# Longterm Course of Mixed Cryoglobulinemia in Patients Infected with Hepatitis C Virus

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*ABSTRACT.* ***Objective.*** To describe epidemiological, clinical, and immunological characteristics and the longterm course of persistent mixed cryoglobulinemia (MC) in patients infected with hepatitis C virus (HCV).

***Methods.*** Retrospective study of HCV infected patients (HCV RNA positive) who had persistent positive MC, with 2 immunochemical typings of MC carried out after 24-month minimum interval.

***Results.*** In total, 125 patients were studied, aged 52 ± 13 years at diagnosis of MC, with duration of HCV infection of 18 ± 10 years. At entry, 60 patients had type II MC, 53 patients had type III, and 12 patients had the oligoclonal type. At the second immunochemical typing, after a mean interval of 45 ± 20 months, MC was type II in 72 patients, type III in 39 patients, and the oligoclonal type in 14 patients. The proportion of cases of MC with the same immunochemical type was higher among patients with type II (78%) than type III (59%) or oligoclonal MC (17%) ($p < 0.01$). The MC that changed turned more to type II (55.5%) than type III (29%) or the oligoclonal type (15.5%) ($p = 0.0002$). MC vasculitis (purpura, arthralgia, peripheral neuropathy, renal involvement) and other extrahepatic manifestations (polyarteritis nodosa, lymphoma) in 60/125 patients was associated with advanced age ($p < 0.01$), a longer duration of infection ($p < 0.05$), type II MC (odds ratio = 5, $p < 0.01$), and a higher MC serum level ($p < 0.01$).

***Conclusion.*** During chronic active HCV infection, type II MC is more stable over time than type III and oligoclonal MC. The oligoclonal type appears to be an intermediate stage in the course of type III changing to type II MC. Symptomatic persistent HCV MC was associated with advanced age, longer duration of HCV infection, type II MC, and a higher MC serum level. (J Rheumatol 2004;31:2199–206)

*Key Indexing Terms:*
HEPATITIS C VIRUS
MIXED CRYOGLOBULINEMIA
EXTRAHEPATIC MANIFESTATIONS
VASCULITIS

It has been well established that mixed cryoglobulinemia (MC) is the first extrahepatic manifestation of hepatitis C virus (HCV) infection, since it is diagnosed in 36% to 55% of patients[1-9]. About 25% of these patients will develop specific clinical symptoms of cryoglobulinemic vasculitis[1,10,11]. The immunochemical type of HCV MC, according to Brouet's classification[12], may be type II, which includes a monoclonal component in 20% to 65% of patients, or type III, defined by the association of polyclonal immunoglobulins in 35% to 80% of patients[3,4,8,13-16]. Another type of MC, the oligoclonal or microheterogeneous type, has been characterized, but seldom reported, and is defined by more than 2 heterogeneous bands of heavy chains on immunoblotting. Only one study reports its frequency in the context of HCV infection, i.e., 34% of MC among HCV infected patients not receiving antiviral treatment, and 23% of MC among patients treated with interferon[17]. Further, the clinical and biological effects of oligoclonal HCV MC and their classification are still unclear. Under anti-HCV treatment, MC becomes undetectable in 60% to 90% of sustained virological responders[10,11,18-20]. However, the course of the immunochemical types of persistent MC and their clinical and biological features remain insufficiently studied. We describe epidemiological, clinical, and immunological characteristics and the longterm course of persistent HCV MC.

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## MATERIALS AND METHODS

*Patients.* We set up a retrospective study among HCV infected patients that were MC positive between January 1989 and December 2000 in the immunochemical laboratory of La Pitié-Salpêtrière Hospital, Paris. Patients were included if they had (1) chronic HCV infection as evidenced by a positive third generation ELISA test and HCV RNA by polymerase chain reaction; (2) positive cryoglobulinemia defined as described[3] by a MC serum concentration ≥ 0.05 g/l; and (3) 2 immunochemical typings of MC carried out during a 24-month minimum interval, which implies the persistence of MC positivity during this interval. All patients with other diseases that produce MC were excluded, i.e., hepatitis B virus infection, human immunodeficiency virus infection, connective tissue diseases, and malignant hematological disorders

(other than HCV associated B cell lymphoproliferative disorders). For each patient, the following data were collected retrospectively: sex, age at the time of MC diagnosis, mode and date of HCV contamination (if known), and symptoms associated with MC syndrome (cutaneous vasculitis, arthralgia, peripheral neuropathy, renal involvement, central nervous system involvement) and other extrahepatic manifestations (polyarteritis nodosa-type vasculitis, lymphoproliferative disease). The diagnosis of peripheral neuropathy was performed by electromyogram in presence of paresthesia, limb pains, or sensory or motor deficit. Urine sediments (hematuria, leukocyturia) and proteinuria were tested in all patients, but the diagnosis of renal involvement was considered only for those who underwent a renal biopsy.

