Hepatitis C Virus Infection in Children Treated for Acute Lymphoblastic Leukemia

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We studied 102 consecutive subjects after their completion of acute lymphoblastic leukemia (ALL)-directed chemotherapy, for evidence of hepatitis C virus (HCV) infection by enzyme immunoassay 2 and 3, second-generation recombinant immunoblot assay and reverse transcription-polymerase chain reaction (PCR) for detection of circulating HCV-RNA. Forty-four patients (43%) had evidence of exposure to HCV; 30 of these were anti-HCV+.

Hepatitis C Virus (HCV) infection plays a significant role in the etiology of liver disease in patients treated for childhood leukemia.1 The recent availability of serologic tests for the detection of antibodies to HCV (anti-HCV) permits a reappraisal of the diagnosis of HCV infection in these patients. However, only limited information, usually obtained by first-generation enzyme immunoassay (EIA 1), is available on the overall prevalence of anti-HCV among patients cured of their leukemia.2 Retrospective analysis of sera collected from on-therapy patients recently demonstrated a 19% prevalence of anti-HCV reactivity.3 However, anti-HCV is not an indicator of active infection. Furthermore, patients receiving immunosuppressive therapy may develop anti-HCV reactivity only after treatment withdrawal.3 Thus, studies of HCV prevalence among patients who undergo immunosuppressive therapy should include those with long-term follow-up, and study methods should be used that allow direct virus detection.

To assess the prevalence of HCV infection among children who had been previously treated for acute lymphoblastic leukemia (ALL), 102 subjects were tested after completion of chemotherapy for evidence of HCV infection with the use of EIA 2 and 3, second-generation recombinant immunoblot assay (RIBA 2), and reverse-transcription polymerase chain reaction (RT-PCR) for detection of circulating HCV-RNA.

Patients

One hundred and two consecutive patients, 56 males and 46 females aged between 2.5 and 21.1 years (median age 10.5 years), who had been treated at our institution between 1977 and 1992 for ALL, were studied after completion of treatment. Details on antileukemic treatment administered in this period are reported elsewhere.5 All the patients except one had received blood derivatives from volunteer donors as part of their antileukemic treatment. Furthermore, all donors were screened for anti-HCV after the introduction of first-generation assay in January 1990. The exposure to blood products is expressed in blood units (one blood unit is defined as one unit of packed red blood cells, platelets, or fresh frozen plasma derived from one donor). The mean number of units transfused was 9.4 per patient (range, 1 to 40 U). All the patients had been prospectively followed-up in our center during and after treatment of their leukemia. Median duration of follow-up after treatment withdrawal was 34 months (range, 1 to 150 months). Between November 1992 and October 1993, all our patients were subjected to periodic physical examinations and blood samplings for evaluation of the following parameters: serum alanine aminotransferase (ALT), anti-HCV antibodies, and serum HCV RNA. All the analyses were performed on the same sample; three patients were HBsAg+ and anti-HBc+.

Methods

Anti-HCV detection. Patients were tested by an EIA 2 (Ortho HCV, 2nd generation; Ortho Diagnostic Systems, Raritan, NJ) that detects antibodies to core antigen and a fusion of the c100-3 and c33-c antigens (c200) of HCV according to the manufacturer’s instructions. Tests were performed in duplicate and positive results repeated. Anti-HCV reactivity by EIA 2 was confirmed in all patients by RIBA 2 (Chiron Co and Ortho Diagnostic Systems, Raritan, NJ), which uses four recombinant HCV antigens (5-1-1, c100-3, c33-c, and c22-3) fused to human superoxide dismutase. Those patients that were Sera anti-HCV negative by EIA 2 were retested by EIA 3 (Ortho HCV 3.0 ELISA Test system; Ortho Diagnostic). This assay also incorporates the NS5 protein, the putative HCV replicase.

