

Review

Mixed cryoglobulinemia and HCV

N. Tassopoulos¹, Johanna N. Papageorgiou²

SUMMARY

Mixed cryoglobulinemia (MC) is a well-documented extrahepatic manifestation of hepatitis C virus (HCV) infection. MC is a systemic vasculitis of small to medium-sized vessels due to vascular deposition of circulating immune-complexes and complement; it is characterized by the presence in the cooled serum of one or more mono- or poly-clonal immunoglobulins that precipitate at temperature below 37° C and redissolve on rewarming. MC is clinically characterized by a triad of purpura, arthralgia and asthenia and sometimes may involve peripheral nerves, the kidneys and the development of B-cell non-Hogkin's lymphoma. The discovery of the relation between HCV infection and MC shows the association between a viral infection, and an autoimmune disease and, thus, a potential link between the systemic autoimmune and lymphoproliferative disorders. The eradication of HCV, by alfa-interferon, is associated with improvement or disappearance of MC-associated clinical manifestations.

Key-words: Mixed cryoglobulinemia, Hepatitis C Virus, non-Hogkin's lymphoma, interferon alfa.

The definition of cryoglobulins is based on an in vitro phenomenon; namely the presence in the cooled serum of one or more immunoglobulins that precipitate at temperature below 37° C and redissolve on rewarming. Cryoglobulinemia is generally classified, according to the

clonal composition of immunoglobulins, into three subgroups¹. Type I cryoglobulins (25%) composed of single monoclonal immunoglobulins and are always associated with a malignant lymphoproliferative disorder (e.g. multiple myeloma, Waldenstrom's macroglobulinemia); type II and III (60%) are mixed cryoglobulins composed of different immunoglobulins with a mono- or poly-clonal IgM rheumatoid factor (RF) activity respectively and are associated with connective tissue disease (e.g. rheumatoid arthritis, lupus, Sjogren syndrome, Wegener's granulomatosis, polyarteritis nodosa), a malignant hematological disorder (type B lymphoproliferation) or infections (EBV, CMV, HIV, Leishmania). The existence of cryoglobulins that do not fit well into any of the three subgroups described above have been reported. The presence of oligocloned IgM with trace amounts of polyclonal immunoglobulins has been described and has been defined as a new cryoglobulin type, the type II-III variant (oligoclonal IgM and polyclonal IgG)^{2,3}. The microheterogeneity of the IgM in these cases probably represents a transition from type III to type II, and may indicate the natural evolution of cryoglobulinemia in some patients. The transformation from poly- to oligo- and finally mono-clonal RF may be induced by the continuous B cell stimulation caused by infections or other exogenous agents. Microheterogeneity has been found in 13% of 157 cases of cryoglobulinemic patients². Moreover, 25 (13%) of 210 cases of mixed Cryoglobulinemia exhibited an additional monoclonal IgG on immunoblot. All of these monoclonal IgG were IgGI (37%) and IgG3 (67%) iso types⁴. However, mixed cryoglobulins are detected in the absence of a demonstrable underlying disease (15%), the syndrome has been designated "essential mixed Cryoglobulinemia" (EMC)^{5,6}.

The etiology of EMC has been attributed to hepatitis C virus (HCV) infection. In particular, Ferri et al⁷, noted that 96% of 26 patients with EMC were anti-HCV positive and 91% were HCVRNA positive with polymer-

¹First Dept of Medicine, Western Attica General Hospital, Athens,

²Transfusion Unit, Patission General Hospital, Athens

Author for correspondence:

Nicholas C. Tassopoulos, First Dept. of Medicine Western Attica General Hospital, 1, Dodecannissou Str., GR 123 51, Agia Varvara, e-mail: ntass53@otenet.gr, fax: (3) 0106844046, (3) 0105301307

ase chain reaction; Agnello et al⁸ detected a high frequency of anti-HCV and HCVRNA in the serum of patients and that both anti-HCV and HCVRNA were concentrated to approximately 10-fold and 1000-fold respectively, in the type II cryoprecipitates. Antibodies to HCV have been found in 420 (73%) of 576 patients with EMG.

This high prevalence of anti-HCV (range 30% to 96%) with rivemia in 63% to 93% of patients and HCVRNA in cryoprecipitate (75% to 100%) in 13 studies, reviewed by Cacaub et al⁹ appears to be strong evidence of the role of HCV in the etiology of EMC. Recent studies of MC and the B lymphocyte compartment in HCV positive patients provided interesting data that are key elements of current understanding: 1) The IgM rheumatoid factors (RFs) in MC derive from a highly restricted set of Immunoglobulin V genes, 2) HCV infection is associated with monoclonal B cell expansion and an increased prevalence of B-cell non-Hodgkin lymphoma (NHL), and 3) MC and B-cell clonal expansion secondary to HCV infection subsides in patients who respond to antiviral therapy.

