Vasculitis with renal involvement in essential mixed cryoglobulinemia: Case report and mini-review

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Abstract

The discovery of a strong association between hepatitis C virus (HCV) infection and mixed cryoglobulinemia (MC) has led to an increasingly rare diagnosis of idiopathic essential MC (EMC). The incidence of EMC is high in regions where there is a comparatively low HCV infection burden and low in areas of high infection prevalence, including HCV. The diagnosis of EMC requires an extensive laboratory investigation to exclude all possible causes of cryoglobulin formation. In addition, although cryoglobulin testing is simple, improper testing conditions will result in false negative results. Here, we present a 46-year-old female patient with a case of EMC with dermatological and renal manifestations, highlighting the importance of extensive investigation to reach a proper diagnosis. We review the need for appropriate laboratory testing, which is often neglected in clinical practice and which can result in false negative results. This review also emphasizes the significance of an extended testing repertoire necessary for better patient management. Despite a strong association of MC with HCV infection and other causes that lead to cryoglobulin formation, EMC remains a separate entity. Correct diagnosis requires proper temperature regulation during sample handling, as well as characterization and quantification of the cryoprecipitate. Inclusion of rheumatoid factor activity and complement levels in the cryoglobulin test-panel promotes better patient management and monitoring. Consensus guidelines should be developed and implemented for cryoglobulin detection and the diagnosis of cryoglobulinemic syndrome, which will reduce variability in inter-laboratory reporting.

Key words: Cryoglobulin characterization; Cryoglobulin detection; Essential mixed cryoglobulinemia; Cryoglobulinemic glomerulonephritis; Hepatitis C virus; Renal manifestations

Core tip: The diagnosis of essential mixed cryoglobulinemia (EMC) requires thorough laboratory investigation to exclude all possible causes of cryoglobulin formation. Although cryoglobulin testing is simple, it requires careful temperature regulation to avoid false negative results. The testing panel should also include cryoglobulin quantification and characterization, rheumatoid factor activity and complement levels, to better facilitate patient management. Furthermore, there is a need to develop and implement consensus guidelines for laboratory and clinical diagnoses of EMC.

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Cryoglobulins (CGs) are abnormal proteins/immunoglobulins (Igs) that precipitate out of serum at temperatures below 37°C [1]. Initially identified by Winthrop and Buell in 1933 and later named by Lerner et al [2], CGs are found in many disorders ranging from autoimmune and infectious diseases to malignancies [3-5]. Cryoglobulinemia, the presence of CGs in blood, is significant only when the associated symptoms are present [6,7]. Mixed cryoglobulinemia (MC) is strongly associated with hepatitis C virus (HCV) infection [8-11]. Other diseases, including autoimmune and infectious disorders, can also lead to MC [12]. When the cause for MC cannot be identified, the disease is termed idiopathic or essential mixed cryoglobulinemia (EMC) [13-15]. An extensive laboratory investigation is required to rule out the known conditions associated with cryoglobulinemic vasculitis to impart a proper diagnosis of EMC [3-4,16]. Here, we present a 46-year-old female patient with EMC and briefly review the current laboratory methods for diagnosing this syndrome.

**INTRODUCTION**

Cryoglobulins (CGs) are abnormal proteins/immunoglobulins (Igs) that precipitate out of serum at temperatures below 37°C [1]. Initially identified by Winthrop and Buell in 1933 and later named by Lerner et al [2], CGs are found in many disorders ranging from autoimmune and infectious diseases to malignancies [3-5]. Cryoglobulinemia, the presence of CGs in blood, is significant only when the associated symptoms are present [6,7]. Mixed cryoglobulinemia (MC) is strongly associated with hepatitis C virus (HCV) infection [8-11]. Other diseases, including autoimmune and infectious disorders, can also lead to MC [12]. When the cause for MC cannot be identified, the disease is termed idiopathic or essential mixed cryoglobulinemia (EMC) [13-15]. An extensive laboratory investigation is required to rule out the known conditions associated with cryoglobulinemic vasculitis to impart a proper diagnosis of EMC [3-4,16]. Here, we present a 46-year-old female patient with EMC and briefly review the current laboratory methods for diagnosing this syndrome.

**CASE REPORT**

A 46-year-old female patient presented to the Department of Nephrology after suffering from anuria and renal failure for ten days. Prior to admission at our institute, the patient had already undergone four sessions of dialysis in another hospital. The only significant medical history for the patient was a laparoscopic cholecystectomy she received two months prior. The patient had no history of hypertension, diabetes mellitus or other renal disease.

