Lesson from an intriguing case of cryoglobulinemia

Michele Barone, Raffaele Licinio, Annabianca Amoruso, Maria Teresa Viggiani, Angela Maria Vittoria Larocca, Alfredo Di Leo

Michele Barone, Raffaele Licinio, Annabianca Amoruso, Maria Teresa Viggiani, Alfredo Di Leo, Department of Emergency and Organ Transplantation, University of Bari, 70100 Bari, Italy
Angela Maria Vittoria Larocca, Department of Biomedical Science and Human Oncology, University of Bari, 70100 Bari, Italy
Author contributions: Barone M, Licinio R, Amoruso A, Viggiani MT and Larocca AMV contributed equally to this work; Di Leo A approved the final version.
Correspondence to: Alfredo Di Leo, Professor, Department of Emergency and Organ Transplantation, University of Bari, Policlinico G. Cesare Sq 11, 70124 Bari, Italy. alfredo.dileo@uniba.it
Telephone: +39-80-5592577 Fax: +39-80-5593251
Received: May 5, 2012 Revised: July 30, 2012
Accepted: August 14, 2012
Published online: January 14, 2013

Abstract
Cryoglobulinemia is a pathological condition usually associated with hepatitis C virus (HCV) chronic liver disease and less commonly with autoimmune or lymphoproliferative disorders. The possible association of cryoglobulinemia with hepatitis B virus (HBV) infection is not widely accepted. In our patient, serum negativity for HCV markers initially led us to consider two other causes of cryoglobulinemia. Myelodysplastic disorders were excluded on the basis of hematological studies, while serum markers for active HBV infection were positive. Surprisingly, the detection of HCV RNA in the cryocrit, even in the absence of anti-HCV antibodies, suggested a pathogenetic role of HCV in this case of cryoglobulinemia. Negative "first level" tests for HCV in the serum do not completely exclude HCV involvement in the pathogenesis of cryoglobulinemia. Analysis of the cryoprecipitate is always essential for diagnosis.

© 2013 Baishideng. All rights reserved.

Key words: Cryocrit; Hepatitis B virus; Hepatitis C virus; Myelodysplastic disorders; Cryoglobulinemia

INTRODUCTION
Cryoglobulinemia is a pathological condition usually associated with hepatitis C virus (HCV) chronic liver disease. Cases of cryoglobulinemia associated with autoimmune or lymphoproliferative diseases are less common, while the association with hepatitis B virus (HBV) infection is not widely accepted, the HBV virus being mostly associated with other immunocomplex-related disorders.

Cryoglobulins are proteins that can precipitate at low temperatures (< 4 °C). The final product of this precipitate, termed the cryocrit, can be characterized on the basis of its composition: polyclonal immunoglobulins, monoclonal immunoglobulins or the presence of rheumatoid activity (RA). Three types of cryoglobulinemia have been identified, namely: type 1, including monoclonal cryoglobulins (IgM, IgG and IgA) without any RA; type 2, that includes a monoclonal component (usually IgM with RA) and a polyclonal component (usually IgM/IgG); type 3, that includes several polyclonal components and a component with RA (usually IgG/IgM).

