Systemic Vasculitis in Patients with Hepatitis C

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ABSTRACT. ***Objective.*** To analyze the main characteristics of patients infected with hepatitis C virus (HCV) presenting with different types of vasculitis syndrome.

Methods. We retrospectively compared 2 groups of patients with HCV presenting with systemic vasculitis: 10 with biopsy proven polyarteritis nodosa-type systemic vasculitis (PAN, Group 1) and 7 with mixed cryoglobulinemia syndrome (MC, Group 2).

Results. Patients of Group 1 presented with different features than Group 2: life threatening systemic vasculitis (10 vs 0; $p < 0.01$), severe multifocal sensorimotor mononeuropathies versus distal moderate sensory polyneuropathies, malignant hypertension (5 vs 0; $p = 0.04$), cerebral angiitis (2 vs 0), ischemic abdominal pain (2 vs 0), kidney and liver microaneurisms (2 vs 0), increased erythrocyte sedimentation rate and C-reactive protein (7 vs 0; $p < 0.01$), renal insufficiency (5 vs 0; $p = 0.04$), HCV genotype 1b (3 vs 6; $p = 0.06$), and lower activity of chronic hepatitis ($p = 0.02$). Neuromuscular biopsies showed lesions of vasculitis in all patients, but the type of vasculitis was different in Group 1 compared to Group 2: medium size artery involvement (7 vs 0; $p < 0.01$), necrotizing vasculitis (10 vs 0; $p < 0.01$), and mononuclear cell infiltrate in perivascular areas (0 vs 7; $p < 0.01$). Using prednisone, plasma exchanges, and interferon-α, complete recovery was obtained in all PAN-type patients except one. In Group 2 patients, interferon-α did not have any effect on the peripheral neuropathy.

Conclusion. HCV infection may be associated with different types of systemic vasculitis, i.e., polyarteritis nodosa or mixed cryoglobulinemia. Because of differences in clinical and pathological features and therapeutic strategy, PAN-type vasculitis should be distinguished from MC-type vasculitis in HCV patients. (J Rheumatol 2001;28:109–18)

Key Indexing Terms:
HEPATITIS C VIRUS
VASCULITIS
MIXED CRYOGLOBULINEMIA
POLYARTERITIS NODOSA

Vasculitides are a heterogeneous group of disorders. Their diverse features, including clinical manifestations that sometimes overlap and involvement of all types of blood vessels, have hindered development of a universally accepted classification system[1]. Although the classification advocated by Lie[2], which basically separated vasculitides into infectious and noninfectious categories, has some clinical relevance, it can be considered dated, as the infectious origin of most of the disorders previously termed "essential" mixed cryoglobulinemia has been identified[3,4]. In addition, some infectious agents may induce different types of vasculitis. For example, hepatitis B virus (HBV) and human immunodeficiency virus are recognized as etiologic factors of both mixed cryoglobulinemia (MC) syndrome and classic polyarteritis nodosa (PAN)[5,6].

Hepatitis C virus (HCV) is a worldwide infection that is the main cause of non-A, non-B hepatitis. Numerous extrahepatic manifestations have been associated with HCV infection, such as MC, glomerulonephritis, porphyria cutanea tarda, sicca syndrome, and systemic necrotizing vasculitis[7-11]. Chronic HCV infection may be associated with different types of vasculitis, i.e., small vessel vasculitis (such as MC) or larger vessel disease (such as PAN). In large clinical studies, anti-HCV antibodies have been found in 60 to 80% of patients with "essential" MC[11-18]. MC are also frequently found (55%) in HCV patients without extrahepatic manifestations[19,20]. The prevalence of anti-HCV antibodies in patients with PAN ranges from 5 to 12%[21-24]. However, since most of these PAN patients infected by HCV also had cryoglobulins in their serum, there is controversy regarding the existence of "authentic" PAN related to HCV infection.

We describe detailed clinical, biological, virological, and histopathological features of 17 patients with HCV presenting with systemic vasculitis manifestations, 10 that fulfilled the criteria for "authentic" PAN-type vasculitis and 7 that only presented symptomatic mixed cryoglobulinemia.

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MATERIALS AND METHODS

Patients. We retrospectively studied the files of patients seen in internal medicine and neurology departments of our university hospital presenting with signs of systemic vasculitis and chronically infected with HCV. All patients had chronic HCV infections defined by anti-HCV antibodies detected by a third generation test (ELISA or recombinant immunoblot assay, RIBA), liver biopsy findings compatible with chronic hepatitis C, and no other identifiable cause of liver dysfunction (i.e., chronic hepatitis B, autoimmune hepatitis, primary biliary cirrhosis).

Patients were separated into 2 groups depending on whether they presented predefined PAN criteria (Group 1) or just symptomatic MC (Group 2). Since some clinical symptoms were common to both PAN-type and MC patients, strict criteria were used to define Group 1 patients. Only patients who fulfilled the American College of Rheumatology criteria for PAN[25] were included in Group 1. They all presented multiple system clinical involvement and histological evidence of mostly medium size vessel necrotizing vasculitis. Necrotizing vasculitis was characterized as an inflammatory process that interrupts and destroys the vascular walls (i.e., transmural inflammatory cell infiltration, segmental necrosis of the wall). Group 1 included 10 patients (6 men, 4 women) with a mean age of 51 ± 15 years (range 30–72). The main features of patients who did not fulfill criteria for PAN were analyzed in the symptomatic MC group, Group 2. Group 2 included 7 patients, 5 men and 2 women, mean age 59 ± 11 years (36–76). No Group 2 patient showed necrotizing vasculitis of medium or small size arteries.

The clinical features were analyzed retrospectively. For each patient the following data were recorded: age, sex, recent weight loss, neurologic involvement (central, peripheral), recent onset hypertension (systolic blood pressure > 160 and/or diastolic blood pressure > 95 mm Hg), cutaneous involvement (Raynaud's phenomenon, purpura, livedo, distal ulcers, or gangrenous changes), arthralgia, myalgia, and signs of clinical hepatic involvement (hepatomegaly, splenomegaly, collateral venous circulation, spider angioma).