Upon routine examination, the patient was pale, icteric, apyrexial and normotensive, with facial and pedal edema. Palpable purpura was present on both hands. No other abnormalities were revealed during the remainder of the systemic examination. Laboratory investigations revealed normochromic and normocytic anemia, elevated total leukocyte counts with neutrophilia, and thrombocytopenia. Renal and liver functions were abnormal and proteinuria was present (+2 on a dipstick) (Table 1). A chest X-ray and an abdominal ultrasound were normal. There was no evidence of malignancy or infections upon bone marrow biopsy, and all requested cultures (blood, urine, and bone marrow) to rule out infections were negative. A provisional diagnosis of vasculitis was made.

In the absence of infections and malignancies, further examinations were carried out (Table 1). An immunological work-up showed low complement levels (C3 and C4) and a negative anti-nuclear antibody test, which remained negative at a 6-mo follow-up. Type II CGs were detected, with a cryocrit of 6% (Figure 1A and B). Tests for anti-HCV antibodies and HCV RNA were negative. Other tests to evaluate vasculitis were also negative (Table 1). Renal biopsy showed evidence of cryoglobulinemic glomerulonephritis (Figure 1C and D).

Before vasculitis was suspected, the patient was empirically managed with antibiotics and hemodialysis, but did not show improvement. After the diagnosis of cryoglobulinemic glomerulonephritis was made, the patient was treated with intravenous somulomedrol (15 mg/kg body weight) for three days, monthly intravenous injections of cyclophosphamide (15 mg/kg body weight) for five months, and plasmapheresis. After 14 plasmapheresis sessions, the patient’s renal functions normalized (serum creatinine 1.2 mg/dL) and the cryocrit decreased to 1%. Cryocrit levels subsequently decreased to less than 1% over a

**Table 1 Laboratory results from a patient with essential mixed cryoglobulinemic syndrome**

| Laboratory investigation | Test results (reference ranges) |
|--------------------------|--------------------------------|
| Complete blood cell counts | Hemoglobin 8.5 g/dL (11.5-15.4 g/dL) |
|                          | White blood cell count 15200 × 10^3/L (4.0-11.0 × 10^3/L) |
|                          | Neutrophils 87% (40%-80%) |
|                          | Lymphocytes 10% (20%-40%) |
|                          | Platelets 22000/L (150-400 × 10^3/L) |
| Coagulation profile      | APPT 28 s (25.8 s) |
|                          | INR 1.98 (< 1.35) |
| Renal functions          | Urea 88 mg/dL (15.39 mg/dL) |
|                          | Creatinine 6.2 mg/dL (0.5-1.5 mg/dL) |
| Liver functions          | Total bilirubin 6.1 mg/dL (0.2-1.0 mg/dL) |
|                          | Direct bilirubin 3.2 mg/dL (0.025 mg/dL) |
|                          | Alkaline phosphatase 202 U/mL (32-92 U/mL) |
|                          | Alanine amino transferase 10 U/mL (10-40 U/mL) |
|                          | Gamma glutamyl transferase 204 U/mL (7-64 U/mL) |
| Bone chemistry           | Calcium 7.5 mg/dL (8.4-10.2 mg/dL) |
|                          | Phosphorus 4.4 mg/dL (2.5-4.0 mg/dL) |
|                          | Albumin 2.2 mg/dL (3.5-5.0 mg/dL) |
| Urine detailed report    | Anuric on admission, later showed +2 proteinuria; no red blood cells, no cast |
| Immunological work-up    | Complement 3 0.4 g/L (0.79-1.52 g/L) |
|                          | Complement 4 0.01 g/L (0.16-0.38 g/L) |
|                          | Anti-nuclear antibodies Negative |
|                          | ANCA Negative |
|                          | Rheumatoid factor activity Positive: 859 IU/mL (cut off < 20 IU/mL) |
|                          | Anti-cyclic citrillinated peptide Negative |
|                          | Anti-extractable nuclear antigens Weakly positive for anti-Ro52 |
|                          | C-reactive protein 0.1 mg/dL (cut off < 0.7 mg/dL) |
| Viral markers            | HbAg Negative |
|                          | Anti-HCV Negative |
|                          | Anti-HIV Negative |
|                          | HCV RNA Not detected |

ANCA: Anti-neutrophil cytoplasmic antibodies; APTT: Activated partial thromboplastin time; HbAg: Hepatitis B surface antigens; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; INR: International normalized ratio.
period of six months. Normal renal function was found to be maintained at the one-year follow-up examination.

