Detection of antibody to envelope (E2) antigen of hepatitis C virus

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Hepatitis C virus (HCV) was the primary etiological agent of parenterally transmitted non-A, non-B hepatitis (1). Serological testing of the blood supply has significantly reduced the risk of parenteral HCV transmission. The laboratory diagnosis of HCV infection depends on detecting antibody to HCV (anti-HCV) by enzyme immunoassays (EIAs) and confirmation by recombinant immunoblot assay (RIBA). A specimen is considered anti-HCV positive when reactive by EIA and RIBA. RIBA is used because of the problem of EIA’s nonspecificity in low risk populations.

Third generation RIBA 3.0 (Chiron Corporation, California) has four HCV antigen bands on each strip – C100p (NS4), C22p (Core), C33 (NS3), and NS5. The absence of reactivity to any of the HCV antigen bands is considered nonreactive. Samples re-
acting with two or more bands are considered HCV positive, whereas those reacting with only one band are deemed indeterminate. The indeterminate test result creates a problem for immediate diagnosis of HCV infection.

Recent studies have shown that a new experimental EIA developed for the detection of antibody to the second envelope protein (E2) of HCV may resolve some of the indeterminate samples (2-5). Antibody to E2 antigen (anti-E2) was found in 88% of RIBA 2.0 indeterminate samples positive for HCV RNA (5). Anti-E2 was also present in 42.4% of core and 15.4% of NS3 indeterminates (2). In another study, 47.6% of patients positive for anti-HCV were also reactive for anti-E2 (5) by EIA. A fluorescent antibody test has also been used for the detection of anti-E2 (6), and 93% of HCV patients were positive. RIBA indeterminate specimens reacting with E2 antigen may be considered positive for anti-HCV because they are reacting with two antigens (E2 and one of the other four antigens on RIBA 3.0 strip). With RIBA 3.0, samples reacting with two of the HCV antigens are considered positive.

In the present study 104 serum samples were tested for anti-E2. These samples were positive for anti-HCV by EIA. By RIBA 3.0, 49, 42 and 13 samples were reactive, indeterminate and nonreactive, respectively. Indeterminate samples were either caused by C100p (12), C33c (15) or C22 (15). The 104 samples tested were submitted to Laboratory for Viral Hepatitis, Ottawa, Ontario, for anti-HCV confirmation from provincial public health laboratories. Risk factors were reported for 26 of 42 indeterminate samples; risk factors included intravenous drug use, hemophilia, employment as a health care worker, transfusion, sexual contact with an infected partner, hemodialysis, transplantation and human immunodeficiency virus (HIV) infection. Anti-E2 results were evaluated for the presence of HCV RNA and different genotypes. Detection of RNA was considered evidence of HCV infection in the absence of serological test for viral antigen.

Anti-E2 antibody was detected by an experimental bead EIA (7) test (Abbott Laboratories, Illinois). The E2 antigen was produced in Chinese hamster ovary cells transfected with HCV E2 plasmid (amino acids 388-664). The antigen was further purified and used for coating beads, as described previously (2). Briefly, the testing was performed at 37°C, 50 µL of the specimen was diluted in 400 µL of diluent and 200 µL of the resulting solution was used for testing. Anti-human immunoglobulin-G labelled with horseradish peroxidase was used for the detection of anti-E2.

HCV RNA was detected by the Amplicor HCV test (Roche Diagnostic System, New Jersey), and genotyping was performed by line probe hybridization assay (Innogenetics, NV Zwijnaarche, Belgium). HCV RNA was detected using the Amplicor HCV protocol and was extracted from 100 µL of serum by guanidinium thiocyanate lysis and isopropanol precipitation. The RNA pellet was suspended in 1 mL of diluent, and 50 µL were used for amplification. The master mix contained rTth DNA polymerase primers (5'-GCGAAGGCTTACGATCCGTTTGGG-3' and 5'-biotinylated CTGCAAGGGCCTACCATCAGGGCTG) UNG, dATP, dCTP, dGTP and dUTP. The primers amplified 244 base pairs (bp) in the 5'-untranslated region. The amplification was carried out in the GeneAmp 9600 thermocycler (Perkin-Elmer-Cetus). Reverse transcription was carried out for 30 mins at 60°C and then amplified (40 cycles). PCR amplification temperature was 90°C for 15 s and 60°C for 20 s. The amplified product was detected by hybridization of the denatured product to solid phase probe specific for HCV. Hybridized complexes were detected with avidin-horseradish peroxidase.