*Detection, isolation, and immunochemical typing of cryoglobulinemia*. Cryoglobulins were precipitated from serum drawn and clotted at 37°C and stored at 4°C, in 0.1 g/l sodium azide, for up to 7 days. The precipitates were washed 5 times at 4°C with 0.15 mol/l NaCl, and the total protein concentration of each precipitate was measured by reading the absorbance at 280 nm of the purified cryoglobulins diluted 1:20 in 0.1 mol/l NaOH. A purified human gammaglobulin preparation (Etablissement de Transfusion Sanguine, Paris, France) was used as a standard. The immunoglobulin (Ig) composition of washed cryoglobulins was determined by immunoblotting according to Musset, *et al*[21]. Briefly, washed cryoglobulins (diluted to a protein concentration of 0.05 g/l) were separated by electrophoresis (37°C) on an agarose gel (Paragon™, Beckman, Gagny, France) and transferred to a nitrocellulose membrane by pressure blotting. After saturation (1 h, 37°C) in a mixed solution of powdered skimmed milk (50 g/l) and NaCl (0.9%), each line was cut out and incubated with goat antiserum monospecific for each heavy (μ, γ, α) and light (κ, λ) chains of human immunoglobulin (Dako AS, Roskilde, Denmark). After washing (15 min) with NaCl 0.9%, blots were revealed using an anti-goat immunoglobulin antiserum labeled with alkaline phosphatase (EC 3.1.3.1; Jackson Immunoresearch, West Grove, PA, USA). Cryoglobulins composed of different Ig were classified according to Brouet, *et al*[12] as type II when containing a monoclonal Ig, type III if containing only polyclonal Ig, or oligoclonal (at least 2 microheterogeneous bands in heavy chains). The cutoff for positive cryoglobulinemia was fixed at 0.05 g/l according to Cacoub, *et al*[3].

*Other tests*. Serum samples were tested for the presence of a rheumatoid factor by latex test (Dade-Behring, Marburg, Germany) and Waaler-Rose test (Polyartitre, Fumouze, France), for total serum hemolytic complement activity (according to Kabat and Meyer's test), and for C3 and C4 components (laser nephelometric essays; Dade-Behring). Alanine aminotransferase (ALT) was measured using standard tests. The determination of HCV genotype was made with the LiPA method (Innogenetics, Brussels, Belgium).

Histological features of liver specimens were scored according to the METAVIR system[22,23]. Liver biopsies > 10 mm length were fixed, embedded in paraffin, and stained with at least hematoxylin and eosin–safran and Masson's trichrome or picrosirius red for collagen. For each liver biopsy, a stage of fibrosis and a grade of activity were established according to the following criteria. Fibrosis was staged on a scale from 0 to 4: 0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, and 4 = cirrhosis. This feature has been shown to be highly reproducible among pathologists[22]. The grading of activity, evaluating the intensity of necroinflammation, was indicated as follows: A0 = no histological activity, A1 = mild, A2 = moderate, and A3 = severe activity.

*Statistical analysis*. All quantitative data were expressed as mean ± standard deviation. Univariate analysis used the chi-square test or Fisher's exact test for comparison of qualitative values. Comparison of qualitative to quantitative data was performed with the Mann-Whitney test (Kruskal-Wallis test for 2 groups) and the multivariate analysis was by logistic analysis. Significance was assessed at $p \leq 0.05$. Statistical analysis was done with Epi Info 2000 v. 1.1.2 software (Centers for Disease Control, Atlanta, GA, USA).

