

Peripheral neuropathy associated with essential mixed cryoglobulinaemia: a role for hepatitis C virus infection?

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Abstract

Background—The prevalence of hepatitis C virus (HCV) infection has been estimated at 43 to 84% in patients with essential mixed cryoglobulinaemia in recent large series. Some of these cases have been successfully treated with interferon- α . The objective was to evaluate the prevalence and the possible role of HCV infection in essential mixed cryoglobulinaemia.

Methods—Fifteen patients (eight men and seven women; mean age: 61.2 (SD 16.5) years) with peripheral neuropathy (10 polyneuropathies and five multifocal mononeuropathies) and essential mixed cryoglobulinaemia were tested for serum anti-HCV antibodies.

Results—Antibodies were found in 10 of 15 patients involving either polyneuropathies (seven patients) or multifocal mononeuropathies (three patients). Electrophysiological studies and teased nerve fibre studies (in seven patients) allowed neuropathies to be classified as predominantly sensory axonopathies. Compared with HCV-negative (HCV -) patients, HCV-positive (HCV +) patients had a more pronounced and more widespread motor deficit; motor nerve conduction velocities in peroneal and median nerves were more impaired in HCV + patients, although significance was not reached except for the mean value of the amplitude of the compound muscle action potentials of the median nerves ($P < 0.05$); necrotising vasculitis was found in two of nine nerve biopsies from the HCV + patients studied and in none of the three HCV - patients. In addition, HCV + patients had more frequent cryoglobulin related cutaneous signs, higher aminotransferase and serum cryoglobulin concentrations, lower total haemolytic complement concentrations, and more frequent presence of rheumatoid factor. A liver biopsy performed in eight HCV + patients disclosed a range of lesions, from chronic active hepatitis (six patients) to persistent hepatitis (two patients). Lastly, treatment with interferon- α conducted over six months in two patients seemed to improve the peripheral neuropathy.

Conclusions—Patients with peripheral neuropathy and essential mixed cryoglobulinaemia should be tested for anti-HCV antibodies to determine the appropriate treatment.

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Keywords: peripheral neuropathy; mixed cryoglobulinaemia; hepatitis C virus infection

Cryoglobulinaemia is a condition characterised by the presence of serum proteins which reversibly precipitate in the cold. They have been classified¹ into three types: type I, single monoclonal immunoglobulin; type II, monoclonal immunoglobulin associated with a polyclonal component; type III, polyclonal immunoglobulin. Cryoglobulinaemia may be idiopathic: essential mixed cryoglobulinaemia corresponding to types II or III, or secondary to various diseases. A significantly high frequency of antibodies to hepatitis C virus (HCV) in essential mixed cryoglobulinaemia was first reported in 1990,² then confirmed by other authors, with a prevalence ranging from 43 to 84%.³⁻¹⁰ In numerous cases, liver biopsy discloses chronic hepatitis, leading to a treatment with interferon- α being proposed.¹¹

On the other hand, peripheral neuropathy associated with cryoglobulinaemia has been widely reported since the first descriptions.¹²⁻¹³ Polyneuropathies are more frequent than multifocal mononeuropathies, and the cryoglobulinaemia is mainly essential mixed cryoglobulinaemia in the patients reported.¹⁴⁻²¹

The aim of this study was to evaluate the prevalence and possible role of HCV antibodies in a group of patients with peripheral neuropathy and essential mixed cryoglobulinaemia.

Patients and methods

PATIENTS

Fifteen patients (1-15) were retrospectively selected in the neurology and internal medicine departments of our university hospital on the basis of (1) a peripheral neuropathy based on clinical and electrophysiological findings and (2) essential mixed cryoglobulinaemia diagnosed as described later. We included only the patients with positive mixed cryoglobulinaemia (corresponding to types II and III) on two successive determinations, with a serum concentration higher than 0.05 g/l. We excluded patients having an underlying disease known to be associated with mixed cryoglobulinaemia: autoimmune disorders, non-viral infectious diseases, and malignant haematological disorders. Also, other causes of peripheral neuropathy were systematically excluded.