HCV-RNA detection. Freshly-collected serum samples stored frozen at −80°C were used for HCV-RNA extraction. Primers used
RESULTS

Forty-four (43%) of the 102 patients studied had evidence of exposure to HCV as shown by the presence of circulating anti-HCV and/or HCV-RNA (Table 1). The three HBsAg-negative patients were all anti-HCV negative. Of 23 patients who were positive for both anti-HCV and HCV-RNA, 16 (69%) had a moderate increase in ALT activity without clinical signs of liver disease. Seven patients were anti-HCV negative in the absence of detectable viremia. Finally, 14 patients were seronegative despite the presence of HCV-RNA in the serum.

Of 58 patients (57%) who had no evidence of HCV infection, ie, anti-HCV negative and HCV-RNA negative, 5 had mild-to-moderate increase in ALT activity. All except one had been transfused, and none were HBsAg negative.

Nine of the 14 patients who were anti-HCV negative and HCV-RNA negative could be re-evaluated for anti-HCV and for HCV-RNA, 2 to 15 months after the first determination. Four patients were HCV-RNA negative also on follow-up sera. Of the remaining five patients, two developed anti-HCV reactivity as detected by EIA 3.

Thirty of the 37 sera that were found to be HCV-RNA positive using primers of the 5' noncoding region of the genome were successfully amplified with primers of the core region for genotype analysis. Twenty-two sera were from anti-HCV positive patients, whereas eight were from anti-HCV negative patients. HCV genotype I (1a) was present in three patients, genotype II (1b) in 10 patients and genotype III (2a) in 14 patients; in three cases, HCV genotype could not be identified (Table 2). No correlation was found between any specific genotype and the absence of anti-HCV seropositivity. Although half of the patients with genotype III had normal ALT value, patients with normal ALT levels were represented in all genotype groups.

There was no significant difference in terms of total number of blood units administered, with a median of 6 U given to each patient in both groups. The median age at first transfusion was comparable (61 ± 63 months, respectively). The transfusion-associated risk of HCV infection in our population decreased over the study period, although this difference did not reach statistical significance (χ², 4.86, not significant).

Table 2. HCV Genotypes, EIA 2 and RIBA 2 Reactivities, and ALT Activity in 27 HCV-RNA Positive Patients After Completion of Antileukemia Chemotherapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>HCV Type</th>
<th>EIA 2</th>
<th>RIBA 2</th>
<th>ALT (× NV)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>-</td>
<td>ND</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
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<tr>
<td>4</td>
<td>I</td>
<td>+</td>
<td>+</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>I</td>
<td>+</td>
<td>+</td>
<td>1.0</td>
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<tr>
<td>6</td>
<td>I</td>
<td>-</td>
<td>ND</td>
<td>1.2</td>
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<td>+</td>
<td>+</td>
<td>1.0</td>
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<tr>
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<td>I</td>
<td>+</td>
<td>+</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
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<td>6.8</td>
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<td>I</td>
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<td>+</td>
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<td>I</td>
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<td>+</td>
<td>1.7</td>
</tr>
<tr>
<td>12</td>
<td>II</td>
<td>-</td>
<td>ND</td>
<td>3.6</td>
</tr>
<tr>
<td>13</td>
<td>II</td>
<td>-</td>
<td>ND</td>
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<tr>
<td>14</td>
<td>III</td>
<td>+</td>
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<td>+</td>
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<td>III</td>
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</table>

Abbreviation: NV, normal value; ND, not determined.

DISCUSSION

The findings reported here show that over 40% of patients who completed leukemia-directed treatment had evidence of
exposure to HCV, and the majority of these showed ongoing HCV infection, as assessed by the presence of circulating HCV-RNA. This is in keeping with a recent study reporting HCV infection, as assessed by the presence of circulating EIA-HCV.

**HCV INFECTION IN CHILDREN**

Previously seropositive for anti-HCV and HCV-RNA with evi-

dently low prevalence of type I, whereas types 11 and 111

About 10% of the patients were anti-HCV⁺ and HCV-

RNA⁻ and had normal liver enzyme activity. These patients

might have had recovered from HCV infection or might have

had low fluctuating levels of viremia below the limits of
detection of the RT-PCR assay.

