AGE AT INFECTION AFFECTS THE LONG-TERM OUTCOME OF TRANSFUSION-
ASSOCIATED CHRONIC HEPATITIS C

Running title:  Outcome of transfusion-associated HCV infection

Regular manuscript

1Eliseo Minola, M.D., 2 Daniele Prati, M.D., 1Fredy Suter, M.D., 1Franco Maggiolo, M.D.,
3 Flavio Caprioli, M.D., 1Aurelio Sonzogni, M.D., 3 Mirella Fraquelli, M.D., PhD.,
3 Silvia Paggi M.D., and 3 Dario Conte, M.D.

1 Infectious Diseases Unit, Ospedali Riuniti, Bergamo; 2 Blood Transfusion and Transplantation
Immunology Center, IRCCS Ospedale Maggiore, Milan; 3 Postgraduate School of
Gastroenterology, IRCCS Ospedale Maggiore, Milan, Italy

Corresponding author (reprint requests):
Dario Conte M.D.
Postgraduate School of Gastroenterology,
IRCCS Ospedale Maggiore,
Via F.Sforza 35,
20122 Milano,
Italy
Phone: ++39 – 02 55033418
Fax: ++39 – 02 55012111
E-mail: dario.conte@unimi.it (Preferred method of acknowledgment of receipt of the manuscript)

Word count:  Abstract: 271
Text: 3190

Supported by a “Research Competition Award 2000” from IRCCS Ospedale Maggiore, Milan,
Italy.
Abstract

Introduction: Before the introduction of hepatitis C virus 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.

Patients and methods: 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 US-guided liver biopsy and were enrolled in the study.

Results: 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 ALT levels at biopsy. The time necessary to have a 50% probability of developing cirrhosis in patients aged 21-30, 31-40 and >40 years was respectively 33, 23 and 16 years. In comparison with those aged ≤20 years at infection, the risk ratio of developing cirrhosis over a period of 30 years for patients aged 21-30 and ≥31 years at infection was respectively 4.51 (95% CI: 1.03-19.76) and 12.29 (95% CI: 3.06-49.40).

Conclusions: 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 in order to prevent the progression to end-stage liver disease.
Introduction

Chronic hepatitis C virus (HCV) infection is the main cause of end-stage liver disease in developed countries [1]. Until the introduction of anti-HCV screening for blood donors, the administration of infected blood components significantly contributed to the spread of the virus. In Italy, more than 10% of the patients receiving blood transfusion during the 1980s developed clinically evident post-transfusion hepatitis [2,3], and the prevalence of anti-HCV reactivity among transfusion-dependent patients starting their transfusion regimens in the same period approached 90%, with 70% of these patients developing chronic HCV infection [4,5].

The current risk of acquiring HCV infection through blood transfusion is negligible in comparison with other routes of viral transmission [4,6], and can be expected to decrease further following the adoption of nucleic acid testing technology for blood screening. However, specialists in various fields of medicine – including hematologists and hepatologists – are now facing the clinical effects of the past epidemic of transfusion-associated HCV. Current recommendations require that individuals receiving blood transfusions before the initiation of second-generation anti-HCV tests undergo anti-HCV screening [1]. Furthermore, an increasing number of patients with transfusion-associated infection are now seeking treatment and counseling.

As detailed in a recent review [7], the majority of studies of the rate of progression of chronic hepatitis C have so far concentrated on community-acquired infections, and only scattered data are available concerning transfusion-associated disease. However, estimates of the real proportion of patients experiencing a more severe clinical course and the definition of the risk factors for disease progression are highly important in order to identify the patients deserving a more aggressive and targeted clinical approach.

The aim of this study was to assess the frequency of liver disease and estimate the rate of progression to cirrhosis in a cohort of patients who acquired HCV infection after a single and precisely-dated transfusion event, and to identify the factors associated with a possibly negative prognosis.
Methods

Patients

A non-concurrent prospective, observational study of an ongoing cohort was carried out at the Department of Infectious Diseases of Ospedali Riuniti, Bergamo, 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 cases (41%); 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 (i.e. HCV-RNA positive) patients was 1185 (81%), 151 of whom had concomitant causes of liver disease, including HBsAg+ (#142), full-blown hemochromatosis (#3) and long-lasting alcohol abuse, arbitrarily defined as a daily alcohol intake >100 g for at least ten years (#6); 642 had either an unknown or non-transfusional source of HCV infection, including 420 IVDUs; the remaining 392 (33%) 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 di Bergamo” (Bergamo, Italy).

