False Positive Tests for Anti-Hepatitis C Antibodies and the Problem of Notifying Blood Donors

SHULAMITH BAR-SHANY,* MANFRED S GREEN** AND EILAT SHINAR*


Background. All donated blood in Israel is tested for anti-hepatitis C virus (HCV) antibodies by enzyme immunoassay (EIA) and donors are notified of the result. There is evidence that at low antibody titres, the percentage of false positives may be high, with consequent labelling of healthy people as being infected with HCV.

Aim. In this study we examined the correlation between anti-HCV antibody titres determined by a second generation EIA test with supplemental EIA tests and evidence of abnormal liver function.

Methods. Blood samples of 201 Israeli civilians who donated blood during 1992 and who were repeat reactive for anti-HCV antibody based on second generation EIA, were tested by a supplemental test. Follow-up data were obtained from the donors and their family physicians.

Results. Results of anti-HCV EIA tests on two separate occasions of blood donation were highly correlated with each other (r = 0.86). Positive supplemental tests and abnormal liver function tests were found only in those subjects with high antibody titres. Furthermore low antibody titres were more prevalent during the winter months, suggesting that seasonal intercurrent infections may increase the percentage of false positives.

Conclusions. A high proportion of blood donors labelled as anti-HCV antibody positive based on low antibody titres, may not be at increased risk of carrying HCV. Since labelling would result in creating unnecessary anxiety among blood donors, it is important to confirm such results with tests such as radioimmunoblot assay (RIBA).

Keywords: blood donors, supplemental EIA tests, liver enzymes, anti-HCV antibody levels

There is evidence that a high percentage of patients who are positive for anti-hepatitis C virus (HCV) antibody have hepatitis C viraemia and are infectious. However, the relatively low specificity of the enzyme immunoassay (EIA) tests for anti-HCV antibodies could result in a large number of healthy donors being falsely labelled as carriers of HCV. The second generation EIA is more specific than the earlier tests, but there is evidence of cross-reaction of the test with antibodies against other viruses. This cross-reaction is usually seen at low levels of positivity on EIA, resulting in a low specificity for HCV infection at these levels. Furthermore, Zhang et al. found that using the second generation EIA, higher antibody titres reflected by an S/C ratio of >5 discriminated well between infective and non-infective subjects.

In this study we analysed the serological and biochemical findings in a sample of anti-HCV positive volunteer Israeli blood donors. The aim of the study was to estimate the extent of possible false positive tests. We determined the correlation between the second generation EIA screening test and supplementary EIA tests, and examined results of liver enzymes at different strengths of positive reactions on screening EIA.

MATERIALS AND METHODS

All blood collected by the Magen David Adom blood services in Israel is obtained from voluntary donors. In addition to testing for hepatitis B surface antigen and anti-HIV antibody, anti-HCV antibody titres are determined by means of a second generation EIA (Abbott, Chicago, Illinois). The test antigen is based on the antigens HC34, HC31 and c100-3.

This is followed by supplemental tests in initially positive subjects. The supplemental assay (Abbott HCV EIA supplemental assay) uses two different recombinant antigens, separately coated onto two different beads. The structural antigen is derived from the core region of
Table 1: Risk groups of Israeli blood donors positive for anti-hepatitis C antibodies by ALT results

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Normal ALT(^*)</th>
<th>Elevated ALT(^*)</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>History of blood transfusion</td>
<td>30</td>
<td>20.4</td>
<td>20</td>
<td>37.0</td>
</tr>
<tr>
<td>Intravenous drug use</td>
<td>3</td>
<td>2.0</td>
<td>6</td>
<td>11.1</td>
</tr>
<tr>
<td>Medical/paramedical occupations</td>
<td>14</td>
<td>9.5</td>
<td>3</td>
<td>5.6</td>
</tr>
<tr>
<td>Tattoos</td>
<td>2</td>
<td>1.4</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>History of hospitalization</td>
<td>31</td>
<td>21.1</td>
<td>8</td>
<td>14.8</td>
</tr>
<tr>
<td>None</td>
<td>67</td>
<td>45.6</td>
<td>16</td>
<td>29.6</td>
</tr>
<tr>
<td>Total</td>
<td>147</td>
<td>100.0</td>
<td>54</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\(^*\) Chi-square = 28.1, \(P < 0.001\), for difference in distribution of risk groups between subjects with normal and abnormal ALT.