HCV serum markers. All sera had anti-HCV antibodies identified by third generation ELISA (Ortho) and confirmed by RIBA (Chiron, Diagnostic Systems, Emeryville, CA, USA). HCV polymerase chain reaction (PCR) was performed on sera that had been kept at –80°C and never thawed to avoid false negativity due to RNA destruction by RNases and false positivity due to contamination. Serum RNA was extracted, reverse transcribed, and amplified as described[17]. HCV genotyping was performed using a second generation line probe assay (LiPA; Innogenetics, Brussels, Belgium), which identifies the main HCV types and subtypes[26].

Other viral markers. Sera were also tested for the presence of hepatitis B surface antigen (HBsAg), anti-HBs antibody, and anti-hepatitis B core antibody (HBcAb) using commercial immunoassays (Abbott Laboratories, Diagnostic Pasteur). HBV DNA was investigated in all sera by molecular hybridization (Genostics, Abbott Laboratories). All sera were negative for anti-HIV antibodies using commercial immunoassays (Abbott Laboratories, Diagnostic Pasteur).

Detection and characterization of cryoglobulins. Cryoglobulins were isolated from patients' sera, purified, and characterized by immunoblotting at 37°C as described[27]. Previous evaluation using this method showed that only 5/131 (3.8%) of healthy blood donors have a MC, all with a level lower than 0.03 g/l[15]. We considered patients had a significant cryoglobulin level when ≥ 0.05 g/l on 2 determinations. Cryoglobulins were then classified according to Brouet, *et al*[28], as either type II MC, which includes a monoclonal component, or type III MC, defined by the association of polyclonal immunoglobulins.

Biochemical evaluation. Using standard laboratory procedures the following were evaluated: blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum alanine aminotransferase and aspartate aminotransferase, alkaline phosphatase, albuminemia, creatinemia, rheumatoid factor (RF), antinuclear antibody, complement components (C3 and C4), and total hemolytic complement activity (CH50). Hematuria and daily proteinuria were also assessed. Antineutrophil cytoplasmic antibodies (ANCA) were tested by an indirect immunofluorescence assay on ethanol fixed neutrophils. Their specificity was determined as described[29].

Electrophysiological studies. Electrophysiological studies were performed with a Viking Nicolet electromyograph as described[30]. Based on the clinical and electrophysiological findings each peripheral neuropathy observed was classified as either a polyneuropathy or multifocal mononeuropathy. The peripheral neuropathies were then classified as axonal or demyelinating using the criteria for chronic inflammatory polyneuropathies of the American Academy of Neurology[31].

Analysis of neuromuscular biopsy specimens. All patients underwent a neuromuscular biopsy of the superficial peroneal nerve and peroneus brevis muscle in the most affected limb, studied as described[32]. Necrotizing vasculitis was diagnosed when vessel walls were interrupted and destroyed by an inflammatory process (transmural inflammatory cell infiltration, segmental necrosis of the wall). Perivascular infiltrate was diagnosed when the inflammatory process did not involve the vascular walls. The type of inflammatory infiltrate was analyzed. It was composed of either mononuclear cells (lymphocytes, monocytes) or both mononuclear cells and polymorphonuclear neutrophils. Affected vessels were assessed for size of the smallest vessel diameter at the outer layer of the media. The diameter of the vessels was measured at a level without lesion by a micrometric slide and controlled the standard diameter of erythrocyte. Medium size vessels were defined as arteries with diameter > 70 µm. Small size vessels included veins, capillaries, venules, and arterioles.

Analysis of liver biopsy specimens. Histologic examination of liver biopsies included qualitative and quantitative analyses of the inflammatory activity and severity of fibrosis, classified using Knodell's score[33].

Statistical analysis. All data were expressed as the mean ± standard deviation (SD). Chi-squared or Fisher's exact tests were used for comparisons of percentages. Mean quantitative values were compared using the unpaired Student t test. Nonparametric analyses were by Mann-Whitney U test. Significance was assessed at $p = 0.05$. All calculated p values were 2 tailed.

RESULTS

Patients with HCV PAN-type vasculitis (Group 1)

Clinical data (Table 1). Disease onset was acute in all cases and patients developed a very severe illness within a few weeks. The peripheral neuropathy was a multifocal mononeuropathy in 9 Group 1 patients, involving either 2 (n = 3) or all 4 limbs (n = 6), and a polyneuropathy involving 4 limbs in one patient. Severe motor deficit was found in 8/10 patients. Both proximal and distal upper and lower limbs were involved in 6 patients and only distal lower limbs in 2 patients. Due to motor deficit severity 3 patients needed a wheelchair. Sensory symptoms were painful in 8/10 patients and appeared simultaneously with the motor signs. No patient complained of autonomic nervous system symptoms or had orthostatic hypotension. Painful symptoms were also due to myalgia (n = 4) and arthralgia (n = 4). Cutaneous involvement was frequent: purpura (n = 6), Raynaud's phenomenon (n = 3), digital ulcers (n = 2), and livedo (n = 1). Severe hypertension (diastolic blood pressure ≥ 100 mm Hg) was noted at entry in 5 patients, but was associated with renal insufficiency in only 2 cases. Two patients had ischemic abdominal pains that explained their rapid and critical weight loss. Arteriography (Patients 1 and 2) showed stenosis of coeliac and mesenteric

Table 1. Clinical and biological features of patients with HCV–PAN-type vasculitis.