Of the enrolled patients, 309 were referred by their family physicians or a primary- or secondary-care centre because of a persistent abnormality in liver enzymes or anti-HCV reactivity; whereas the anti-HCV reactivity in 83 was first recognized during serological screenings for blood donations after 1992. Liver biopsy was not indicated in 100 patients because of persistently normal ALT levels (# 56), age >65 years (# 13), chronic ischemic heart disease (# 7), severe cardiomiopathy (# 3), obstructive lung disease (#4), concomitant mental disorder (#2), or platelet counts of less than 80,000/µl (#15). In this last group, one patient had chronic autoimmune thrombocytopenic purpura, two had connective disorder, and one EDTA-dependent pseudothrombocytopenia contraindicating
liver biopsy because of medico-legal concerns; of the remaining 11 patients, eight had clinical, biochemical and/or US 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 (AST, normal values 0-35 IU/L), alanine aminotransferase (ALT, normal values 0-35 IU/L), alkaline phosphatase (normal values 30-120 IU/L), total serum protein concentration (normal values 5.5-8.0 g/dL) and serum ferritin (normal values 10-200 ng/mL). Anti-HCV reactivity was detected by means of a second- or third-generation enzyme linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Raritan, NJ), and confirmed by third generation recombinant immunoblot assay (RIBA) (RIBA III; Ortho Diagnostic Systems, Raritan, NJ), the results of which were classified as positive (reaction to at least two bands), indeterminate (single-band reactivity) or negative. Hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV) were tested by means of enzyme immunoassays (Murex, Dartford, UK). HCV-RNA was determined by means of a qualitative reverse-transcription-polymerase chain reaction (RT-PCR) assay, which has an analytical sensitivity of 1,000 genome equivalents/mL (Beckman Analytical, Milan, Italy). The results were confirmed by at least two 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-eosin for reticulin and Masson’s trichrome or picrosirius red for collagen. All of the histological examinations were performed by a single pathologist (A.S.), the results being classified according to Knodell et al. [8]
from 1986 to 1996; in 1997, the classification system proposed by Ishak et al. [9] 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 chi-squared test and one-way analysis of variance (ANOVA) were respectively used to compare the non-parametric and parametric data. Univariate and multivariate analyses (multivariate regression model) were used in order to assess the independent associations between a histological diagnosis of liver cirrhosis and gender, 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% CI 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 chi-squared test for multiple-group comparison and the Cox-Mantel test for two-sample comparisons [12,13]. All of the tests were two-sided and p-values of <0.05 were considered statistically significant. The statistical analyses were made using a personal computer and the SPSS statistical software package (SPSS Inc.; Chicago, Illinois, USA).

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 (Tab.1).
Table 1. Demographic and baseline characteristics of 392 patients with chronic HCV infection related to single transfusion event, by enrolment (see text).

<table>
<thead>
<tr>
<th>All patients (# 392)</th>
<th>Biopsy performed (# 268)</th>
<th>Biopsy not performed (# 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male / female (#)</td>
<td>142/126</td>
<td>81/43</td>
</tr>
<tr>
<td>Duration of HCV infection (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (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>
Of the biopsied patients, 54 (20.1%) were found to have definite cirrhosis a median of 18.4 years (range 4.2-43.1) after blood transfusion.

Univariate analysis showed that all of the examined variables (gender, 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 ($p=0.001$), age at liver biopsy ($p=0.011$), serum ALT levels at biopsy ($p=0.003$) and the duration of follow-up (i.e. the time from transfusion) ($p=0.002$) were independent predictors of the presence of cirrhosis, which was not significantly associated with gender 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 ($R=0.6$, $P<0.0001$) and significantly correlated with both the staging and grading of liver disease ($P <0.005$ for both).

In order to investigate this aspect further, the patients were divided into five 10-year age classes (i.e. 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 (respectively 30, 47, 89, 59 and 43) and the duration of follow-up was adequate (at least 41 years unless all of the patients had reached the endpoint). The course of chronic HCV infection was negatively influenced by age at infection (Figure 1), as is indicated by the finding of three cases of cirrhosis among the 77 patients aged $\leq$ 20 years at infection as against 51 among the 191 older patients (chi-squared test: 14.17; $p <0.0002$). These findings were supported by the estimate of the time necessary to have a 50% probability of remaining free from cirrhosis. This could not be calculated for the patients aged $\leq$ 20 years at infection, but was respectively 33, 23 and 16 years among the patients aged 21-30, 31-40 and >40 years. Furthermore, in comparison with those aged less than 20 years at infection, the risk ratio of developing cirrhosis over a period of 30 years was 4.51 (95% CI: 1.03-19.76) in the patients aged 21-30 and 12.29 (95% CI: 3.06-49.40) in those aged $\geq$ 31 years.
Figure 1 Kaplan-Meier curves of progression to cirrhosis by different age classes at infection.