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PERIPHERAL NEUROPATHY

All patients underwent clinical and electrophysiological studies. The MRC scale was used for evaluation of motor deficit. A neuromuscular biopsy was performed in 12 patients.

Electrophysiological studies

Electrophysiological studies were performed with a Viking Nicolet electromyograph. Motor nerve conduction velocities (MNCVs) were measured with supramaximal percutaneous nerve stimulation, whereas the compound muscle action potentials (CMAPs) were recorded with surface electrodes. For all patients, median, ulnar, and common peroneal nerves were examined bilaterally. Distal motor latencies and evoked motor response amplitudes (baseline to negative peak) were measured in all nerves. The F wave latencies were measured after distal supramaximal stimulation (at least 16 stimuli for each nerve) in median, ulnar, and peroneal nerves.

Sensory nerve conduction velocities (SNCVs) and amplitudes were measured in the median, ulnar, and sural nerves. For all nerve conduction studies, skin temperature was maintained at 36°C.

Electromyographic recordings were performed with a concentric needle electrode. Four muscles were tested in all patients: right and left tibialis anterior, first dorsal interosseus, and abductor pollicis brevis. Patients suspected of having a multifocal mononeuropathy underwent other recordings in related muscles.

On the basis of clinical and electrophysiological findings, peripheral neuropathy was classified as polyneuropathy or multifocal mononeuropathy, then classified as axonal or demyelinating peripheral neuropathy according to the American Academy of Neurology criteria for chronic inflammatory demyelinating polyneuropathies (CIPDs).

Neuromuscular biopsies

Twelve patients underwent a neuromuscular biopsy in the superficial peroneal nerve and peroneus brevis muscle of the most affected lower limb, following the already fully described techniques.²² For patient 1, only a muscle biopsy was performed. Briefly, for muscle biopsy, after immediate sampling and fixation of a thin fascicle for electron microscopy, two short fascicles (5 mm wide, 6 mm long) were removed for histochemistry and immunohistochemistry. A larger and longer fascicle (7–13 mm), or a few fascicles of the same volume, were fixed in 3.7% neutral formaldehyde for cross section and longitudinal paraffin embedding. Six to 10 semiserial sections were prepared. Another fragment was dry cooled and stored at –80°C for biochemistry. For nerve biopsy, a 10–20 mm long fragment of the whole section of the nerve was immediately fixed in 2.5% glutaraldehyde for the preparation of teased nerve fibres, semithin cross sections, and electron microscopy. A fragment of the same length was embedded in paraffin for transverse and longitudinal sectioning (six to eight

sections). A 5 mm long segment was frozen for immunocytochemistry. Small fragments were used for biochemistry or cell cultures. Specimens of the superficial peroneal nerve, paraffin embedded for semiserial sections, were then stained with haematoxylin-eosin, Masson's trichrome, and Congo red. Vascular lesions were considered as necrotising vasculitis only if fibrinoid necrosis and cell infiltrates were seen in the walls of large arteries displaying a diameter greater than 80 µm. A histogram of the distribution of myelinated fibres was performed on 2 µm thick epoxy embedded transverse sections stained with toluidine blue. Teased fibre preparations were studied in seven cases. The samples were selected at random from among the nerve fascicles: 100 fibres with more than four internodes were evaluated, except in two severely affected patients. The fibres were classified as normal, affected by axonal degeneration (myelin ovoids and balls), regeneration (short regular internodes), segmental demyelination (paranodal or internodal), or remyelination (excessive variability of myelin thickness and short irregular internodes).

CRYOGLOBULINAEMIA

Blood samples were kept at 37°C until complete coagulation and analysed for the presence of cryoglobulinaemia. The cryoglobulinaemia was purified and then characterised by an immunoblotting method, as previously described.²³

