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Non-organ-specific autoantibodies in chronic hepatitis C patients: Association with histological activity and fibrosis

P. Chrétien [a,*], M. Chousterman [b], I. Abd Alsamad [c], V. Ozenne [b], I. Rosa [b], C. Barrault [b], T. Lons [b], H. Hagège [b]

[a] Laboratoire d'immunologie, Centre Hospitalier intercommunal de Créteil, 40 avenue de Verdun, 94010 Créteil Cédex, France
[b] Service de gastroentérologie, Centre Hospitalier Intercommunal de Créteil, 40 avenue de Verdun, 94010 Créteil Cédex, France
[c] Laboratoire d'anatomopathologie, Centre Hospitalier Intercommunal de Créteil, 40 avenue de Verdun, 94010 Créteil Cédex, France

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ABSTRACT

Background: Non-organ-specific autoantibodies (NOSAs) are frequently found in the sera of patients with Hepatitis C Virus (HCV) infection. However, no conclusive answers have been produced concerning the clinical relevance of these antibodies.

Aim: To determine whether a relationship might exist between the presence of NOSA and the severity of liver disease in chronic hepatitis C.

Methods: 186 treatment-naïve chronic hepatitis C patients were studied consecutively for autoantibodies. Liver biopsies were analyzed according to the Metavir score.

Results: NOSAs were present in 75 patients (40%). Anti-nuclear antibodies were found in 32% of patients (speckled pattern), anti-smooth muscle in 15% without F-actin specificity, anti-mitochondria in 0.5%, and anti-LKM1 in 0.5%, respectively. No liver-cytosol1 or soluble liver antigen antibodies were detected. There was a highly significant correlation between the positivity of NOSA and the degree of inflammation and hepatocellular injury ($p = 0.001$) and also with the degree of fibrosis ($p < 0.0001$). The presence of NOSA was associated with higher aspartate aminotransferase, γ-glutamyl-transpeptidase, γ-globulin and immunoglobulin G levels. By contrast, no differences were observed regarding age, gender, route of infection, duration of disease, HCV genotypes or viral load.

Conclusion: NOSAs were associated with the most severe forms of chronic HCV infections.

1. Introduction

Hepatitis C Virus infection frequently causes chronic infection leading to liver damage, cirrhosis and hepatocellular carcinoma [1].

Organ-specific and non-specific autoantibodies were first described in autoimmune disorders [2], but many of them may also be found during viral infections. Hepatitis C Virus (HCV) seems to be highly autoimmunogenic because numerous autoantibodies have been detected in HCV-infected patients [3].

Thus the reported prevalence of non-organ-specific autoantibodies (NOSAs) ranges from 6% to 21% for anti-nuclear antibodies (ANA), from 12% to 66% for smooth muscle autoantibodies (SMA), and from 0% to 10% for liver kidney microsomal autoantibodies (LKM) [3–7]. Furthermore, patients with chronic hepatitis C may have extrahepatic manifestations or syndromes, some of which are considered to be of immunologic origin, such as: sicca syndrome, autoimmune thyroiditis, mixed cryoglobulinemia, rheumatoid symptoms and glomerulonephritis [8,9].

However, there have been no conclusive findings concerning the clinical relevance of NOSA in chronic hepatitis C, although biochemical evidence of liver disease has been reported in association with NOSA [4,5,10].

Furthermore, the relationship between their positivity and the severity of liver damage in such patients still remains controversial [4,5,10,11].

The aim of this cohort study was thus to assess the prevalence, type and clinical importance of NOSA in chronic hepatitis C patients and the correlations observed between NOSA positivity and histological characteristics prior to any treatment.

2. Materials and methods

2.1. Study population

Between 1999 and 2004, 186 patients with chronic hepatitis C and referred consecutively to the Digestive Diseases Unit at our

* Corresponding author. Tel.:+331 57022224; fax: +331 57022219.
E-mail address: pascale.chretien@chicreteil.fr (P. Chrétien).

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hospital (Créteil, France), were routinely tested for the presence of NOSA. All patients had serum positivity for anti-HCV and HCV-RNA. All treatment-naïve, adult patients with histologically-proven chronic hepatitis were included. Serum samples were obtained within 6 months of a liver biopsy. No patient had anti-HIV antibodies. Patients with other causes for their liver disease, including hepatitis B and drug or alcohol abuse, were excluded. Additional causes for exclusion were decompensated liver disease or hepatocellular carcinoma. The demographic variables collected included age, gender, and risk factors for HCV infection. The duration of HCV infection was calculated by considering the date of the first injection of illicit drugs or any blood transfusion before 1992 as the time of infection. Study of this cohort was carried out in accordance with the guidelines issued by our institution.

