Peripheral neuropathy in hepatitis C virus infection with and without cryoglobulinaemia

R Nemni, L Sanvito, A Quattrini, G Santuccio, M Camerlingo, N Canal

Objectives: Hepatitis C virus (HCV) infection is often associated with cryoglobulinaemia (CG). Peripheral neuropathy (PN) is a comparatively common complication of CG associated with HCV infection and it is thought to be attributable to nerve ischaemia. Only few HCV patients with PN have been reported. The recent finding of HCV RNA in nerve biopsy specimens has suggested a possible direct role of HCV in the pathogenesis of PN. The authors studied 51 HCV patients to determine the prevalence of CG and to clarify the possible mechanism by which HCV determines the PN.

Methods: All the patients were studied clinically, by laboratory tests and electrophysiologically. Twenty eight patients underwent sural nerve biopsy where both morphological and morphometric evaluation of the biopsy specimen was performed, as well as statistical analysis.

Results: CG was found in 40 of 51 cases (78%). Polyneuropathy was significantly prevalent in CG+ patients compared with CG− (18 of 40 compared with 1 of 11 patients; p=0.01). HCV CG− patients more frequently developed well defined mononeuropathy or multiple neuropathy when compared with HCV CG+. Differential fascicular loss of axons was found in 10 of 25 CG+ patients and in 3 of 11 HCV CG− patients: epineurial vasculitis was present in 8 of 25 HCV CG+ (32%) and in 2 of 3 HCV CG−. Differential fascicular loss of axons was found in 10 of 25 CG+ (40%) and 1 of 3 CG−, signs of both demyelination and axonal degeneration were present in 7 of 25 CG+ (28%). No significant difference was found in neuropathological features, while histometrical analysis disclosed more severe involvement in CG+ patients.

Conclusions: These findings suggest that the presence of CG is a negative predictive factor for the associated PN. Morphological findings in the sural nerve from HCV CG− and CG+ are consistent with an ischaemic mechanism of nerve damage and are against a direct role of the virus in causing the associated PN.

Cryoglobulinaemia (CG) is a condition characterised by the presence of serum proteins that reversibly precipitate in the cold. According to the molecular composition, cryoglobulins are classified into three types: type I, isolated monoclonal Ig; type II, monoclonal IgM rheumatoid factor (RF) associated with a polyclonal component; type III, polyclonal Ig. Types II and III are classically referred to as “mixed cryoglobulinaemia”.

CG may be idiopathic (essential mixed cryoglobulinaemia, EMC) or secondary to other diseases, such as lymphoproliferative disorders, collagen diseases, and chronic infections. It has been reported that 46%–54% of patients with chronic hepatitis C virus (HCV) infection show detectable CG, although most of them do not have CG related symptoms.1,2

According to different reports, peripheral neuropathy (PN) is present in variable proportions in patients with symptomatic CG, related or not to HCV.4–4 PN usually occurs in type II and type III CG, rather than type I, and may clinically present as a mononeuropathy, multiple mononeuropathy, or polyneuropathy. Nerve biopsy shows mainly axonal degeneration.4–7 Two main pathogenetic mechanisms have been suggested: interference of the vasa nervorum microcirculation by intravascular deposits of cryoglobulins and vasculitis induced ischaemia.5–7,10 A third mechanism, an immunologically mediated demyelination, was seldom reported and has not been supported by subsequent studies.

Recently some HCV+ patients with PN and persistent negativity for CG have been reported.4–6 It has also been reported the finding of HCV RNA in homogenates of nerve biopsy specimens in five patients by in situ RT-PCR.6 These studies suggested a possible direct role of HCV in the pathogenesis of PN.

We examined a series of 51 consecutive HCV neuropathic patients to assess the prevalence of CG and to clarify the pathogenetic mechanism by which HCV determines PN.

METHODS

Patients
We examined 51 consecutive patients with HCV infection and neuropathy, referred to our department in the past eight years. Other causes of PN, except for the presence of monoclonal gammopathy, were excluded (diabetes, alcohol misuse, renal failure, vitamins deficiency, thyroid disorder, neoplasm, toxicity). The patients were all studied clinically, serologically, and electrophysiologically; 28 patients accepted to undergo nerve biopsy. The time lapse of the follow up was of four years or longer; all the patients at the time of the study were untreated. Peripheral nerve involvement was classified as mononeuropathy and/or multiple neuropathy, cranial neuropathy, and polynueropathy.

