Chronic Hepatitis C Virus Infection After Treatment for Pediatric Malignancy

By Simone Cesaro, Maria Grazia Petris, Flavio Rossetti, Riccardo Cusinato, Corrado Pipan, Maria Guido, Lucia Masiero, Giuseppe A. Botta, Giovanni A. Meloni, and Luigi Zanesco

Sera of 658 patients who had completed treatment for pediatric malignancy were analyzed by a second-generation enzyme-linked immunosorbent assay and recombinant immunoblot assay test to assess the prevalence of hepatitis C virus (HCV)-seropositivity. All HCV-seropositive patients underwent detailed clinical, laboratory, virologic, and histologic study to analyze the course of HCV infection. One hundred seventeen of the 658 patients (17.8%) were positive for HCV infection markers. Among the 117 anti-HCV+ patients, 41 (35%) were also positive for markers of hepatitis B virus infection with or without delta virus infection markers, 51 (77.8%) had previously received blood product transfusions, and 25 (21.4%) showed a normal alanine aminotransferase (ALT) level during the last 5-year follow-up (11 of them never had abnormal ALT levels). The remaining 92 patients showed ALT levels higher than the upper limit of normal range. Eighty-one of 117 (70%) anti-HCV+ patients were HCV-RNA+, with genotype 1b being present in most patients (54%). In univariate analysis, no risk factor for chronic liver disease was statistically significant. In this study, the prevalence of HCV infection was high in patients who were treated for a childhood malignancy. In about 20% of anti-HCV+ patients, routes other than blood transfusions are to be considered in the epidemiology of HCV infection. After a 14-year median follow-up, chronic liver disease of anti-HCV+ positive patients did not show progression to liver failure. © 1997 by The American Society of Hematology.

Detection of HBV and HCV Infection

Serologic markers of chronic hepatic viral infection. HBV markers (HBsAg, HBsAb, HBeAg, HBcAb) and anti-hepatitis delta virus (HDV) antibodies were tested in fresh serum by commercial radioimmunoassay (Abbott Laboratories, North Chicago, IL). Antibodies to HCV (anti-HCV) were detected by the Ortho-ELISA (ELISA II) test (Ortho Diagnostic Systems, Raritan, NJ) following the manufacturer’s instructions. To define the specificity of the results obtained by ELISA, all positive sera were also investigated by RIBA II (Chiron Corp, Emeryville, CA, and Ortho Diagnostics Systems), according to the manufacturer’s instructions: sera showing only a band or at least two bands were considered undetermined or positive, respectively.

Detection of HCV-RNA and HCV genotyping. Sera of all anti-HCV+ patients were assessed for HCV-RNA by polymerase chain reaction (PCR). This assay was repeated at least once after 4 to 6 months in those patients whose sera was HCV-RNA+ at the first test. Total RNA was extracted by the guanidium thiocyanate-phenol-chloroform method. Synthesis of cDNA was performed using M-MLV Reverse Transcriptase (GIBCO-BRL, Grand Island, NY) and random Hexamer (Boehringer Mannheim, Mannheim, Germany) as primer.

PATIENTS TREATED for a pediatric malignancy are at high risk for parenterally transmitted viral hepatitis. Blood product transfusions are the major risk factors. Moreover, when compared with immunocompetent patients, the immunodepression caused by chemotherapy increases the chronicity rate of viral hepatitis. During the last two decades, screening blood donors for the hepatitis B virus (HBV) has resulted in a remarkable reduction of posttransfusion B-virus hepatitis; thus, non-A, non-B hepatitis has become the major form of parenterally transmitted hepatitis. The successful cloning of hepatitis C virus (HCV) genome and the development of serologic markers of HCV infection showed that HCV was responsible for 85% to 90% of parenterally transmitted non-A, non-B hepatitis. The prognosis of chronic HCV is a matter of controversy. HCV could worsen the outcome of successfully treated pediatric oncology patients because a progression rate to cirrhosis of 20% has been documented in 20-year follow-up studies in HCV-infected adults with no other disease. Furthermore, recent studies have shown that HCV infection is a risk factor for hepatocellular carcinoma. On the other hand, Seef et al. after an average follow-up of 18 years, reported a low incidence of deaths related to chronic HCV infection acquired from blood transfusion.

