Hepatitis C Virus Infection and Chronic Liver Disease in Children With Leukemia in Long-Term Remission

By Anna Locasciulli, Gianluca Gornati, Alessandro Tagger, Maria Lisa Ribero, Daniela Cavalletto, Luisa Cavalletto, Giuseppe Masera, Howard M. Shulman, Bernard Portmann, and Alfredo Alberti

Antibody to the recently identified hepatitis C virus (HCV) was investigated in sera of 50 leukemic children who had chronic liver disease (CLD), observed for 1 to 12.6 years after therapy withdrawal. All patients were tested for anti-HCV at regular intervals: Ortho-enzyme-linked immunosorbent assay (ELISA) test was performed in all cases. Reactive sera were also tested by recombinant immunoblotting assay to define the specificity of the results obtained by ELISA. Twelve cases (24%) were persistently positive (group A), 11 (22%) were transiently anti-HCV positive (group B), and 27 (54%) were negative. Mean SGPT peak during follow-up was significantly higher in group A (P = .014, A v B and P < .00001, A v C). SGPT normalized off-therapy in 1 of 12 cases (group A), 10 of 11 (group B), and 19 of 27 (group C) (P = .0004, A v B and P = .012, A v C). Accordingly, liver histology, available in 37 patients, showed signs of chronic hepatitis in all patients in group A while most patients in group B and C had less severe liver lesions. These results indicate that HCV plays a significant role in the etiology of chronic hepatitis in leukemic patients and that persistent anti-HCV activity correlates with a more severe CLD, which could jeopardize the final prognosis of children cured of leukemia.

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CHRONIC HEPATITIS frequently develops in patients with leukemia. This occurs during1-5 or after chemotherapy6 and may become a major long-term problem in patients potentially cured of their leukemia. Indeed, previous studies indicated that at least 1/3 of patients who have undergone antileukemic therapy have liver disease, which is persistent in more than half of the patients after treatment withdrawal.7 In addition to the direct hepatotoxic effects of drugs,6 parenterally acquired hepatitis viruses certainly play an etiologic role. The role of hepatitis B virus (HBV) has clearly been proven.8-10 In other patients with HBV-negative liver disease, non-A, non-B agents have been the proposed cause.11

Recently, a new RNA virus named hepatitis C virus (HCV) has been identified as the major cause of parenterally acquired non-A, non-B hepatitis worldwide.12 An assay has been developed to detect serum antibodies against a nonstructural epitope of HCV (anti-HCV).13 There is evidence that this is often a marker of ongoing infection rather than of postinfection. To define the possible role of hepatitis C in patients successfully treated for leukemia, we have studied patterns of anti-HCV positivity in a series of 50 children with leukemia who had chronic liver disease (CLD) and were observed on a long-term basis after cessation of antileukemic therapy.

MATERIALS AND METHODS

To determine the role of HCV infection in CLD of children surviving leukemia, we adopted the following inclusion criteria: (1) Biochemical evidence of CLD during chemotherapy (a threefold or higher increase in serum transaminase levels confirmed by serial testing to last more than 6 months). (2) Off-therapy follow-up for at least 1 year. (3) A series of stored sera that included at least three samples taken while on therapy and two during follow-up, at least 6 months apart from blood transfusion, to avoid positivity due to passive antibody transfer. (4) Liver function monitoring at least every 6 months for the whole period of observation.

Patients. Three hundred twenty consecutive children treated for various forms of leukemia at the Department of Pediatric Hematology, University of Milano, Monza, Italy, between 1969 and 1989 were reviewed. Of them, 146 were excluded from the present study because of relapse of leukemia either during treatment (n = 103) or within the first year off-therapy (n = 43); in this group 90 cases (61.6%) would otherwise have been candidates as they experienced CLD while on therapy. The remaining 174 patients who completed chemotherapy in first remission and had at least 1 year of observation after therapy withdrawal formed the basis of this study. CLD on treatment occurred in 108 of 174 (62%) patients. Nine of 108 children were lost to liver disease follow-up. Forty-nine of 108 patients, although tested for anti-HCV at least once, were excluded from the analysis secondary to lack of a consistent number of serum specimens either during (n = 29) or after therapy (n = 20). Fifty children who fulfilled all the inclusion criteria were evaluated. There were 30 boys and 20 girls. Mean age at onset of leukemia was 5.8 years (range: 9 months to 16.6 years). Forty patients had acute lymphoblastic leukemia, eight had acute myeloid leukemia, while a refractory anemia with excess of blasts (RAEB) and a chronic myeloid leukemia were diagnosed in the remaining two cases. All patients had been treated with polychemotherapy protocols for at least 2 years (range: 2 to 5 years). Thirteen of them also received an allogeneic bone marrow transplantation. Mean follow-up from diagnosis was 9.3 years (SD = 4.1 years, range: 3 to 17.2 years), and after therapy withdrawal was 6.2 years (SD = 3.4 years, range 1 to 12.6 years).