2.2. Detection of autoantibodies

Anti-nuclear antibodies were detected by indirect immunofluorescence (IIF) on HEp2 slides (Kallestadt ™, Bio-rad, Marnes-la-Coquette, France) in 1/80 phosphate buffered saline (PBS) diluted sera. The slides were revealed using a fluorescein-labeled anti-human IgG, IgA and IgM antiserum (Bio-rad, Marnes-la-Coquette, France). The cutoff titer for positivity was fixed at 1/80. When sera were positive at 1/80 they were titrated.

Anti-smooth muscle (ASMA), anti-liver-kidney microsomal (LKM), anti-mitochondria (AMA) antibodies and anti-liver cytosol1 (LC1) were routinely detected using IIF on rodent rat liver–kidney–stomach sections (Kallestadt™, Bio-rad, Marnes-la-Coquette, France), as previously described [12]. The initial serum dilutions were 1/40 in PBS, and were titrated if necessary. The cutoff value was fixed at 1/40 except for ASMA, where positivity was fixed at 1/80. All sera positive for ASMA (>1/80), AMA (>1/40), LKM (>1/40) or LC1 (>1/40) were tested respectively for anti-F actin antibodies, anti-pyruvate dehydrogenase complex, anti-cytochrome P4502D6, and anti-formiminotransferase cyclodeaminase (FTCD) using immunodot (D-TEK® blue dot Liver, Diasorin, Antony, France).

Anti-soluble liver antigen (SLA) levels were also determined by immunodot (D-TEK® blue dot Liver, Diasorin, Antony, France). Anti-SLA has only been detected since 2001, so only 125 sera could be tested for these antibodies.

Anti-thyroperoxidase and anti-thyroglobulin antibodies were studied using enzyme-linked immunoassay (Orgentec SAS, Trappes, France).

2.3. Biochemistry

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transpeptidase (GGT), alkaline phosphatase (ALP) and serum γ globulins were measured using standard techniques.

IgG, IgA and IgM levels in sera were obtained by immunonephelemetry using Siemens® reagents on BN Propec (Siemens®, Dortmund, Germany).

Cryoglobulins were tested in 154 patients. Blood sampling, clotting and centrifugation were performed at 37 °C. Cryocrit determinations and cryoglobulin characterizations were performed routinely at +4 °C after 7 days. Protein levels in the cryocrit were quantified under immunofixation (Sebia, Evry, France) to enable the identification of cryoglobulins.

2.4. Virological assays

Serological evidence of HCV infection was provided by detecting antibodies to HCV by 2nd and 3rd generation enzyme-linked immunoassays (Ortho Diagnostic System Raritan, NJ, USA). HCV-RNA levels were obtained for all patients using a commercial quantitative polymerase chain reaction technique (PCR, Amplicor HCV, Roche Mannheim, Germany). HCV genotypes were obtained using the INNO-LiPA HCV reverse hybridization method (Innogenetics, Ghent, Belgium).

2.5. Staging of liver disease

A liver biopsy specimen was obtained from all patients. A single liver pathologist reviewed all tissue specimens without prior knowledge of autoantibody results and then graded individual histological features in accordance with the Metavir score [13]. Liver biopsies were scored in separate reports for grading (A0: no activity, A1: mild, A2: moderate, A3: severe) and staging (F0: no fibrosis, F1: portal fibrosis without septa, F2: portal fibrosis with rare septa, F3: numerous septa without cirrhosis, F4: cirrhosis).

2.6. Statistical analysis

All data were analyzed using SPSS (version 15.0, SPSS Inc., Chicago, IL). Statistical analysis was performed using the t test or Mann–Whitney test, corrected χ^2test or Fischer exact test and Spearman correlation, when appropriate. The results are expressed as counts and percentages for qualitative variables and as medians and ranges for discrete variables. A p-value <0.05 was considered to be statistically significant. Data are presented as the mean ± SEM in the tables and text.

Data are expressed as counts and percentages for qualitative variables and as medians and ranges for discrete variables.

3. Results

186 unselected, adult, untreated patients with chronic hepatitis C were evaluated consecutively. Their mean age was 50 ± 13 years, and 52% of them were female. Injection drug use and transfusion were the risk factors for HCV infection in 22% and 28% of cases, respectively. 120 patients (65%) were infected with HCV genotype 1, and 60 patients (32%) had a F3–F4 fibrosis score.

3.1. Prevalence of autoantibodies

NOSA could be found in 75 patients (40%): ANA occurred in 60(32%), ASMA in 28 (15%), AMA in 1 (0.5%) and LKM in 1 (0.5%) patients, respectively. Concomitant positivity for two antibodies: ANA and ASMA, was observed in 15 of these 75 cases. Neither LC1 nor SLA was present in any patients in the population tested ($n = 125$). The antibody levels observed are mainly low (Table 1). An

Table 1
Prevalence of autoantibodies in patients with chronic hepatitis C infection.

Autoantibodies		N	%
ANA		60	32
1/180	22		
1/160	16		
1/320	9		
1/640	7		
1/1280	6		
ASMA		28	15
1/80	21		
1/160	5		
1/320	2		
AMA		1	0.5
LKM		1	0.5
LC1		0	
SLA		0	