All the patients had serum anti-HCV antibodies detected by enzyme linked immunosorbent assay, confirmed by the more specific recombinant immunoblot assay. Laboratory studies included routine blood tests, immunological tests (latex test

Abbreviations: CG, cryoglobulinaemia; HCV, hepatitis C virus; PN, polyneuropathy; SCV, sensory conduction velocity; SAV, sensory action potential

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for IgM rheumatoid factor, C4 values, autoantibodies), detection and characterisation of cryoglobulins according to Broquet et al. Cryoglobulin determinations were performed at least three times during observation.

Electrophysiological studies
Electrodiagnostic tests were performed with standard electro-myographic equipment (Medelec MS20 Mystro); all nerve conduction studies were carried out at a constant cutaneous temperature of 33°C under automatic control with a DISA type 15 H 0.2 temperature regulator system.

We studied sensory conduction of the right median and ulnar nerve and of the sural nerve of both sides in all patients. For each nerve were considered sensory conduction velocity (SCV) and sensory action potential (SAP) amplitude. Sural and ulnar SAPs were recorded antidromically at the ankle and wrist respectively, whereas SAPs of the median nerve were obtained by anterodromal stimulation at wrist and elbow to determine separately wrist to finger and elbow to wrist SCV. Computer averaging was used to determine the size of low amplitude sensory responses.

We also studied motor conduction of median, ulnar and deep peroneal nerves; for each nerve we evaluated motor conduction velocity (MCV), compound motor action potential (CMAP) amplitude and distal latency (DL). To detect changes of proximal conduction we recorded F waves (20 times for each test) from ulnar and deep peroneal nerve at the elbow and above the knee respectively: the shortest F wave latency was considered.

Muscles that underwent EMG needle examination were tibialis anterior and medial gastrocnemius. Electrophysiologica parameters of the patients were considered abnormal if outside twice the standard deviation of mean values obtained from healthy age matched controls.

Neuropathological studies
We studied 28 sural nerve biopsy specimens, 25 from PN CG+ and three from PN CG− patients.

The sural nerve biopsy specimens were taken just proximal to the lateral malleolus. A portion was fixed in 10% formalin, embedded in paraffin wax, and sections were stained with haematoxylin and eosin, Congo red, alcian blue, and PAS. A second portion was quickly frozen in liquid nitrogen and either frozen or paraffin wax embedded; 6 µm sections were used for direct immunocytochemical study using peroxydase-conjugated goat antihuman affinity purified antibodies (IgG, IgA, IgM, 1:500 dilutions). A third portion was fixed in 2% buffered glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon 812. Semithin sections were stained with toluidine blue and examined under the light microscope to estimate the distribution and severity of myelinated fibre abnormalities. Quantitative histometrical analysis of myelinated fibres were obtained from micrographs, at a magnification of ×1000 of three fascicles for each patient. Fifty teased myelinated fibres were studied in each patient.

The sections were analysed for the presence of signs of axonal degenerations or demyelination. Particular attention was focused on the presence of perivascular and interstitial inflammatory infiltrates; other vascular abnormalities, such as hyperplasia of muscular and endothelial cell layers, were noted. Epineurial vasculitis was diagnosed according to the criteria of Dyck et al when intramural infiltration with necrosis of the vessel walls was present.

Statistical methods
Statistical analysis of the results were performed using a χ² test (associated to Yates’s correction when necessary) and Student’s t test for unpaired data. p Values <0.01 and <0.05 were considered significant.

RESULTS
Fifty one HCV infected patients, 25 men and 26 women, were studied. Table 1 summarises their clinical, laboratory, and neuropathological features. Their ages at time of diagnosis ranged from 39 to 81 years; mean (SD) age 62 (10). Detectable cryoglobulins (with a cryocrit greater than 0.1%) were found in the serum in 40 of 51 patients (78%). Cryoglobulins were type III and type II. No patient had type I cryoglobulins.

Clinical examination disclosed four different patterns of peripheral nerve involvement: PN, mononeuropathy and/or multiple neuropathy (MN), cranial neuropathy (CN), polyneuropathy combined with cranial neuropathy (PN + CN). PN was a symmetrical sensormotor neuropathy with predominant sensory features in some cases. Subgroups of multiple mononeuropathy and cranial neuropathy as well as cranial neuropathy associated with clinical polyneuropathy, were all considered to be clinical expression of an ischaemic nerve damage as other causes of focal nerve damage were excluded by inclusion clinical criteria.
Of the 40 CG+ patients, 18 had PN (45%), 16 had MN (40%), 3 had CN (7.5%), and 3 had combined PN+CN (7.5%). Among the 11 CG− patients, the clinical features were as follows: five patients had CN (46%), four patients had MN (36%), one patient had PN (9%), and one patient had combined PN+CN (9%) (table 1).

