



HCV and cryoglobulinemic vasculitis

C. FERRI, MD, A.L. ZIGNEGO*, MD, D. GIUGGIOLI, MD, M. SEBASTIANI, MD, M. CAZZATO, MD,
A. ANTONELLI, MD, L. LA CIVITA, MD, P. FADDA, MD, G. LONGOMBARDO, BS, S. PILERI**, MD

■ CRYOGLOBULINEMIA

The presence in the serum of one (monoclonal cryoimmunoglobulinemia) or more immunoglobulins (mixed cryoglobulinemia), which precipitate at temperatures below 37 °C and redissolve on re-warming¹⁻³ is termed “cryoglobulinemia.” This is an *in vitro* phenomenon; the actual mechanism(s) of cryoprecipitation remains obscure. It could be secondary to intrinsic characteristics of both mono- and polyclonal immunoglobulin (Ig) components; it can be caused as well by the interaction among single components of the cryoprecipitate.

Monoclonal cryoimmunoglobulinemia is almost invariably associated with a well-known hematological disorder, and it is frequently asymptomatic *per se*. Similarly, circulating mixed cryoglobulins are commonly detected in a great number of infectious or systemic disorders.¹⁻³ On the contrary, “essential” mixed cryoglobulinemia (MC) represents a distinct disorder¹ characterized by leukocytoclastic vasculitis of small- and medium-sized vessels and frequent multiple organ involvement (**Table 1**).

Cryoglobulinemia is usually classified into three subgroups: type I, composed by a single monoclonal immunoglobulin, usually a paraprotein; type II and III, characterized by polyclonal IgG and mono- or polyclonal IgM RF, respectively.² Cryoglobulinemia type I is mainly found in patients with overt lymphoid tumors (ie, immunocytoma/Waldenström’s macro-globulinemia, multiple myeloma, etc.). MC type II and III can be associated with different infectious, immunological or neoplastic diseases.¹⁻³ The analysis of cryoprecipitates is generally carried out by means of immunoelectrophoresis or immunofixation. Using more sensitive methodologies (immunoblotting or two-dimensional polyacrylamide gel electrophoresis), type II MC shows a microheterogeneous composition; in particular, oligoclonal IgM or a mixture of polyclonal and monoclonal IgM can be detected. This particular serological subset, termed type II-III MC, could represent an intermediate, evolutive state from type III to type II. In any case, this serological condition agrees with

the most recent molecular studies showing the presence of oligoclonal B-lymphocyte proliferation in liver and bone marrow biopsies in the majority of type II MC patients.⁴

■ CRYOGLOBULINEMIC VASCULITIS

The so-called “essential” MC was first described by Meltzer et al in 1966.⁷ Originally, this term was referred to as autonomous disease when other well-known systemic, infectious, or neoplastic disorders had been ruled out by means of a wide clinico-serological work-up. MC syndrome is characterized clinically by a triad—purpura, weakness, arthralgias—and by a series of pathological conditions,¹⁻⁴ including chronic hepatitis, membranoproliferative glomerulonephritis, peripheral neuropathy, skin ulcers, diffuse vasculitis, and less frequently by lymphatic and hepatic malignancies.¹⁻⁴ The prevalence of MC manifestations reported in **Table 1** regards an Italian patient population referred to a rheumatology-immunology division. A variable patient recruitment at different specialist centers together with racial differences among patient series is often responsible for some contrasting data present in the literature.¹⁻⁵ The prevalence of MC presents great geographic heterogeneity; the disease is more common in Southern Europe than in Northern Europe or Northern America. The disease is considered to be a relatively rare disorder; however, as yet there are no adequate epidemiological studies regarding its overall prevalence. Given its clinical polymorphism, a single manifestation (skin vasculitis, hepatitis, nephritis, peripheral neuropathy, etc.) is often the only apparent or clinically predominant feature, so that MC patients are often referred to different specialties. A correct diagnosis might thus be delayed or overlooked entirely. Consequently, the actual prevalence of MC is probably underestimated.

