PAPER

Prevalence and characteristics of peripheral neuropathy in hepatitis C virus population

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Objective: To assess the prevalence of peripheral neuropathy (PN) and its correlation with cryoglobulinemia (CG) in an unselected, untreated referral hepatitis C virus (HCV) population.
Patients and Methods: Two hundred and thirty four patients (120 women and 114 men) with untreated HCV infection were consecutively enrolled by seven Italian centres. Clinical neuropathy was diagnosed when symptoms and signs of peripheral sensory or motor involvement were present. Median, ulnar, peroneal, and sural nerves were explored in all patients and distal symmetric polyneuropathy was diagnosed when all explored nerves or both lower limb nerves were affected. Mononeuropathy and mononeuropathy multiplex were diagnosed when one nerve or two non-contiguous nerves with asymmetrical distribution were affected. Screening for CG was done in 191 unselected patients.
Results: Clinical signs of PN were observed in 25 of the 234 patients (10.6%). Electrophysiological PN was found in 36 (15.3%). CG was present in 56/191 patients (29.3%). The prevalence of CG increased significantly with age ($p<0.001$) and disease duration ($p<0.05$). PN was present in 12/56 (21%) patients with CG and 18/135 (13%) without CG (p = NS). PN increased significantly with age ($p<0.001$) and logistic regression analysis confirmed age as the only independent predictor of PN (OR 1.10 for each year; 95% CI 1.04 to 1.15; $p<0.001$).
Conclusions: Electrophysiological examination detected subclinical neuropathy in 11 patients (4.7%). Statistical analysis showed that CG was not a risk factor for PN whereas PN prevalence increased significantly with age.

Hepatitis C virus (HCV) is a parenterally transmitted, hepatotropic, and lymphotropic RNA virus. HCV infection affects approximately 170 million people worldwide[1] and is the major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The infection may be associated with mixed cryoglobulinaemia (CG), lymphoproliferation, and a variety of extrahepatic manifestations including membranoproliferative glomerulonephritis, sicca syndrome, porphyria cutanea tarda, thyroiditis, and peripheral neuropathy (PN).[2] A subacute, distal sensory-motor polyneuropathy frequently associated with CG is the commonest neurological complication, but mono and multiple mononeuritis have also been reported.[3–5]

The pathophysiology of HCV related PN remains largely speculative; vascular deposition of HCV RNA containing CG,[6] direct viral infection[7] or perivascular mononuclear inflammatory cells[8 9] may be at the origin of HCV associated inflammatory vascular lesions. However, it is likely that HCV neuropathy results from virus triggered immune mediated mechanisms rather than from direct nerve infection and in situ replication.[10] The clinical and electrophysiological spectrum of HCV associated PN has been mainly explored in HCV patients with CG, and only a few papers have investigated the prevalence of the PN in an unselected HCV population.[11 12] The present study is a multicentre prospective study and it was designed to assess clinically and electrophysiologically the prevalence and the characteristics of PN in an unselected, untreated referral HCV population, and the correlation between PN and CG.

PATIENTS AND METHODS

Patients with HCV infection were consecutively enrolled by seven Italian centres (Genoa, Lodi, Messina, Monza, Naples, Padua, and Siena) from January 2001 to December 2003. These were all secondary or tertiary centres with a sound electrophysiological background and easy access to a gastroenterology/hepatology service. In each centre, a neurologist participating in the study contacted the local gastroenterologists/hepathologists to identify potentially eligible patients. Care was taken to enroll only patients with untreated HCV infection. The inclusion criteria were: (1) HCV infection assessed by ELISA and recombinant immunoblot, and (2) no specific immunomodulating or antiviral therapy. Exclusion criteria were any other causes of PN (diabetes, alcohol abuse, renal failure, vitamin deficiency, thyroid disorders, neoplasm, toxic agents), which were ruled out through history and ad hoc laboratory investigations.[13] The duration of HCV positivity was assessed from the first laboratory detection of HCV infection.

