Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C

Eliseo Minola, Daniele Prati, Fredy Suter, Franco Maggiolo, Flavio Caprioli, Aurelio Sonzogni, Mirella Fraquelli, Silvia Paggi, and Dario Conte

Before the introduction of hepatitis C virus (HCV) screening for blood donors, the risk of acquiring HCV infection as a result of a transfusion was about 10%. The aim of this study was to assess the frequency and rate of progression to cirrhosis in patients with transfusion-associated chronic HCV infection and identify possibly negative prognostic factors. Of 2477 consecutive patients with clinical or laboratory evidence of liver disease, 392 (16%) were anti-HCV– and HCV-RNA–positive, had anamnestic evidence of a single and precisely dated transfusion event, and showed no other causes of chronic liver disease; 268 (68%) underwent ultrasound-guided liver biopsy and were enrolled in the study. After a mean interval of 18.4 years, 54 patients (20.1%) had cirrhosis, which multivariate analysis showed to be independently associated with the duration of follow-up, age at infection and at the time of liver biopsy, and serum alanine aminotransferase levels at biopsy. The time necessary to have a 50% probability of developing cirrhosis in patients aged 21-30, 31-40, and more than 40 years was 33, 23, and 16 years, respectively. In comparison with those aged 20 years or less at infection, the risk ratio of developing cirrhosis over a period of 30 years for patients aged 21-30 and at least 31 years at infection was, respectively, 4.51 (95% confidence interval, 1.03-19.76) and 12.29 (95% confidence interval, 3.06-49.40). In patients with transfusion-associated chronic hepatitis C, the risk of cirrhosis is related to age at infection and disease activity. Our findings suggest that an aggressive therapeutic approach should be adopted in patients infected by HCV at an older age to prevent the progression to end-stage liver disease.

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Patients and methods

Patients

A nonconcurrent prospective, observational study of an ongoing cohort was carried out at the Department of Infectious Diseases of Ospedali Riuniti, Bergamo, Italy, after having been approved by the pertinent ethics committee. Between February 1986 and December 1999, 2477 patients with clinical or laboratory evidence of liver disease came to our observation. Miscellaneous causes of chronic liver disease were detected in 1014 (41%) cases; the remaining 1463 (59%) were positive for anti-HCV at enzyme-linked immunosorbent assay, at diagnosis, or during follow-up.
when anti-HCV testing became available. The overall number of viremic (ie, HCV-RNA–positive) patients was 1185 (81%), 151 of whom had concomitant causes of liver disease, including hepatitis B surface antigen positivity (n = 142), full-blown hemochromatosis (n = 3), and long-lasting alcohol abuse, arbitrarily defined as a daily alcohol intake of more than 100 g for at least 10 years (n = 6); 642 had either an unknown or nontransfusional source of HCV infection, including 420 intravenous drug users; the remaining 392 (37.9%) reported a single transfusion event in their medical history and represented the target population of this study.

Of these 392 cases, 39 (9.9%) had evidence of a single transfusion event between 1948 and 1964 in their medical history and/or personal copies of medical records; the remaining 353 cases were treated in 1965 or later, and the pertinent medical records were fully accessible on microfilm at Ospedali Riuniti.

Of the enrolled patients, 309 were referred by their family physicians or a primary or secondary care center because of a persistent abnormality in liver enzymes or anti-HCV reactivity, whereas the anti-HCV reactivity in 83 was first recognized during serologic screenings for blood donations after 1992. Liver biopsy was not indicated in 100 patients because of persistent normal liver transaminases (ALT) levels (n = 56), age more than 65 years (n = 13), chronic ischemic heart disease (n = 7), severe cardiomyopathy (n = 3), obstructive lung disease (n = 4), concomitant mental disorder (n = 2), or platelet counts of less than 80 × 10^9/L (80 000/μL) (n = 15). In this last group, 1 patient had chronic autoimmune thrombocytopenic purpura, 2 had connective disorder, and 1 had ethylenediamine tetraacetic acid–dependent pseudothrombocytopenia contraindicating liver biopsy because of medicolegal concerns; of the remaining 11 patients, 8 had clinical, biochemical, and/or ultrasound findings consistent with underlying liver cirrhosis.

At the end of the selection process, a liver biopsy was proposed to the remaining 292 patients, 268 of whom (91.8%) gave their written informed consent to the procedure.

