Definition of False-Positive Reactions in Screening for Hepatitis C Virus Antibodies

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The rate of false-positive hepatitis C virus enzyme immunoassay results was determined to be at least 10% among 1,814 reactive serum samples based on (i) negative results in an independent confirmation assay, (ii) negative PCR results, and (iii) no patients developing clinical or biochemical signs of hepatitis during a 1-year follow-up.

In daily laboratory routine, reliable diagnosis of hepatitis C virus (HCV) infection is not always possible by sole use of an HCV enzyme immunoassay (EIA), since it is well known that for a number of patients this assay produces false-positive results (4, 8, 17). Therefore, results obtained by EIA need to be confirmed by additional testing. However, the commercially available assay RIBA 2.0 (Chiron Corporation) does not fulfill the criteria defining a confirmation assay since it consists of recombinant proteins identical to those in the EIA (1, 5, 9). HCV PCR cannot be used for confirmation of positive EIA results, since a negative PCR result does not exclude the possibility of HCV infection with low-level viremia (below the limit of detection). Furthermore, PCR is too laborious and expensive to be used regularly as a confirmatory assay. Therefore, we have established an HCV strip immunoblot assay (SIA) (Universitäts-Krankenhaus Eppendorf [UKE] SIA) consisting of four recombinant proteins, derived from the core and three nonstructural regions (NS3, NS4, and NS5) of HCV, which are different from those used in the HCV EIA (5).

In the present study we compared the results of a second-generation HCV EIA with those of the UKE SIA for 2,283 serum samples. The aim was to assess the significance of positive results in the HCV EIA to define criteria for the performance of further tests to reliably diagnose HCV infection in the daily laboratory routine. Sera were drawn from 2,283 persons living in northern Germany around the city of Hamburg. They were sent to our laboratory under suspicion of HCV infection due to either elevated liver enzyme values (alanine aminotransferase, >45 U/liter) or clinical signs of hepatitis (jaundice and upper abdominal pain) or risk factors for percutaneously transmitted diseases, such as chronic hemodialysis, blood transfusion, or intravenous drug use. At the time of investigation they tested negative for acute infection with HAV (anti-HAV immunoglobulin M antibodies) and HBV (hepatitis B surface antigen). Repeated examinations were performed as follow-up every 3 months for 1 year. For serological screening a second-generation HCV EIA (Abbott Laboratories, North Chicago, Ill.) was performed. For confirmation of HCV EIA results, sera were tested in parallel by the UKE SIA as previously described (5). The immunoblot assay was considered positive when antibodies to at least two different recombinant proteins were detectable. Reactivity against only a single protein was rated as an indeterminate result. For detection of HCV RNA reverse transcription-PCR was performed as previously described (6, 7).

The HCV EIA was negative for 469 samples, of which 456 (97%) were also negative by UKE SIA. For 13 samples the UKE SIA was considered indeterminate. All 469 of these sera were negative by HCV PCR, and none of the patients developed clinical or biochemical signs of hepatitis during the follow-up. Moreover, these patients did not develop clinical or biochemical signs of hepatitis. This indicates that in at least these 180 samples (10%), false-positive results occurred. We must assume that the EIA was also false positive in the specimens for which no suitable material for PCR was available, since the UKE SIA remained negative and none of the patients developed clinical or biochemical signs of hepatitis during the follow-up. This indicates that as long as no better screening assays are commercially available every positive HCV EIA result must be confirmed.

An indeterminate result in the UKE SIA was observed with

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180 of the 1,814 EIA-positive samples (10%). Suitable specimens for HCV PCR were obtained for 134 of these 180 samples, and HCV RNA could be detected in 58 of them. During the follow-up serum viremia was observed in four patients. All of them initially revealed antibody directed against the NS3 protein of the UKE SIA. In follow-up samples, reactivity against additional recombinant proteins emerged. These results support the previous assumption that antibody reactivity against NS3 plays an important role in the early serological detection of HCV infection (5). Furthermore, a particularly high correlation has been found between HCV viremia and antibody reactivity against the c33c antigen of the commercially available RIBA (2). Samples with a positive result by HCV EIA and an indeterminate result by immunoblot assay must be subjected to PCR, since we detected HCV RNA in 43% of samples (58 of 134). The percentage of indeterminate results by the UKE SIA is remarkably low compared to that by RIBA 2.0 or 3.0 (2, 3, 11, 16). One reason for this might be that local isolates were used to establish the UKE SIA, since serological tests containing recombinant proteins of local isolates have been shown to have better sensitivity and specificity than commercially available assays (5, 12). However, this is unlikely to be the only reason, since the UKE SIA was evaluated with serum samples containing a variety of HCV genotypes as previously described (5).

The diagnosis HCV positive has a deep impact on the life of the afflicted person. Therefore, it must be reached as reliably as possible. Our data indicate that the widely used HCV EIA produces a high percentage (10%) of false-positive results. Compared to other screening assays, e.g., human immunodeficiency virus EIAs, this is unacceptably high. Therefore, confirmation of every positive HCV EIA result by supplemental tests is mandatory. As we have shown with our in-house UKE SIA, one possibility for improving the reliability of HCV diagnosis is to introduce proteins into the confirmation assay which are different from those used in the screening assay.

REFERENCES

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<tr>
<th>UKE SIA result</th>
<th>No. (%) of samples with HCV EIA result</th>
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<tbody>
<tr>
<td></td>
<td>Positive (n = 1,814)</td>
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<tr>
<td>Positive</td>
<td>1,394 (77)</td>
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<tr>
<td>Indeterminate</td>
<td>180 (10)</td>
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<td>Negative</td>
<td>240 (13)</td>
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