Clinical examination

Eligible patients were evaluated by an expert neurologist. A diagnosis of clinical neuropathy was made when symptoms (weakness, sensory disturbances) and signs (weakness and atrophy and/or sensation abnormalities and/or reduced/absent tendon reflexes) of peripheral sensory and/or motor involvement were present. Asymmetrical or symmetrical distribution of neurological signs was also recorded. Patients with symptoms such as pain, burning paresthesia, and fatigue, but no signs of peripheral nerve involvement, were not considered as affected by clinical PN.

Abbreviations: CG, cryoglobulinaemia; CMAP, compound motor action potential; DL, distal latency; HCV, hepatitis C virus; MCV, motor conduction velocity; NCS, nerve conduction study; PCR, polymerase chain reaction; PN, peripheral neuropathy; SAP, sensory action potential; SCV, sensory nerve conduction velocity.

Laboratory investigations

HCV infection was assessed by ELISA and recombinant immunoblot; quantitative assays of hepatitis C virus level was performed in 113 patients by polymerase chain reaction (PCR). Routine blood and serum chemical tests including serum thyroid hormones, vitamins (B12 and E), tumoral markers, and (when indicated) toxicological investigations, were run for all patients.[13] Screening for CG was done in 191 unselected patients; blood samples were kept at 37°C until complete coagulation and analysed for the presence of CG.

Electrophysiological study

All patients underwent an electrophysiological examination according to a simplified nerve conduction study (NCS) protocol to define the presence of distal symmetrical neuropathy.[14] Motor conduction velocity (MCV), compound motor action potential (CMAP) amplitude, and distal latency (DL) of the right median, right ulnar, and left peroneal nerves were recorded. To detect changes in nerve conduction in proximal nerve segments, the F-wave from median, ulnar, and deep peroneal nerves was recorded at wrist and ankle. Antidromic sensory nerve conduction velocity (SCV) was measured along the right median, right ulnar, and left sural nerves, and sensory action potential (SAP) amplitude was evaluated. If a response was absent for any of the explored nerves (sensory or motor) or clinical examination suggested an asymmetrical involvement, NCS was repeated in the contralateral nerves. All nerve conduction studies were done using surface electrodes and skin limb temperature was kept at least 32°C. For each nerve, the electrophysiological values were considered abnormal if more than 2.5 standard deviations (SD) from the means for healthy age matched controls in each laboratory.

A peripheral nerve was electrophysiologically defined as affected when at least two parameters were found to be abnormal (prolonged DL, reduced MCV and/or SCV, reduction of SAP and/or CMAP amplitude, prolonged F-wave latency). Distal symmetric PN was diagnosed when all explored nerves or when both lower limb nerves were affected.[14] Mononeuropathy and mononeuropathy multiplex were diagnosed when one nerve or two non-contiguous nerves with asymmetrical distribution were affected. The neuropathy was considered subclinical when the patient had no symptoms nor clinical signs of peripheral nerve involvement. Isolated mononeuropathies of the median nerve were also checked but were excluded from further analysis because of their high prevalence in the general population.

Statistical analysis

The results were analysed using the χ^2 test and Student's *t* test for unpaired data. Multivariate analysis (conditional stepwise backward logistic regression) was also done to assess the association of PN with CG after adjusting for age, sex, duration of HCV infection, and centre. The limit of statistical significance was set at 5%.

RESULTS

Two hundred and thirty four patients—120 women and 114 men—were studied. Mean age (SD) was 52.3 (13.7) years (range 18–89). Median duration of HCV infection was 36 months (range 1–156). CG were detectable in the serum of 56/191 patients (29.3%) and CG typing, performed in 28% of them, showed in all a type II mixed cryoglobulinaemia. The main demographic, clinical, and laboratory features of patients with and without electrophysiologically defined PN are shown in table 1.