Methods
A medical history was taken of all of the patients, who also underwent a physical examination. The biochemical tests pertinent to this study included hemogram, aspartate aminotransferase (normal values 0-35 U/L), ALT (normal values 0-35 U/L), alkaline phosphatase (normal values 30-120 U/L), total serum protein concentration (normal values 55-80 g/L [5.5-8.0 g/dL]), and serum ferritin (normal values 10-350 μg/L [10-200 ng/mL]). Anti-HCV reactivity was detected by means of a second- or third-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ) and confirmed by third-generation recombinant immunoassay (RIBA III; Ortho Diagnostic Systems), the results of which were classified as positive (reaction to at least 2 bands), indeterminate (single-band reactivity), or negative. Hepatitis B surface antigen and antibodies to human immunodeficiency virus were tested by means of enzyme immunoassays (Murex, Dartford, United Kingdom). HCV-RNA was determined by means of a qualitative reverse transcription–polymerase chain reaction assay, which has an analytical sensitivity of 1000 genome equivalents per milliliter (Beckman Analytical, Milan, Italy). The results were confirmed by at least 2 different assays. The different HCV genotypes were determined using the Typing C kit (Beckman Analytical). The liver biopsies were obtained under ultrasonographic control by means of Tru-Cut needles (gauge 16), and the specimens were fixed, paraffin-embedded, and stained with hematoxylin and eosin for reticulin and Masson trichrome or picrosirius red for collagen. All of the histologic examinations were performed by a single pathologist (A.S.), the results being classified according to Knodell et al⁶ from 1986 to 1996; in 1997, the classification system proposed by Ishak et al³ was introduced, and the previously collected samples were reclassified. Cirrhosis was diagnosed when a staging score of 5 or 6 was observed, in accordance with accepted criteria.¹⁰

Statistical analysis
Conventional descriptive statistics were used to analyze the data. The χ² test and one-way analysis of variance were respectively used to compare the nonparametric and parametric data. Univariate and multivariate analyses (multivariate regression model) were used to assess the independent associations between a histologic diagnosis of liver cirrhosis and sex, follow-up duration, age at liver biopsy, age at HCV infection, HCV genotype, and ALT levels at biopsy. The relative risk of developing cirrhosis was estimated, and 95% confidence intervals (CIs) were obtained by means of log transformation. Survival analysis was made using the Kaplan-Meier (product limit) method,¹¹ with the duration of follow-up being defined as the time between the single transfusion event and the time of the liver biopsy. The statistical analysis was stopped when fewer than 20% of the patients remained under observation. The survival inferences were based on the χ² test for multigroup comparison and the Cox-Mantel test for 2-sample comparisons.¹²⁻¹³ All of the tests were 2-sided, and P < .05 was considered statistically significant. The statistical analyses were made using a personal computer and the SPSS statistical software package (SPSS, Chicago, IL).

Results
The single transfusion events for the 392 eligible patients were precisely dated between 1948 and 1992 (median 1977). A total of 336 (86%) patients had increased serum ALT levels at the time of liver biopsy. The demographic and baseline characteristics of the 268 patients included in the study did not differ from those of the 124 patients who did not undergo liver biopsy (Table 1). Of the biopsied patients, 54 (20.1%) were found to have definite cirrhosis a median of 18.4 years (range, 4.2–43.1 years) after blood transfusion.

Univariate analysis showed that all of the examined variables (sex, follow-up duration, age at liver biopsy, age at HCV infection, HCV genotype, and ALT levels at biopsy) were associated with the presence of cirrhosis. At multivariate analysis, age at infection (P = .001), age at liver biopsy (P = .011), serum ALT levels at biopsy (P = .003), and the duration of follow-up (the time from transfusion) (P = .002) were independent predictors of the presence of cirrhosis, which was not significantly associated with sex or HCV genotype. However, when analyzed as a function of time using the Cox-Mantel regression test, only age at infection retained its negative statistical prognostic significance (K = 0.6, P < .0001) and significantly correlated with both the staging and grading of liver disease (P < .005 for both).