DETECTION OF ANTI-HCV ANTIBODIES

Serum samples were assayed for anti-HCV antibodies with a second generation enzyme linked immunosorbent assay (ELISA, Ortho Diagnostic Systems, Roissy, France), then confirmed by a recombinant based immunoblot assay (RIBA Chiron, Emeryville, CA; Diagnostic Systems), as previously reported.¹⁰ Samples were considered reactive when the ELISA ratio was repeatedly above 1. The RIBA was considered reactive if two or more bands were observed, indeterminate if only one band was present, and non-reactive if there was no band with an intensity greater than that of low positive controls. In four HCV + patients, serum samples and cryoprecipitates that had been kept at –80°C to avoid false negative results due to RNA destruction by RNases and false positive results due to contamination, were assayed for HCV RNA using the polymerase chain reaction (PCR). Serum or cryoprecipitate RNA was extracted, reverse transcribed, and amplified, as previously reported.²⁴

Serum samples were also assayed for the presence of hepatitis B virus infection by detection of hepatitis B surface antigen (HBs Ag), anti-HBs antibodies (anti-HBs Ab), and anti-hepatitis B core antibodies (anti-HBc Ab) with commercially produced immunoassays (Abbott Laboratories, Abbott Park, IL; Diagnostic Pasteur), and in one patient by detection of hepatitis B viral DNA by means of molecular hybridisation (Genostics, Abbott Laboratories). Screening for HIV1 and HIV2 antibodies was also carried out.

LIVER: PATHOLOGICAL EXAMINATION

A transperietal or transjugular liver biopsy was performed in eight patients. Periportal necrosis, intralobular necrosis, portal inflammation, and fibrosis were quantified by Knodell's score.²⁵

COMPARISON OF PATIENTS WITH AND WITHOUT HCV INFECTION

Clinical, electrophysiological, and neuropathological data of peripheral neuropathy were analysed with regard to their relevance to the HCV infection. One way analysis of variance was used to compare mean values of nerve conduction velocity between the two groups.

Results

PERIPHERAL NEUROPATHY (TABLE)

There were 15 patients (eight men, seven women, mean age: 61.2 (SD 16.5); range: 32–80 years). Age at onset of peripheral neuropathy ranged from 32 to 80 (mean: 59.8 (SD 14.5) years). The onset of peripheral neuropathy was subacute (one week to four months before diagnosis) in three patients and chronic (six months to five years before diagnosis) in 12 patients.

The peripheral neuropathy was a distal polyneuropathy in 10 patients (67%) and a multifocal mononeuropathy in the five others (33%). The table summarises the clinical data. Sensory symptoms and signs were found in all patients and were always present at the onset of the peripheral neuropathy. Sensory symptoms were painful in 50% of patients, the most often reported symptoms being burning, numbness, and pricking. Decreased sensation to pinprick was found in a stocking distribution in polyneuropathies, and in at least one affected limb in multifocal mononeuropathies. On the other hand, vibration and joint position sensation were impaired in only three patients, always in the distal lower limbs. No patient had ataxia. None complained of symptoms of autonomic nervous system distur-

bance or had orthostatic hypotension. A motor deficit was found in 11 patients: distal lower limbs in nine patients, distal upper limbs and lower limbs in one patient, and both distal and proximal upper and lower limbs in one patient. Ankle jerks were abolished in six patients, were diminished in five, and were normal in four.

The peripheral neuropathy was associated with weight loss in five patients, fever in two, purpura in four, Raynaud's phenomenon in two, skin necrosis in three cases, arterial thrombosis in two, arthritis in two and, hepatomegaly in two. Electrophysiological studies showed all the neuropathies to be axonopathies: in the lower limbs, motor nerve conduction velocities either could not be elicited or were reduced in proportion to the denervation in 13 of 15 patients (86%). In the upper limbs they were reduced in only five of 15 patients (33%). The mean value of the MNCV in the peroneal nerve was 39.5 (SD 4.8) (range 30–44) m/s (lower control value of our laboratory: 42 m/s) and in the median nerve it was 50.8 (SD 6.4) (range) 40–62 m/s; (lower control value: 48 m/s). The mean value of F wave latencies measured in the peroneal nerve was 57 (SD 4.1) ms (upper control value 55 ms). The mean value of F waves in the upper limbs (measured on median or ulnar nerves) was 32 (SD 3.7) ms (upper control value: 33 ms).