DISCUSSION

Brouet et al. [17] immunochemically classified CGs into three types. Type I CGs are characterized by the presence of only a monoclonal component, mostly IgMκ, and are usually found in patients with lymphoid malignancies such as multiple myeloma. Mixed CGs are comprised of type II, characterized by the presence of polyclonal Igs with a monoclonal component, and type III, which contain only polyclonal Igs [17]. Mixed CGs are also associated with rheumatoid factor (RF) activity [7,18,19].

MC with or without HCV infection

Before the discovery of HCV infection, most cases of MC were defined as EMC, without an underlying identifiable cause [20]. In the early 1990s, HCV became recognized as the major cause of CG formation, with approximately 90% of MC patients having the virus [3]. It has been reported that approximately 50% of HCV-infected patients develop CGs in their blood [3]. The viral particles have also been detected in cryoprecipitates [21,22]. Since this discovery, the diagnosis of EMC has become rare [10,16,23].

Autoimmune or connective tissue diseases [13,14,24,25], lymphoproliferative disorders [26] and other infections such as bacterial, viral and parasitic [27] infections, have been attributed to MC development. However, 5%-48% of cases with cryoglobulinemic syndrome have still been reported as EMC [28,30]. The incidence of EMC is higher in regions with a low prevalence of HCV infection, and lower in areas with high HCV prevalence [3]. In Pakistan, more than ten million people are infected with HCV [31,32] and there is a high burden of other infections, especially in immunocompromised populations [33], which makes the risk for EMC lower. Therefore, an extensive and thorough examination is required to rule out other causes of cryoglobulinemia and properly assign an EMC diagnosis [34,35].

Symptomatic MC

Cryoglobulinemic syndrome was first described by Meltzer et al. [18] in 1966 as a triad of symptoms including purpura, arthralgia and weakness. The syndrome may also involve the visceral organs, including hepatospleno-megaly [16], glomerulonephritis [37] and lymphadenopathy [30]. The presence of CGs is not always associated with clinical features [24], and low levels of CGs have been found in HCV-infected patients without associated symptoms [3,6,7]. During renal replacement therapy for end stage renal disease, low levels of CGs have been detected in a substantial number of non-infected and HCV-infected patients, with and without symptoms [39].

Renal manifestations in MC

The incidence and presentation of renal manifestations
in HCV-associated cryoglobulinemia is well document-
ed[10,11]. In non-HCV cryoglobulinemia, including EMC, the evidence of renal manifestations is predominantly confined to case reports[20,26,42]. It is important to note that most of the data on renal manifestations in EMC have come from studies on French populations[16,28,30,34]. In a multicenter French study comprised of 20 HCV-negative patients with renal manifestations, 50% of the patients had EMC[40]. In this study, microscopic hematuria and hypertension were the most common presenting clinical features, followed by nephrotic range proteinuria and renal insufficiency. In a separate French study, which contained 33 patients with type II CGs, 13 (39%) of the patients had EMC. Of these patients, eight (62%) had renal involvement, with four of those suffering from renal insufficiency[30]. In a Dutch study of 22 non-HCV patients with type II CGs, seven (32%) were found to have EMC, of which six patients had renal involvement[45].

CG detection and testing repertoire
Detection of CGs requires stringent temperature control, a fact that cannot be stressed enough. It is imperative to maintain the sample temperature at 37 °C from the time the sample is collected until separation of the serum[45]. Failure to keep samples at 37 °C will result in false negative results[4,44,45]. In our own experience, centrifuges even those lacking a heater-with a wide temperature range (e.g., 0 °C–40 °C) can be used to separate serum at 37 °C. This can be achieved by preheating the instrument with an initial dry run at >37 °C and then immediately transferring the samples from a 37 °C incubator to the centrifuge. Once centrifugation is complete, the samples should be immediately taken out of the centrifuge and placed in a 37 °C incubator until the supernatant can be separated. Adherence to this protocol helps to avoid red blood cell contamination, which can hinder the visibility of CGs.

Although cryoglobulinemic syndrome was first identified in 1933, the significance of a proper CG detection assay and awareness for the condition is still not fully recognized. A large European survey of 140 laboratories revealed that only 37 (26%) were following the appropriate standard procedure for CG detection[40]. The reporting of CG positive results should include quantification and characterization of these proteins, RF activity and complement (C3 and C4) levels[14,44]. Although CG concentration does not correlate well with disease activity, CG levels, along with RF activity and complement levels, are useful in monitoring the disease[14].