The prevalence of PN was significantly higher in CG+ patients compared with CG− patients (45% versus 9%; p=0.01, \( \chi^2 \) test). CN was significantly higher in CG− patients (46% versus 7.5%; p=0.01, \( \chi^2 \) test). There was no significant difference in the proportion of mononeuropathy/multiple neuropathy and cranial neuropathy combined to polyneuropathy.

Considering CN, MN, and CN+PN as expression of a mononeuritic process, we found a significant difference between CG+ and CG− groups (p<0.03, \( \chi^2 \) test): CG− patients more frequently developed a well defined mononeuritic process (10 of 11, 90%) when compared with CG+ patients (22 of 40, 55%).

CG+ patients showed significantly higher proportion with rheumatoid factor positivity (87.5% versus 18%; p>0.001, \( \chi^2 \) test) and lower C4 levels (92.5% versus 45.5%; p=0.001, \( \chi^2 \) test). Increased transaminase activities (ALT>70 U/l), as possible expression of HCV cytopathic effect, were found in 67.5% of CG+ patients compared with 45.5% of CG− patients; no significant difference was found between the two groups (\( \chi^2 \) test).

Table 2 gives the electrophysiological findings. Analysis of the data showed a significant difference only for one parameter: MCV of deep peroneal nerve in CG+ compared with CG− patients. The other neurophysiological parameters were suggestive of a wider and more severe involvement of peripheral nerve in CG+ patients, even if no significant differences were found.

Nerve biopsy was performed in 25 CG+ patients: epineurial vasculitis (fig 1) was present in 8 of 25 cases (32%), differential fascicular loss of axons (fig 2) was found in 10 cases (40%), signs of both demyelination and axonal degeneration were present in seven cases (28%). Three of the 11 CG− patients underwent sural nerve biopsy: two patients had epineurial vasculitis and one showed a differential fascicular loss of axons. In all cases, teased fibre preparation demonstrated axonal degeneration without evidence of primary demyelination. Comparison of neuropathological features disclosed no significant difference between CG+ and CG− groups (\( \chi^2 \) test). Table 3 gives the histometrical data of sural nerve biopsy specimens. We found a significant fibre loss in CG+ compared with CG− patients (p<0.005, t test), as well as in

<table>
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<th>Table 2</th>
<th>Motor and sensory nerve conduction studies in CG+ and CG− patients (mean [SD])</th>
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<tbody>
<tr>
<td>Controls</td>
<td>CG+</td>
</tr>
<tr>
<td>Deep peroneal nerve</td>
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</tr>
<tr>
<td>MCV [m/sec]</td>
<td>51.5 (3.4)</td>
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<tr>
<td>DL [msec]</td>
<td>4.1 (0.5)</td>
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<tr>
<td>Amp [mV]</td>
<td>11.5 (3.7)</td>
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<tr>
<td>F wave [msec]</td>
<td>40.5 (2.6)</td>
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<td>Median nerve</td>
<td></td>
</tr>
<tr>
<td>MCV [m/sec]</td>
<td>58.1 (3.3)</td>
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<tr>
<td>DL [msec]</td>
<td>3.4 (0.3)</td>
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<tr>
<td>Amp [mV]</td>
<td>17.0 (3.9)</td>
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<td>Ulnar nerve</td>
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<tr>
<td>MCV [m/sec]</td>
<td>59.6 (4.2)</td>
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<tr>
<td>DL [msec]</td>
<td>2.5 (0.3)</td>
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<tr>
<td>Amp [mV]</td>
<td>16.8 (2.5)</td>
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<tr>
<td>F wave [msec]</td>
<td>22.3 (0.2)</td>
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<td>Sural nerve</td>
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<tr>
<td>SCV [m/sec]</td>
<td>50.8 (3.7)</td>
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<tr>
<td>SAP ampl [mcV]</td>
<td>22.9 (12.2)</td>
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<td>Median nerve</td>
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<td>WF SCV [m/sec]</td>
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<td>SAP ampl [mcV]</td>
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<td>Ulnar nerve</td>
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<tr>
<td>SCV [m/sec]</td>
<td>55.6 (4.6)</td>
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<tr>
<td>SAP ampl [mcV]</td>
<td>35.0 (18.8)</td>
</tr>
</tbody>
</table>

* p<0.05 CG+ group compared with CG− group (Student’s t test for unpaired groups).