Our clinics follow a large number of patients who were treated for malignancy when they were children and when there were no tests available for detecting HCV in blood products. Such tests became available in 1990, allowing us to determine the prevalence, virologic pattern, and clinical course of chronic HCV hepatitis in a cohort of children and young adults who had been treated for malignancy before 1990.

MATERIALS AND METHODS

Patient Selection

As of August 31, 1995, the Division of Pediatric Hemato/Oncology was following 658 patients who had begun treatment for malignancy before 1990 and who completed chemotherapy before August 31, 1994. We analyzed the sera of all patients for antibodies to HCV using second-generation enzyme-linked immunosorbent assay (ELISA) and recombinant immunoblot assay (RIBA) tests as described below. All anti-HCV seropositive patients underwent detailed clinical, laboratory, and virologic study, including a retrospective count of how many blood (red cell and platelet) transfusions they had undergone. Liver biopsy was performed in most patients with elevated alanine aminotransferase (ALT) levels.

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primers at 42°C for 45 minutes and 99°C for 5 minutes, and rapidly chilled on ice. The cDNA samples were amplified using published primers chosen from the highly conserved 5′ noncoding region nucleotide sequence of the HCV genome. 15, 16

Moreover, the prevalence of HCV genotypes was investigated in the HCV-RNA+ patients following the method of Cha et al. 17 with little modification. Briefly, the cDNA was synthesized from extracted RNA and amplified using two sets of primers for the 5′UTR and E region of the HCV genome. Afterward, the amplification of the PCR products was analyzed by Southern blot hybridization using four 32P-labeled genotype-specific oligonucleotide probes. This assay allows the identification of four genotypes (1a, 1b, 2, and 3) according to the classification of Simmonds et al. 18

**Definition of Chronic Liver Disease in Anti-HCV+ Patients**

We used a standard definition for chronic liver disease (CLD); i.e., the elevation of serum ALT (normal range, 5 to 55 IU/L) for more than 6 months 19 after the completion of chemotherapy for childhood malignancy. Patients classified as anti-HCV+ were consequently evaluated for other potential causes of CLD using the analysis of serum α1-antitrypsin, ferritin, copper, ceruloplasmin, and autoimmune hepatitis markers (i.e., serum anti-nuclear, -smooth muscle, -mitochondrial, and -liver/kidney microsome auto-antibodies). Other causes of liver disease (i.e., alcoholism, hepatotoxic drugs) were excluded by medical history. In addition, all anti-HCV+ patients underwent screening for hepatocellular carcinoma (serum α-fetoprotein determination and hepatic ultrasound examination) and were monitored for ALT, serum bilirubin, γ-glutamyltransferase (GGT), and prothrombin time at least once every 6 months.

To define patients with persistent normal ALT level, the transaminase level was determined every 3 months for at least 1 year. For statistical analysis purposes, anti-HCV+ patients were divided into subgroups of those with and without CLD.

**Liver Histology**

Liver biopsy was performed only in patients with CLD. After the patient’s or parental consent, all biopsies were performed using a Menghini needle. All specimens, at least 1.5 cm long, had been fixed in 5% buffered formalin and embedded in paraffin. Five-micrometer sections had been routinely stained with hematoxylin-eosin, periodic acid-Schiff (PAS) before and after diastase digestion, Van Gieson method for collagen, Gomori method for reticulin, and Perl’s method for iron. All liver biopsy specimens were reviewed by one of us (M.G.), blind of any clinical information. Portal, periportal, and intralobular necro-inflamatory lesions were semiquantitatively scored and an overall histological activity index (HAI) was attributed. The final diagnosis of chronic hepatitis (CH) was based on both grade and stage of liver disease. The grade of CH was defined as mild (grade 1), moderate (grade 2), or severe (grade 3) based on the severity of portal/periportal and lobular activity. Fibrosis was assessed as absent, portal (stage 1), septal (stage 2), septal with architectural distortion (stage 3), or septal with cirrhosis (stage 4). 19