RFs are a sine qua non of MC. IgM RF isolated from MC type II and MC type III do not cryoprecipitate alone, but are cryoprecipitable when mixed with unrelated monomeric or aggregated polyclonal human IgG, with human Fc fragments, or in some cases, with rabbit IgG^{1,2,5,6}. The three major constituents of HCV-associated mixed cryoprecipitate are IgM RF, polyclonal IgG, and HCVRNA (Table 1). In addition, IgA and Clq have been detected in 13% and 56% of MC respectively¹⁰. The composition of 4 type III cryoprecipitates were 29% to 45% IgG, 8% to 11% IgA and 46% to 61% IgG¹¹, although IgA was detected in only 3 of 19 type III cryoprecipitates in another study¹². Several studies have found that IgG anti-HCV antibodies are essentially always present in HCV-associated cryoprecipitates^{10,13} where they comprised of one-fourth of the serum anti-HCV IgG activity of MCII patients¹⁰. Most IgM molecules in mixed cryoprecipitates are IgMRF, which have been reported to

cross-react with cellular but not plasma fibronectin¹⁴, and not with HCV proteins in RIBA^{13,15}.

Lipoprotein VLDL molecules might also be present in cryoprecipitate, because some HCVRNA are precipitable by anti-beta lipoprotein antibody¹⁵.

In conclusion, the principal structure of HCV-induced mixed cryoglobulin consists of HCV-IgG complexes whose IgG Fc regions are bound by cryoprecipitable IgM RF. Thus, sufficient cryoprecipitable IgM RF is an essential requirement for mixed cryoglobulins formation. IgG anti-HCV are also important because their specificities and subclasses can affect the structure, abundance, and RF binding of IgG-HCV complexes. It is likely that HCV-IgG complexes are a stimulus for RF production, and their persistence is a reason why RFs are so frequent in HCV positive patients^{16,17}. It is conceivable that, if IgG responses to new HCV quasispecies can generate new immune complexes and initiate a fresh RF response, then mixed cryoglobulin production in HCV positive patients might reflect a series of acute RF responses against a background of chronicity.

Mixed cryoglobulinemia is a systemic vasculitis of small and medium-sized blood vessels due to the deposition of circulating immune complexes (CIC) and complement. The pathologic hallmark of cutaneous and visceral manifestations of the disease is a leukocytoclastic vasculitis (Figure 1) secondary to vascular deposition of CIC, mainly the cryoglobulins and complement². Skin biopsy specimens showed immunohistochemical evidence of HCV-associated antigens¹⁸.

Clinically, MC is characterized by a clinical triad-purpura, asthenia, arthralgias - and occasionally associated with glomerulonephritis, peripheral neuropathy, and chronic hepatitis. It appears to represent the consequence of an immune-complex-type vasculitis and is supported by clinical features, analysis of the cryoglobulins, the depressed level of complement (particularly C4) during the active phase of the disease, and the deposition of immunoglobulins and complement in the lesions. Possible mechanisms include specific immune interactions with viral antigens, perhaps cross-reacting with the host, polyclonal activation of B and/or T cells, host genetic make-up and other environmental factors.

There is a geographic heterogeneity in the prevalence of the mixed cryoglobulinemias among different countries; the disease is more frequent in Southern Europe than in Northern Europe or Northern America. Adequate epidemiologic studies have not been performed; however, it is considered to be a relatively rare disorder.

Table 1. The composition of HCV-associated mixed cryoprecipitates (MC)

Constituent	Role in MC formation
IgMRF	Binds IgG Fc
IgG	Binds to HCV virions Is bound by IgMRF
HCV	Bound by IgG anti-HCV antibody
IgA	Unknown
Clq	Binds Fc in CIC



Figure 1. Skin changes of MC associated with hepatitis C virus infection consisting of purpura on the legs (courtesy of Dr. Kontochristopoulos).

Cryoglobulins were detected significantly more frequently in patients with chronic HCV infection than in healthy controls (38/89 or 42,6% vs 2/42 or 4,7% $p < 0,0001$). In particular, EMC was observed in 68,4% (13/19) of patients with HCV liver cirrhosis, which was nearly twice the figure of HCV non-cirrhotic patients with elevated (18/47 or 38,3%) or persistently normal enzymes (7/23 or 30,4%; $p < 0,05$)¹⁹. Only 13% of patients with EMC were symptomatic. It seems that the presence of symptoms in immune-complex-mediated diseases depends on the number of erythrocyte C3b receptors. In 10% of the population the expression of this receptor is reduced, and clinical symptoms of EMC seem to appear only in this subgroup of the population. EMC was also detected in 30 (48%) of 62 prospectively followed patients with chronic HCV infection. Patients with EMC were, on average older ($52,3 \pm 12,8$ vs $40,8 \pm 12,1$ years, $p < 0,02$), showed an apparently longer duration of infection ($13,5 \pm 8,8$ vs $9,1 \pm 5,8$ years, $p < 0,05$), suffered more frequently from arthralgias (20% vs 9,3%, $p < 0,05$) had clinically advanced liver disease more frequently (13,3% vs 3,1%, $p < 0,02$), accompanied by a significant increase of total serum IgM concentration $4,4 \pm 1,7$ vs $1,9 \pm 1,1$ g/L,