Table 3 | Histometry of sural nerve biopsy specimens (mean [SD]) |
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<tbody>
<tr>
<td>Controls</td>
<td>CG+</td>
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<tr>
<td>Fibre density (n/mm²)</td>
<td>5.400–8.600</td>
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<tr>
<td>Fibres &gt;8 µm (%)</td>
<td>22.8–34.6</td>
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<tr>
<td>Total number of clusters</td>
<td>&lt;38</td>
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</table>

* p<0.005; ** p<0.05 (Student’s t test for unpaired groups).

Figure 1 Transverse section of sural nerve. The non-uniform pattern of myelinated fibre loss is shown in four different fascicles (A, B, C, and D) from the same CG+ patient. (Epon embedded semithin section, toluidine blue. Bar =20 µm.)
degeneration. Oclusion causes fascicular ischaemia that results in axonal complement fixation and RF deposition. The vasculitis or vascular induced by longstanding cryoglobulin precipitation with com-

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epineurial vessels changes caused by occlusion or vasculitis CG. It was postulated that nerve damage is secondary to characterised by axonal damage and it is usually associated with CG. It was postulated that nerve damage is secondary to epineurial vessels changes caused by occlusion or vasculitis induced by longstanding cryoglobulin precipitation with complement fixation and RF deposition. The vasculitis or vascular occlusion causes fascicular ischaemia that results in axonal degeneration.

It has been supposed that HCV may have a direct role in the pathogenesis of neuropathy; it could induce nerve damage by a direct cytopathic effect or by an immunomedi
duced chronic stimulation of the immune system.

Peripheral neuropathy associated with HCV is mainly char-

The association between HCV infection and CG is well estab-

DISCUSSION

The association between HCV infection and CG is well estab-

percentage of large myelinated fibres (p<0.05, t test). Total number of clusters, representing regenerating fibres, was sig-

In our series of patients affected by HCV infection and neu-

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In summary, our study disclosed a more severe impairment in HCV CG+ patients as compared with CG−, either by clinical, electrophysiological, and histometrical analysis. The mechanism of peripheral nerve damage seems to be vasculitic in both CG+ and CG− patients, as supported by the clinical and morphological findings. The presence of CG in the serum is predictive of a more severe and widespread neuropathic involvement, but there is evidence that cryoglobulins are not the unique factor involved in the vasculitic process. Further studies are needed to clarify the role of HCV in innate complement activation and the relevance of the “in situ” HCV induced chronic stimulation of the immune system.

Figure 2  [A] Transverse section of sural nerve of a CG− patient. Vasculitis of small sized artery in the epineurium: inflammatory infiltration of the vessel wall and perivascular space is present. (Paraffin wax embedded section, haematoxylin and eosin. Bar=50 µm.) (B) Immunostaining demonstrates that inflammatory cells are lymphocytes. (Cryosection stained with peroxidase conjugated antibodies to lymphocytes. Bar=50 µm.)

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Peripheral neuropathy in HCV infection

Pubcrawler: www.pubcrawler.ie

Neuromyelitis optica: a review

Peripheral neuropathy in HCV infection

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A

ll of us suffer from significant difficu-

ty in keeping up to speed with

the neurological literature. One study suggested that a dedicated doctor

would have to read no less than 17 scientific articles a day, in order to keep pace

with the medical literature. Quite simply having the time to keep up to date with

your own area of subspecialist interest is a significant issue. One solution to this very

real clinical problem is a website called

Pubcrawler (www.pubcrawler.ie). PubCrawler is a free “alerting” service set up

by the genetics department of Trinity College, Dublin, that is specifically used for

neurological articles. It is essentially a website called PubCrawler that allows you to

subscribe to Medline criteria that one wants to search under, and PubCrawler will email you to

notify of any updates in the literature at a

preset time. I have used PubCrawler for

over 4 years now, I have it set up to

perform searches on Parkinson’s disease, multiple system atrophy, and progressive supranuclear palsy on a weekly basis. On a
	typical week, I will be emailed about 40 citations, which I can quickly skim through (in less than 5 minutes), and select the abstracts that are of specific interest to me.

One criticism relates to the graphical look of the website, which is not particu-
larly appealing, and the users will need to be confident in their use of Medline search criteria. However, these are minor issues, this really is a website that I would strongly recommend to any clinician or neuroscientist. The PubCrawler system may not be perfect (although it comes close), but it must have saved me hours of work in the library. As the web-

site’s logo affectionately states “It goes to the library, you go to the pub”.

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