The putative HCV genome and the non-structural antigen from sequences in the NS3 and NS4 regions. The results are expressed as the ratio of the actual EIA test reading (sample = S) to the cutoff (C) value for the test (S/C ratio). During 1992, of 161,813 blood units (from 130,122 donors) tested at the Tel Hashomer blood service facility, 896 (0.55\%) were anti-HCV repeat reactive. The S/C ratios of the positive tests were distributed as follows (only the lower bound is inclusive): 42\% were between 1 and 2, 14\% between 2 and 3, 6\% were between 3 and 4 and 37\% were \(\geq 4\). Of 97,965 units donated by civilians, 585 (0.6\%) were found to be anti-HCV positive. During the first half of 1992, only subjects whose blood tested strongly positive for anti-HCV antibodies (S/C > 3) were notified of their results and subsequently all subjects testing positive were notified. During the whole year, a total of 303 positive subjects were notified and given a referral letter to their family physicians, which recommended that they undergo a general medical check-up and liver function tests. The first author (S.B-S.) attempted to contact by telephone all these donors for risk factor history. The subjects were classified by risk groups according to possible previous exposure to contaminated blood or blood products. Those who fell into more than one risk category were classified by what was considered by the interviewer to be the more likely exposure according to the following hierarchy, previous blood transfusion being the most important exposure to previous hospitalization as being the least important. Attempts were made to contact the physicians of all subjects. The family physicians of 201 subjects were contacted, the findings on the patients discussed and liver enzyme test results (aspartate aminotransferase) recorded. For technical reasons, the physicians of the remainder of the subjects could not be located, but there was no reason to suspect any bias resulting from the failure to obtain data on these subjects. In order to test the consistency of the test results, an additional study was made of anti-HCV positive donors who had previously donated blood since HCV antibody testing was first implemented and the test results on the two occasions were compared. Finally, for the entire study group, the prevalence of anti-HCV antibodies was also analysed by month of blood donation. Correlations between different tests were estimated using Pearson correlation coefficients. The Mantel-Haenszel extension test was used to test for significance of trend in the seasonality of the prevalence of positive results. An approximate randomization test was used for comparing several percentages where some cells have very small numbers.

RESULTS
Of the 201 subjects in the study, 47 (23\%) were women. This proportion is similar to that in the whole population of blood donors. The average age was 43.5 years (range 18–65 years). When examined by region of origin, 40\% were born in Israel, 18\% were from the previous USSR, 10\% were from Eastern Europe, 9\% from Western Europe, and less than 5\% were from any other specific country. The characteristics of the anti-HCV antibody positive subjects by risk category are given in Table 1. Those with a history of blood transfusion or intravenous drug use were more likely to have elevated serum alanine aminotransferase (ALT). The graph of the EIA anti-HCV antibody results of the first and subsequent tests in a group of 45 initially antibody positive subjects is shown in Figure 1. The correlation coefficient was 0.86.
The prevalence of positivity of the supplemental anti-HCV antibody tests by level of positivity of EIA is shown in Figure 2. Only at levels of S/C > 4 were most of the tests positive for both the structural and non-structural antigens and at levels of S/C < 3, only 1 out of 71 was positive for both antigens. The association of the level of the EIA tests with elevated liver enzymes in anti-HCV antibody positive blood donors is shown in Table 2. At levels equal to or exceeding an S/C ratio of 4, nearly 50% had elevated enzymes whereas none with an S/C ratio < 3 had elevated enzymes. Thus for an S/C ratio ≥ 3, 83.6% (102/122) were positive on both the structural and non-structural antigens, and 42.5% (54/127) had elevated liver enzymes. The prevalence of positive EIA antibody titres at two levels, by calendar month, is shown in Figure 3. For the lower titres there is a significant trend for increased prevalence of seropositivity during November and December (P < 0.001 for trend).