Clinical PN was diagnosed in 25 out of 234 patients (10.6%). All these patients had electrophysiological evidence of PN. Moreover, electrophysiological examination disclosed a subclinical PN in 11 additional patients (4.7%). In summary, electrophysiological examination detected PN in 36 out of 234 patients (15.3%). An axonal sensory-motor polyneuropathy or mononeuropathy multiplex was diagnosed in 19/36 (52.8%) and 17/36 patients (47.2%), respectively. No electrophysiological signs of demyelination or cranial nerve involvement was found.

Table 1 Demographic, clinical, and laboratory features of patients with and without electrophysiologically defined peripheral neuropathy

	Neuropathy			
	Yes		No	
Variable	n	%	n	%
Total	36	15	198	85
Sex				
Men	19	17	95	83
Women	17	14	103	86
Age (years)*				
<45	3	4	65	96
45–54	5	9	53	91
55–64	18	28	46	72
>65	10	23	34	77
Duration of infection (months)				
<12	10	12	76	88
12–60	9	14	57	86
>60	17	21	65	79
Cryoglobulinemia				
Yes	12	21	44	79
No	18	13	117	87

*Mantel-Haenszel χ^2, 18.1; $p<0.001$.

Thirty patients complained of symptoms consisting of pain, burning paresthesiae, and fatigue but did not show any clinical or electrophysiological signs of PN.

The patients tested for CG did not differ significantly from those who were not tested, for age, sex, centre, duration of infection, and electrophysiological findings. The prevalence of CG tended to increase significantly with age. CG was present in 15.5% of patients aged <45 years, and in 25%, 35.4%, and 51.6% of those aged 45–54 years, 55–64 years, and >65 years (Mantel-Haenszel χ^2, 14.7; $p<0.001$). CG was also related to disease duration; CG was present in 15% of patients with disease duration <12 months, and in 32% and 40% of those with 12–60 or more than 60 months (Mantel-Haenszel χ^2, 10.4; $p<0.05$).

PN was present in 12/56 patients with CG (21%) as compared to 18/135 (13%) without CG (Pearson χ^2, 1.9; p = NS). PN was also unrelated to sex and centre, but tended to increase significantly with age; it was present in 4.4% of patients aged <45 years, and in 8.6%, 28.1%, and 22.7% of those aged 45–54 years, 55–64 years, and >65 years (Mantel-Haenszel χ^2, 18.1; $p<0.001$). PN was unrelated to duration of infection (Pearson χ^2, 2.8; p = NS). Logistic regression analysis confirmed age as the only independent predictor of PN (OR 1.10 for each year; 95% CI 1.04 to 1.15; $p<0.001$).

The median HCV RNA level in the blood was 1.8 million of copies/ml (range 1800–42 000 000). No correlation was found between HCV-RNA levels and the presence of PN (Pearson χ^2, 0.9; p = NS) or CG (Pearson χ^2, 2.2; p = NS).

DISCUSSION

The current study is the first large prospective survey of a fairly unselected consecutive series of untreated patients with HCV infection which underwent systematically both clinical and electrophysiological investigation to assess the prevalence and the characteristics of PN and the correlation between PN and CG.

The prevalence of electrophysiological PN in this population was 15.3% and the prevalence of CG was 29.3%. Clinical PN was present in 10.6% of all enrolled patients (25/234) and in 69% (25/36) of patients with electrophysiological evidence of PN.

Only two similar studies have been published, so far. In the first, only 36 patients were clinically and electrophysiologically investigated and PN was detected in 8% of them[11]; in the other large prospective study, Cacoub and colleagues diagnosed a peripheral neuropathy in 9% of 321 HCV patients on the basis of clinical symptoms only.[12] The prevalence of PN in our study (10.6%), if based on clinical assessment only, is very close to that of Cacoub and colleagues.[12] However, the electrophysiological examination revealed a subclinical neuropathy in 11 additional patients (4.7%). Therefore, pure clinical assessment tends to underestimate peripheral nervous system involvement in the HCV general population. As pointed out by England and colleagues,[14] polyneuropathy occurs with a combination of multiple symptoms, signs, and abnormal electrodiagnostic studies, whereas symptoms alone have relatively poor diagnostic accuracy in predicting the presence of polyneuropathy. According to these criteria, PN could not be confirmed in the 30 patients complaining of pain, burning paresthesiae, and fatigue in the absence of electrophysiological abnormalities. On the other hand, these symptoms are frequently described in HCV patients and they can be due to non-neurological (for example, rheumatological) causes or to a small fibre neuropathy. A small fibre neuropathy could not be ruled out in our patients either by clinical examination or by conventional nerve conduction studies, but this was not an aim of the present study.