There are no available diagnostic criteria for MC. In 1989, the Italian Group for the Study of Cryoglobulinemias proposed preliminary criteria for MC classification. A revised version of these criteria, including pathological and virological findings, has been recently proposed.⁴ Circulating mixed cryoglobulins, low C4, or thostatic skin purpura, and leukocytoclastic vasculitis are the hallmarks of the disease. Leukocytoclastic vasculitis, involving medium- and, more often, small-sized blood vessels (arterioles, capillaries, and venules) is responsible for MC tissue injury.^{3,4} Cryoglobulinemic vasculitis (CV) is secondary to vascular deposition of circulating immune complexes (CIC), mainly cryoglobulins, and complement,

From Dipartimento di Medicina Interna, Reumatologia, Università di Pisa, Pisa; *Dipartimento di Medicina Interna, Università di Firenze, Firenze; **Servizio di Anatomia Patologica ed Ematopatologia, Università di Bologna, Bologna, Italy.
Address correspondence to Prof. Clodoveo Ferri, Dipartimento Medicina Interna-Reumatologia, Via Roma 67, 56126 Pisa, Italy.
E-mail: c.ferri@int.med.unipi.it

with the possible contribution of both hemorheological and local factors.¹⁻⁵ Due to its clinical and histological features, CV is classified among systemic vasculitides, in the subgroup of small-vessel vasculitides, which also includes cutaneous leukocytoclastic vasculitis and Schönlein-Henoch purpura.¹⁻⁵

■ HCV AND CRYOGLOBULINEMIC VASCULITIS

Following the discovery of hepatitis C virus (HCV) as the major etiologic agent of non-A non-B chronic hepatitis,⁶ the role of this virus in the pathogenesis of MC has been definitely established on the basis of numerous clinicoepidemiological and virological studies.⁷⁻¹² A direct involvement of HCV antigens in CIC responsible for cryoglobulinemic vasculitis has also been demonstrated.⁹ Therefore, the term “essential” no longer seems to be appropriate for the majority of MC patients.^{4,9-12} On the other hand, numerous epidemiological studies demonstrated low levels of circulating mixed cryoglobulins in over 50% of HCV-infected individuals, while overt cryoglobulinemic syndrome develops in only a minority of patients.⁴ The large diffusion of HCV infection worldwide contrasts with the geographical heterogeneity observed in the prevalence of HCV-related MC.⁴ Thus, a role for particular HCV genotypes, unknown environmental and/or genetic factors may contribute to the pathogenesis of MC.¹¹ However, few and often contrasting studies are available, and the actual role of the above co-factors remains to be demonstrated.⁴

An increasing number of autoimmune disorders have been observed in individuals with chronic HCV infection,^{4,5,10-12} suggesting that the same virus might be responsible for different hepatic and extrahepatic immune-mediated disorders. **Table 2** summarizes the main organ or systemic diseases variably associated with HCV infection. Interestingly, different HCV-related diseases show a clinico-serological overlap.^{4,10} In particular, MC can represent a crossroads between some classic autoimmune disorders (autoimmune hepatitis, sicca syndrome, glomerulonephritis, thyroiditis, etc.) and malignancies (B-cell lymphomas, hepatocellular carcinoma, thyroid cancer).^{4,8,10,12,13}

■ HCV, CV, AND LYMPHOPROLIFERATION

HCV has been recognized to be both an hepato- and lymphotropic virus, as suggested by the presence of active or latent viral replication in the peripheral lymphocytes of patients with type C hepatitis or MC.^{8,14} The infection of lymphoid tissues could represent an HCV reservoir contributing significantly to viral persistence; moreover, the quasispecies nature of HCV permits it to escape immune surveillance and favors the persistence of infection in the host.⁸ These biological characteristics may explain the appearance of a constellation of both autoimmune and lymphoproliferative disorders in HCV-infected individuals.^{4,5,10-12} It has been proposed that HCV infection exerts a chronic stimulus on the immune system, which facilitates the development and selection of abnormal clones.^{4,8} Patients with type II MC may develop a B-cell lymphoma, usually after a long-term follow-up.^{4,8,10} This complication may be related to peripheral B-cell expansion

TABLE 1
DEMOGRAPHIC, CLINICO-SEROLOGICAL, AND VIROLOGICAL FEATURES OF 190 MC PATIENTS

Age, mean $\pm$ SD yrs (range)*	50 $\pm$ 10 (29-74)
Female/male ratio	3
Disease duration, mean $\pm$ SD yrs (range)	12 $\pm$ 6.5 (1-34)
Purpura	91%
Weakness	90%
Arthralgias	80%
Arthritis (nonerosive)	9%
Raynaud's phenomenon	35%
Sicca syndrome	33%
Peripheral neuropathy	39%
Renal involvement**	34%
Liver involvement	71%
B-cell non-Hodgkin's lymphoma	7%
Hepatocellular carcinoma	2%
Cryocrit, mean $\pm$ SD %	3.2 $\pm$ 8
Type II/type III mixed cryoglobulins	2/1
CH50, mean $\pm$ SD units (normal 160-220)	85 $\pm$ 60
C3, mean $\pm$ SD mg/dl (normal, 60-130)	80 $\pm$ 29
C4, mean $\pm$ SD mg/dl (normal, 20-55)	9 $\pm$ 14
Antinuclear antibodies	25%
Antimitochondrial antibodies	12%
Anti-smooth muscle antibodies	25%
Anti-extractable nuclear antigen antibodies	8%
Anti-HCV antibodies	92%
HCV RNA	88%
Anti-HBV antibodies	42%
HBsAg	4%