To investigate this aspect further, the patients were divided into 5 10-year age classes (1–10, 11–20, 21–30, 31–40, and > 40 years) on the basis of their age at infection. The number of patients in each age class was comparable (30, 47, 89, 59, and 43, respectively), and the duration of follow-up was adequate (at least 41 years unless all of the patients had reached the end point). The course of chronic HCV infection was negatively influenced by age at infection (Figure 1), as indicated by the finding of 3 cases of cirrhosis among

Table 1. Demographic and baseline characteristics of 392 patients with chronic HCV infection related to single transfusion event, by enrollment

<table>
<thead>
<tr>
<th>All patients (n = 392)</th>
<th>Biopsy performed (n = 268)</th>
<th>Biopsy not performed (n = 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>142/126</td>
<td>81/43</td>
</tr>
<tr>
<td>Mean duration of HCV infection, y (95% CI)</td>
<td>26.8 (25.2–28.3)</td>
<td>22.6 (19.6–25.6)</td>
</tr>
<tr>
<td>Distribution (%) of HCV genotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (a, b)</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>2 (a, b, c)</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>3 (a)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Years of follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>19.4 (18.2–20.6)</td>
<td>22.3 (20.4–24.3)</td>
</tr>
</tbody>
</table>
the incidence of nontransfusional sources in the preceding decades, the developing cirrhosis over a period of 30 years was 4.51 (95% CI, with those aged less than 20 years at infection, the risk ratio of 21-30, 31-40, and more than 40 years. Furthermore, in comparison was, respectively, 33, 23, and 16 years among the patients aged

The relevance of age at infection is further supported by the finding of a progressively decreasing median time to end-stage liver disease from 33 years to the 16 years observed in the patients aged more than 40 years at infection, who developed cirrhosis at a median age of 62 years. In this regard, it is worth mentioning some recent data relating to children and women. In the first prospective series of 67 patients with chronic HCV infection due to infected blood supply at a mean age of 2.8 years, the infection resolved in 30 (45%) after a mean follow-up of 20 years; of the remaining 37 patients, only 1 had abnormal liver enzymes, and only 3 showed signs of histologic damage although they also had additional risk factors for liver damage.\(^\text{17}\) The second series included a highly selected cohort of Irish women who had received HCV-infected anti-D immune globulin at a mean age of 28 years: after a median of 17 years, most of the 376 viremic subjects showed minimal changes at liver biopsy; of the 7 with cirrhosis (2%), 2 reported concomitant chronic alcohol abuse.\(^\text{18}\) Importantly, however, an unexpectedly high proportion (45%) of the infected women recovered and were no longer viremic 20 years after the infection, which may suggest that low viral titers or attenuated strains were present in the infected immunoglobulins due to manufacturing procedures. The data provided by Poynard et al,\(^\text{19}\) who studied a large cohort of patients who acquired chronic hepatitis C from different sources, are also consistent with the hypothesis of an inverse correlation between age at infection and progression to cirrhosis.

The same 3 studies indicate a rate of progression to cirrhosis ranging from nil to 18% 12 to 20 years after infection.\(^\text{17-19}\) The noticeably higher frequencies in other series (up to 46%)\(^\text{20-22}\) may be due to the heterogeneity of the sources of infection, the concomitance of relevant comorbidity, and/or the lack of age stratification.

Various factors may be responsible for the age-related differences in the clinical course of chronic HCV infection. First, structural, biochemical, and/or immunologic differences have been described in the livers of children and adults, mainly concerning fibrogenic mechanisms; eg, the reduced availability of antioxidant systems could be responsible for the age-related sensitivity of the liver to the oxygen-reactive compounds and organic aldehyde radicals formed during lipid peroxidation, which can induce the expression of profibrotic and proinflammatory cytokines and increase collagen synthesis by hepatic stellate cells.\(^\text{23-25}\)

Second, a number of reports have described the key role of alcohol intake in both the severity and course of HCV-related chronic infections; eg, in the series studied by Poynard et al,\(^\text{19}\) the cumulative frequency after a mean follow-up of 18.4 years would be 1 per 54.3. This would lead to no more than 5 cases (1.84%) being inappropriately classified as “posttransfusional.”

