Acta Derm Venereol (Stockh) 1995; 75: 234–236

# Cryoglobulinemic Vasculitis Associated with Hepatitis C Virus Infection

## *A Report of Eight Cases*

FRANCISCO REVENGA ARRANZ[1], ROSA DÍAZ DÍAZ[1], LUIS IGLESIAS DÍEZ[1], BEGOÑA CASSIS HERCE[2], FERNANDO SÁNCHEZ GÓMEZ[2] and ANTONIO FUERTES ORTIZ[3]

*Departments of [1]Dermatology, [2]Gastroenterology and [3]Microbiology, Hospital Universitario 12 de Octubre, Madrid, Spain*

**Cryoglobulinemia has an unknown etiology in many cases. In the last years, hepatitis C virus has been known to be involved as a causative agent of so called essential mixed cryoglobulinemia.**

**We report 8 patients suffering from cutaneous vasculitis and chronic hepatitis C virus infection. Their sera showed cryoglobulinemia (6 cases mixed, 2 cases not typified), rheumatoid factor and complement consume. Their hepatitis C virus infection was confirmed by PCR. Hepatitis C virus RNA was found by PCR in 6/6 cryoprecipitates and 5/6 supernatants. The patients were followed for 0.5–12 years. Three of them developed renal affection (2 membranoproliferative glomerulonephritis, 1 not biopsied). Two patients were treated with alpha interferon. One patient noted a marked improvement in her cutaneous vasculitis with interferon treatment, lasting for 18 months after the end of treatment.**

**We recommend the search of hepatitis C virus infection in every case of mixed cryoglobulinemia. Interferon therapy may be useful in some of these cases.** ***Key words: cryoglobulins; HCV; immunotherapy.***

(Accepted October 25, 1994.)

Acta Derm Venereol (Stockh) 1995; 75: 234–236.

F. Revenga Arranz, Servicio de Dermatología, Hospital Universitario 12 de Octubre, Crta. de Andalucía s/n, 28041 Madrid, Spain.

Cryoglobulins are serum immunoglobulins that have the property of reversibly precipitating in the cold (1). Brouet et al. (1) divided cryoglobulinemia into three types according to the composition of cryoglobulins. Type I is composed of monoclonal cryoglobulins and is commonly associated with lymphoid malignancies. Cryoglobulinemia cases related to infections are usually type II or III, also called mixed cryoglobulinemia (MC).

A large number of MC are of unknown etiology and are called essential mixed cryoglobulinemia (EMC). Levo et al. (2) suggested that hepatitis B virus (HBV) could be an etiologic agent of some EMC and pointed out that other viruses could also be involved. Since hepatitis C virus (HCV) was cloned in 1989 (3), it has been related to several dermatoses (Table I). MC and porphyria cutanea tarda show the strongest association.

## MATERIALS AND METHODS

### *Patients*

Eight patients (2 males and 5 females, aged 37 to 66 years) consulted our department of dermatology for recurrent bouts of purpuric cutaneous lesions located on both legs. Their main clinical and serological data appear in Table II.

The patients had suffered from cutaneous purpuric lesional bouts associated with arthralgias and in some cases with mild fever from 3 months to 10 years prior to their first consultation at our department. The lesions involved mainly the legs and feet but could extend to the thighs and even to the upper limbs and abdomen. The lesions were usually purpuric papules and macules, but we could also observe ulcers, bullae, nodules and necrotic lesions.

Three patients (cases 2, 3, 5) had been diagnosed of chronic liver disease before their consultation at our department. Three patients (cases 4, 7, 8) had been transfused several years before; the others did not remember any possible source of infection. Physical examination, including a thorough search for adenopathies, did not show any important data, except ascites and hepato-splenomegalia in case 3. A biopsy from skin lesions was diagnosed of leukocytoclastic vasculitis in all the patients. ALT and AST were above the normal limits at least one occasion in all patients, but only case 3 had analytical data of hepatocellular dysfunction. The diagnostic profile revealed the presence of cryoglobulins, rheumatoid factor activity and consummation of complement in all cases. Cryoglobulins were typified as type II (monoclonal IgM-kappa, polyclonal IgG) in six cases and could not be typified in cases 2 and 3. Renal function was measured by creatinine clearance, urine biochemistry, 24 h proteinuria and 12 h Addis test. It was within normal limits in all cases at diagnosis of vasculitis, except in case 3, who showed a nephrotic syndrome. Unfortunately a renal biopsy could not be done in this patient due to bleeding diathesis. One patient (case 7) showed a monoclonal peak in her plasma protein electrophoresis. A bone marrow biopsy and a thoraco-abdominal CT ruled out the presence of a lymphoid malignancy. ANA, ENA and dsDNA were negative in all the patients. HCV infection was diagnosed by ELISA and RIBA-4 (Ortho Diagnostics) and confirmed by nested PCR (4). Two patients (cases, 2, 6) had markers of a past HBV infection but were HBsAg- and HBeAg-negative. Cryoprecipitate was carefully separated from supernatant in 6 patients. Nested PCR for HCV was found in 6/6 cryoprecipitates and in 5/6 supernatants. The patient in whose supernatant HCV-RNA was not found (case 4) had been previously treated with alpha 2b interferon for 12 months and until 18 months before been tested.