**Statistical Analysis**

Data collected by the dBase-4 program (Ashton-Tate Corp, USA) were analyzed using the SAS for Windows package (SAS Institute Inc, Cary, NC) to determine risk factors related to the development of CLD. We compared the frequency for the following putative risk factors in patients with and without CLD: sex, age at diagnosis, diagnosis of malignancy, number of blood transfusions, concomitant positivity for HBV markers with or without HDV infection, and follow-up duration. Risk factors significantly different at P < .1 were entered into a stepwise logistical regression model. The chi-squared test was used for the analysis of contingency tables and the Wilcoxon nonparametric score test was used to determine a different distribution for a quantitative variable. The results are those as of August 31, 1995.

**RESULTS**

**Prevalence of Anti-HCV+ Tests in the Study Cohort**

One hundred seventeen of 658 (17.8%) patients were determined to be anti-HCV+ by ELISA, and confirmed by the RIBA II test. There were 70 males and 47 females; their ages at diagnosis of malignancy ranged from 4 months to 19.7 years (mean, 5.7 years; median, 4.9 years), and the follow-up period ranged from 5.1 to 24.8 years (mean, 14.3 years; median, 14 years). The diagnosis of malignancy was leukemia or lymphoma in 74 of 117 (63%) patients and solid tumor in 43 of 117 (37%) patients. The prevalence of HCV positivity was 17.2% (74 of 431) among leukemia/lymphoma patients, and 18.9% (43 of 227) among patients with solid tumor.

**Analysis of 117 Anti-HCV+ Patients**

CLD. Eleven anti-HCV+ (9.4%) patients always had normal ALT levels during the entire follow-up period, whereas 14 (12%) had normalized ALT levels in a median time of 4.6 years (mean, 4.6 years; range, 1.2 to 9.4 years). CLD was found in 92 of 117 (78.6%) patients; 3 of these patients (2 with concomitant HBsAg positivity) had prothrombin time below the normal range (70% to 110%, 12 to 14 seconds) and 5 (all of them anti-HCV+ only) had elevated GGT and bilirubin levels. Albumin level was normal in all 117 anti-HCV+ patients. Thirty-two of 117 patients (27%) showed IgG levels higher than the upper limit of normal range (3 of 25 [12%] in the group without CLD and 29 of 92 [31.5%] in the group of anti-HCV+ patients with persistent CLD).

No anti-HCV+ patient had biochemical markers of other possible causes of CLD (hemochromatosis, α1-antitripsin deficit, Wilson disease, autoimmune hepatitis) or developed hepatocellular carcinoma (HCC).

**Blood product transfusions.** Ninety-one anti-HCV+ patients (77.8%) received at least one blood product transfusion. Blood transfusion support was more frequent in the leukemia/lymphoma group (67 of 74, 90.5%) than in the solid-tumor group (24 of 43, 55.8%) (P < .001). The median number of transfused blood product units was 4 per patient (mean, 8; range, 0 to 43). The median number of blood product units in the leukemia/lymphoma group was 4 (mean, 9; range 0 to 43) versus 2 in the solid-tumor group (mean, 3; range, 0 to 12) (P < .0001).

**Serological markers of HBV and HDV infection.** Forty-one of 117 (35%) anti-HCV+ patients were also positive for markers of previous HBV infection, and 9 of them (8% of 117 anti-HCV+, and 22% of 41 patients with HCV-HBV markers) were also positive for anti-HDV antibodies. Among the 41 patients with HBV infection markers, 29 (70.7%) were HBsAg+ (including all 9 anti–HCV-HBV-HDV+ patients) and 12 were HBsAg+ . All of them were negative for HBV markers at diagnosis of malignancy; thus, seroconver-
CHRONIC HCV INFECTION AFTER PEDIATRIC TUMOR

Table 1. Data on 117 Anti-HCV Patients Regarding Markers of HBV and HDV Infection