* at presumed disease onset; ** invariably, membranoproliferative glomerulonephritis.

sion and to lymphoid infiltrates observed in the liver and bone marrow of MC patients.^{4,8,10} In particular, these infiltrates have been regarded by some authors as “early lymphomas,” since they are sustained by lymphoid components indistinguishable from those of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL) and immunocytoma (Ic).⁸

The HCV-related lymphoproliferation, varying from the benign polyoligoclonal B-cell expansion frequently observed in MC to overt malignant lymphoma, is a multifactorial and multistep process for which multiple genetic aberrations are probably necessary.^{4,8} The recent identification of HCV envelope protein E2 able to bind CD81 molecule expressed on both hepatocytes and B lymphocytes¹⁵ could help to clarify the pathogenesis of HCV-related autoimmune and lymphoproliferative diseases. In fact, CD81 is a cell-surface protein, which, on B cells, is part of a complex along with CD21, CD19, and Leu 13.

This complex reduces the threshold for B-cell activa-

TABLE 2
ASSOCIATION BETWEEN HEPATITIS C VIRUS INFECTION AND DISEASES

1—Proven association

Cryoglobulinemic vasculitis
Porphyria cutanea tarda

2—Significant association

Autoimmune hepatitis
B-cell NHL
Monoclonal gammopathies

3—Possible association

Sicca syndrome
Polyarteritis nodosa
Poly/dermatomyositis
Chronic polyarthritis
Lung fibrosis
Diabetes mellitus
Thyroiditis
Thyroid cancer
Lichen planus
Mooren corneal ulcer

- 1 – HCV infection in the majority of patients.
2 – Found in a significant percentage of patients if compared to general population.
3 – Suggested but unproven association.

tion by bridging antigen-specific recognition and CD21-mediated complement recognition. It can be hypothesized that the interaction between HCV-E2 and CD81 may increase the frequency of VDJ rearrangement in antigen-reactive B cell. One possible consequence could be the bcl-2 recombination observed in a significant number of HCV-infected individuals and particularly in MC patients.¹⁶ This proto-oncogene is able to inhibit the apoptosis, leading to extended cell survival. The aberration of bcl-2 may explain, at least in part, the B-lymphocyte expansion and the wide autoantibody production observed in HCV-infected individuals.^{4,8} Other mechanisms such as molecular mimicry may be involved in B-lymphocyte activation responsible for different autoimmune disorders. On the other hand, prolonged B-cell survival can expose these cells to other genetic aberrations^{4,8} leading to overt malignant lymphoma.

■ TREATMENT OF CV

Due to its complex etiopathogenesis, the treatment of CV is particularly challenging. For a correct therapeutic approach we must deal with three important factors: HCV infection, autoimmune disorders, and neoplastic complications. Following the cascade of events leading from HCV infection to cryoglobulinemic vasculitis we can treat the disease at different levels by means of different—etiologic, pathogenetic, symptomatic—therapies.^{4,8} Since HCV represents the triggering factor of the disease and probably exerts a chronic stimulus on the immune system,

an attempt at HCV eradication should be done in all cases of HCV-associated MC. Unfortunately, the beneficial effect observed with this drug is often transient and not rarely associated with important immune-mediated complications—in particular, peripheral sensory-motor neuropathy.^{4,8} There are no parameters available for predicting this harmful complication; thus, alpha-interferon therapy should be avoided at least in those patients with clinically evident peripheral neuropathy. On the whole, the usefulness of alpha-interferon treatment in MC patients is limited by the low rate of responders and frequent side effects. The association of alpha-interferon and ribavirin might achieve the eradication of HCV infection in a rather significant number of treated subjects, as recently demonstrated in patients with type C chronic hepatitis.⁴

Hopefully, with the rapid growth of molecular biology, a vaccine against HCV may be available in the near future. The recent identification of the interaction between HCV envelope protein E2 and CD81 on both hepatocytes and lymphocytes^{4,15} suggests the possibility of interfering with HCV binding to target cells.