Patient	1	2	3	4	5	6	7	8	9	10
Clinical features										
Age/sex	45 M	31 F	49 M	57 M	65 F	51 M	30 M	41 F	69 M	72 F
Weight loss (kg)	10	12	5	16	5	0	5	0	0	6
Type of neuropathy	MM, 2 limbs	MM, 4 limbs	MM, 4 limbs	MM, 4 limbs	MM, 4 limbs	MM, 4 limbs	MM, 4 limbs	MM, 2 limbs	PN, 4 limbs	MM, 2 limbs
Deficit	SM	SM	S > M	S > M	S > M	SM	S > M	S	S	SM
Abnormal nerves on EMG	Peron, median	Peron, radial	Peron, ulnar	Peron, ulnar, radial	Peron, ulnar	Peron, median	Peron, median, ulnar	Peron	LL	Peron, radial
Blood pressure (mm Hg)	165/105	240/140	190/100	170/90	170/80	220/120	170/110	110/60	120/80	210/80
Cutaneous signs	Purpura	Ulcers	Purpura	0	Purpura, RP	0	Purpura, ulcers	Purpura, livedo	Purpura, RP	RP
Arthralgia/myalgia	Yes/Yes	No/No	Yes/No	No/No	No/No	Yes/Yes	Yes/Yes	No/Yes	No/No	No/No
Ischemic abdominal pain	Yes	Yes	No	No	No	No	No	No	No	No
Hepatomegaly/splenomegaly	No/Yes	No/No	Yes/No	Yes/No	No/No	No/No	Yes/No	No/No	No/No	No/No
Biological features										
Liver tests	N	Abnormal	Abnormal	Abnormal	N	Abnormal	Abnormal	Abnormal	N	Abnormal
Creatinemia (µmol/l)	89	318	115	83	163	165	82	60	140	245
Proteinuria (g/d), abnormal sediment	0/No	0.85/Yes	0/No	0/No	0/No	0.80/No	0/Yes	0/No	0/No	ND/Yes
ESR (mm)/CRP (mg/l)	61/77	90/70	45/60	64/1	30/13	7/ND	13/28	14/13	1/ND	ND/ND
Cryoglobulin level (g/l)	0.92	1.32	0.66	0.08	0.63	0.16	0.10	0.32	1.3	1.23
Cryoglobulin type	III	II Mk	II Mk	II Mk	II Mk	II Mk	II Mk	II Mk	II Mk	II Mk
C3,C4 (g/l)/CH50 (U/ml)	0.75, 0.36/34	0.56, 0.04/19	0.99, 0.12/19	1.21, 0.35/36	1.2, 0.48/31	0.56, 0.03/33	0.59, 0.08/17	0.65, 0.10/31	0.69, 0.10/19	0.45, 0.10/30
Rheumatoid factor	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes
HBsAg/HBc Ab/HBV DNA	0/+/0	0/+/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/+/0	0/0/0
HCV PCR positivity, genotype	Yes, 3a	Yes, 4	Yes, 1b	Yes, 4	Yes, 1b	Yes, 1b	No, ND	Yes, 3a	Yes, 2a/2c	Yes, 5a

Peron: peroneal, LL: lower limbs, RP: Raynaud's phenomenon, SM: sensorimotor, MM: multifocal mononeuropathy, PN: polyneuropathy, S: purely sensory, S > M: predominantly sensory, N: normal, ND: not done.

arteries, as well as renal and hepatic microaneurysms (Figures 1, 2). In 2 patients (Patients 2 and 5) the peripheral neuropathy was associated with a cerebral angiitis leading to troubles of consciousness, stroke, hemiballism, and numerous large hyperintense lesions in the white matter on cerebral magnetic resonance imaging suggestive of vasculitis.

Biological and pathological data (Tables 1, 2). Although 3 patients had anti-HBc antibodies none tested positive for HBV DNA. HCV PCR was positive in all patients except one. The distribution of HCV genotype was heterogeneous: type 1b (n = 3), type 3a (n = 2), type 4 (n = 2), type 2 (n = 1), type 5a (n = 1), and was not determined in one case. Liver biopsies revealed moderate chronic hepatitis with an average Knodell score of 5.1 ± 2.4 (range 2 to 8), without frank cirrhosis. Recent onset renal insufficiency was noted in 5 patients (creatinemia 140–318 µmol/l) and was usually associated with microscopic hematuria and moderate proteinuria (< 1 g/dl), but only one patient underwent renal biopsy. This patient (Patient 2) rapidly developed endstage renal insufficiency leading to hemodialysis within a few weeks. The renal biopsy showed ischemic cortical necrosis without immunoglobulin or complement glomerular deposits. All patients in Group 1 had a mixed cryoglobulinemia: type II with an IgM kappa (n = 9) and type III (n =

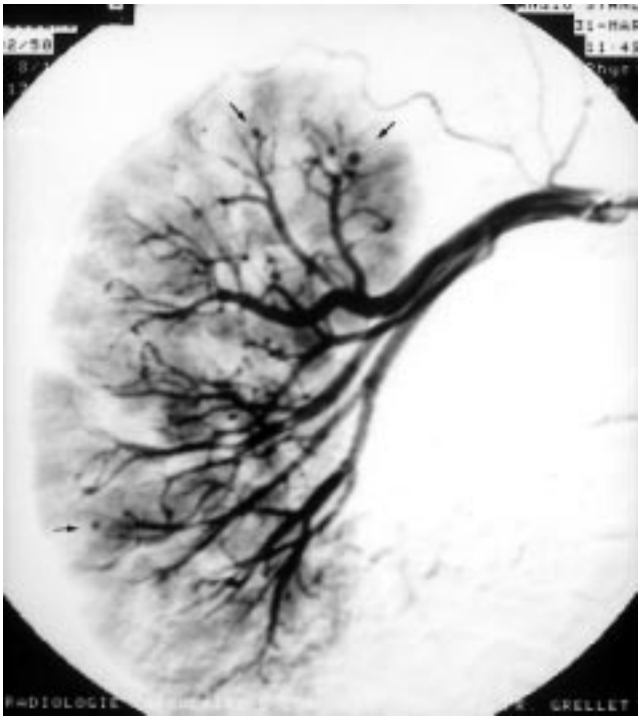

Figure 1. Renal arteriography of Patient 1 with HCV–PAN-type vasculitis showing numerous intrarenal microaneurysms.