<table>
<thead>
<tr>
<th></th>
<th>Anti-HCV</th>
<th>Anti–HCV-HBV</th>
<th>Anti–HCV-HBV-HDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>88</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>M/F</td>
<td>54/34</td>
<td>11/9</td>
<td>5/4</td>
</tr>
<tr>
<td>LL/ST (%)</td>
<td>(62.5/37.5)</td>
<td>(55/45)</td>
<td>(89/11)</td>
</tr>
<tr>
<td>Median age* (mean; range)</td>
<td>18.4</td>
<td>23.3</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td>(18.8; 6.5-30.8)</td>
<td>(24.2; 13.4-32.6)</td>
<td>(22.1; 17.4-24.1)</td>
</tr>
<tr>
<td>Median follow-up* (mean; range)</td>
<td>13.5</td>
<td>18.2</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>(13.2; 5.2-22.5)</td>
<td>(17.8; 12-24.8)</td>
<td>(17.3; 14.2-19.2)</td>
</tr>
<tr>
<td>Prior BT (yes/no) (% yes)</td>
<td>67/21</td>
<td>15/5</td>
<td>9/0</td>
</tr>
<tr>
<td></td>
<td>(76%)</td>
<td>(75)</td>
<td>(100)</td>
</tr>
<tr>
<td>Median BT units² (mean; range)</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(9.5; 0-43)</td>
<td>(5; 0-24)</td>
<td>(4; 1-9)</td>
</tr>
</tbody>
</table>

Abbreviations: *+, seropositive; M, male; F, female; LL, leukemia/lymphoma; ST, solid tumor; BT, blood transfusions.

* In years, as of August 1995.
† Quantity of RBCs and/or platelets transfused.

sion to anti–HBsAg-positivity was found in 29.3% (ie, in 12 of 41 patients). Among the 29 HBsAg+ patients, 19 (65.5%) were HBeAg+ (14 of 20 patients with HCV-HBV chronic infection and 5 of 9 anti–HCV-HBV-HDV+), and 10 were anti-HBeAg-.

Liver histology in patients with CLD. Fifty-one of 92 (55.4%) patients underwent liver biopsy at a median time of 10.4 years (mean, 10.2; range, 0.8 to 15.9) from the date of CLD diagnosis. The median interval between biopsy and last follow-up (as of August 31, 1995) was 4.8 years (mean, 6.6). Twenty-two of 51 (43%) patients underwent biopsy after a median interval of 2.4 years (mean, 2.6) from the serologic diagnosis of HCV infection, whereas 29 of 51 (57%) patients underwent biopsy before that diagnosis (when still classified as non-A, non-B chronic viral hepatitis patients). The remaining 41 patients either refused liver biopsy or have not yet undergone liver biopsy at the date of this study. The patients who underwent biopsy included those who had abnormal prothrombin time or bilirubin level, and 3 of 10 patients with CLD and HCV-RNA negativity. Fourteen patients had double chronic infection B and C (two were also anti-HDV+).

Inactive cirrhosis and severe fibrosis (stage 4) were observed in 3 of 14 (21.5%) patients with HCV-HBV chronic infection versus 1 of 37 (2.7%) anti–HCV- only patients. HAI score ranged between 2 and 7. The three HCV-RNA- patients with CLD scored mild as grading, 1, 1, 2 as staging, 2, 3, 4 as HAI.

A second liver biopsy was performed in 9 of 29 patients who underwent biopsy before the serologic diagnosis of HCV infection. All of them were anti–HCV- only. The median time from the first biopsy was 5.3 years (mean, 5), and that from the serologic diagnosis of HCV infection was 4 years (mean, 3.6). The histologic staging improved in 1 patient, was unchanged in 5, worsened slightly in 2 (from stage 1 to 2 and from stage 2 to 3, respectively), and worsened more severely in 1 patient (from stage 2 to stage 4).

Table 2 shows the reviewed histologic diagnosis and the distribution regarding grading, staging, HAI, and serologic markers.