Normally, cryoglobulinemia type 1 is associated with lymphoproliferative/myelodysplastic diseases such as multiple myeloma, Waldenstrom macroglobulinemia, chronic lymphatic leukemia, non-Hodgkin lymphoma, etc. Cryoglobulinemia type 2 is associated not only with lymphoproliferative diseases and plasma cellular dyscrasias but also with infectious and autoimmune diseases (rheumatoid arthritis, Sjogren's syndrome, etc.). Finally, cryoglobulinemia type 3 is frequently associated with autoimmune or infectious diseases. The mechanism responsible
for cryoglobulin formation during lymphoproliferative or autoimmune diseases is known, but the etiopathogenesis of the forms defined as “essential cryoglobulinemias”, which occur as isolated events, has still to be clarified. Among the essential forms, the most common variant is cryoglobulinemia type 2. The amount of cryoglobulins, expressed as a percentage of the serum volume, can vary significantly among patients and may change over time. It is therefore useful to evaluate the serum cryocrit, also during follow-up, in order to assess prognosis and treatment. Cryoglobulinemia characterization by protein electrophoresis and immunofixation is equally important to define the type26. In 1966, Meltzer et al 7 described a clinical triad characterized by palpable purpura, arthralgia and asthma associated with nephropathy and neuropathy. It is widely accepted that presentation of the complete triad is rare in clinical practice. In fact, most patients are asymptomatic and purpura, often fleeting, is the only clinical manifestation. The frequent association of HCV infection with the great majority of cryoglobulins, initially defined as essential, suggested the involvement of this virus in the pathogenesis of mixed cryoglobulinemia. Actually, mixed cryoglobulinemia (type 2 or 3) is found in 50% of patients with chronic HCV infection8,9. Therefore, in patients with mixed cryoglobulinemia, the possibility of HCV infection should always be considered, and serum tests should be performed for the detection of anti-HCV antibodies and HCV RNA.

CASE REPORT

Our patient was in apparent good health until the age of 73 when he underwent percutaneous angioplasty for acute non-Q myocardial infarction, after which he was prescribed anticoagulant and antihypertensive therapy. At the age of 77, he had an outpatient medical visit because of the first manifestation of purpura of the lower limbs, arthralgia and a sense of postural instability, raising the suspicion of a cryoglobulinemic syndrome. At this time, the cryocrit was positive (35%), and the patient underwent several serological and molecular investigations that excluded HCV (negative anti-HCV and HCV RNA) and HIV infection and demonstrated chronic HBBV infection (surface antigen of the hepatitis B virus (HBsAg) 11 700 IU/mL, anti-HBsAg negative, hepatitis B core antibody positive, anti-hepatitis Be antibody positive, HBV-DNA 2 410 000 IU/mL, anti-HDV negative). Immediately after, he was admitted to our unit because of the onset of a hypertensive crisis that was not controlled by the administration of calcium antagonists, beta-blockers and diuretics. Renal function monitoring demonstrated the following values: creatinine 1.85 mg/dL, creatinine clearance 20 mL/min and 24-h proteinuria 1.3 g/24 h. Echotomographic examination showed ultrasongraphic signs of cirrhosis, and hepatic elastometry yielded a stiffness value of 47.2 kPa. Other laboratory tests demonstrated aspartate aminotransferase × 6.7 upper limit of normal (ULN), alanine aminotransferase × 4.9 ULN and gamma-glutamyl transeptidase × 5.2 ULN, and total bilirubin 1.8 mg/dL. On the basis of these data, the patient was started on antiviral treatment (0.5 mg entecavir every 72 h) and prednisone, 50 mg/d, to be tapered to 10 mg/d. Although the hypertensive crisis could presumably be ascribed to a cryoglobulin-induced nephropathy attributable to HBV, considering that the serum β2-microglobulin levels were markedly increased (7.2 mg/L, < 2 ULN) and gamma-globulin showed a monoclonal peak, we also considered the possibility of cryoglobulinemia associated with a myeloproliferative disorder. The characteristics of the cryoprecipitate were therefore evaluated in order to define the type of cryoglobulinemia. We found rheumatoid factor positivity, lower C3 and C4 levels, 24% cryocrit, while serum immunofixation showed a K monoclonal IgM component, and cryocrit immunofixation a K monoclonal IgM component with polyclonal IgG. On the basis of these elements we made a diagnosis of mixed cryoglobulinemia type 2, that did not exclude a myeloproliferative disorder, so a peripheral blood smear and bone marrow biopsy were performed, and these excluded a myeloproliferative disease. During the period of hospitalization we observed an improvement in renal function (creatinine decreased to 1.4 mg/dL, 24-h creatinine clearance increased to 35 mL/min, and 24-h proteinuria decreased to 0.9 g) and a striking reduction of the cryocrit (6.1%). Surprisingly, cryoprecipitate characterization demonstrated the presence of HCV RNA (807 copies/mL, by m2000 Real Time System, Abbott, IL, United States, Figure 1) and for this reason, we rechecked the serum anti-HCV antibodies and HCV RNA, and the absence of both was confirmed (HCV RNA undetectable using 50 IU/mL as cut-off).