This case report highlights the importance for proper and thorough investigation of a patient in order to diagnose cryoglobulinemic syndrome in the absence of infections (including HCV) and other known causes of MC. Furthermore, the quantification and characterization of CGs is important, as the decrease in cryocrit level in the present case correlated well with clinical remission. Although repeated anti-nuclear antibody tests were negative, there was a weak positivity for anti-Ro52 antibodies, which can be found in various conditions, including cryoglobulinemia[46]. Similarly, a low C-reactive protein has also been observed in the serum of patients with MC, not related to systemic lupus erythematosus[9,47].

CG quantification can be done in various ways, including total protein or immunoglobulin content of cryoprecipitates, cryocrit determination[45], gel-based semi-quantitative tests[46] and other methods[49,50]. Determination of levels using a cryocrit is less cumbersome and more informative, though it should be noted that a cryocrit is less sensitive and specific than quantification of washed cryoproteins[44]. CG characterization provides valuable information that aids in long-term patient management[46,47]. Patients with type II CGs should be closely monitored for B cell lymphoma development, especially in patients that are difficult to treat[6,14]. Lack of standardization and an incomplete testing repertoire inadvertently lead to distrust of laboratory results by the treating physicians and undermine the significance of CG detection in suspected cases, which may poorly affect patient management[4,45,49,50]. This is very important given that better treatment options are increasingly becoming available to manage this rare yet troublesome disorder[49,51,52].

Diagnosis of cryoglobulinemic syndrome: Diagnostic versus classification criteria
Varied clinical presentation and insignificant correlation of CG concentration with clinical manifestations have made the diagnosis of cryoglobulinemic syndrome quite difficult. Thus, various diagnostic and classification criteria have been proposed to facilitate in the diagnosis of this condition[7,54]. Diagnostic criteria are based on major and minor laboratory and clinical features[14], and preliminary classification criteria have been proposed, including a questionnaire regarding symptoms, laboratory findings (including CG types, complement levels, RF activity and HCV presence) and organ involvement[1]. Diagnostic and classification criteria indicate that the presence of CGs in serum is the gold standard for a cryoglobulinemic vasculitis diagnosis. Moreover, the presence of clinical features consistent with this syndrome in the absence of CGs warrants repeat testing for these proteins given the possibility for false negative results[7,33].

In conclusion, despite a strong association of MC with HCV infection and other causes that can lead to CG formation, EMC remains a separate entity. Accurate diagnosis requires a thorough laboratory investigation to rule out the known causes of MC. Correct laboratory diagnosis not only requires proper handling of the samples for CG detection, but also quantification of the amount of cryoprecipitate and identification of the type of CGs present. Other tests, such as RF activity and complement levels (C3 and C4), should be included in the testing panel to ensure better patient management and monitoring. There is a need to develop and implement consensus guidelines for the detection of CGs and cryoglobulinemic syndrome to reduce variability in inter-laboratory reporting and to establish the diagnostic criteria for this clinical syndrome.
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COMMENTS

Case characteristics
A 46-year-old female presented with anuria, acute renal failure and palpable purpura.

Clinical diagnosis
The patient was diagnosed with essential mixed cryoglobulinemic syndrome.

Differential diagnosis
Vasculitis causing acute renal failure was ruled out.

Laboratory diagnosis
The following laboratory tests were acquired from the patient: negative cultures, anti-nuclear antibodies, anti-neutrophil cytoplasmic antibodies, anti-hepatitis C virus (HCV) and HCV RNA, and positive cryoglobulins (CGs) with low C3 and C4 levels and high rheumatoid factor (RF) activity.

Imaging diagnosis
The patient had normal chest X-ray and abdominal ultrasound reports.

Pathological diagnosis
Hematoxylin and eosin staining, in addition to periodic acid-Schiff stains from a renal biopsy, showed evidence of cryoglobulinemic glomerulonephritis.

Treatment
The patient was treated with intravenous injections of solumedrol and cyclophosphamide and was discharged on prednisolone and azathioprine.

Related reports
Essential mixed cryoglobulinemia (EMC) is rare and requires a thorough examination to rule out other known causes. A complete work-up for cryoglobulinemic syndrome, including quantification and characterization of cryoprecipitates, complement levels and RF activity, is required for proper management and monitoring.

Term explanation
Cryoglobulinemia is a disorder in which CGs precipitate out of the blood at temperatures lower than 37°C and is often associated with hepatitis C virus infection.

Experiences and lessons
Early recognition, extensive diagnostic work-up and proper patient management, including regular follow-ups and immunological monitoring, resulted in a favorable patient outcome in this study.

Peer review
This case report with a mini-review highlights the importance of a thorough investigation for the rare and often neglected diagnosis of EMC. This article will be of great benefit to clinicians as it increases the awareness regarding the clinical utility and proper testing and interpretation of cryoglobulin detection assays.

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