## RESULTS

*Epidemiological, clinical, virological, and liver histological features*. Among the 1010 HCV infected patients with positive MC diagnosed between January 1989 and December 2000, 125 patients (63 men, 62 women) who had a persistent MC with no other cause of MC were included in this study (Figure 1). Seventy-seven were followed in the Department of Hepatogastroenterology, and 48 in the Department of Internal Medicine. Patients were followed by the same physician in each department at 4 to 6-month intervals, without a predefined protocol. The main features of the study population are reported in Table 1. The mean age at contamination was 31.6 ± 12.7 years. Patients infected by blood transfusion were significantly older than those infected from intravenous drug use [ages 33.8 ± 12.2 vs 20.7 ± 5.8 yrs; p = 0.00001]. At the time of MC diagnosis, the mean age was 51.9 ± 13.2 years (both sexes), and the mean duration of HCV infection was 18.4 ± 10.1 years (range 3.4–43 yrs) with a trend toward a shorter duration in male than in female patients (16.1 ± 8.5 vs 20 ± 10 yrs, respectively; p = 0.06). Mean ALT level was 2.4 ± 1.8 times the upper limit of the normal value, being higher among men than women (2.8 ± 2.3 vs 2 ± 1, respectively; p = 0.08). A liver biopsy was carried out in 115 patients after a mean duration of infection of 17.4 ± 9.6 years. The mean METAVIR fibrosis score was 1.97 ± 1.3, and 22 of 115 (19%) patients had cirrhosis (METAVIR F4). The mean METAVIR activity score was 1.2 ± 0.8. Sixty patients (48%, 32 women) presented clinical symptoms related to MC vasculitis and other extrahepatic disorders: peripheral neuropathy (39/125 = 31%), arthralgia (37/125 = 29.6%), cutaneous vasculitis with purpura (29/125 = 23%), recent onset hypertension (11/125 = 8.8%), renal involvement (7/125 = 5.6%) with 4 membranoproliferative glomerulonephritis and 3 renal polyarteritis nodosa vasculitis, polyarteritis nodosa systemic vasculitis in 9/125 patients (7%), B cell non-Hodgkin lymphoma (B-NHL) in 9/125 patients (7%), and cerebral vasculitis in 3/125 patients (2.4%) (Table 2).

*Immunochemical features*. At the first immunochemical typing investigation, 48% (60/125) of patients had a type II MC, 42.4% (53/125) type III MC, and 9.6% (12/125) the oligoclonal type MC (Figure 2). For type II MC, the monoclonal immunoglobulin was more frequently Mκ (39/60 = 65%) than Mλ (6/60 = 10%), Gλ (3/60 = 5%), or Gκ (1/60 = 1.7%) ($p < 0.00001$). Eleven patients had 2 immunoglobulin monoclonal components (Mκ + Mλ in 3 patients, Mλ + Gκ in one patient, Mλ + Gλ in 2 patients, Mκ + Gκ in 3 patients, Mλ + Gλ in 2 patients). The second immunochemical typing of MC was carried out after a mean of 45 ± 20 months. The proportion of type II MC increased to 57.6% (72/125), whereas the type III MC proportion decreased to 31.2% (39/125), and the oligoclonal MC type remained stable at 11.2% (14/125). The type II MC remained predominantly the Mκ type (69%).

The course of each type of MC between the 2 immunochemical typings was then analyzed (Figure 2). For type II MC, 78.3% (47/60) remained type II, whereas only 15% (9/60) and 6.7% (4/60) turned into the oligoclonal type or type III, respectively ($p < 0.000001$). Type II MC turned more

