The potential role of hepatitis C virus in the pathogenesis of the neurological syndrome in chronic hepatitis C

C Caudai, D Maimone, P Almi, P Annunziata, I Bastianoni, C A Boggiano, G C Guazzi, M Padula, P E Valensin

Abstract
A 72 year old man developed chronic sensory neuropathy (CSN) during chronic hepatitis C (HCV) infection. Neurological symptoms began one year after acute HCV hepatitis and slowly worsened over three years. No conventional cause for CSN was found. Circulating antinnervous tissue antibodies (including anti-Hu) and inflammatory infiltrates in sural nerve biopsy specimens were absent. However, the presence of anti-HCV antibody and HCV-RNA in cerebrospinal fluid indicated that HCV had reached the intrathecal compartment, suggesting the direct viral involvement in the pathogenesis of CSN.

Keywords: hepatitis C virus; RT-PCR; cerebrospinal fluid

Case report
A 72 year old man (patient A) with chronic sensory neuropathy (CSN), complained of unsteadiness on walking and numbness in the hands and feet with prickling paresthesias. Clinical symptoms began one year after acute hepatitis C virus (HCV) infection and slowly worsened over a period of three years leading to apparent stabilisation. Objective signs included ataxic gait, absence of deep tendon reflexes, loss of kinesthetic and vibratory senses, and a mild reduction in tactile and thermal senses. Increased conentrations of serum aspartate aminotransferase (AST) (214 U/l; normal <40), serum alanine aminotransferase (ALT) (208 U/l; normal <40), serum IgM (360 mg/ dl; normal <250), and erythrocyte sedimentation rate (ESR) (19 mm/hour) were found. Cell blood count, glucose concentration, electrolytes, kidney and liver function tests, serum immunoelectrophoresis, antinuclear antibody (ANA), extractable nuclear antigen (ENA; anti-Ro and anti-La), antimitochondrial, and anti-LKM-1 (liver-kidney-microsomal) antibodies were all normal. Screening tests for neoplasms, alcoholism, vitamin B6 overdose, Sjögren’s syndrome (performed using Schirmer’s test), and cryptoglobulinaemia were all negative. Cerebrospinal fluid (CSF) analysis revealed mild elevation of IgG index (0.74; normal <0.71) and a few oligoclonal bands, which suggested the occurrence of an intrathecal synthesis of immunoglobulins. Sural nerve biopsy was indicative of severe axonal neuropathy with extensive loss of myelinated and moderate reduction in unmyelinated fibres. Serum anti-Hu, anti-Purkinje cell, and antisulphatide antibodies were undetectable. Repeated testing of serum aminotransferases (ALT) after recovery from acute HCV hepatitis confirmed the chronicity of hepatic disease. In our patient, no risk factor of HCV infection was noted. Other screening tests ruled out all further pathological conditions which could be associated with sensory neuropathy.

Anti-HCV antibody was found in serum and CSF by using a second generation enzyme immunoassay (HCV-EIA, Abbott Laboratories, Abbott Park, Illinois, USA) and Immunoblot (Chiron RIBA HCV 3.0, Ortho, Raritan, New Jersey, USA). Antibodies to all four peptides (C-100–3, C-33c, C-22–3 and NS5) were detected in both samples.

HCV-RNA was extracted from 100 µl serum and 200 µl CSF using the guanidine thocyanate method. HCV-RNA in serum and CSF was detected by reverse transcription polymerase chain reaction (RT-PCR) which was performed using two sets of oligonucleotide primers selected from the highly conserved 5’ non-coding region of the HCV genome and which amplified a 146 bp long internal sequence. Three different types of negative controls were included in RT-PCR analysis to exclude false positive results due to contamination: reaction mixture plus water; anti-HCV and HCV-RNA negative human serum; and human CSF from a patient (patient B) with chronic hepatitis C and serum positive for hepatitis C virus.
### TABLE 1  
**HCV genotype and HCV-RNA levels in CSF and serum samples**

<table>
<thead>
<tr>
<th>Patient</th>
<th>HCV-RNA Serum (g.eq/ml)</th>
<th>HCV-RNA CSF (g.eq/ml)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Chronic sensory neuropathy and chronic hepatitis</td>
<td>Detected ($2 \times 10^3$)</td>
<td>Detected ($10^2$)</td>
<td>1b</td>
</tr>
<tr>
<td>B Chronic hepatitis and no neurological symptoms</td>
<td>Detected ($2 \times 10^3$)</td>
<td>Not detected (0)</td>
<td>1b</td>
</tr>
</tbody>
</table>

HCV-RNA, but without neurological symptoms. Using ethidium bromide-agarose gel electrophoresis a 146 bp band was visualised in both serum and CSF from patient A and in the positive control serum of patient B (fig 1). The HCV-RNA concentration of each sample was quantified in genome equivalents per ml (g.eq/ml) by a semiquantitative RT-PCR assay (table 1). HCV genotype 1b was determined by INNO-LiPA (Innogenetics, Nuclear Laser Diagnostics, Belgium).

**Discussion**

This is a case of CSN of unknown origin. Our screening tests ruled out all pathological conditions which can be associated with sensory neuropathies. CSN may be idiopathic or associated with neoplasms, Sjögren’s syndrome, dysproteininaemias, drug intoxication, or vitamina B overdose. Antineuronal or antiperipheral nerve autoimmunity has been frequently implicated in CSN, but antibodies to dorsal root ganglia, cerebellum, or peripheral nerves were not found in the serum of our patient. However, we cannot rule out the involvement of other autoantibody types. Various neurological syndromes can occur during the course of hepatitis virus infection, but only one case of sensory neuropathy with chronic non-A, non-B hepatitis has been described to date. Guillain-Barré syndrome (GBS) is the most frequent nervous system disorder associated with viral hepatitis. Peripheral neuropathies associated with essential mixed cryoglobulinaemia have been related to HCV infection, but cryoprecipitable serum immune complexes were not found in our patient. The presence of HCV-RNA in CSF was detected in individuals infected by HIV-1, but blood-CSF barrier damage and immunosuppression in such patients may be determinants for HCV penetration into the central nervous system. The lack of a similar condition in patient A allows us to hypothesise that HCV can be a direct cause of viral neurological syndrome. The elevated IgG index and the occurrence of a few CSF oligoclonal bands also pointed to a persistent antigenic stimulation within the intrathecal compartment. No correlation between HCV-RNA levels in the serum samples and a neurological syndrome was found. The correlation between 1b HCV genotype and the pathology of the nervous system warrants further study.

The characteristic of HCV tropism in hepatocytes and peripheral blood monocyte cells and its persistence in these cells is known, but HCV neurotropism is not. Our report invites further research into HCV infection markers in patients with a neurological syndrome of unknown origin and encourages us to continue to investigate the molecular basis of the potential role of HCV in neurological syndromes.