The overall proportion of viremic patients who developed cirrhosis was 20.1%. However, it is notable that the patients with resolved infection (HCV-RNA–negative) were not included in this computation. Because these patients usually have a normal histologic pattern on liver biopsy,\(^\text{15,16}\) the actual proportion of cirrhosis among the initially infected patients could be slightly lower. On the other hand, we excluded from the histologic evaluation 8 patients with clinical evidence of cirrhosis because of the presence of thrombocytopenia: this leads to an underestimate in the frequency of liver cirrhosis that, however, was not statistically significant. At multivariate regression analysis, age at infection and biopsy, disease activity at biopsy, and the duration of clinical follow-up were all independently associated with the risk of developing end-stage liver disease. However, in the older age groups (Figure 1d,e) few patients were available for analysis after 25 years of follow-up, and so the data after this period should be cautiously interpreted.

By way of conclusion, we believe that the longitudinal study of patients infected as newborns clearly demonstrates that chronic HCV infection might evolve to irreversible liver disease at any age and that the risk of developing cirrhosis in infected children is comparable with that of adults. Therefore, all infected children should be carefully monitored in order to detect early signs of liver damage.

**Discussion**

Our series included patients with chronic HCV infection due to a single transfusion event between 1948 and 1992. In accordance with the aim of the study, other possible cofactors for chronic liver disease (eg, alcohol abuse, hepatitis B virus and/or human immunodeficiency virus coinfection, iron overload) were carefully excluded, thus minimizing biases in patient recruitment. The 16% of the total cohort of 2477 consecutive patients attending the tertiary care liver unit in Bergamo who fulfilled the enrollment criteria represent a valid population for studying the natural history of transfusion-associated chronic hepatitis C. We are reasonably confident in attributing most of these cases to blood transfusion because, until the end of the 1980s, approximately 10% of the patients receiving blood transfusions in Italy developed posttransfusion hepatitis\(^\text{2}\) and most were subsequently diagnosed as having HCV infection. Conversely, the incidence of community-acquired HCV infection calculated in the same area in the 1990s was 1 per 10 000 person-years.\(^\text{14}\) Even if we hypothesize a 10 times higher incidence of nontransfusional sources in the preceding decades, the
liver biopsy findings were significantly worse in the patients consuming at least 50 g/d of alcohol than in those drinking smaller amounts and in teetotallers. An increase in lipid peroxidation and free-radical formation, or a promoting effect on HCV replication, may account for the synergistic effect of alcohol on HCV-related liver damage.26,27 In our series, alcohol intake may have influenced the results in different ways: we excluded patients with a daily alcohol intake of at least 100 g, but alcohol-related toxicity has been reported for much lower doses, and a dose-dependent effect has been repeatedly observed in both HCV- and hepatitis B virus–related liver damage.28 In this context, the less favorable course of chronic HCV infection in older individuals may be attributable to the increasing duration of alcohol consumption.

Third, a further factor that may be responsible for the age-related worsening of the clinical course of chronic HCV infection is hepatic steatosis. Hourigan et al have recently demonstrated that fibrosis in chronic hepatitis C correlates with body mass index and steatosis,29 and another study by Adinolfi et al30 found steatosis in about 50% of 221 patients with chronic HCV infection. The degree of liver fat infiltration significantly correlated with liver biopsy staging (fibrosis score) and grading (histologic activity index), and a further interesting finding was the relationship between the degree of fibrosis and age. Our data suggest that HCV genotypes are not related to the degree of histopathological damage and are in line with data previously published by our group31 and confirmed by a recent meta-analysis by Pagliaro et al32; however, a relationship has been found in other series.33-35 The prevalence of cirrhosis calculated in the present study among patients with transfusion-associated infection (20.1%) was similar to that observed in our series of 334 patients with the community-acquired form who underwent liver biopsy (19.3%, 64 cases; data not shown). However, the date of infection could not be precisely identified in this group. Importantly, it needs to be pointed out that our study focused on patients who acquired HCV infection through the transfusional route, and that such patients have a number of particular characteristics that may influence their clinical outcome. These include a high titer of viral particles in the original inoculum and, possibly, deranged clinical conditions and/or immune status at the time of transfusion. Furthermore, transfused individuals have a higher rate of mortality due to the disease for which the transfusion was administered. Our conclusions, therefore, do not necessarily apply to patients with the community-acquired form of the disease.

Our data on the outcome of transfusion-associated hepatitis C suggest that the risks and benefits of antiviral treatment should be weighed on the basis of the accelerated course of liver damage with increasing age at infection. Thus, patients who acquired HCV infection at an older age would require a more aggressive and timely therapeutic approach to prevent disease progression.

References

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