Forty-eight children had been transfused with red blood cells or platelet concentrates during therapy with a mean of 10.6 ± 9.5 U. All transfused patients were tested for anti-HCV antibody more than 12 months after the last transfusion (range: 1 to 17 years, mean 7.6 years).

Blood components administered to the patients were obtained from the Division of Pediatric Hematology, University of Milano; Monza: Clinica Medica II, University of Padova; Institute of Virology, University of Milano; Institute of Hygiene, University of Milano, Italy; the Department of Pathology, Fred Hutchinson Cancer Research Center, Seattle, WA; and the Department of Pathology, Liver Unit, King's College Hospital and Medical School, London, UK. Submitted October 29, 1990; accepted May 3, 1991.

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from hepatitis B surface antigen (HBsAg)-negative volunteer donors, before screening for anti-HCV was introduced.

Assessment of liver disease. The 50 children underwent clinical and biochemical assessment of liver disease, including serum tests for glutamic pyruvic transaminase (SGPT), alkaline phosphatase, bilirubin, albumin, and prothrombin time, which were performed at 3- to 6-month intervals. Serum samples, taken at the same time intervals, were kept frozen at −20°C to be tested for viral markers.

Thirty-seven children underwent percutaneous liver biopsy for diagnostic purposes. Biopsy was performed, with parents' informed consent, using a Menghini needle. Three children had follow-up biopsies.

Lever specimens were fixed in 10% formalin and embedded in paraffin. Four-micron sections were stained using standard histologic techniques (hematoxylin and eosin, Van Gieson, and/or chromotrope aniline blue, Shikata's orcein Perl's method for hemosiderin, and Gordon and Sweet silver impregnation for reticulin). Two of the investigators (B.P. and H.M.S.) independently reviewed these biopsies without prior knowledge of clinical and serologic results.

Assessment of HBV and HCV markers. HBV markers, including HBsAg, anti-HBs, and anti-HBc, were tested in serum by commercial radioimmunoassay (Abbott Laboratories, North Chicago, IL). Antibodies to HCV (anti-HCV) were detected by the Ortho Enzyme-linked immunosorbent assay (ELISA) test (Ortho Diagnostic Systems, Raritan, NJ). Sera were diluted 1:10 and the method and the evaluation of results were performed according to the manufacturer's instructions. To define the specificity of the results obtained by ELISA, reactive sera were also investigated by the recombinant immunoblotting assay (RIBA). For this purpose, sera were analyzed by second-generation RIBA (Chiron Corporation, Emeryville, CA and Ortho Diagnostics Systems), following the manufacturer's instructions. Accordingly, sera without bands of reactivity with viral antigens were defined negative, those with only one band of reactivity were defined indeterminate, and those reacting with at least two viral antigens were defined positive.

This investigation was approved by local institutional human research committees.

RESULTS

Prevalence of anti-HCV positivity by ELISA and by RIBA. The 50 children were serially tested for anti-HCV (mean = 7 tests/patient; range: 5 to 21) during 2 to 17 years of follow-up. The initial screening of these sera was performed by ELISA, using samples obtained at least 12 months after last transfusion to avoid detection of passive antibodies. On the basis of ELISA results patients were classified into three groups: 12 cases (24%) remained constantly anti-HCV negative, 24 (48%) had transient anti-HCV positivity at some point during follow-up, while the remaining 14 (28%) were persistently anti-HCV positive throughout the observation period.

To assess the specificity of anti-HCV reactivity, all patients with anti-HCV ELISA positivity were tested by RIBA. Patients with transient anti-HCV positivity were tested by RIBA at the time of ELISA reactivity peak. As shown in Table 1, positivity was confirmed by RIBA in 11 of 24 cases (46%) with transient anti-HCV positivity and in 12 of 14 (86%) cases with persistent anti-HCV positivity. Mean ELISA optical density (OD) was significantly lower in ELISA-positive/RIBA-negative samples compared with ELISA-positive/RIBA-positive samples (0.9 ± 0.5 vs. 1.8 ± 0.9, P = .007).

Anti-HCV positivity and liver disease. The pattern of liver disease seen in the 50 children during and after chemotherapy is shown in Table 2. A clinically overt hepatitis occurred in seven cases (14%): in five children during chemotherapy and in two 3 and 5 months after its withdrawal. The five patients with overt hepatitis on therapy showed a biochemical and clinical improvement, although no major changes were made in treatment schedules. Forty-three patients remained asymptomatic for the whole period of observation, and high transaminase values were the only biochemical abnormality detected.