**DISCUSSION**

We found a very high correlation between second generation EIA tests on repeated blood donations, indicating stability of the results over a period of months. Thus a finding of either high or low titre can be regarded as a consistent finding over a short period. The Abbott EIA supplemental test was positive almost exclusively at second generation EIA S/C ratios ≥ 3, suggesting that at S/C ratios < 2, the specificity of the standard EIA test may be very low. The findings in this study indicate that evidence of active hepatitis (as reflected by abnormal enzymes) is only present in blood
donors with high anti-HCV antibody titres. This is consistent with the findings of Kurosaki et al.\(^9\) who observed that patients with increased liver enzymes had higher levels of HCV RNA by PCR than those with normal enzymes and concluded that active liver disease was correlated with the level of viraemia. Romeo et al.\(^{10}\) found that in anti-HCV positive blood donors with normal liver enzymes, in only a minority was HCV viraemia detectable.

The more specific radioimmunoblot assay (RIBA) has been licensed as a confirmatory anti-HCV test, but is not universally available because of its high cost. Another limitation of the RIBA is the high per cent of indeterminate results. Sayers et al.\(^{11}\) found that 34% (52/153) of repeat reactive samples of blood donors were RIBA indeterminate. Yun et al.\(^{12}\) found that RIBA was positive in about 46% of those with anti-HCV EIA antibodies. HCV RNA was found in 95% of the RIBA positive patients, in 21% of the RIBA indeterminate, and in none of the RIBA negative patients. In addition, HCV RNA was found in the serum of all but one of those with chronic active or persistent hepatitis. They observed that HCV viraemia can be present in patients who have antibodies to only one HCV antigen, particularly the C22 epitope. In another study, HCV EIA-2 correctly identified all RIBA-confirmed specimens.\(^{13}\) However, 80% of HCV EIA-1 positive who were RIBA negative or had an S/C ratio < 2 in EIA-1 were negative by EIA-2 and they suggested that in such cases donors can be reassured.

The higher prevalence of anti-HCV positives at low EIA titres observed during the winter months, suggests that there is cross-reactivity with other antibodies, possibly to respiratory viruses which are prevalent in winter. This phenomenon has also been observed in African subjects by Hyams et al.\(^7\) In two studies of blood donors who received influenza vaccine, multiple false-positive ELISA were obtained for HIV, HTLV-I and HCV.\(^{14,15}\) This phenomenon appears to persist longer for HCV.\(^{14}\) In the present study, the higher prevalence of anti-HCV antibodies in winter is explained mainly by an excess of subjects with S/C ratios < 3, which strengthens the impression that the specificity of the EIA tests for anti-HCV antibodies may be much reduced at low antibody titres.

In summary, blood donors who are identified as positive for anti-HCV antibodies on second generation EIA are much more likely to be true positive if they have high antibody titres and consequently at increased risk for chronic liver disease. Low antibody titres may be non-specific and could result from cross-reaction with other antigens. Consequently, a more reassuring notification can be sent to donors found to be positive at S/C ratios < 3, and a limited deferral time before repeat donation of blood should be considered. It is well-established that RIBA is the standard confirmatory test and should be used whenever possible. However, we suggest that where RIBA confirmatory assays are not readily available, the strength of the EIA anti-HCV test (S/C ratio) should be included in donor notification. Donors with high ratios (S/C > 3) should be urged to see their physicians for long-term follow-up, and be advised not to donate blood in the future. However, donors with low antibody titres should be notified that the result may be non-specific and they should be followed-up by their physicians for repeat testing.

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**REFERENCES**


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