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Abstract **Case Report**

Cryoglobulins are circulating immunoglobulins or immunoglobulin complexes that precipitate under cold conditions and resolubilize on rewarming. Persistant presence of cryoglobulins in serum is known as Cryoglobulinemia. It is of three types- Type I, II, III. Type II and III are known as Mixed cryoglobulinemia. We recently encontered special case during routine examination of complete blood cell count of a 67 years old male patient which showed pseudoleucocytosis on Coulter DXH 800 automatic haematology analyzer and WBC histogram showed decreasing trend. We examined peripheral blood smear to rule out other causes of psuedoleucocytosis like platelet clumps or nRBCs which were absent on smears studied. However large ammount of lumpy grey-blue deposits were observed. On warming blood at 37^0C lumpy grey blue deposits reduced on smear but did not disappear. With these findings we suspected cryoglobulinemia and took detailed patient history which revealed triad of weakness, arthralgia and purpuric rash suggesting possibility of cryoglobulinemia. For quick confirmation RA factor and complement levels were analyzed. RA factor was positive and C4 was reduced with normal C3 levels characteristically indicating mixed cryoglobulinemia. Finally for confirmation blood sample was recollected in pre warmed syringe and under temperature control plasma was separated and kept for cold incubation at 4-8^0 C which showed cryoprecipitate at bottom of tube after 12 hrs. It confirmed mixed cryoglobulinemia. Further Serum Protein electrophoresis revealed distorted gamma region but no monoclonal band was seen. This confirmed polyclonal nature of cryoglobulin indicating mixed cryoglobulinemia. Hence though rare disorder (Incidence 1 in 100000) simple clue of erroneous leucocyte count, and WBC histogram pattern followed by blood smear examination can help easily identify unsuspected cases of cryoglobulinemia.

Keywords: Mixed Cryoglobulinemia, WBC histogram, Pseudoleukocytosis.

INTRODUCTION

Cryoglobulins are circulating immunoglobulins or immunoglobulin complexes that precipitate under cold conditions and resolubilize on rewarming. Persistant presence of cryoglobulins in serum is known as Cryoglobulinemia. Cryoglobulins have been reported in various conditions like infectious, renal, hepatic, hematologic, autoimmune and neoplastic diseases. However they can also occur without any apparent disease [1, 2]. There are three types of cryoglobulins.- Type I, II & III. These are classified on the basis of presence or absence of monoclonality and rheumatoid factor activity (Table I). Type I cryoglobulins are monoclonal but lack rheumatoid factor activity. These are associated with certain hematological malignancies (e.g. multiple myeloma). Type II and III are known as Mixed cryoglobulinemia as they contain complexes of both IgG and IgM antibodies. The IgM component in both type II and III possess rheumatoid factor activity. They present commonly with vasculitis. Ninety percent patients with cryoglobulinemic vasulitis presents with characteristic hypocomplementemia where C4 is markedly reduced than C3 [2, 3]. Hematology autoanalyzers may give falsely elevated WBC or platelet count or nRBC flag and characteristic decreasing trend in WBC histogram

due to presence of cryoglobulins in blood. Peripheral blood smear also reveals pale grey-blue lumpy deposits in smear. These Hematology analyzer findings are the clue to suspect cryoglobulinemia [1, 4, 5].

Table 1: Classification of Cryoglobulinemia [6]

Types of cryoglobulinemia	Type I	Type II	Type III
Clinical presentation	Hematological malignancy (e.g multiple myeloma, B-cell lymphoma)	Weakness, arthralgia, purpura (skin rash)	Weakness, arthralgia, purpura (skin rash)
Monoclonal or polyclonal nature of cryoglobulins.	Monoclonal	Monoclonal or oligoclonal IgM and Polyclonal immunoglobulin (mainly IgG)[6]	Polyclonal (all isotype) [6]
Rheumatoid factor ativity	Absent	Present	Present

Cryoglobulinemia is rare disorder with prevalence of 1: 1,00,000 with male to female ratio (3:1) [6]. Patient commonly present with Meltzer's triad composed of Purpuric lesions, arthralgias and weakness [3]. These symptoms are nonspecific and can be found in many other disorders (e.g. viral fever, autoimmune disorders) and hence high degree of suspicion is needed in order to diagnose cryoglobulinemia due to its rarity. Cryoglobulinemia may involve multiple organs and patient keep consulting different physicians to get symptomatic relief. Here comes the role of laboratory diagnosis. Simple artifact like falsely elevated WBC count (pseudoleucocytosis) or platelet count (pseudothrombocytosis) on automated hematology analyzer may help in suspecting this disorder. Eyes see what the mind knows. Along with characteristic WBC histogram and thorough peripheral blood smear examination one may get clue to start further investigation for cryoglobulinemia. We came across an interesting case where patient had classical triad along with peripheral neuropathy since 3 years however cryoglobulinemia was not suspected due to its rare incidence.