Table 2. Pathological features in patients with HCV–PAN-type vasculitis.

Patient	1	2	3	4	5	6	7	8	9	10
Kidney	ND	Ischemic cortical necrosis	ND	ND	ND	Large artery angiitis	ND	ND	ND	ND
Skin	ND	ND	ND	ND	Normal	ND	Vasculitis	Vasculitis	Vasculitis	ND
Liver (Knodell score)	3	2	7	6	6	ND	ND	8	2	7
Neuromuscular										
Axonal degeneration	Severe	Severe	Severe	Moderate	Severe	Severe	Severe	Moderate	Moderate	ND
Small regenerating fibers	—	—	—	+	—	—	—	+	+	—
Site of inflammatory process	Nerve, muscle	Nerve, muscle	Nerve	Nerve, muscle	Nerve	Muscle	Muscle	Nerve	Nerve	Muscle
Type of vessel involved	Arteries	Arteries	Arteries, veins	Arterioles, veins	Arteries, venules	Arteries	Veins	Arteries	Arterioles, veins	Arteries, arterioles
Size of vessel involved	Medium, small	Medium, small	Medium, small	Small	Medium, small	Medium	Small	Medium, small	Small	Medium, small
Necrotizing vasculitis	+	+	+	+	+	+	+	+	+	+
Occlusion of the lumen	+	–	+	+	+	–	–	–	+	+
Fibrinoid necrosis	–	+	–	+	–	+	+	–	–	+
Perivascular infiltrate, type	+, mixed	+, mixed	+, mono	+, mixed	+, mixed	+, mixed	+, mixed	+, mixed	+, mono	–, mixed

ND: not done.

1). Cryoglobulinemia levels ranged from 0.08 to 1.32 g/l. RF activity was found in 7 patients. We found low levels of C3 (n = 6), C4 (n = 7), and CH50 levels (n = 8). No patient had ANCA.

In all patients, neuromuscular biopsies showed lesions of necrotizing vasculitis (Figure 3), localized in the nerve (n = 7) and/or muscle (n = 6). Whatever the vessel's size, necrotizing vasculitis was always found showing vessel wall interruption and destruction by an inflammatory process and leading to occlusion of the lumen (n = 6) or fibrinoid necrosis (n = 5). The infiltrate was composed in 2 cases of only mononuclear cells and in 8 cases in association with polymorphonuclear neutrophils. Severe and moderate axonal degeneration was noted in 6 and 3 cases, respectively.

Treatment and followup. Five patients were treated with a combination of prednisone (1 mg/kg/day), plasma exchanges, and interferon-α. They each received a total of 12 plasma exchanges and the dosage of steroids was rapidly reduced within 4 weeks to 10 mg/day. Interferon-α was administered at 3 million IU three times a week over 18 to 36 months. All 5 patients showed complete recovery (except for endstage renal failure in Patient 2), including great neurological and general state improvement, normalized blood pressure, and regression of ischemic abdominal pain, skin purpuric lesions and fever. Patient 1 received a combination of prednisone (5 mg/day) and interferon-α during 18 months. Then steroids were stopped and interferon-α was maintained for 10 months. He remained clinically asymptomatic with persistent negative HCV viremia. Two months after withdrawal of interferon-α, he had a PAN relapse concomitant with positive viremia. He responded rapidly to plasmapheresis, interferon-α, ribavirin, and low dose steroids, with resolution of viremia and remission of vasculitis. For the other 4 patients, we did not try to stop

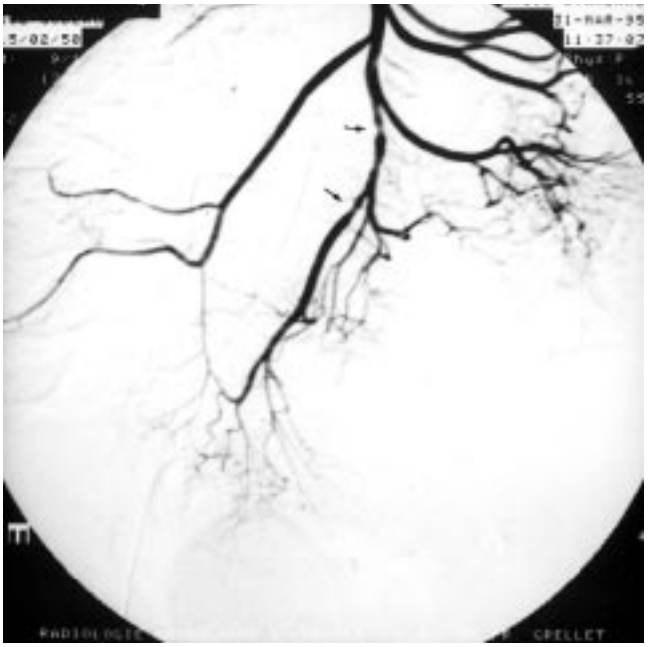

Figure 2. Mesenteric arteriography of Patient 1 with an HCV–PAN-type vasculitis showing inflammatory stenosis of the proximal superior mesenteric artery. This lesion disappeared within 6 months after treatment with low dose steroids, plasma exchanges, and interferon-α.

Table 3. Clinical and biological features in patients with symptomatic HCV–mixed cryoglobulinemia-type vasculitis.