Sensory potentials were abnormal in the lower limbs in all patients, whether polyneuropathies or multiple mononeuropathies: they were absent or reduced in amplitude in the upper limbs in six of 10 patients with polyneuropathies and five of five patients with multiple mononeuropathies.

Electromyographic recordings showed fibrillation potentials at rest in at least two studied muscles in four patients.

The examination of semithin sections of the nerve, confirmed by the teased fibre study, available in seven patients, disclosed a predominant axonal degeneration in six patients: the percentage of fibres showing axonal degen-

Summary of patients

Patient no	Age at onset	Sex/age (y)	Anti-HCV antibodies	Cryoglobulinaemia type, monoclonal component	Type of neuropathy	Other cryoglobulin related signs
1	80	F/80	+	II, IgMk	MM	Radial, peroneal SM S S > M S
2	61	M/76	+	II, IgMk	PN	
3	43	F/45	+	III	PN	
4	58	M/59	+	II, IgMk	PN	
5	70	F/73	+	II, IgMk	PN	S S > M
6	79	F/80	+	II, IgMk	PN	
7	48	F/48	+	II, IgMk	MM	
8	56	M/57	+	III	PN	S > M S > M SM
9	53	M/76	+	II, IgMk	PN	
10	50	M/50	+	II, IgMk	MM	
					Peroneal and ulnar bilateral, median	S > M S S > M
11	67	F/73	-	II, IgAk	PN	
12	47	M/51	-	III	MM	
13	27	M/32	-	II, IgMI	MM	Ulnar bilateral, peroneal S > M
14	66	F/80	-	II, IgMk	PN	
15	35	M/39	-	III	PN	S > M None

MM = Multifocal mononeuropathy; PN = polyneuropathy; SM = sensorimotor; S = purely sensory; S > M = predominantly sensory.

eration ranged from 40% (patient 8) to 95% (patients 3 and 4). Only patient 2 had pronounced segmental demyelination (43% of examined fibres) associated with mild axonal degeneration.

CRYOGLOBULINAEMIA

Cryoglobulinaemias were identified as type III in four patients and as type II cryoglobulin in 11 patients: in the latter patients, the monoclonal component was an IgM kappa in nine patients, an IgM lambda in one, and an IgA kappa in one. The mean cryoglobulinaemia concentration was 0.42 (SD 0.45) g/l, ranging from 0.05 to 1.5 g/l.

FREQUENCY OF HCV SERUM ANTIBODIES

Antibodies to HCV were found in 10 of 15 patients (67%). They were present both in polyneuropathies (seven of 10 patients) and multifocal mononeuropathies (three of five patients). HCV RNA screening was performed by PCR in four of 10 HCV + patients (3, 4, 7, and 8), of whom three (4, 7, and 8) were positive.

FREQUENCY OF SERUM ANTIBODIES TO OTHER VIRAL INFECTIONS

All patients studied were negative for HIV1 and HIV2 antibodies. Screening for HBs Ag, anti-HBs Ab, and anti-HBc Ab was negative in all HCV - patients. In HCV + patients HBs Ag was always negative, anti-HBs Ab was present, but with an absence of anti-HBc Ab in patients 5 and 10; both anti-HBs Ab and anti-HBc Ab were present in patients 3 and 9, and in patient 8 only anti-HBc Ab was present, but HBV DNA, assayed using PCR, was absent (this is consistent with the absence of HBV chronic hepatitis).

COMPARISON BETWEEN HCV POSITIVE AND HCV NEGATIVE PATIENTS

Clinical data on the peripheral neuropathy

There was no significant difference between the HCV + and HCV - patients in terms of age, sex, and duration and type of peripheral neuropathy. However, HCV + patients seemed to be more severely affected. The motor deficit was more pronounced in HCV + patients: three of them had a sensorimotor neuropathy, whereas all HCV - patients had a predominantly sensory neuropathy; in addition, four HCV + patients (40%) had an MRC score lower than 3 in at least one affected muscle, whereas none of the HCV - patients had an MRC score < 4. Moreover, the onset of the motor deficit was subacute (< four months) in three HCV + patients, whereas it was slowly progressive (> six months) in each of the HCV - patients. Lastly, the motor deficit concerned both lower and upper limbs in two HCV + patients, whereas it was not present in the upper limbs in the HCV - patients.