CASE REPORT

A 64 year male operated for Carcinoma Prostate 14 years back and is on regular follow up without any evidence of recurrence. We came across his blood sample for complete blood cell count (CBC) as routine checkup. CBC results measured using Beckman Coulter DxH 800 automatic hematology analyzer were WBC $10.5x10^3$/uL, Uncorrected WBC (UWBC) $15.1x10^3$ /uL , RBC $5.03x10^6$/uL, Hb 11.8 g/dL, PLT $168x10^3$/uL. Patient had microcytic hypochromic anemia.WBC histogram showed decreasing trend (Fig 1). Based on UWBC count we examined peripheral blood smear thoroughly for platelet clumps and nRBCs which were absent on smear examination. However pale greyish blue amorphous material deposits noted on smear examination indicating cryoglobulins (Fig 2). We repeated Coulter examination and microscopic examination of peripheral blood smear after incubating sample at 37^0C for 30 minutes. WBC histogram improved with normal pattern (fig 1) and pale grey blue deposits reduced in number but not completely dissolved.

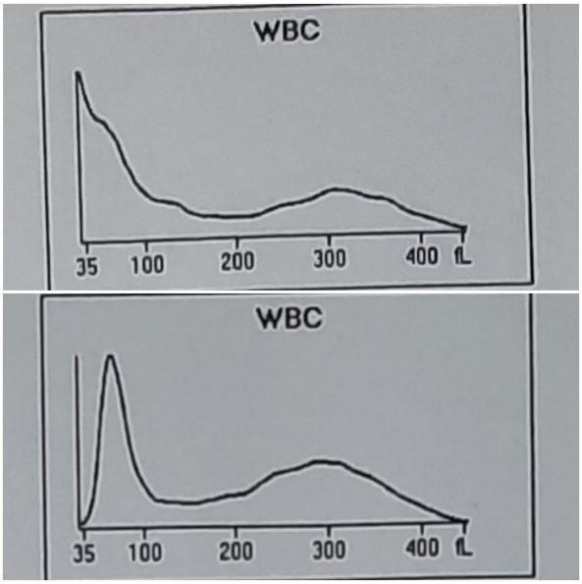

Fig 1: Decreasing trend on WBC histogram due to cryoglobulins (upper curve) which on incubation at 37^0C disappeared and became normal curve (lower curve)