The prevalence of CG in our neuropathic patients was lower than in other series.[5 10 15] The simplest explanation of this difference may be the modality of the enrollment, which in our study was based on the presence of HCV infection, independently of the signs or symptoms of PN and CG, duration of infection, severity of liver disease, and HCV viral load. However, the prevalence of CG in the present study is half of that reported by Cacoub (29% *v* 56%).[12] We do not have an exhaustive explanation of this difference, though a wide variability of CG prevalence in the HCV patients has been well documented.[16]

As in Cacoub's study, we found no significant association between PN and CG. Vice versa an association between PN and CG was found in two other studies. However, in both studies patients were selected according to the presence of neuropathy or CG.[5 15] Moreover the absence of CG related symptoms in one third of neuropathic patients,[15] or the similar neuropathological features in nerve biopsy specimens in both CG+ and CG− patients,[5] made questionable the direct role of CG in the pathogenesis of neuropathy.[15] In fact, different mechanisms unrelated to the presence of CG, but possibly due to the direct or indirect effects of HCV infection, have been largely proposed in the pathogenesis of nerve damage.[7–10] Inflammatory vascular lesions and axonal degeneration, supporting an ischaemic mechanism of nerve damage more than a direct role of the virus in HCV related PN, have been described in sural nerve biopsy of HCV patients both with and without CG.[5 10] Under this assumption, the lack of correlation between type of PN and CG is also in keeping with the current hypothesis that HCV related vascular nerve damage could be due to virus triggered immune mediated mechanisms rather than longstanding CG precipitation.[10] The lack of correlation between HCV viral load and PN or CG further supports this hypothesis.

Statistical analysis showed a strong correlation between older age and PN but not between PN and the known duration of HCV positivity. At first sight, this latter observation might seem contradictory but the duration of HCV positivity was assessed from the first laboratory detection of HCV infection and it is likely different from the true duration of HCV infection, which might have actually occurred several years earlier.

Some authors have already noted that older age is a major risk factor for the clinical and biological extrahepatic manifestations of HCV.[15 17] In keeping with these data, we found a strong correlation between older age and both PN and CG, which may be interpreted in the light of the emerging hypothesis of an immune mediated pathological mechanism of HCV related clinical manifestations.[10]

The study has several limitations. Firstly, although we elected to enroll consecutive patients regardless of the presence of CG to prevent selection bias towards more severe infection and patients with neurological complications, this is not a population based study. For this reason, our findings cannot be extended to HCV patients who do not seek care in secondary and tertiary centres. Secondly, although we screened all patients to detect other causes of PN, CG was tested only in 82%. Although there were no significant differences between patients tested and not tested, we cannot exclude that their physicians decided to test patients at higher risk for PN. Thirdly, although our sample is fairly large, it may still be too small to detect a difference in the risk of PN between CG+ and CG− patients. Even with these limitations, however, we can conclude that in our fairly unselected, untreated HCV referral population the prevalence of PN is lower than that observed in the diabetic population (overall prevalence of distal symmetric polyneuropathy of 34%)[18] and higher than that reported in neoplastic patients (2–5%)[18] or in the general population (2.4%–8%).[19 20] Moreover, we can also conclude that CG is not a risk factor for neuropathy. However, an electrophysiological examination should always be done to avoid underestimating PN, particularly in older HCV patients.