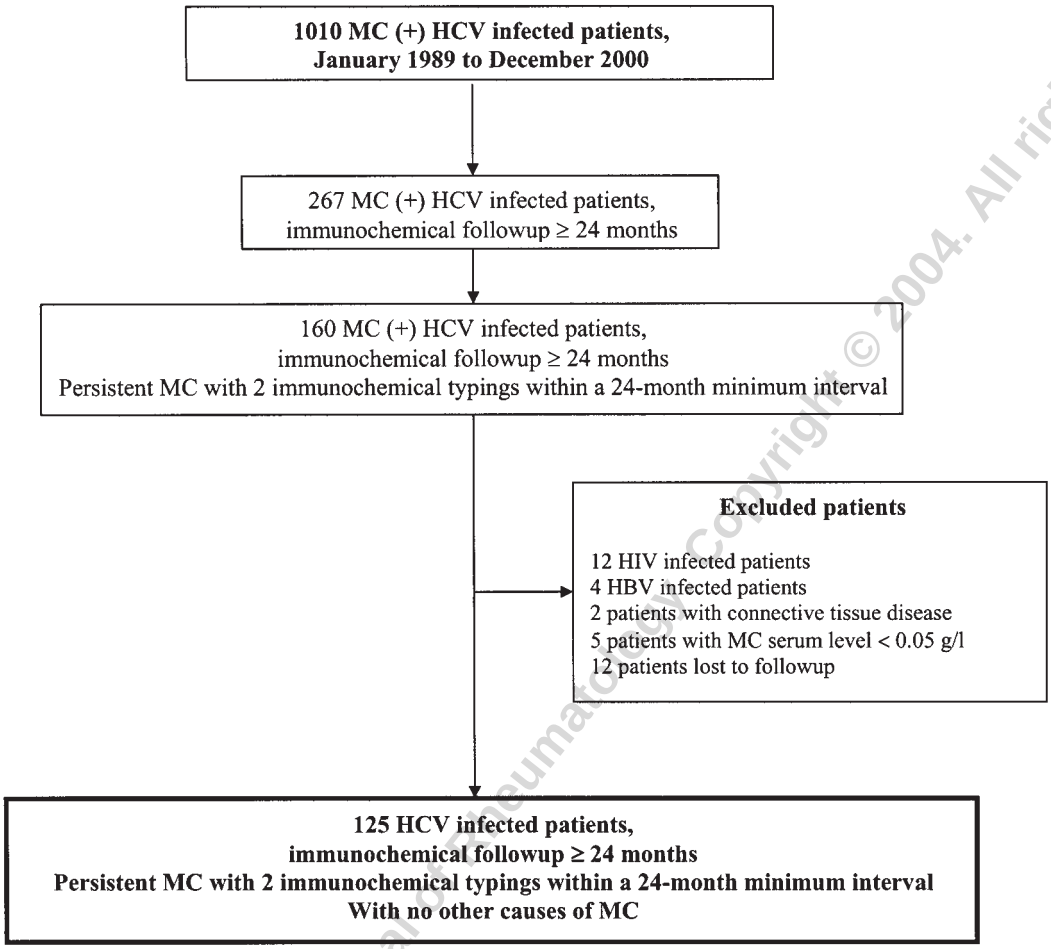


*Figure 1*. Selection of HCV infected patients with persistent positive MC.

*Table 1*. Epidemiological, virological, and liver histological features of patients.

| Feature | N = 125 |
|---|---|
| Sex ratio (M/F), n | 63/62 |
| Age at MC diagnosis, yrs | 52 ± 13 |
| Route of HCV infection, n (%) | 67/125 (54) |
| Blood transfusion | 53/67 |
| IV drug use | 13/67 |
| Acupuncture | 1/67 |
| HCV infection duration, yrs | 18 ± 10 |
| HCV viral genotype, n (%) | 115/125 |
| 1 | 64 (56) |
| 2 or 3 | 36 (33) |
| 4 or 5 | 15 (11) |
| Liver biopsy, n (%) | 115 (92) |
| Cirrhosis (METAVIR F4), n (%) | 22/115 (19) |

frequently into the oligoclonal type (9/13 = 69%) than type III (4/13 = 31%) (p = 0.05). For type III MC, 58.5% (31/53) remained type III, whereas 35.8% (19/53) and 5.7% (3/53) turned into type II (p = 0.019) and the oligoclonal type (p < 0.000001), respectively. Type III MC turned more frequently into type II (19/22 = 86.4%) than the oligoclonal type (3/22 = 13.6%) (p = 0.000001). For the oligoclonal type MC, the course was mainly to another type in 83.3% of patients (10/12; 6/12 became type II and 4/12 type III; p = nonsignificant), whereas only 16.7% (2/12) remained identical (p = 0.001). Overall, the cases of type II MC were more stable (78.3%) than the type III (58.5%) and the oligoclonal type (16.7%) (p = 0.0001). The MC having changed immunochemical types (13 type II, 22 type III, and 10 oligoclonal types) turned mainly to type II (25/45 = 55.5%) rather than type III (13/45 = 29%) or the oligoclonal type (7/45 = 15.5%) (p = 0.0002).

The mean MC serum level at the time of diagnosis was 0.39 ± 0.79 g/l (0.05–6.8) and tended to be higher for type II MC (0.54 ± 1.11 g/l) than type III MC (0.28 ± 0.15 g/l) and the oligoclonal type MC (0.24 ± 0.25 g/l) (p = 0.3). This trend was confirmed at the second immunochemical typing with a MC serum level of 0.57 ± 0.82 g/l for the type II MC compared to 0.25 ± 0.03 g/l for type III and the oligoclonal MC (p = 0.03).

*Table 2.* Distribution of HCV associated extrahepatic manifestations according to the immunochemical type of MC.

| | MC II, 60 Patients | MC III, 53 Patients | Oligoclonal MC, 12 Patients | p |
|---|---|---|---|---|
| Peripheral neuropathy, n (%) | 28/60 (47) | 9/53 (17) | 2/12 (17) | 0.002 |
| Arthralgia, arthritis, n (%) | 26 (43) | 9 (17) | 2 (17) | 0.005 |
| Purpura, n (%) | 24 (40) | 5 (9) | 0 | < 0.0001 |
| Recent onset hypertension, n (%) | 9 (15) | 3 (6) | 0 | 0.05* |
| Lymphoma, n (%) | 9 (15) | 0 | 0 | 0.004** |
| Renal involvement, n (%) | 6 (10) | 1 (2) | 0 | 0.03*** |
| Cerebral vasculitis, n (%) | 3 (5) | 0 | 0 | 0.2† |

* 9/60 (15%) versus 3/65 (4.6%), p = 0.05. ** 9/60 (15%) versus 0/65 (0%), p = 0.004. *** 6/60 (10%) versus 1/65 (0.23%), p = 0.03. † 3/60 (5%) versus 0/65 (0%), p = 0.2.