$p < 0,02$) and RF activity ($114,3 \pm 228,3$ vs. $19,8 \pm 9,5$ IU/L, $P < 0,05$). The HCV genotype distribution among patients with EMC was not different from that found in patients without cryoglobulinemia²⁰, confirming previous data²¹ and suggesting that HCV genotypes are not responsible for the development of EMC, despite the report from Italy that found genotype 2a to be more common in HCV infected patients with type II EMC²². A similar prevalence (29/62 or 42%) of EMC was found in a VAMC in the USA; presence of cryoglobulins was unrelated to a history of intravenous drug abuse and excessive alcohol ingestion which appear to have no role in the development of EMC²³. The apparent episodic presence of cryoglobulins in study patients (10 of 19) with chronic HCV infection may signify fluctuation in cryoglobulin level below detectable levels of fluctuation in stimulatory factors. Isolated cases of EMC have been reported after orthotopic liver transplantation in 6 of 31 HCV positive transplanted patients and in none of HCV-negative transplanted patients (6/31 or 19% vs. 0/21 or 6%, $p = 0,036$). The only parameter associated with cryoglobulins in the HCV positive group was rheumatoid factor (11/31 or 36% vs. 4/21 or 19%, $p < 0,036$). Pretransplantation records showed that none of the 6 EMC patients had a history of purpura and/or vasculitis or glomerulonephritis before OLT, suggesting that EMC developed de novo after transplantation²⁴.

HCV-associated type III and II mixed cryoglobulinemias (MCs) are comparable with regard to organ involvement and clinical course, with the exception of their potential evolution to malignancy. They might represent two different steps of the same disorder. MC type III may lead to benign lymphoproliferative disorder with monoclonal IgG component, the MC type II a preneoplastic condition which in some individuals can develop to a frank B-cell NHL, usually after a long-term follow-up period²⁵.

MC is a peculiar condition in which a chronic infection coexists with a number of autoimmune and lymphoproliferative disorders²⁵⁻²⁷. Cryoglobulinemic vasculitis is the most frequent extrahepatic complication of HCV infection with a variable spectrum of autoimmune manifestations. HCV can be the triggering factor of immunology alteration that subsequently may become self-perpetuating, as is the case with classic autoimmune diseases. MC, however, represents a benign lymphoproliferative disorder, characterized by different immune system alterations; serum cryo- and noncryo-immune complexes and autoantibodies, chiefly the Wa RF, clonal expansion of IgMK-bearing B cells, lymphocyte and plas-

mocytoid cell-infiltrates in the bone-marrow and frequent Bcl-2 proto-oncogenes translocation. This clinically indolent lymphoproliferation can switch over to frank B cell NHL. The above clinico-serological and pathological observations indicate that there is a continuum between chronic HCV infection, MC and other autoimmune-lymphoproliferative disorders.

Therapy: The traditional treatment for MC (pre-isonolone, immunosuppressive drugs, plasmapheresis) has been found ineffective in inducing long-term remission. Alpha-interferon (IFN) is now clearly the drug of choice for treatment of this disease and the results of IFN treatment of patients in this setting represent an indirect proof for the etiopathogenetic link between EMC and HCV infection.

In the first four studies, a six-month treatment of cryoglobulinemia patients with 3MU IFN had a good end-of-treatment response (42-73%), but disappointingly low sustained response rate 0-22% in patients^{7,28-30}. Symptomatic patients tend to respond less well than asymptomatic ones and immunological responses tend to mirror viral responses, implying that the success of IFN relies on its antiviral rather than effects on immune function. Marginal improvements in the sustained responses have been achieved with a 12-month regimen of IFN treatment. The end-of-treatment (EOT) and the sustained virological (SVR) responses were 42-44% and 12-14% respectively³¹⁻³². A symptomatic patients tended to have significantly higher EOT than symptomatic ones (15/25 or 60% vs. 7/25 vs. 28%, $p < 0.05$) and higher SVR (20% vs. 4%)³³. No clinical improvement has been observed in patients with neurological manifestations. Combination treatment of IFN-ribavirin in previous IFN-relapsers has shown good results in 5 (38,5%) of 13 patients with sustained virological and immunological responses in 80% of cases³³.

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