The relation between anti-HCV reactivity and liver disease was analyzed after therapy withdrawal (Table 3). In 11 of 12 patients with persistent anti-HCV positivity confirmed by RIBA, transaminases remained persistently elevated during the entire observation period (group A). On the other hand, transaminase normalization was observed in 10 of 11 patients with transient anti-HCV positivity confirmed by RIBA (group B) and in 19 of 27 anti-HCV-negative patients (group C) (A v B = .0004; A v C = .0012;
Table 4. Liver Histology in 37 Children With Leukemia and CLD According to HCV Serology

<table>
<thead>
<tr>
<th>Histologic Diagnosis</th>
<th>No. Patients/No. Biopsies</th>
<th>NSRH</th>
<th>CLH</th>
<th>CPH</th>
<th>CAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Persistently positive</td>
<td>11/14</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>B: Transiently positive</td>
<td>7/9</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>C: Negative</td>
<td>19/19</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Abbreviations: NSRH, nonspecific reactive hepatitis; CLH, chronic lobular hepatitis; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis.

B v C = not significant). The transaminase profile was also analyzed in patients positive by ELISA while negative by RIBA. In the two patients with persistent ELISA reactivity SGPT normalized during follow-up as it also did in 11 of 13 patients with transient anti-HCV positivity.

Mean peak SGPT levels observed during the off-therapy follow-up were significantly higher in patients with persistent anti-HCV positivity compared to cases with transient (P = .014) or negative (P < .00001) anti-HCV reactivity.

These results indicate that CLD was more long-lasting and severe in the presence of anti-HCV persistence. In agreement with these conclusions were the liver histology findings, which were available in 37 patients (Table 4). Signs of chronic hepatitis, either persistent or active, were present in the liver of all patients with confirmed persistent anti-HCV positivity while most patients with transient positivity had chronic lobular hepatitis. Among those negative for anti-HCV, 1/6 had mild unspecific changes.

HBV markers in relation to anti-HCV serology. Fourteen of 50 patients (28%) were HBsAg-positive during follow-up: anti-HCV was always negative in 7 patients, transiently positive in 3, and constantly positive in 4. Two of 14 children (one anti-HCV-negative and one with transient anti-HCV positivity) were persistent carriers of the antigen. Seventeen additional patients (36%) were positive for antibodies to HBV (anti-HBs and/or anti-HBc): anti-HCV was negative in 10 patients, transient in 5, and persistently positive in 2. The prevalence of HBV markers was therefore high in these patients, but no association with a specific anti-HCV profile was observed.

Two examples of our patients, one with transient anti-HCV positivity and the other with persistent anti-HCV, shown in Figs 1 and 2, underlie the different severities of liver disease.

**DISCUSSION**

In this study we have documented a persistent and RIBA-confirmed anti-HCV positivity in the majority of children with leukemia in first complete remission showing persistent signs of CLD after chemotherapy withdrawal. The clinical relevance of the anti-HCV assay is still somewhat controversial in terms of its relation to past or ongoing infection, but there are data suggesting that this antibody reactivity, when confirmed by RIBA, is often a marker of virus presence.14,15 We have found a correlation between persistent anti-HCV positivity and a more severe liver involvement as assessed by transaminase levels and histologic findings. Moreover, the long-term outcome of liver disease was also influenced by the behavior of anti-HCV, as most patients with continuing anti-HCV positivity had

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**Fig 1.** Serologic and biochemical profile in a leukemic child with a transient anti-HCV positivity: SGPT normalized off-therapy, while anti-HCV reappeared transiently during follow-up. A liver biopsy taken shortly before cessation of chemotherapy showed features of nonspecific reactive hepatitis. LB, liver biopsy; OT, off-therapy.

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**Fig 2.** Biochemical profile during and after antileukemic therapy in a girl with persistent anti-HCV positivity: SGPT never normalized, while a liver biopsy taken during therapy showed a chronic active hepatitis. LB, liver biopsy; OT, off-therapy.
persistent serum biochemical abnormalities, while remission often occurred during follow-up when anti-HCV was transient. These findings are in agreement with those obtained in patients with acute and chronic non-A, non-B hepatitis, in whom recovery or remission of liver disease is associated with reduction in anti-HCV activity. In leukemia-cured patients antibodies to HCV appear as markers of ongoing infection and may be useful to predict the severity and course of CLD.

It should be noted that SGPT levels were also abnormal in several of our patients who remained constantly seronegative for anti-HCV. Normalization of liver tests was less frequent here compared to patients with transient antibody positivity. This may reflect the existence of other etiologic factors as the cause of persistent liver damage in anti-HCV-negative patients. On the other hand, the evaluation of HCV infection by the anti-HCV test is still of limited accuracy due to low sensitivity and risk of unspecificity. "False-positive" pitfalls were avoided in our study by the use of the RIBA test, but it is instead possible that some of our patients were "false-negative" due to immunosuppression induced by leukemia and chemotherapy. Previous data have demonstrated that the degree of immune deficiency may influence the course and clinical and serologic pattern of hepatitis B, and similar false-negative results may occur with HCV infection. The possibility of HCV variants unreactive with current serologic tests may also be considered.

The development of more direct and sensitive tests of virus presence will certainly improve the diagnostic approach to HCV infection in this particular patient population. If this new information can be coupled with more effective antiviral therapy (eg, α-interferon), then the long-term outlook of children already cured of leukemia will surely be enhanced.

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