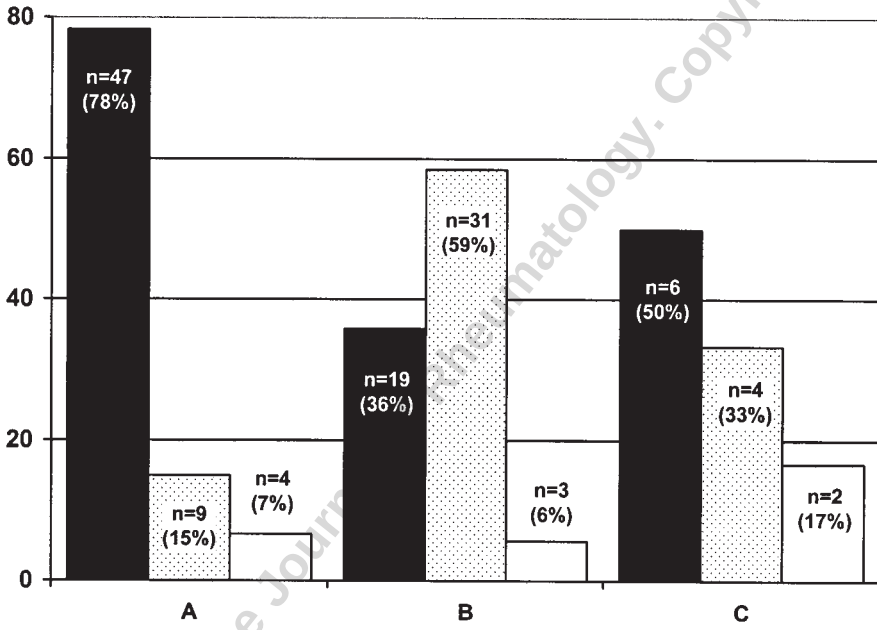


*Figure 2.* Course of each immunochemical type of MC between the 2 typing investigations. A: Cases of MC that were type II at the first immunochemical typing. B: Cases that were type III at the first immunochemical typing. C: Cases that were the oligoclonal type at the first immunochemical typing. Black bars: proportion of MC cases that remained unchanged or turned into type II MC. Shaded bars: cases that remained unchanged or turned into type III MC. White bars: cases that remained unchanged or turned into oligoclonal type MC at the second immunochemical typing.

Tests for the total serum hemolytic complement activity (CH50) and its C3 and C4 components were carried out among 42 patients (32 patients with type II MC, 8 with type III, and 2 with the oligoclonal type). Serum complement level was low for the C4 component (≤ 0.14 g/l) in 28 patients (66.7%), and for CH50 (≤ 35 CH50/ml) in 34 patients (81%), whereas the C3 component was generally normal or raised (≥ 0.52 g/l; 36/42 = 85.7%). A low serum C4 level was more frequent with the type II (25/32 = 78%) than the type III or oligoclonal type (3/10 = 30%) (p = 0.015). The CH50 was low in 26/32 patients (81%) with type II MC, 7/8 patients (87.5%) with type III, and 1/2 patients with the oligoclonal type. Positive rheumatoid factor was found in 25/29 patients with type II MC (86%), 6/7 patients with type III (87%), and in the only oligoclonal-type patient who was tested.

Eighty-seven of 125 patients received anti-HCV therapy (39 patients interferon-alpha alone, and 48 patients a combination of interferon-alpha and ribavirin). Using these therapeutic regimens, the majority of patients were virological nonresponders (74/87 = 85%). But this was expected, as patient selection in this study was based on the presence of persistent MC, without consideration for the therapeutic status. Similarly, among the 60 patients with severe HCV related extrahepatic manifestations (i.e., MC vasculitis, lymphoma), 45 received anti-HCV treatment, of whom only 9 had a sustained virological response. Fifteen patients did not

receive antiviral therapy: 4 patients were treated with prednisone + cyclophosphamide + plasmapheresis, one with corticosteroids only, and 10 were not treated (low severity of vasculitis). Probably due to the heterogeneity of the treatments used, the rate of virological sustained response did not appear to be influenced by either the viral genotype [6/49 genotype 1 (12%), 3/12 genotype 2 (25%), 1/13 genotype 3 (8%), 1/6 genotype 4 (17%), 1/3 genotype 5 (33%), 1/4 "non-genotyped" patients (25%)] or by the severity of liver fibrosis: 6/28 patients with severe fibrosis (METAVIR F3F4) versus 7/55 patients without severe fibrosis (METAVIR F0F1F2) ($p = 0.5$).