We are now organising a prospective study of HCV patients to assess the incidence of neuropathy, its outcome, and the effects of specific therapies.

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REFERENCES

1 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001;**345**:41–52.
2 **Gumber SC**, Chopra S. Hepatitis C: a multifaceted disease. Review of extrahepatic manifestations. *Ann Intern Med* 1995;**123**:615–20.
3 **Apartis E**, Leger JM, Musset L, *et al*. Peripheral neuropathy associated with essential mixed cryoglobulinaemia: a role for hepatitis C virus infection? *J Neurol Neurosurg Psychiatry* 1996;**60**:661–6.
4 **Lidove O**, Cacoub P, Maisonobe T, *et al*. Hepatitis C virus infection with peripheral neuropathy is not always associated with cryoglobulinaemia. *Ann Rheum Dis* 2001;**60**:290–2.

5 **Nemni R**, Sanvito L, Quattrini A, *et al*. Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia. *J Neurol Neurosurg Psychiatry* 2003;**74**:1267–71.
6 **Agnello V**, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992;**327**:1490–5.
7 **Agnello V**, Abel G. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. *Arthritis Rheum* 1997;**40**:2007–15.
8 **Bonetti B**, Scardoni M, Monaco S, *et al*. Hepatitis C virus infection of peripheral nerves in type II cryoglobulinaemia. *Virchows Arch* 1999;**434**:533–5.
9 **Bonetti B**, Invernizzi F, Rizzuto N, *et al*. T-cell-mediated epineurial vasculitis and humoral-mediated microangiopathy in cryoglobulinemic neuropathy. *J Neuroimmunol* 1997;**73**:145–54.
10 **Authier FJ**, Bassez G, Payan C, *et al*. Detection of genomic viral RNA in nerve and muscle of patients with HCV neuropathy. *Neurology* 2003;**60**:808–12.
11 **Ripault MP**, Borderie C, Dumas P, *et al*. Peripheral neuropathies and chronic hepatitis C: a frequent association? *Gastroenterol Clin Biol* 1998;**22**:891–6.
12 **Cacoub P**, Renou C, Rosenthal E, *et al*. Extrahepatic manifestations associated with hepatitis C virus infection. A prospective multicenter study of 321 patients. The GERMIVIC. Groupe d'Etude et de Recherche en Medecine Interne et Maladies Infectieuses sur le Virus de l'Hepatite C. *Medicine (Baltimore)* 2000;**79**:47–56.
13 **McLeod JG**. Investigation of peripheral neuropathy. *J Neurol Neurosurg Psychiatry* 1995;**58**:274–83.
14 **England JD**, Gronseth GS, Franklin G, *et al*. Distal symmetrical polyneuropathy: definition for clinical research. *Muscle Nerve* 2005;**31**:113–23.
15 **Zaltron S**, Puoti M, Liberini P, Antonini L, *et al*. High prevalence of peripheral neuropathy in hepatitis C virus infected patients with symptomatic and asymptomatic cryoglobulinaemia. *Ital J Gastroenterol Hepatol* 1998;**30**:391–5.
16 **Cacoub P**, Hausfater P, Musset L, *et al*. Mixed cryoglobulinemia in hepatitis C patients. GERMIVIC. *Ann Med Interne (Paris)* 2000;**151**:20–9.
17 **Cacoub P**, Poynard T, Ghillani P, *et al*. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999;**42**:2204–12.
18 **Martyn CN**, Hughes RA. Epidemiology of peripheral neuropathy. *J Neurol Neurosurg Psychiatry* 1997;**62**:310–18.
19 **Hughes RA**. Peripheral neuropathy. *BMJ* 2002;**324**:466–9.
20 **Beghi E**, Monticelli ML. Chronic symmetric symptomatic polyneuropathy in the elderly: a field screening investigation of risk factors for polyneuropathy in two Italian communities. Italian General Practitioner Study Group (IGPST). *J Clin Epidemiol* 1998;**51**:697–702.