Patient	11	12	13	14	15	16	17
Clinical features							
Age/sex	65 F	64 M	58 M	57 M	63 M	36 M	76 F
Weight loss (kg)	0	0	0	0	2	4	0
Type of neuropathy	PN, 2 limbs	PN, 2 limbs	PN, 2 limbs	PN, 2 limbs	PN, 2 limbs	PN, 4 limbs	PN, 2 limbs
Deficit	S > M	S > M	S	S	S	S > M	S
Abnormal nerves on EMG	LL	UL, LL	UL, LL	LL	LL	UL, LL	LL
Blood pressure (mm Hg)	140/90	135/70	200/105	130/70	125/70	130/80	145/80
Cutaneous signs	Purpura	Purpura	Purpura, Ry, livedo	Purpura	Purpura	0	0
Arthralgia/myalgia	0	0	0	0	0	0	0
Ischemic abdominal pain	No	No	No	No	No	No	No
Hepatomegaly/splenomegaly	No/No	Yes/Yes	No/No	Yes/Yes	No/No	No/No	No/No
Biological features							
Liver tests	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	N	Abnormal
Creatinemia (µmol/l)	83	84	115	99	80	112	85
Proteinuria (g/d), abnormal sediment	ND/No	0/No	0/No	ND/No	0/No	0/No	0/No
ESR (mm)/CRP (mg/l)	20/44	23/8	18/13	ND/ND	14/13	24/16	24/1
Cryoglobulin level (g/l)	2.0	0.95	1.33	0.15	0.42	0.08	0.45
Cryoglobulin type	II Mk	II Mk	II Mk	II Mk	II Mk	II Mk	III
C3,C4 (g/l)/CH50 (U/ml)	0.83, 0.9/19	0.56, 0.32/25	0.88, 0.04/22	1.4, 0.08/47	0.3, 0.03/19	0.6, 0.98/28	0.79, 0.31/32
Rheumatoid factor	Yes	No	Yes	Yes	Yes	ND	Yes
HBsAg/HBc Ab/HBV DNA	0/0/0	0/+/0	0/+/0	0/0/0	0/0/0	0/0/0	0/+/0
HCV PCR positivity, genotype	Yes, 1b	Yes, 1b	Yes, 1b	Yes, 1b	Yes, 1b	Yes, 3c	Yes, 1b

Abbreviations as in Table 1.

interferon-α, considering the good tolerance of treatment and the risk of relapse of vasculitis.

Interferon-α was tested alone in 3 patients (3 million IU three times weekly) for 6 to 12 months, but it had efficacy on skin lesions only. In 2 patients (Patients 3 and 6), HCV infection was diagnosed 3 and 5 years after treatment for PAN. However, HCV antibodies were detected retrospectively in frozen sera taken at the first onset of PAN-type vasculitis, that is, before any treatment for PAN or blood transfusion. These 2 patients had had good response to the usual treatment of severe systemic vasculitis that included steroids, plasma exchange, and IV cyclophosphamide.

After a mean followup of 45 ± 28 months (range 13–96), all patients were alive and none had had a vasculitis relapse. However, 5/10 patients were still receiving low doses of steroids (< 10 mg/day) plus interferon-α (3 million IU three times weekly) at the end of the study.

Patients with HCV–symptomatic mixed cryoglobulinemia (Group 2)

Clinical data (Table 3). The predominant extrahepatic symptom was a subacute distal polyneuropathy, preceding the diagnosis by a few months. No patient had life threatening disease at entry or during the course of study. Sensory symptoms with electromyographic signs were present in all patients from the onset of their neuropathy. No patient complained of autonomic nervous system disturbances or had orthostatic hypotension. A moderate motor deficit was found in 3 patients. There were no cases of multifocal mononeuropathy or signs of cerebral vasculitis in Group 2. One patient had disability due to severe sensory ataxia. The other 6 patients had only moderate disabilities after a long followup. Five patients had purpuric skin lesions of the lower limbs. Two patients had hepatosplenomegaly. A single patient (Patient 13) had hypertension and moderate renal insufficiency, with no proteinuria or abnormal urinary sediment. No arthralgia, myalgia, or ischemic abdominal pain were noted.

Biological and pathological data (Tables 3, 4). Liver biological tests were abnormal in 5/7 patients, with no signs of hepatocellular insufficiency. All patients had PCR positive for HCV. HCV genotypes were mainly of type 1b (n = 6). One case was type 2. Three patients had anti-HBc antibodies, but none had HBsAg or HBV DNA. Liver biopsies showed signs of chronic active hepatitis, frequently associated with signs of cirrhosis (5/7). The mean Knodell score was 11.8 ± 2.4 (range 7–14). Only one 64-year-old hypertensive patient had a moderate renal insufficiency, with creatinemia 115 µmol/l. His renal biopsy showed absence of cryoglobulinemic nephritis and presence of arteriosclerosis. MC was found in all patients, including type II IgM kappa in 6 patients (0.08 to 2.0 g/l) and type III in one patient. RF activity was found in 5/7 patients. Serum complement components were usually abnormal, with low C3 (n = 4), C4 (n = 4), and CH50 (n = 7) levels.