The amplified products were subtyped by a line probe hybridization assay. In this assay oligonucleotides derived from the 5' noncoding region act as a specific probe for each subtype. The specific probes are immobilized as parallel lines on nitrocellulose strips. The amplified product from Amplicor HCV PCR is denatured and hybridized with the probe on strips. Following hybridization and washing, streptavidin labelled with alkaline phosphatase was used to detect the biotinylated hybrid. Colour development was achieved by using the substrate nitroblue tetrazolium-5-bromo-4-chloro-3-idolylphosphate. The pattern of reactivity to different probes was noted, and subtypes were identified.

All 49 HCV RNA positive samples were positive for anti-E2. The assay was not influenced by the HCV heterogeneity because all genotyped samples – 1a (11 of 11), 1b (11 of 11), 2a (nine of nine), 2b (seven of seven) and 3a (11 of 11) – were anti-E2 positive. This further extends the previous observation (2) that anti-E2 was detected in high proportion (97.3%) in HCV RNA positive specimens (subtype 1a). Testing of different subtypes of HCV for anti-E2 reactivity has not been reported before. This testing was performed to evaluate the effect of hypervariability of E2 region on anti-E2 assay.

The anti-E2 results for RIBA 3.0 indeterminate and negative specimens are given in Table 1. Twenty per cent (three of 15) of C33c and 33% (five of 15) of C22p indeterminate samples were positive for anti-E2 and HCV RNA. However, among

<table>
<thead>
<tr>
<th>RIBA 3.0 result</th>
<th>Samples tested (number)</th>
<th>HCV RNA positive</th>
<th>HCV RNA negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-E2 positive (%)</td>
<td>Anti-E2 negative (%)</td>
</tr>
<tr>
<td>C100p</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C33c</td>
<td>15</td>
<td>3 (20)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>C22p</td>
<td>15</td>
<td>5 (33)</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

E2 Second envelope protein; HCV Hepatitis C virus; RNA Ribonucleic acid
samples indeterminate by C100p only, two (16.6%) were positive for anti-E2, and all were negative for HCV RNA. Overall, anti-E2 was detected in 67% (eight of 12) of RNA positive, and in 30% (nine of 30) of RNA negative indeterminate samples. Among EIA positive and RIBA 3.0 negative samples, two (15%) were positive for anti-E2 and nonreactive for HCV RNA.

Sixty-seven per cent (eight of 12) of indeterminate samples were positive for HCV RNA and anti-E2 antibody. The presence of HCV RNA confirmed that these patients were infected with HCV despite their indeterminate status. This suggests that the testing for anti-E2 may help resolve indeterminate samples. If the C100p indeterminates are excluded, an even higher percentage (27%) of samples were positive for HCV RNA and anti-E2. A high percentage (19%) of the indeterminate samples were positive for HCV RNA because 62% of patients were from a high risk group; four were intravenous drug users, two were HIV-positive, one was a hemophiliac and one had a needle stick from a HCV positive patient.

The results also showed that a higher proportion of C22p (67%) and C33c (60%) indeterminates were positive for anti-E2 than C100p intermediates (16.6%) (P<0.05). None of the C100p indeterminates were positive for HCV RNA, and this may indicate that C100p is not a good marker for infectivity. Our observation that C100p is not a good marker for infectivity is similar to those reported previously (5), where 224 C100p (?) indeterminate samples were all negative for HCV RNA. Nine per cent (four of 42) of indeterminate samples were positive but negative for anti-E2. Probably these patients were at an early stage of infection, where the antibody to E2 antigen had not developed.

On the other hand, nine (21%) indeterminate samples were positive but negative for HCV RNA. This may be explained by the fact that in some cases HCV infection does resolve and in others the concentration of the virus is below the detection limit of PCR (5).

Among RIBA negative samples, two were positive for anti-E2. This may be due to increased sensitivity of anti-E2 assay or due to nonspecificity. Alter (8) reported that the E1/E2 proteins appear to be the most antigenic of HCV recombinant proteins, reacting with the sera of about 90% of HCV-infected individuals.

The number of indeterminate samples tested for anti-E2 was small; however, these data suggest that this assay may resolve some RIBA 3.0 indeterminate samples as HCV antibody positive or negative.

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