*Univariate analysis*. Considering the immunochemical types of MC, type II MC were more often symptomatic than type III or oligoclonal MC: 68% (41/60), 30% (16/53), and 25% (3/12), respectively ($p < 0.0001$). Cases of the type II MC, compared to the type III and oligoclonal MC, were more frequently associated with peripheral neuropathy [46.7% (28/60) vs 17% (9/53) and 16.7% (2/12), respectively; $p = 0.002$], arthralgia [43.3% (26/60) vs 17% (9/53) and 16.7% (2/12), respectively; $p = 0.005$], cutaneous vasculitis [40% (24/60) vs 9.4% (5/53) and 0% (0/12), respectively; $p < 0.0001$], renal involvement [10% (6/60) vs 0.23% (1/65) for MC III and oligoclonal together; $p = 0.03$], and B-NHL [15% (9/60) vs 0% (0/65); $p = 0.004$] (Table 2). Type III MC became more frequently symptomatic when turning to type II MC. Thus, among 19 patients in whom type III turned into type II MC, 8 patients (42%) became symptomatic compared to 6/31 patients (19.4%) in whom type III MC did not change ($p = 0.08$).

Patients with the following symptoms more frequently had type II than type III or oligoclonal type MC: peripheral neuropathy [72% (28/39) vs 23% (9/39) and 5% (2/39), respectively; $p < 0.0001$], purpura [83% (24/29) vs 17% (5/29) and 0%, respectively; $p < 0.0001$], arthralgia [70.3% (26/37) vs 24.3% (9/37) and 5.4% (2/37), respectively; $p < 0.0001$], and renal involvement [86% for type II (6/7) vs 14% for type III (1/7); $p = 0.03$]. Moreover, all 7 patients with non-Hodgkin lymphoma had type II MC. There was no significant difference in the distribution of the immunochemical types among the 22 patients with cirrhosis (METAVIR F4): type II 6/22 (27%), type III 11 patients (50%), and oligoclonal-type MC 5 patients (23%).

Analysis of the correlation between MC serum levels and clinical symptoms also highlighted interesting results. Thus, MC serum levels were higher among patients presenting cutaneous vasculitis (purpura; $0.92 \pm 1.5$ g/l vs $0.23 \pm 0.15$ g/l; $p = 0.0001$), arthralgia ($0.77 \pm 1.4$ g/l vs $0.23 \pm 0.15$ g/l; $p = 0.001$), and peripheral neuropathy ($0.72 \pm 1.3$ g/l vs $0.25 \pm 0.2$ g/l; $p = 0.001$). MC serum levels also tended to be higher among patients with B cell lymphoma ($1.18 \pm 2.15$ g/l vs $0.33 \pm 0.55$ g/l; $p = 0.06$) and renal involvement ($0.97 \pm 1.7$ g/l vs $0.35 \pm 0.7$ g/l; $p = 0.7$].

Overall, the symptomatic (i.e., MC vasculitis) compared to the nonsymptomatic patients (MC positive patients without signs of vasculitis) were older at the time of MC diagnosis ($55.6 \pm 13.6$ vs $48.5 \pm 11.8$ yrs; $p = 0.002$), tended to have a longer duration of HCV infection ($20.6 \pm 10.9$ vs $16 \pm 7.7$ yrs; $p = 0.067$), and had a more frequent type II MC [68.3% (41/60) vs 29.2% (19/65); $p < 0.0001$] and a higher MC serum level ($0.58 \pm 1.1$ g/l vs $0.22 \pm 0.15$ g/l; $p = 0.001$). They also more frequently had low C4 [75.7% (28/37) vs 0% (0/5); $p = 0.004$] and low CH50 serum levels [86.5% (32/37) vs 40% (2/5); $p = 0.06$] (Table 3).

*Multivariate analysis*. For multivariate analysis, we chose the 4 variables for which a statistically significant difference was found by univariate analysis when comparing the symptomatic to the asymptomatic patients (excluding the C4 and CH50 levels). Thus, in multivariate analysis, we identified 4 factors that appeared to be positively associated with the symptomatic character of MC, i.e., advanced age at the time of MC diagnosis ($p = 0.002$), type II MC ($p = 0.0003$, odds ratio = 4.6, 95% confidence interval = 2–11), a high MC serum level at the time of diagnosis ($p = 0.007$), and a longer duration of HCV infection ($p = 0.04$) (Table 3). Considering each MC vasculitis symptom, type II MC appeared to be positively associated with peripheral neuropathy (OR 3, 95% CI 1.3–7.5), cutaneous vasculitis (OR 7, 95% CI 2–23), arthralgia (OR 3.4, 95% CI 1.4–8.5), and renal involvement (OR 9.8, 95% CI 1.2–81). The level of MC in serum was also positively associated with peripheral neuropathy ($p = 0.03$), cutaneous vasculitis ($p = 0.0007$), and arthralgia ($p = 0.002$).