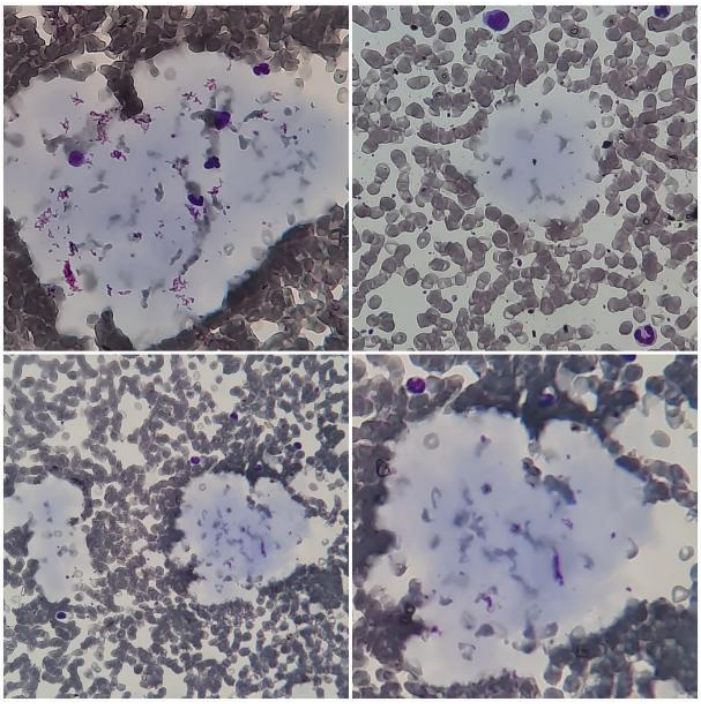

Fig 2: Presence of pale gray blue deposits indicating presence of cryoglobulins

Based on these findings we suspected Cryoglobulins and detailed patient history has been taken. Patient revealed following complaints: Skin rash (purpuric rash) on hands and feet which exacerbates in cold season (It was being treated as psoriasis), weakness, joint pain (Arthralgia) and in addition tingling numbness and burning sensation in hands and feet. For quick confirmation, we did run test for Rheumatoid factor which was positive (426.7 IU/ml) but Anti CCP was negative and we noticed hypocomplementemia with Normal C3 (94 mg/dL) and characteristically markedly reduced C4 (1 mg/dL). For confirmation of cryoglobulin, we collected one more sample from patient in pre warmed syringe in laboratory premises in order to take all due precaution for cryoglobulinemia testing. The blood sample was centrifuged at 1500g for 15 min and plasma was separated and stored at 4-8^{0}C. A control sample was also kept alongside. Patient's plasma, at 12 hours showed gel like precipitate at bottom of tube (Fig 3). Serum protein electrophoresis revealed distorted gamma region and no monoclonal band seen confirming polyclonal nature of cryoglobulins (Fig 4). So we reached conclusion of mixed cryoglobulinemia. As patient visited laboratory we were able to confirm purpuric rash (Fig 5). Renal function test (RFT), liver function test (LFT) were within normal limits and HCV tested negative (Table 2). Patient was advised bone marrow and skin biopsy to complete work up. Being a standalone laboratory we lost the patient to follow up.

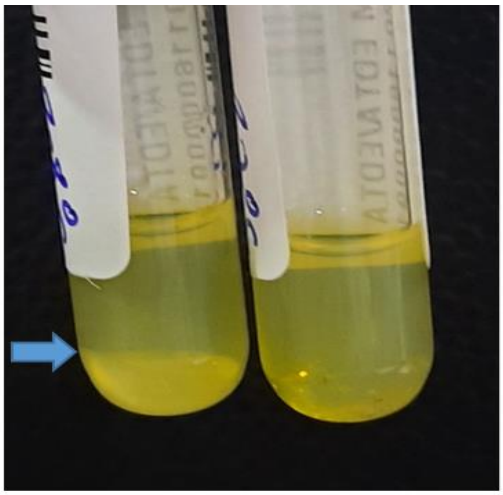

Fig 3: Cryoglobulin precipitate on cold incubation (4-8^0 C) indicated by arrow. Control tube accompanying it showed no precipitate

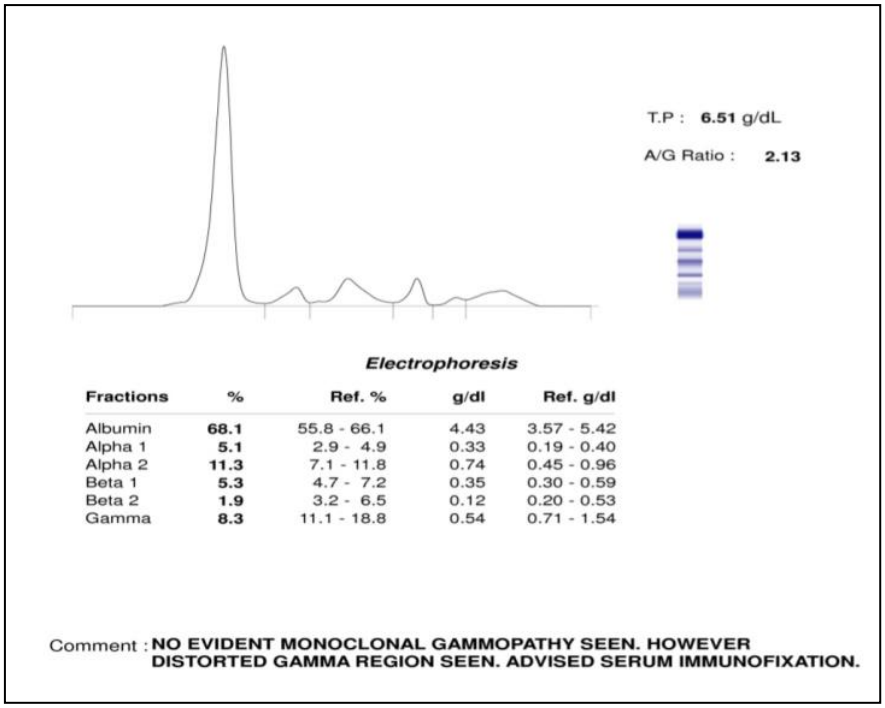

Fig 4: Serum protein electrophoresis showed no evidence of monoclonal gammopathy. However distorted gamma region noted indicating polyclonal nature

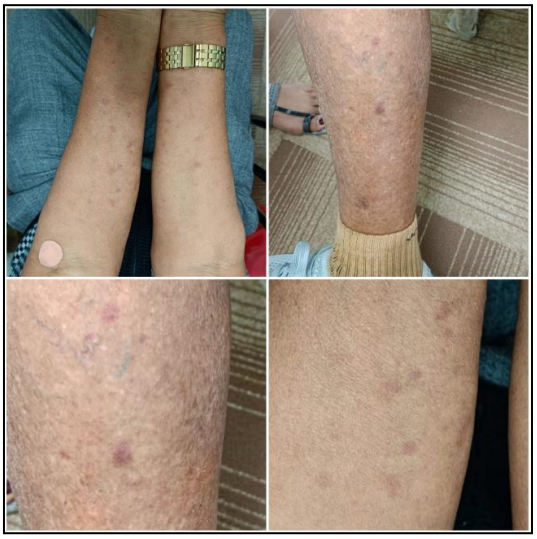

Fig 5: Multiple purpuric skin rash (cryoglobulinemic vasculitis) on hands and feet of patient