Immunosuppressive treatment is still the first-line intervention in cases of non-HCV-associated MC. For HCV-associated MC, immunosuppressive treatment should be considered, especially in patients who have failed to respond to alpha-interferon. This includes steroids, low-antigen-content (LAC) diet, plasma exchange, and immunosuppressors, mainly cyclophosphamide.^{4,8,10} A reduction in circulating immune-complex levels can be achieved by means of plasmapheresis therapy including both traditional plasma exchange and double-filtration plasma exchange.⁴ The use of oral cyclophosphamide (50-100 mg/day for 2-6 weeks) during the tapering of apheresis sessions can reinforce the beneficial effect of plasma exchange; moreover, it can prevent the rebound phenomenon that may be observed after the discontinuation of apheresis. Plasma exchange is useful in severe MC complications, and particularly in active cryoglobulinemic nephropathy. LAC diet has been employed in some immune-complex-mediated disorders such as MC and IgA-nephropathy.^{4,10} In MC patients, this particular dietetic treatment can improve the CIC clearance by restoring the activity of the reticulo-endothelial system, overloaded by large amounts of circulating cryoglobulins.⁴ LAC diet and/or low dosage of steroids may be sufficient to improve mild to moderate manifestations of MC. As commonly observed, MC patients with mild to moderate symptoms, such as palpable purpura, are particularly sensitive to the smallest variations of daily steroid dosage (1-2 mg). On the whole, MC treatment should be tailored for the individual patient according to the severity of clinical symptoms.⁴ Therefore, patients with severe vasculitic manifestations must be promptly treated with high doses of steroids and/or plasma exchange and/or cyclophosphamide, whereas clinically asymptomatic patients usually do not need any treatment, even in the presence of high levels of cryocrit. Careful clinical monitoring of the disease is mandatory in all cases, with particular attention to neoplastic complications.^{4,8,13}

■ REFERENCES

1. Meltzer M, Franklin EC, Elias K, et al. Cryoglobulinemia: a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; 40:837-856.
2. Brouet JC, Clouvet JP, Danon F, et al. Biologic and clinical significance of cryoglobulins. *Am J Med* 1974; 57:775-88.
3. Gorevic PD, Kassab HJ, Levo Y, et al. Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am J Med* 1980; 69:287-308.
4. Ferri C, Zignego AL, Pileri S. Cryoglobulins (review). *J Clin Pathol* 2002; 55:4-13.
5. Lamprecht P, Gause A, Gross WL. Cryoglobulinemic vasculitis (review). *Arthritis Rheum* 1999; 42:2507-2516.
6. Choo GL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A non-B viral hepatitis genome. *Science* 1989; 244:359-361.
7. Ferri C, La Civita L, Longombardo G, et al. Hepatitis C virus and mixed cryoglobulinemia (review). *Eur J Clin Invest* 1993; 23:399-405.
8. Ferri C, Pileri S, Zignego AL. Hepatitis C virus, B-cell disorders, and non-Hodgkin's lymphoma. Chapter 19; p. 349-368. In: *Infectious Causes of Cancer. Targets for Intervention*. Goedert JJ, ed. National Cancer Institute (NIH). The Humana Press Inc. Totowa, New Jersey; 2000.
9. Abel G, Zhang QX, Agnello V. Hepatitis C virus infection in type II mixed cryoglobulinemia (review). *Arthritis Rheum* 1993; 36:1341-1349.
10. Ferri C, Zignego AL. Relation between infection and autoimmunity in mixed cryoglobulinemia. *Curr Opin Rheumatol* 2000; 12:53-60.
11. Zignego AL, Ferri C, Giannini C, et al. Hepatitis C virus genotype analysis in patients with type II mixed cryoglobulinemia. *Ann Intern Med* 1996; 124:31-34.
12. Gumber S, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations (review). *Ann Intern Med* 1995; 123:615-20.
13. Antonelli A, Ferri C, Fallahi P. Thyroid cancer in patients with hepatitis C infection. *JAMA* 1999; 281:1588.
14. Ferri C, Monti M, La Civita L, et al. Infection of peripheral blood mononuclear cells by hepatitis C virus in mixed cryoglobulinemia. *Blood* 1993; 82:3701-3704.
15. Pileri P, Uematsu Y, Campagnoli S, et al. Binding of hepatitis C virus to CD81. *Science* 1998; 282:938-941.
16. Zignego AL, Giannelli F, Marocchi ME, et al. T(14;18) translocation in chronic hepatitis C virus infection. *Hepatology* 2000; 31:474-479.