## DISCUSSION

In this study of 125 HCV infected patients with mixed cryoglobulinemia, we have highlighted that persistent MC may change its immunochemical type during the course of the chronic active infection, with the majority of cases of MC turning to type II. Common factors of persistent MC are more frequent clinical symptoms of MC vasculitis, particularly in patients with type II MC; a high MC serum level; advanced age; and a long duration of HCV infection.

The distribution of the immunochemical types of HCV MC at the time of diagnosis (type II 48%, type III 42%) is comparable with that reported in the literature[4,8]. In addition to polyclonal immunoglobulins (IgG, IgM, IgA), type II MC consists of a monoclonal immunoglobulin, usually IgMκ (65% to 69%)[5,21]. Patients with type II MC may have 2 monoclonal immunoglobulins in 9.7% to 18% of cases. Cases of the oligoclonal type of MC are often ignored or regarded as type II or type III MC in the majority of laboratories, and their classification remains unclear. They represent 10% of MC in our study at the time of diagnosis, and this proportion remained stable nearly 45 months later. Comparative data for type II/type III MC and oligoclonal MC are scarce. Musset, *et al* found 21 cases of oligoclonal-type MC among 149 MC of numerous etiologies (14%)[21]. Tridon, *et al* found higher proportions of oligoclonal type MC, 27% (7/26) to 34% (19/56)

*Table 3.* Mean differences between symptomatic (i.e., MC type, PAN-type vasculitis*, lymphoma) and non-symptomatic patients with persistent MC-HCV according to epidemiological and immunochemical indicators.

| | Symptomatic | Asymptomatic | Univariate p | Multivariate Analysis p |
|---|---|---|---|---|
| Number (%) | 60 (48) | 65 (52) | 0.5 | — |
| Sex ratio (M/F) | 28/32 | 35/30 | 0.4 | — |
| Age at MC diagnosis, yrs | 55.6 ± 13.6 | 48.5 ± 11.8 | 0.002 | 0.03 |
| Infection duration**, yrs | 20.6 ± 10.9 | 16.1 ± 7.7 | 0.07 | 0.04 |
| Type II MC, n (%) | 41 (68.3)*** | 19 (29.2) | < 0.0001 | 0.0003 |
| Type III MC, n (%) | 16 (26.7) | 37 (56.9) | < 0.001 | — |
| Oligoclonal type MC, n (%) | 3 (5) | 9 (13.8) | 0.09 | — |
| MC serum level, g/l | 0.58 ± 1.1 | 0.22 ± 0.15 | 0.001 | 0.007 |

* Cutaneous vasculitis, arthralgia, arthritis, peripheral neuropathy, renal involvement, recent onset hypertension, central nervous system involvement (PAN: polyarteritis nodosa). ** Infection duration: estimated time between contamination and the diagnosis of MC. *** Type II MC was significantly more frequent among symptomatic patients compared to the type III or oligoclonal type (68.3% vs 26.7% and 5%, respectively; $p < 0.0001$).

in HCV infected patients[17]. Our results confirm that MC serum levels of type II are higher than those of type III or oligoclonal MC[1,5,17,26,27]. Type III and oligoclonal MC have similar serum levels (0.28 ± 0.15 g/l vs 0.24 ± 0.25 g/l) as reported by Musset, *et al*[21].

Regarding the course of MC types, we found that type II cases were the most stable type during chronic HCV infection compared to type III and oligoclonal MC (78% vs 58.5% and 17%, respectively; $p < 0.05$). The main trend in variation of all MC is towards the type II MC. The oligoclonal type represents nearly 10% of HCV MC and is very close to type III, both at the serum level and in terms of clinical effect. With an 83% conversion rate, oligoclonal MC represent the most variable type, converting most frequently to type II MC. Although there is to our knowledge no report of longterm followup of bone marrow or liver immunohistopathology of HCV infected patients at each stage of the immunological disorders, we can hypothesize, as proposed by Schifferli, *et al*[24], that during chronically active HCV infection, the progression of immunoglobulin disorders may start with the appearance of type III MC. Second, the type III MC could evolve toward the oligoclonal type. As reported by Musset, *et al*[21], this oligoclonal type of MC is characterized by multiple clonal bands, previously presented as type II MC, and it may represent a possible expansion of multiple B cell clones[25]. Finally, this oligoclonal type might evolve toward type II MC, following the trend to oligoclonal and/or monoclonal selection of B lymphocytes in bone marrow and/or in liver tissue. Indeed, in type II MC, the immunohistopathologic analysis of bone marrow and/or liver biopsies often reveals a clonal and more frequently an oligoclonal B cell expansion[25,26]. At this final stage, it is important to investigate the appearance of clinical manifestations including malignant B cell lymphoproliferative disease. HCV associated MC probably represents an indolent B cell lymphoproliferation[25,26]. Recent data with B cell-target therapy, such as anti-CD20 drugs, have described the disappearance of the monoclonal component[27,28], with clear improvements in clinical and biological manifestations of MC vasculitis.