A liver biopsy was performed in 4 patients (cases 2–5). It was diagnosed as chronic active hepatitis in 3 cases and as cirrhosis in one case.

### *Follow-up and treatment*

We have followed these 8 patients for 0.5–12 years. During this time, patients have experienced recurrent bouts of lesions. They were mainly triggered by standing. No patients have noted any exacerbation in cold weather months. Two patients (cases 1, 2) developed renal affection during the follow-up period. A renal biopsy was done and was diagnosed in both patients as membranoproliferative glomerulonephritis (MP Gn). Case 1 has maintained a baseline renal function for 6 years after MP Gn was diagnosed. Renal function in case 2 began to worsen 6 weeks after alpha 2b interferon treatment, at a dose of $3 \times 10^6$ U/sc 3 days/w, was introduced and evolutioned to acute renal failure complicated with sepsis by *Pseudomonas aeruginosa* and death.

Three patients died, two of them due to complications of their chronic hepatitis (cases 3, 5) and one due to complications of her acute glomerulonephritis (case 2).

The patients were treated with bed rest, non-steroidal anti-inflammatory drugs and/or oral corticosteroids, with variable success. Two patients were treated with alpha 2b interferon (cases 2, 4). Case 2 was treated with $3 \times 10^6$ U/sc 3 days/w. Treatment was badly tolerated by this patient and was stopped when an acute glomerulonephritis was



 ISSN 0001-5555

Table I. *Dermatosis related to hepatitis C virus*

Well-defind association:
- Porphyria cutanea tarda (8)
- Mixed cryoglobulinemia (6, 9–12)

Poorly defined association:
- Lichen planus (13)
- Urticaria (14)
- Panniculitis (15,16)
- Sjögren's syndrome (17)
- Malacoplakia (18)

detected 6 weeks after interferon therapy had begun. This patient did not have anti-liver-kidney microsomal antibodies or any other marker of autoimmunity. Case 4 was treated for 12 months at a dose of $5 \times 10^6$ U/sc 3 days/w. This patient noted a significant reduction in the number and intensity of her cutaneous vasculitis a few weeks after interferon therapy had begun. This improvement has continued 18 months after it was stopped, but a second liver biopsy at the end of the treatment did not show any difference in the hepatitis activity index.

## DISCUSSION

A large number of MC are of unknown origin. They are called essential mixed cryoglobulinemia (EMC). In 1977, Levo et al. (2) were the first authors to note that HBV could be involved in some cases of EMC, as they found an active or past HBV infection in 74% of 19 cases of EMC. In their article they pointed out that other viruses could also be involved in EMC (2). It is considered that cryoprecipitable serum in these viral related-EMC is composed of the viral particle, antibodies against it and a monoclonal immunoglobulin with rheumatoid factor activity. This rheumatoid factor would be produced by a lymphocyte B clonal expansion, induced by the viral infection (6).

HCV is a single stranded RNA virus, first cloned in 1989 (3). Current data show that this virus is the main etiologic agent for sporadical and parenteral transmitted chronic hepatitis. An HCV infection runs a chronic course in 50% of cases and, in large number of them, leads to liver cirrhosis and, even, to a hepatocarcinoma (7). Its diagnosis remains in serological data, as this virus has not still been cultured. Some advanced laboratories can detect it by PCR (4). HCV has been related to several dermatoses (Table I).