DISCUSSION

In the present case, the detection of HCV components in the cryocrit cast some doubt on the pathogenesis of the cryoglobulinemia that we initially attributed to HBV infection. It has been reported that in chronic HCV patients with mixed cryoglobulinemia, HCV RNA may be undetectable in the plasma (due to the entrapment of HCV RNA in the cryoprecipitate) and the diagnosis of HCV infection is based on the presence of anti-HCV antibodies8. However, in our patient anti-HCV antibodies were undetectable in the serum. This could be explained by interference between the two viruses that may even lead to inhibition of HCV replication in patients with chronic hepatitis C with a superimposed HBV infection.

This experience suggests that negative tests for HCV detection in the serum (anti-HCV antibodies and HCV RNA) do not completely exclude the involvement of HCV in the pathogenesis of cryoglobulinemia, and show that analysis of the cryoprecipitate is always essential, since detection of HCV RNA in the cryoprecipitate is the most sensitive method. Once HCV infection has been excluded, investigations must take into account other causes

---

Barone M et al. An intriguing case of cryoglobulinemia

WJG | www.wjgnet.com 305 January 14, 2013 | Volume 19 | Issue 2
of cryoglobulinemia, such as autoimmune and myelo- or lymphoproliferative disorders and HBV infection.

REFERENCES
1. Ferri C, La Civita L, Longombardo G, Greco F, Bombardieri S. Hepatitis C virus and mixed cryoglobulinaemia. *Eur J Clin Invest* 1993; 23: 399-405 [PMID: 8397090 DOI: 10.1111/j.1365-2362.1993.tb00782.x]
2. Trendelenburg M, Schifferli JA. Cryoglobulins are not essential. *Ann Rheum Dis* 1998; 57: 3-5 [PMID: 9536813 DOI: 10.1136/ard.57.1.3]
3. Johnson RJ, Couser WG. Hepatitis B infection and renal disease: clinical, immunopathogenetic and therapeutic considerations. *Kidney Int* 1990; 37: 663-676 [PMID: 1968522 DOI: 10.1038/ki.1990.32]
4. Liang TJ. Hepatitis B: the virus and disease. *Hepatology* 2009; 49: S13-S21 [PMID: 1939811 DOI: 10.1002/hep.22881]
5. Cakir N, Pamuk ON, Umit H, Midilli K. Successful treatment with adefovir of one patient whose cryoglobulinemic vasculitis relapsed under lamivudine therapy and who was diagnosed to have HBV virologic breakthrough with YMDD mutations. *Intern Med* 2006; 45: 1213-1215 [PMID: 17139120 DOI: 10.2169/internalmedicine.45.1816]
6. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974; 57: 775-788 [PMID: 4216269 DOI: 10.1016/0002-9343(74)90852-3]
7. Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia--a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; 40: 837-856 [PMID: 4956871]
8. Bichard P, Ounanian A, Girard M, Baccard C, Rolachon A, Renversez JC, Cordonnier D, Seigneurin JM, Debru JL, Zarski JP. High prevalence of hepatitis C virus RNA in the supernatant and the cryoprecipitate of patients with essential and secondary type II mixed cryoglobulinemia. *J Hepatol* 1994; 21: 58-63 [PMID: 7525697]
9. Alberti A, Pontisso P, Chemello L, Fattovich G, Benvengù L, Belusi F, De Mitri MS. The interaction between hepatitis B virus and hepatitis C virus in acute and chronic liver disease. *J Hepatol* 1995; 22: 38-41 [PMID: 7602074]
10. Sagnelli E, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; 36: 1285-1291 [PMID: 12395342]

**Figure 1** Real time polymerase chain reaction amplification plot for hepatitis C virus RNA. The presence of viral RNA started to become evident after 19.4 cycles and the amount of RNA was 807 copies/mL.