Type II MC was one of the main factors favoring the occurrence of clinical manifestations of MC. Moreover, when type III turns into type II, patients also tend to develop clinical signs of vasculitis more frequently. Nearly 50% of our patients had extrahepatic manifestations, mainly related to MC vasculitis. This is higher than the 25% to 28% reported by others[1,10,11]. But these authors reported the prevalence of clinical symptoms among all HCV infected patients having MC, with no selection for persistent MC over time. In our study, we selected patients having persistent MC with a mean followup of 5 years. This MC persistence appears to expose patients to a higher risk of vasculitis symptoms. In multivariate analysis, we observed 4 independent factors associated with the occurrence of clinical manifestations of such vasculitis: advanced age at the time of MC diagnosis, duration of HCV infection, type II MC, and the MC serum level. Advanced age has already been identified as a risk factor for MC in HCV infected patients[6], as well as a risk factor for virological nonresponse to antiviral treatment[29,30]. Cases of type II MC were more frequently symptomatic than type III or oligoclonal MC for the main clinical manifestations associated with MC (68% vs 30% and 25%, respectively; $p < 0.00001$)[5,21,27,28,31-33]. We also found a close association between high MC serum levels and the development of clinical manifestations such as purpura, peripheral neuropathy, and arthralgia. The prevalence of peripheral neuropathy (31%), arthralgia (30%), and cutaneous vasculitis (23%) was concordant with rates reported by others[3,6,7,11,16,31,33,34]. Nevertheless, in our study, only a few patients (5.6%) experienced renal involvement. This relatively low prevalence is explained in part by the fact that only patients with histological proof of renal disease were considered for study. The differences between the diagnostic criteria for MC glomeru-

lonephritis (hematuria, proteinuria, and/or histological proof) in numerous studies are emphasized by the large range in the prevalence of glomerulonephritis (2% to 50%) in patients with MC[35].

Another questionable result in our study concerns the low frequency of sustained virological responders (15%) undergoing anti-HCV therapy compared to 40% to 50% success reported with the combination course of interferon and ribavirin[29]. But this result is not surprising since our study includes only patients with persistent MC regardless of antiviral therapy. It has been found that the course of MC parallels the viral load, i.e., in patients with neutralization of HCV viremia MC usually disappears, and conversely persistent HCV viremia under antiviral treatment is associated with persistent MC[10,19,36,37].

The main limitations of our study are its retrospective design and the technical variability in the diagnosis and immunochemical typing of MC. To minimize the effect of the latter, all the immunochemical typing for MC was carried out in the same laboratory. As for the immunoblotting, Musset, *et al*[21] demonstrated that this technique is more efficient than standard immunofixation or immunoelectrophoresis for the immunochemical typing of MC and especially the detection of the microheterogeneous or oligoclonal type. Another typing method, 2-dimensional polyacrylamide gel electrophoresis, could be useful for detection of transition forms between type III and type II (tentatively named II-III) and perhaps oligoclonal types[38,39], but because of limited studies and lack of comparative studies its benefit compared to immunoblotting has not been proved. The duration of the interval defining persistent MC was arbitrarily fixed at 24 months. There is to date no clearly established duration for evaluation of the immunochemical course of MC, and our study is the first to explore the course of immunochemical types of MC among HCV infected patients. The hypothesis of MC transition remains unproven, although supported by our results, and it needs to be confirmed by molecular analysis of B cell clonal expansion in liver and bone marrow samples from MC positive patients infected with HCV.

Our results suggest that type II mixed cryoglobulinemia may represent the final evolutionary form of the majority of cases of persistent MC during HCV infection, since it is more likely to be invariant, and the majority of the other types of MC (type III and oligoclonal) convert to type II. As persistent MC may become symptomatic in nearly half of patients, we observed 4 factors closely associated with the occurrence of clinical manifestations — type II MC, high MC serum level, advanced age at the diagnosis of MC, and long duration of HCV infection.

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