Pascual et al. (9) reported in 1990 the first case of EMC in a patient with serological markers of HCV infection. Later, some case reports and reviews dealing with the association of EMC (type II and III) and chronic HCV hepatitis have been published (6, 10–12), most of them in internal medicine journals. We believe that dermatologists should be aware of this process, as many patients initially consult for the cutaneous lesions induced by their cryoglobulinemia. Five of our 8 patients did not know that they were infected by HCV when they first consulted our department for cutaneous vasculitis. Three of them were the subject of a previous article (5), in which we described 25 cases of cryoglobulinemic vasculitis, before HCV infection tests were available. Eight of those 25 patients had a chronic liver disease; 5 of them had died before the present work began, and the others could be tested for HCV infection with a positive result (cases 1, 4, 6).

Studies by Agnello et al. (10) and Johnson et al. (19) have reinforced the association between HCV and MC, as they did quantitative PCR studies and found a HCV-RNA concentration 10–1000 higher in cryoprecipitate than in non-cryoprecipitable serum (supernatant). We searched for HCV-RNA by nested PCR in cryoprecipitate and supernatant from 6 patients (5). It was found in 6/6 cryoprecipitates and 5/6 supernatants. The patient (case 4) in whose supernatant HCV-RNA could not be amplified had been previously treated with alpha 2b interferon. None of the other 5 patients had received this therapy. This could indicate that alpha 2b interferon treatment induced a lodging of HCV in cryoprecipitable serum.

The clinical course of 6 of our 8 patients was indolent. They had recurrent bouts of cutaneous vasculitis for 2–12 years, but we did not observe any visceral affection. Three patients died (cases 2, 3, 5). Death was due to liver failure in 2 patients. Case 2 died one month after a membranoproliferative glomerulonephritis, possibly related to her MC, had been diagnosed. Her death was caused by a *Pseudomonas* sepsis. On the other hand, case 2 developed a membranoproliferative glomerulonephritis 6 years after MC was diagnosed and had maintained a stable renal function for 6 years, without the need of any additional therapy.

Several authors have found a favourable response to alpha interferon in an important number a patients with MC, whether it was related HCV or not (20, 21). Interferon could exert its beneficial action as an antiviral agent against HCV, by its immunomodulating properties on lymphocytes and natural killer cells or by enhancing immunocomplex clearance. Nevertheless, pa-

Table II. *Clinical and serological data*

| Case | Sex/Age | Cryoglobulin type | ELISA/ RIBA-4 | HCV PCR serum | HCV PCR sup/cryop (a) | Liver biopsy | Renal affection | Follow-up (years) | Death (cause) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M/53 | II | +/+ | + | +/+ | Not done | MP Gn (d) | 12 | |
| 2 | F/48 | Not done | +/+ | + | Not done | C.A.H.(c) | MP Gn | 1 | MP Gn |
| 3 | M/60 | Not done | +/+ | + | Not done | C.A.H. | Yes(e) | 1/2 | Cirrhosis |
| 4 | F/53 | II | +/+ | + | −/+ (b) | C.A.H. | No | 8 | |
| 5 | F/66 | II | +/+ | + | +/+ | Cirrhosis | No | 2 | Cirrhosis |
| 6 | M/50 | II | +/+ | + | +/+ | Not done | No | 10 | |
| 7 | F/58 | II | +/+ | + | +/+ | Not done | No | 2 | |
| 8 | F/37 | II | +/+ | + | +/+ | Not done | No | 8 | |

(a) supernatant/cryoprecipitate; (b) after treatment with alpha 2b interferon; (c) chronic active hepatitis; (d) membranoproliferative glomerulonephritis; (e) renal biopsy not done.

tients successfully treated with alpha interferon often worsen both in their visceral and cutaneous affection when this therapy is stopped. We have treated 2 patients with alpha 2b interferon (case 2, 4). Treatment was badly tolerated in case 2 and was complicated by a glomerulonephritis 6 weeks after it was started. This patient did not have autoimmunity data that have been related with a bad response to interferon treatment. We have found two other cases (22, 23) of HCV-related MC that showed a worsening in their vasculitis and neuritis, respectively, after interferon treatment had begun. Case 4 was treated for one year with a marked improvement in her cutaneous vasculitis, which has persisted for 18 months after the end of treatment, but without any improvement in her chronic hepatitis.

Data derived from our series support the reported association between HCV infection and MC. We highly recommend the study of the HCV status in every case of EMC. In future years many of the cases considered as "essential" up to now may have revealed a viral etiology, as occurred in 3 of our 8 patients.