Neuromuscular biopsies showed an inflammatory

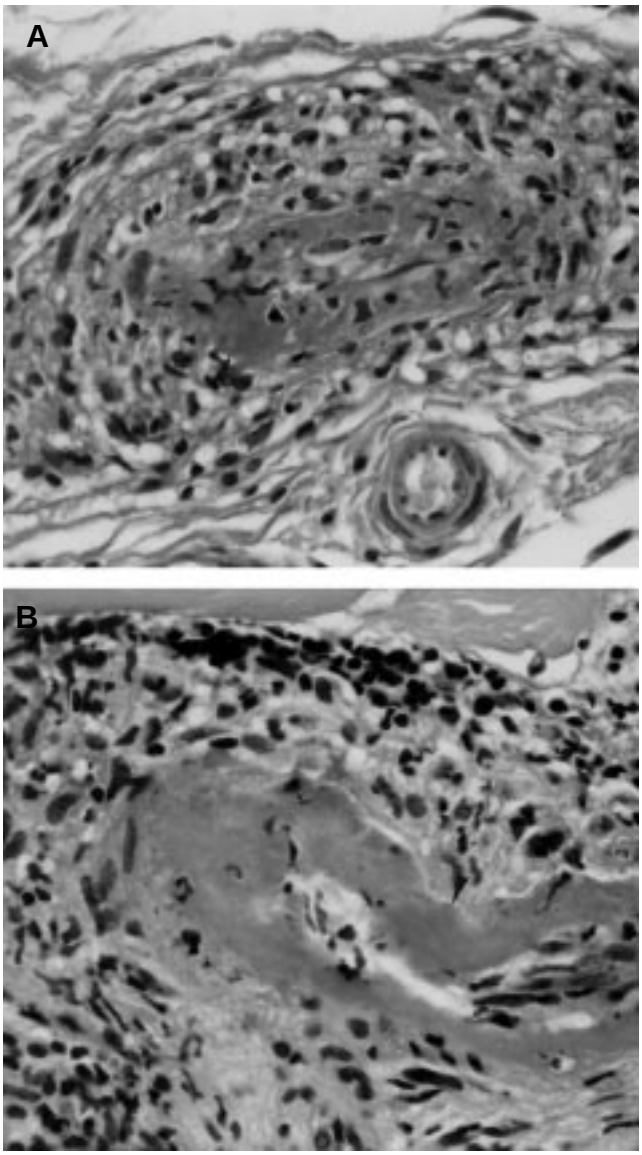

Figure 3. Neuromuscular biopsy of Patient 2 reveals HCV–PAN-type vasculitis. Paraffin embedded tissue; transversal section of muscle biopsy; H&E stain. Medium size arteries show a transmural inflammatory process. The vessel wall is interrupted and destroyed by a mixed polymorphonuclear and mononuclear cell infiltrate. Segmental fibrinoid necrosis can be seen (arrow, panel A and B). A ×320, B ×460.

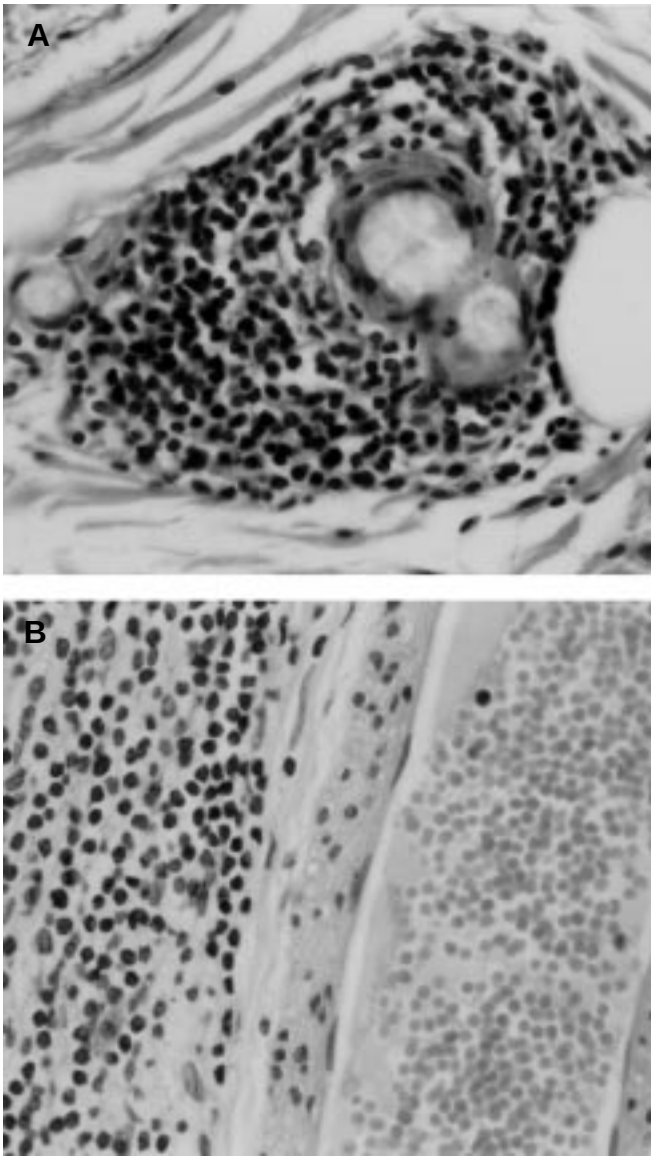

Figure 4. Neuromuscular biopsy of a patient with HCV–MC (Patient 12). Paraffin embedded tissue; transversal section of muscle biopsy; H&E stain. A. Transversal section of superficial peroneal nerve (×320). In the perineurium, the infiltrate of mononuclear cells surrounds vein and small vessels (venules or arterioles). The abundant infiltrate is exclusively composed of small lymphocytic cells. Fibrinoid necrosis is not visible. B. Longitudinal section of superficial peroneal nerve (×460). Infiltrate of mononuclear cells surrounds vein without involvement of the vascular wall.

process of small vessels in all patients. The infiltrate consisted exclusively of mononuclear cells (n = 6) and it was predominantly localized in perivascular areas (Figure 4). The vessel walls were intact and unaffected by the inflammatory process. Veins and/or veinules were frequently involved but medium size arteries were not. All patients had axonal degeneration that was moderate (n = 5) or severe (n = 2) and was usually associated with small regenerating fibers.

Treatment and followup. Five patients received interferon-α (3 million IU three times weekly), alone (n = 3) or associated with plasma exchanges (n = 1), or with steroids plus plasma exchanges (n = 1). Two patients received only low dose steroids (< 15 mg/day). None of these treatments was clearly effective for the extrahepatic manifestations of HCV. Moreover, 3 patients had slow progression of their peripheral neuropathy. All Group 2 patients were alive after a mean followup of 31 ± 17 months (range 15–42), but unlike patients of Group 1, they all endured a permanent disability without neurologic recovery.

Table 4. Pathological features in patients with symptomatic HCV–mixed cryoglobulinemia-type vasculitis.