Electrophysiological data

In the electrophysiological study, MNCVs were more often abnormal in HCV + patients than in HCV - patients. The MNCVs in per-

oneal nerves could not be elicited in five of 10 HCV + patients compared with one of five HCV - patients; in the remaining patients, the mean value of MNCV was lower (38.2 (SD 2.3) m/s) and the mean value of distal motor latencies was higher (7.5 (SD 2.3) ms) in HCV + patients than in HCV - patients (respectively 45 (SD 1.7) m/s and 5.05 (SD) 0.5 ms), although significance was not reached. In median nerves, the mean value of MNCV was lower in HCV + patients than in HCV - patients (49.3 (SD 2.3) v 53.5 (SD 2.7) m/s, but not significantly), and the mean value of the amplitude of the CMAP was significantly lower in HCV + patients than in HCV - patients (4.9 (SD 0.4) v 8.1 (SD 0.8) mV, $P < 0.05$). In addition, fibrillation potentials, indicating active denervation, were seen in three HCV + patients, compared with only one HCV - patient.

Pathological data on neuromuscular biopsies

Necrotising vasculitis was found in two of nine of the HCV + patients studied (muscle only: one patient, nerve only: one patient), but not in any of the three HCV - patients studied. In addition, perivascular infiltrates of mononuclear cells without involvement of the vascular wall, indicating an inflammatory process, were seen in five of nine HCV + patients (both nerve and muscle: three patients, nerve only: two patients), but were not present in any of the three HCV - patients. In the nerve, the infiltrates were seen both in the endoneurium and in the epineurium.

On the other hand, there was no significant difference in the mean density of myelinated fibres between the two groups: 3174 (SD 2460) in the seven HCV + patients studied, compared with 3310 (SD 2278) in the three HCV - patients studied (normal control value used in our laboratory: 9300 (SD 2300)).

Other features associated with cryoglobulinaemia

Other cryoglobulin related signs were more frequent in HCV + patients than in HCV - patients (nine of 10 and one of five, respectively), mainly for cutaneous signs (five of 10 and none of five, respectively). In addition, the mean cryoglobulin serum concentration was significantly higher in HCV + patients than in HCV - patients: 0.62 (SD 0.43) g/l v 0.08 (SD 0.04 g/l) ($P < 0.05$). Compared with HCV - patients, the HCV + patients had a lower mean total haemolytic complement (27.5 (SD 7) C_{H50} /ml v 40.1 (SD 5.7) C_{H50} /ml), and a rheumatoid factor more often positive (eight of 10 v one of five).

Liver: serological and pathological data

Abnormalities in liver function tests were found in all HCV + patients: alanine aminotransferase concentrations were 1.2 to seven times above the upper normal limits, whereas they were normal at repeated examinations in HCV - patients. Among the HCV + cases, seven had a risk factor for HCV transmission: blood transfusion ($n = 2$), intravenous drug addiction ($n = 1$), and surgery ($n = 4$), whereas none of the HCV - patients had risk

factors for HCV infection. The eight HCV + patients who underwent a liver biopsy had a range of liver histological lesions ranging from chronic active hepatitis (n = 6) to persistent hepatitis (n = 2). No correlation could be found between the severity of liver pathological lesions and the severity of the peripheral neuropathy or the neuromuscular vasculitis lesions.

Response to treatment

Four of the five HCV – patients have been treated with a series of 10 to 15 plasma exchanges, resulting in slight to mild improvement of the sensory symptoms. Four of the 10 HCV + patients were treated with steroids and/or cyclophosphamide, associated in three patients with plasma exchanges, for six to 18 months: two patients had a mild response and one had a good response to this treatment. The other six patients received interferon- α (three million units three times a week) because of the presence of active hepatitis: four of them could not be evaluated because the duration of treatment was too short (< four months) or due to drop out. In the remaining two patients interferon treatment, conducted over six months to one year, led to a dramatic improvement in the peripheral neuropathy coupled with the disappearance of cryoglobulinaemia.