Approximately one third of HCV-infected patients did not
have serologic evidence of infection. Specificity and repro-
ducibility of the PCR results were confirmed by follow-up
evaluation of nine patients, of whom four were repeatedly
HCV-RNA⁻. It must be emphasized that HCV-RNA levels
may fluctuate considerably as a function of time, thus pro-
viding an explanation for the five patients who were found
to be PCR⁻ upon follow-up evaluation. Indeed, two of them
seroconverted to HCV by third generation ELISA. Locasci-
ulli et al¹⁶ have reported delayed appearance of anti-HCV in
a population of viremic children who have been infected
early in the course of antileukemic chemotherapy. All pa-

ten except one seroconverted after a median observation
period of 10 months after treatment withdrawal. Indeed, the
median follow-up period was not significantly different from
that of our study population. However, in that study, patients
were retrospectively selected for the presence of anti-HCV,
and search for HCV-RNA was not systematically performed.
The high number of seronegative infections observed in our
patients is similar to that reported for HCV-infected organ

transplant recipients¹⁵ or vertically infected newborns.¹⁶ Al-
though this phenomenon may be related to some extent to
a long-term immunosuppressive effect of antileukemic ther-

apy, it is not easily explainable because immunocompetence
should have been fully restored after treatment withdrawal
in the vast majority of our patients. Chronic hepatitis with
defective serologic expression of HBV has been previously
reported in leukemic children.¹,¹² Moreover, a pattern of sero-
negative HCV viremia has been occasionally found in immu-
nocompetent blood donors,¹⁸ as well as in nonleukemic
children with chronic hepatitis, even though evaluated with
first-generation assay.¹⁹

All the viremic patients, except one who lacked anti-HCV,

had normal transaminase activity, suggesting mild or absent
liver damage. However, this does not exclude the presence
of chronic liver disease in our patients, because it has been
reported that viremic patients with normal transaminase lev-

els may occasionally have chronic liver lesions.²⁰ Our find-
ings confirm that transaminase determination is inadequate
to predict HCV infection, as has been previously reported
in other risk categories.¹¹,²¹,²²

Analysis of HCV genotypes in our patients showed a rela-
tively low prevalence of type I, whereas types II and III

were equally represented. The proportion of the various ge-
notypes was not significantly different from other anti-HCV

patient groups.¹¹ However, genotype III was more frequently
associated with the absence of biochemical indicators of liver
damage, as shown for other patient categories.²²

The era of treatment was related to the risk of HCV infec-
tion in our series. Sixty percent of the patients treated up to
1984 were HCV⁺, and the infection rate decreased to about
29% after the introduction of donor screening for anti-HCV
in 1990. However, two new HCV infections were found after
blood transfusion since the introduction of EIA 2 as a donor-
screening test. In view of the recently reported decline of the
transfusion-associated risk of HCV infection,²⁰ it is possible
that the very high rate of HCV infection in these patients
may not only depend on clearly documented parenteral ex-
posure but also on other still unrecognized routes of transmis-
sion, possibly favored by associated conditions such as im-
munosuppressive therapy.

In conclusion, our study documents the magnitude of HCV
infection in childhood ALL survivors, which is presently
responsible for the majority of cases of non–hepatitis-B vi-
rus—related chronic liver disease in these patients. Whereas
serologic screening appears to identify over 70% of patients
with ongoing HCV infection, genuine infection may be pres-
ent in the absence of a detectable humoral immune response
to the virus, even several years after discontinuation of che-
motherapy. Based on this observation, determination of HCV
infection status by highly sensitive methods such as HCV-
RNA detection by PCR or anti-HCV detection by EIA 3
immunobosay is recommended in patients in prolonged re-
mission after childhood leukemia. Normal ALT levels did
not exclude the presence of HCV infection in more than half
of our viremic patients. Thus, long-term prospective studies
of HCV infection in children, particularly in those cured of
their leukemia, are mandatory to depict the current incidence
and the natural history of liver disease in these subjects. The
indications to perform liver biopsy in subjects with subclini-
cal HCV infection must be carefully considered, in acor-
dance with current therapeutic options.

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