HCV-RNA data. Eighty-one of 117 anti–HCV+ patients (69.2%) had serum that was HCV-RNA+: 8 of 14 patients12 of 41 patients). Among the 29 HBsAg+ patients, 19 (65.5%) were HBeAg+. The prevalence of HCV-RNA positivity was 77.2% (68 of 88) in anti–HCV- only patients, 50% (10 of 20) in anti–HCV-HBV+ patients, and 33% (3 of 9) in anti–HCV-HBV-HDV+ patients.

HCV genotyping was assessed in 65 of 81 (80.2%) HCV-RNA+ patients. Genotype 1a was observed in 8 patients (12%), genotype 1b in 35 patients (54%), and genotype 2 in 3 patients (5%); in 19 patients (29%) the genotype was not identifiable.

Risk Factor Analysis

In univariate analysis, variables such as sex, age at diagnosis, diagnosis of malignancy, number of blood-product trans-
Table 3. Univariate Analysis of Putative Risk Factors for CLD in 117 Anti-HCV Patients

<table>
<thead>
<tr>
<th></th>
<th>Patients With CLD</th>
<th>Patients With Normal ALT Levels</th>
<th>P Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>92 (76.6%)</td>
<td>25 (21.4%)</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (63%)</td>
<td>12 (48%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>43 (37%)</td>
<td>13 (52%)</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (in yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>5.7</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.9</td>
<td>4.9</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Range</td>
<td>0.3-19.7</td>
<td>0.6-17</td>
<td></td>
</tr>
<tr>
<td>Follow-up duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>14.5</td>
<td>13.4</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Median</td>
<td>14.2</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5.1-24.8</td>
<td>5.9-20</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td>&gt;.1</td>
</tr>
<tr>
<td>L/L</td>
<td>55 (59.8%)</td>
<td>19 (76%)</td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>37 (40.2%)</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>Blood transfusions, no. of patients</td>
<td>70 (76%)</td>
<td>21 (84%)</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>No</td>
<td>22 (34%)</td>
<td>4 (16%)</td>
<td></td>
</tr>
<tr>
<td>No. of transfused blood units</td>
<td></td>
<td></td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Mean</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0-43</td>
<td>0-38</td>
<td></td>
</tr>
<tr>
<td>No. of HBV+ patients</td>
<td>17 (18.4%)</td>
<td>3 (12%)</td>
<td>&gt;1</td>
</tr>
<tr>
<td>No. of HDV+ patients</td>
<td>9 (10%)</td>
<td>0</td>
<td>.1</td>
</tr>
</tbody>
</table>

fusions, concomitant positivity to HBV (with or without HDV) infection markers, and follow-up duration did not significantly influence the biochemical signs of liver disease (Table 3).

**DISCUSSION**

The major findings of this study are the high prevalence of HCV infection in patients treated for a childhood malignancy (in particular, in leukemia/lymphoma patients) during the pre-HCV marker era, and a relatively benign clinical course with neither liver failure nor HCC after a 14-year median follow-up.

In this study, the overall prevalence of chronic HCV infection was 17.8% in a group of patients that was off chemotherapy after the treatment of a pediatric malignancy. This figure is high considering a 0.36% prevalence of HCV infection among Italian children (0.2% in our geographic area), but it is relatively low considering other individuals who undergo transfusion on a long-term basis (such as patients with hemophilia or thalassemia) or those in other risk groups (drug addicts, dialysis patients). In pediatric patients treated for malignancy, a 19% to 40% prevalence of HCV infection has been reported. Such disparity could depend on different prevalence of HCV in many geographic areas, in different groups of blood donors, and, among them, on different prevalence of chronic seronegative HCV infection despite HCV-RNA positivity in their sera. In the years following the availability of HCV infection markers, the incidence of this infection significantly decreased in our patients: of 172 patients, only 1 was anti-HCV+ before the diagnosis of malignancy and another one became positive during the follow-up.