Discussion

Peripheral neuropathy may be associated with all types of cryoglobulinaemias. However, the more often described association is that of essential mixed cryoglobulinaemia, and either polyneuropathies or multifocal mononeuropathies: a personal review of 45 documented cases reported in the medical literature shows that this association is found in 84% of patients.^{14–21} The clinical and electrophysiological profile of the peripheral neuropathies in our patients did not differ from that previously reported: polyneuropathies were more frequent than multifocal mononeuropathies, as in the study of Gemignani *et al*²¹; paresthesiae and pain were classic symptoms.^{21–26} Electrophysiological findings and sural nerve biopsy specimens were consistent with predominant axonal degeneration in most cases. Vascular lesions, including vasculitis and alterations of the endoneurial microvessels, were commonly seen in the peripheral nerves, as in previously documented cases.^{18–20} However, the different histopathological aspects may reflect various stages of the disease, rather than the different forms of a pathological process.^{28–29}

On the other hand, the high prevalence of (1) anti-HCV antibodies in the serum, and (2) HCV RNA in the serum and/or cryoprecipitates, in patients with essential mixed cryoglobulinaemia, suggests a direct role of HCV in the production of cryoglobulinaemia. The prevalence of anti-HCV antibodies in our selected group, using second generation HCV tests, was higher than that (52%) found in a series of unselected patients with essential mixed cryoglobulinaemia,¹⁰ but lower than

that reported by Authier *et al*²⁶ in a recent study of 10 patients similar to ours. However, systematically screening for HCV RNA by PCR may raise the proportion of detected patients.^{10–27}

We wished to know whether the presence of anti-HCV antibodies may allow the characteristic features in peripheral neuropathy associated with essential mixed cryoglobulinaemia to be determined. When HCV + patients were compared with HCV – patients, the peripheral neuropathy in HCV + patients seemed to be more severe, mainly because of motor involvement. These clinical findings were confirmed in our study by some of the results of the electrophysiological studies. In addition, direct or indirect signs of vasculitis were more frequent and more severe in the HCV + patients studied. More precisely, necrotising vasculitis was seen in two of our 10 HCV + patients, and could be related to a direct role of HCV infection, in the absence of associated HBV active infection. On the other hand the patient recently reported by Khella *et al*³⁰ of peripheral neuropathy associated with mixed cryoglobulinaemia and hepatitis C infection, had a primarily axonal neuropathy with a vasculitic destruction of the epineurial vessels, but fibrinoid necrosis was not found: these authors suggest that the absence of hepatitis C virus particles from their patient's nerve supports the hypothesis of indirect damage to the nerve, perhaps by the cryoglobulins or the inflammatory cells.

Considering the non-neurological manifestations, Cacoub *et al*¹⁰ showed that patients with HCV have more frequent cryoglobulin related cutaneous involvement, higher alanine aminotransferase concentrations, higher serum cryoglobulin concentrations, lower CH50, and more frequent presence of rheumatoid factor, than patients without HCV. Moreover, liver histological lesions are very often found in HCV + patients. A role for HCV in the development of mixed cryoglobulinaemia has recently been suggested: a possible explanation for their association could be an antigenic cross reaction between HCV and the liver, particularly if damaged, with the production of antibodies cross reacting with both antigens.¹⁰

Consequently, the presence of chronic active hepatitis in liver biopsies led us to propose a treatment with interferon- α , which seemed to be efficacious in hepatic lesions and could also improve the peripheral neuropathy. Khella *et al*³⁰ treated their patient with interferon α for nine months, which improved the symptoms and cleared the hepatitis C viral RNA and cryoglobulin from the serum.

In conclusion, a high proportion of patients with peripheral neuropathy associated with essential mixed cryoglobulinaemia are suspected of having serum anti-HCV antibodies, and these patients seem to have a more severe neurological condition. Patients with peripheral neuropathy and essential mixed cryoglobulinaemia have to be evaluated for anti-HCV antibodies so that the degree of hepatic impairment can be evaluated and a suitable

treatment proposed. Prospective studies are needed to determine whether interferon- α may also be considered as a treatment for peripheral neuropathy.

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