## REFERENCES


1. 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.
2. Levo Y, Gorevic PD, Kassab HJ, Zucker-Franklin D, Franklin EC. Association between hepatitis B virus and essential mixed cryoglobulinemia. N Engl J Med 1977; 296: 1501–1504.
3. Choo QL, Kuo G, Weiner AJ. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244: 359–362.
4. Bréchot C. Polymerase chain reaction for the diagnosis of hepatitis B and C viral hepatitis. J Hepatol 1993; 17: S35–S41.
5. Díaz Díaz RM, Mestre Bauza F, Jaen Olasolo P, Vanaclocha Sebastián F, Iglesias Díez L. Vasculitis leucitoclástica asociada a crioglobulinemia. Aportación de 25 casos. Actas Dermo-Sifiliograficas 1988; 79: 291–298.
6. Levey JM, Bjornsson B, Banner B, Kuhns M, Malhotra R, Whitman N, et al. Mixed cryoglobulinemia in chronic hepatitis C infection. A clinicopathologic analysis of 10 cases and review of recent literature. Medicine 1994; 73: 53–67.
7. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander J, et al. The natural history of community-acquired hepatitis C in the United States. N Engl J Med 1992; 327: 1899–1905.
8. Herrero C, Vicente A, Burgera M, Ercilla MG, Barrera JM, Vidal J, et al. Is hepatitis C virus infection a trigger of porphyria cutanea tarda? Lancet 1993; 341: 788–789.
9. Pascual M, Perrin L, Giostra E, Schifferli JA. Hepatitis C virus in patients with cryoglobulinemia type II. J Infect Dis 1990; 162: 569–570.
10. Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. N Engl J Med 1992; 327: 1490–1495.
11. Misiani R, Bellavista P, Fenili D, Borelli G, Marchesi D, Massazza M, et al. Hepatitis C virus infection with essential mixed cryoglobulinemia. Ann Intern Med 1992; 117: 573–577.
12. Cacoub P, Fabiani FL, Musset L, Perrin M, Frangeul L, Leger FM, et al. Mixed cryoglobulinemia and hepatitis C virus. Am J Med 1994; 96: 124–132.
13. Jubert C, Pawlotsky JM, Pouget F, Andre C, DeForges L, Bretagne S, et al. Lichen planus and hepatitis C virus-related chronic active hepatitis. Arch Dermatol 1994; 130: 73–76.
14. Reichel M, Mauro TM. Urticaria and hepatitis C. Lancet 1990; 336: 822–823.
15. Patoux-Pibouin M, Le Gall F, Jouanolle H, Le Hir I, Deugnier Y, Chevrant-Breton J. Dermohypodermite subintrante associée a une hépatite chronique active due au virus C. Ann Dermatol Venereol 1991; 118: 854–855.
16. Domingo P, Ris J, Martínez E, Casas F. Erythema nodosum and hepatitis C. Lancet 1990; 336: 1377.
17. Haddad J, Deny P, Munz-Gotheil C, Ambrosini JC, Trinchet JC, Pateron D, et al. Lymphocytic sialoadenitis of Sjögren's syndome associated with chronic hepatitis C virus liver disease. Lancet 1992; 339: 321–322.
18. Bodokh J, Lacour JP, Perrin C, Rainero C, Lebreton E, Grosshans E, et al. Malakoplakie cutanée associée à une hépatite chronique due au virus de l'hépatite C. Ann Dermatol Venereol 1993; 120: 808–810.
19. Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi C, Hartwell P, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N Engl J Med 1993; 328: 465–470.
20. Casato M, Laganá B, Antonelli G, Dianzani F, Bonomo L. Long-term results of therapy with interferon alpha for type II essential mixed cryoglobulinemia. Blood 1991; 78: 3142–3147.
21. Misiani R, Bellavita P, Fenili D, Vicari O, Marchesi D, Sironi PL, et al. Interferon alpha-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994; 330: 751–756.
22. Ferri C, Marzo E, Longombardo F, Greco F, Bombardieri S. Alpha interferon in the treatment of mixed cryoglobulinemia patients. Eur J Cancer 1991; 27: S81–S82.
23. Zimmermann R, Köning V, Bauditz J, Hopf U. Interferon alpha in leukocytoclastic vasculitis, mixed cryoglobulinemia and chronic hepatitis C. Lancet 1993; 341: 561–562.