Patient	11	12	13	14	15	16	17
Liver (Knodell score)	8	14	11	12	9	8	13
Neuromuscular							
Axonal degeneration	Severe	Moderate	Severe	Moderate	Moderate	Moderate	Moderate
Small regenerating fibers	+	+	–	+	–	+	+
Site of inflammatory process	Nerve	Nerve	Nerve	Nerve	Nerve	Muscle, nerve	Muscle, nerve
Type of vessel involved	Veins, arterioles	Veins, capillaries	Veins	Veins, arterioles	Veins, capillaries	Veins, arterioles	Veins, arterioles
Size of vessel involved	Small	Small	Small	Small	Small	Small	Small
Necrotizing vasculitis	—	—	—	—	—	—	—
Occlusion of the lumen	—	—	—	—	—	—	—
Fibrinoid necrosis	—	—	—	—	—	—	—
Perivascular infiltrate, type	+, mono	+, mono	+, mono	+, mixed	+, mono	+, mono	+, mono

Comparison between Groups 1 and 2

Comparative clinical, biological, virological, and pathological features of HCV patients presenting with symptoms of systemic vasculitis, PAN, or MC are summarized in Table 5. Patients with HCV–PAN-type vasculitis presented with different clinical features than those with HCV–MC: life threatening systemic vasculitis (10 vs 0; $p < 0.01$), severe multifocal sensorimotor mononeuropathies versus distal moderate sensory polyneuropathies, cerebral angiitis (2 vs 0), ischemic abdominal pain (2 vs 0), malignant hypertension (5 vs 0; $p = 0.04$), and kidney and liver microaneurisms (2 vs 0). Biological features were also different in Group 1 versus Group 2: increased ESR or CRP levels (7 vs 0; $p < 0.01$), thrombocytopenia (0 vs 43%), renal insufficiency (5 vs 0; $p = 0.04$), HCV genotype 1b (3 vs 6; $p = 0.06$). Liver biopsy specimens revealed lower activity of chronic hepatitis in Group 1 than in Group 2 (Knodell score 5.1 ± 2.4 vs 11.8 ± 2.4; $p = 0.02$). Neuromuscular biopsies showed

Table 5. Comparative features of HCV patients with PAN type or mixed cryoglobulinemia vasculitis. Data are number (%), unless specified.

	HCV–PAN	HCV–MC
Age (mean ± SD), yrs	51 ± 15	59 ± 11
M/F	6/4	5/2
Clinical features, n (%)		
Poor general condition	8 (80)	0 (0)
Neurologic involvement	Multifocal mononeuropathy Motor > sensory (90)	Distal polyneuropathy Sensory >>> motor (100)
Cerebral vasculitis	2 (20)	0 (0)
Severe hypertension	5 (50)	1 (14)
Purpura-livedo	7 (70)	5 (71)
Arthralgia-myalgia	4 (40)	0 (0)
Ischemic abdominal pain	2 (20)	0 (0)
Biological features, n (%)		
Abnormal ESR/CRP	6 (60)	0 (0)
Recent onset renal insufficiency	5 (50)	0 (0)
Cryoglobulin type II/III	9/1 (90/10)	6/1 (86/14)
Low C3-C4 or CH50	8 (80)	7 (100)
Rheumatoid factor	7 (70)	5 (71)
HCV infection, n (%)		
Abnormal liver tests	7 (70)	5 (71)
HCV PCR positivity	9 (90)	7 (100)
HCV genotype 1b	3 (30)	6 (86)
Knodell score, mean ± SD	5.1 ± 2.4	11.8 ± 2.4
Neuromuscular biopsy		
Type of vessel involved	Medium size arteries (> 70 mm)	Small arteries (≤ 70 mm), arterioles, capillaries, veins
Necrotizing vasculitis	Yes (100)	No (0)
Perivascular infiltrate	+ (90)	+++ (100)
Type of infiltrate	Mononuclear cells + polymorphonuclear neutrophils (90)	Mononuclear cells (86)

lesions of vasculitis in all patients, but the type of vasculitis was different in Group 1 versus Group 2, with medium size artery involvement (7 vs 0; $p < 0.01$), necrotizing vasculitis (10 vs 0; $p < 0.01$), and inflammatory infiltrate composed of only mononuclear cells and predominantly localized in perivascular areas (0 vs 7; $p < 0.01$). We did not find mixed forms of pathology in patients of either group. At the time of the study, no patient in Group 2 had the clinical, laboratory, or pathologic features of Group 1.

DISCUSSION

Numerous extrahepatic manifestations have been associated with chronic HCV infection[7-11]. The most frequent is represented by mixed cryoglobulinemia, an immune complex vasculitis involving preferentially small size vessels (i.e., venules, capillaries, arterioles)[10,11]. Studies on medium size vessel vasculitis of PAN type and HCV infection association have been controversial. The prevalence of anti-HCV antibodies in patients with proven PAN-type vasculitis has been reported to be from 5 to 12%[21-24]. Except for a few case reports of skin, neurologic, or renal necrotizing vasculitis[34,35], there are few published data on the prevalence of systemic vasculitis in patients with HCV[10]. Differentiation between PAN-type and MC vasculitis can be difficult, since they may share the same clinical manifestations and pathologic lesions[1,2], including peripheral neuropathy[30], purpuric skin lesions[36], arthralgia, myalgia, renal involvement[13], and characteristics of vascular inflammatory infiltrate.

In this series, possible confusion between PAN-type and MC vasculitis arises from the presence of a mixed cryoglobulin in the serum of most patients in both groups. Consequently, it could be argued that the HCV patients of Group 1, of which 9/10 had MC in their serum, presented a particularly severe form of MC vasculitis. This biological finding, however, is insufficient to corroborate all clinical extrahepatic manifestations with the mere presence of MC. Many data substantiate the existence of PAN-type vasculitis in these HCV patients. First, MC are very often found in patients with chronic HCV infection (55–86%), although only a minority are symptomatic (< 15%)[19,20]. MC are also frequently found in connective tissue diseases, with a prevalence range of 19 to 45%, such as in rheumatoid arthritis[37,38], lupus, Sjögren's syndrome[37], Wegener's granulomatosis, and "classic" PAN[39]. The presence of cryoglobulin in serum of patients with a systemic vasculitis syndrome is insufficient to prove the correlation between cryoglobulin and vasculitis. A necrotizing vasculitis associated with HCV infection has been successfully treated with interferon-α, despite the persistence of MC as well as complement consumption[34]. This suggests that qualitative rather than quantitative change of the MC reduced their pathogenic properties.