Estimated rate of progression to cirrhosis in 268 patients with chronic HCV infection as a consequence of a single transfusion event, by age at infection. The number of patients still under observation at a given time is shown at the bottom. The figures in parentheses refer to the patients who developed cirrhosis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of follow-up (years)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>30 30 24 (1) 12 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>47 41 25 (1) 10 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>89 76 (5) 46 (6) 16 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>59 (1) 48 (8) 25 (10) 2 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>43 (3) 25 (9) 4 (1) 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Group a: 0-10 years
- Group b: 11-20 years
- Group c: 21-30 years
- Group d: 31-40 years
- Group e: > 40 years
Discussion

Our series included patients with chronic HCV infection due to a single transfusion event between 1948 and 1992. In according with the aim of the study, other possible co-factors for chronic liver disease (e.g. alcohol abuse, HBV and/or HIV coinfection, iron overload) were carefully excluded, thus minimising 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 the vast majority of these cases to blood transfusion because, until the end of the 1980s, approximately 10% of the patients receiving blood transfusions in Italy developed post-transfusion hepatitis [2] and the vast majority were subsequently diagnosed as having HCV infection. Conversely, the incidence of community-acquired HCV infection calculated in the same area in the 1990s was 1/10,000 person-years [14]. Even if we hypothesise a 10 times higher incidence of non-transfusional sources in the preceding decades, the cumulative frequency after a mean follow-up of 18.4 years would be 1/54.3. This would lead to no more than five cases (i.e. 1.84 %) being inappropriately classified as “post-transfusional”.

The overall proportion of viremic patients who developed cirrhosis was 20.1%. However, it needs to be noted that the patients with resolved infection (i.e. HCV RNA negative) were not included in this computation. As these patients usually have a normal histological pattern on liver biopsy [15,16], the actual proportion of cirrhosis among the initially infected patients could be slightly lower. On the other hand, we excluded from the histological evaluation eight patients with clinical evidence of cirrhosis because of the presence of thrombocytopenia: this leads to an underestimate in the frequency of liver cirrhosis which, 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, it needs to be pointed out that in the older age groups (i.e. $d$ and $e$, Figure 1),
few patients were available for analysis after 25 years of follow-up, and so the data after this period should be cautiously interpreted.

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 >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 one had abnormal liver enzymes, only three showed signs of histological damage although they also had additional risk factors for liver damage [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 seven with cirrhosis (2%), two reported concomitant chronic alcohol abuse [18]. However, it needs to be underlined that an unexpectedly high proportion (i.e. 45%) of the infected women recovered and were no longer viremic 20 years after the infection, which may suggest that low viral titres or attenuated strains were present in the infected immunoglobulins due to manufacturing procedures. The data provided by Poynard et al. [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 three studies indicate a rate of progression to cirrhosis ranging from nil to 18%, 12-20 years after infection [17-19]. The noticeably higher frequencies in other series (up to 46%) [20-22] may be due to the heterogeneity of the sources of infection, the concomitance of relevant co-morbidity 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 of all, structural, biochemical and/or immunological differences have been described in the livers of children and adults, mainly concerning fibrogenic mechanisms: e.g. the
reduced availability of anti-oxidising 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 pro-fibrotic and pro-inflammatory cytokines and
increase collagen synthesis by hepatic stellate cells [23-25].

Secondly, a number of reports have described the key role of alcohol intake in both the severity and
course of HCV-related chronic infections: e.g. in the series studied by Poynard et al. [19], the liver
biopsy findings were significantly worse in the patients consuming ≥50 g/day of alcohol than in the
teetotallers and those drinking smaller amounts. 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 ≥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 HBV-related liver damage [28]. In this context, the less
favourable course of chronic HCV infection in older individuals may be attributable to the
increasing duration of alcohol consumption.

Thirdly, 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 BMI and steatosis [29], and another study by Adinolfi
et al. [30] 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
(histological 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 group [31] and
confirmed by a recent meta-analysis by Pagliaro et al. [32]; 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. 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 titre of viral particles in the original inoculum and, possibly, deranged clinical conditions and/or immune status at the time of transfusion. Furthermore, it is known that 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 in order to prevent disease progression.
References


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

Information about reproducing this article in parts or in its entirety may be found online at: http://www.bloodjournal.org/site/misc/quot;rights.xhtmlquot;&quot;#quot;repub_requests"

Information about ordering reprints may be found online at: http://www.bloodjournal.org/site/misc/quot;rights.xhtmlquot;&quot;#quot;reprints"

Information about subscriptions and ASH membership may be found online at: http://www.bloodjournal.org/site/subscriptions/index.xhtml

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 300, Washington DC 20036. Copyright 2011 by The American Society of Hematology; all rights reserved.