The prevalence of HCV infection did not show any significant change in the distribution between leukemia/lymphoma and solid-tumor patients, even if the former group had a higher exposure to risk factors for HCV infection (ie, more frequent blood-product transfusions and invasive diagnostic procedures such as bone marrow biopsy or aspiration). In this study, 20% of HCV+ patients, in particular solid-tumor patients, did not receive any blood-product transfusion and were infected by another route. We assume that the use of nondonorable materials until the early 1980s (eg, needles for bone marrow aspirates or biopsy), intravenous high-dose Igs as anti-infection prophylaxis, and surgical procedures played major roles in this group of patients.

More than one third of anti-HCV+ patients showed a concomitant positivity to HBV markers, with or without HDV infection. We cannot determine exactly when our patients became infected because all of them were anti-HCV+ at the first assessment.

Spontaneous resolution of chronic HCV infection is a possible, but rare, event and requires a stable ALT normalization along with serum and liver HCV-RNA negativity. Therefore, in such cases the HCV-RNA negativity needs to be confirmed by a longer and more frequent follow-up and, possibly, by PCR detection of viral RNA in the liver.

HCV genotyping has recently raised considerable clinical interest because different HCV genotypes may affect the results of chronic HCV hepatitis treatment. Although the prevalence of different HCV genotypes may vary in each geographic area, there is evidence of a relatively more fre-
quent infection with type 1b in southern and eastern Europe.\textsuperscript{28} The higher prevalence of genotype 1b is also confirmed in our patients, reflecting the distribution in our blood-donor population. Genotype 1 could be more resistant to interferon therapy. If this hypothesis were true, most of our HCV-RNA\textsuperscript{+} patients should not be eligible for interferon therapy. Moreover, to date there is no firm evidence that HCC is related to infection with a particular HCV genotype.

HCV infection can raise concerns on the outcome of children successfully treated for a pediatric malignancy. Cirrhosis and liver failure have been reported in up to 20\% of the chronic HCV patients followed-up for more than 10 years.\textsuperscript{10,11} A superinfection or a co-infection with other hepatotropic viruses might worsen the initial prognosis.\textsuperscript{29,30} Furthermore, an association between chronic HCV infection and the development of HCC has been shown.\textsuperscript{15} The relative risk of HCC is higher (up to threefold) among individuals with chronic HCV hepatitis than in those with chronic HBV hepatitis, in patients infected early in life (presumably because of prolonged exposure), and in patients with underlying cirrhosis. Nevertheless, data from Seef et al\textsuperscript{13} indicate that chronic posttransfusion non-A, non-B (mainly, HCV) hepatitis does not affect the overall mortality rate after an 18-year median follow-up; in that group of patients, the mortality rate related to liver disease could have been influenced by alcoholism.\textsuperscript{13}

In this study, regarding patients who were HCV-infected during treatment of a pediatric malignancy, we did not observe progression to liver failure after a 14-year follow-up. Furthermore, no death or transplant caused by liver failure or hepatocellular carcinoma has been observed during the entire follow-up period in this group of patients. Actually, during the last 20 years, we observed only one case of HCC: a patient with 11-year-long chronic HBV-HDV infection.\textsuperscript{31}

Unfortunately, we have not performed a liver biopsy on every patient to histologically confirm this clinical course. In fact, only a group of patients with longer chronic hepatitis underwent liver biopsy. Although a second biopsy in nine patients showed a significant worsening of staging just in one case, the results of biopsy could be biased by the fact that 40\% (20 of 51) of patients underwent biopsy more than 5 years before the last follow-up and in the meantime some of them may have progressed to cirrhosis. The reviewed histological diagnosis showed more severe fibrosis mainly in patients with double infection (HCV plus HBV), but further investigation is needed to assess the exact weight of this finding. Actually, concomitant positivity to HBV (with or without HDV) failed to show any significant influence on the biochemical signs of CLD.

In conclusion, CLD in this group of anti-HCV\textsuperscript{+} patients has thus far had a relatively benign clinical course. However, we believe, given the young age of these patients, that it is too early to be optimistic. A longer follow-up will help to better define the evolution of chronic HCV infection in this setting.

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