Table 2: Investigation summary

Creatinine	**0.97 mg/dl (Normal)**
Urea	21.9 mg/dl (Normal)
RA factor (Rheumatoid factor)	Increased 426.7 IU/ml (Positive)
Anti CCP	Negative
C3	94 mg/dL (Normal 90-180)
C4	1 mg/dL (Reduced markedly) (Normal 10-40)
HCV	Non-Reactive (Negative)
PSA	0.006 ng/ml (<4 is Normal)
Peripheral blood smear microscopy Automated blood cell counter	Pale grey-blue amorphous deposits. Psedoleukocytosis
Cryoglobulin test	Positive
Serum protein electrophoresis	Suggestive of Polyclonal nature of immunoglobulin.

DISCUSSION

B lymphocyte cell proliferation lies at root of cryoglobulinemia and hence immunosuppression by corticosteroids and newer biologics like Rituximab (anti CD 20) will give significant improvement of condition and hence it is important to suspect this entity. Also Cryoglobulins may present in blood before actual disease predominates clinically (eg: smoldering myeloma) [6].

In this case patient had mixed cryoglobulinemia, however physician did not suspect it due to its rarity. Incidental findings of pseudoleucocytosis (erroneous WBC counts) and peculiar pattern of WBC histogram and its reversal on heating sample at 37^0C were important clue to suspect cryoglobulins. Very few case reports mentions utility of automated hematology analyzer in case of cryoglobulins [1, 2, 4]. More over these findings are not emphasized in any textbooks due to rare occurrence of disease itself. However these findings are easy to pick up and are important clue for further investigation.

Qian *et al.*, reported similar findings on Beckman Coulter DxH 800 hematology analyzer [4]. According to Fohlen Walter *et al.*, and Qian *et al.*, falsely elevated WBC or Platelet count are due to interference by cryoglobulin particles itself or RBC and cryoglobulin forms complexes and in WBC chamber these complexes are broken down into fragments of various sizes. These fragments based on their sizes falsely counted as WBC, platelets or nRBCs. Qian *et al.*, experimented this by creating same mixture as that of WBC chamber, as per manufacturers instruction and evident many RBC-Cryoglobulin complexes microscopically [1, 4].

Cryoglobulin particles which are smaller in size than lymphocytes generates characteristic decreasing trend in WBC histogram. Automated blood cell Counter falsely identifies these particles as WBC but smaller in size and hence WBC graph starts at high on Y axis instead of starting from baseline X axis. The common differentials for such artifact are platelet clumps, large platelets or nRBC which are excluded by microscopic examination of Leishmann stained blood film. Fohlen-Walter *et al.*, studied this phenomena on different cell counters and found similar results [1].

Followed by early clues of pseudoleucocytosis and WBC histogram; testing for Rheumatoid factor and complements will aid in classification and rapid confirmation of this entity. Presence of Rheumatoid factor and low C4 and normal C3 helped us to classify our case to mixed cryoglobulinemia. However Cold incubation at 4-8^0C upto 72 hours (maximum upto 7 days) remains confirmatory test to evidence cryoglobulins [1]. Further clonality can be determined by serum protein electrophoresis.

Only few reports on manifestations of cryoglobulins on blood films have been published [1, 2, 4].Cryoglobulins can present as extracellular or intracellular material in various forms. Extracellular materials reported are grayish precipitates, needle shaped crystals, faintly basophilic droplets or aggregates. Intracellular cryoglobulins are rare [1]. In our case cryoglobulins are extracellular and are pale gray-blue lumpy aggregates of various sizes. Few RBCs traped inside them and RBCs around them showed some morphological distortion [2].

CONCLUSION

Present case study indicates that characteristic anomalies in automated blood cell counts may be related to presence of cryoglobulins. Though it is well described in literature, remains still unfamiliar due to its rare occurrence. The observation of such abnormal findings and histogram must prompt microscopic examination of fresh blood sample and stained blood films, which may permit the visualization of precipitates. In such cases physician should be informed promptly, as such anomalies may be the first indication of presence of cryoglobulinemia.

AKNOWLEDGEMENT

Authors want to Aknowledge our technical team Mr. Sachin Suryavanshi, Mrs Ankita Deshmukh, Mr Sunil and Mr Amit Nargacche and entire Team Metropolis for all their skills, presence of mind and cooperation throughout follow up of this case.

Conflicts of Interest: Nil.

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