Second, all patients with HCV–PAN-type vasculitis had very severe acute clinical manifestations, with polyvisceral failure that was often life threatening[40], unlike those with HCV–MC-type vasculitis that mainly showed subacute moderate skin and neurological symptoms[12,15,17,30]. In the majority of Group 1 patients, the neurological involvement was a severe acute sensorimotor multifocal mononeuropathy involving all limbs, whereas in Group 2, it was only a moderate subacute sensory distal polyneuropathy of the lower limbs, which caused great disability but was never life threatening[4,28,41]. In their series of 86 patients with MC, Brouet, *et al*[28] described that in 4 patients, "the neurologic symptoms appeared abruptly and progressed in an asymmetric course," and that "in three of these cases muscle biopsy showed pictures of PAN." In addition, Gorevic, *et al* described in an MC series 3 patients who also had acute multifocal mononeuropathy[4]. It is therefore possible that in series where so-called "essential" MC were identified (the majority of which are now known to be secondary to HCV infections), some patients had HCV related PAN-type vasculitis. The high prevalence of neuropathy in both groups is partly due to the recruitment of patients from internal medicine and neurology departments, but we did not exclude patients presenting with rheumatologic, dermatologic, or renal signs and symptoms. Such frequent involvement of the peripheral nervous system is reported in many series of systemic vasculitis[40]. Central nervous system involvement was noted in 2 patients of Group 1, a feature rarely reported in MC patients[42,43]. Petty, *et al*[44] reported 2 cases of cerebral ischemia in young women with HCV and MC. In one patient, cerebral angiography showed narrowing of the supraclinoid internal carotid and middle and anterior cerebral arteries. This suggests an inflammatory process in large or medium size arteries as seen in patients with PAN, rather than a vasculitic process associated with MC that involves small arteries[45].

Third, pathologic lesions were different in patients of Groups 1 and 2 (Table 5). The classic PAN involves medium and small size vessels with a mixed inflammatory infiltrate of monocytes, lymphocytes, and polymorphonuclear neutrophils[1] and a necrotizing angiitis[2], as we found in patients of Group 1 (Figure 3). In contrast, mixed cryoglobulins involve small size vessels (arterioles, venules, capillaries) with an inflammatory infiltrate composed of only monocytes and lymphocytes, without necrotizing angiitis[1,2], as we found in Group 2 patients. The inflammatory process preserved the integrity of the vessel walls and was particularly abundant around veins and venules, with no lesions of necrotizing vasculitis (Figure 4). Of interest, the same differences in pathological aspects were noted in the skin biopsies, i.e., the predominant finding of mononuclear perivascular infiltrates in Group 2 patients.

Finally, some distinctive features found in Group 1 patients are common in PAN but have not been reported in MC. They include microaneurysms (found in 2 Group 1

patients)[40,46] and renal cortical necrosis secondary to occlusion of medium size arteries (one patient in Group 1), a known feature of PAN and unreported in MC. In contrast to other small vessel vasculitis, ANCA are not helpful in the distinction between "classic" PAN and MC vasculitis, since they are rarely found in either of them[1,47].

The mechanism(s) leading to PAN or MC-type vasculitis in HCV patients remains unclear. It should be noted, however, that Group 2 patients seem to have more severe and long lasting liver diseases than Group 1 patients. Group 2 patients presented more frequently with 1b genotype infections (83 vs 33%), thrombocytopenia (43 vs 0%), and severe chronic active hepatitis (Knodell score 11.8 ± 2.4 vs 5.1 ± 2.4) (Table 5). This may imply that symptomatic MC could be a complication following long lasting HCV infections with rather severe chronic liver diseases, whereas PAN-type vasculitis (as observed in patients with hepatitis B virus infections)[40] is an earlier complication of HCV patients with only moderate liver disease. This hypothesis is reinforced by the fact that most patients of both groups did not previously receive immunosuppressive treatments that could have accelerated liver disease.

Our treatment of PAN-type vasculitis related to HCV infection was based on that proposed by investigators of PAN related to HBV infections[48]. Initially, corticosteroids were used to rapidly control the most severe life threatening manifestations of systemic vasculitis, followed by a rapid decrease of steroids to prevent excessive HCV replication. Plasma exchanges were undertaken to control the course of vasculitis by clearance of immune complexes without stimulating viral replication. Interferon-α was used to directly control HCV replication. Five patients with PAN-type vasculitis related to HCV infections received prednisone, plasma exchanges, and interferon-α for a period of 4 weeks. Thereafter, the maintenance treatment consisted of low prednisone doses and interferon-α. This treatment allowed rapid control of the main symptoms. Relatively moderate maintenance treatment after 10 to 18 months' delay permitted complete recovery in 4 of the 5 patients without significant side effects. Of note, one patient with HCV–PAN relapsed shortly after interferon-α withdrawal with a parallel course of HCV viremia and clinical signs of vasculitis, suggesting that good efficacy of interferon was mediated by its antiviral effect. In Group 2 patients, although interferon-α had great efficacy on purpuric skin lesions, it had no effect on the peripheral neuropathy, even when associated with steroids plus plasma exchanges.

HCV infection may be associated with different types of systemic vasculitis — polyarteritis nodosa or mixed cryoglobulinemia. Because of the differences in clinical and pathological features and therapeutic strategy, PAN-type vasculitis should be distinguished from MC-type vasculitis in patients with HCV infection.

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