Combined Hepatitis C Virus (HCV) Antigen-Antibody Detection Assay Does Not Improve Diagnosis for Seronegative Individuals with Occult HCV Infection

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A combined hepatitis C virus (HCV) antigen-antibody assay was evaluated for 115 seronegative individuals with occult HCV infection. The assay was reactive in one patient and negative to weakly reactive in three others (all four gave indeterminate results by supplemental assay) but failed to detect HCV in the remaining patients. Despite increased sensitivity the combined assay does not improve serodiagnosis of occult HCV infection.

Occult hepatitis C virus (HCV) infection has been recently identified in patients with persistently abnormal results of unknown etiology in liver function tests (2, 3). This type of infection is evidenced only by the detection of HCV RNA in liver, because patients consistently test negative for antibodies to HCV and HCV RNA in serum. Diagnosis of occult HCV infection is possible only by liver biopsy, which is not routinely performed due to associated morbidity. Thus, the availability of an assay with increased sensitivity for HCV infection will greatly improve the diagnosis of occult HCV infection. A combined HCV antigen-antibody assay that simultaneously detects capsid antigen and specific HCV antibodies in serum has been developed (4). This combined assay has shown increased sensitivity compared with classical anti-HCV assays (1, 4). Our aim has been to assess whether the combined HCV antigen-antibody assay allows HCV serodiagnosis in patients with occult HCV infection.

Over the past 4 years 115 patients have been diagnosed with occult HCV infection according to published criteria (2): they were serum anti-HCV negative (Innotest-HCV Ab IV; Inno-genetics, Gent, Belgium) and serum HCV RNA negative (sensitivity of 50 IU/ml; specificity of 99%; Amplicor HCV version 2.0; Roche Diagnostics, Branchburg, NJ) and presented sustained abnormal results of unknown etiology in liver function tests prior to undergoing a liver biopsy which demonstrated the presence of hepatic HCV RNA. Patients were monitored, and blood samples were collected at each visit. Serum samples were tested by use of Monolisa HCV Ag-Ab Ultra (Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the supplier’s instructions; sample-to-cutoff absorbance (SCO) ratios equal to or greater than 1 were considered reactive. HCV seroreactivity was confirmed by supplemental testing for anti-HCV antibodies by immunoblot assay (DeciScan HCV Plus; Bio-Rad).

The combined HCV antigen-antibody assay was evaluated using sera from 115 seronegative individuals with occult HCV infection. The assay was reactive (SCO ≥ 1) in only one patient (0.9%). Use of a more sensitive SCO threshold of 0.5 (1, 4, 8) led to the identification of three more patients (3.5% positive overall). However, the remaining 111 patients had SCO ratios less than 0.3 and were scored negative by the combined assay. In the supplemental immunoblot assay, all four of the positive samples gave indeterminate results. Table 1 summarizes the characteristics of these four patients. In addition, as shown in Fig. 1, weak positivity continued to be detected by the combined assay in sequential samples from one reactive patient (patient no. 1 in Table 1), whereas in another patient (patient no. 3 in Table 1) HCV responses remained within the gray zone, with ratios of 0.5 to 1 during the follow-up. The explanation for these findings is not clear. The mechanisms that regulate humoral immune responses in HCV infection are not well known. Thus, HCV-specific antibody responses persist for decades...
in patients with chronic hepatitis C but gradually decrease and eventually disappear after recovery from HCV infection (10, 11). Patients with occult HCV are similar to those who have previously been considered to have recovered because they consistently test HCV RNA negative without detectable HCV-specific humoral responses (10).

The combined HCV antigen-antibody assay has allowed serodiagnosis in four patients with occult HCV infection who have repeatedly tested negative by commercial assays. However, the slight increase in sensitivity achieved using the combined assay does not improve the routine serological diagnosis of occult HCV infection. Despite the permanent lack of detectable anti-HCV antibodies using commercial enzyme-linked immunoassays, we have found that some of these samples from occult HCV-infected patients react with HCV proteins on immunoblot assays. Weak reactions can be observed in immunoblot testing in cases of negative screening reaction by enzyme-linked immunosorbent assay. Such profiles have been confirmed in follow-up samples from prison inmates (6) despite repeated nonreactive HCV results in enzyme-linked immunosorbent assays, likely reflecting a low level of specific antibodies indicating exposure to HCV (7, 9). In fact, we have observed that HCV responses remained weak in the follow-up samples of two patients with occult HCV infection (Fig. 1). Therefore, low to weakly reactive SCO ratios appear to indicate the presence of anti-HCV antibodies at very low levels, as suggested previously (9). These findings emphasize that current serodiagnostic reagents are not sufficient for occult HCV diagnosis, and thus, liver biopsy remains the “gold standard” for reliable diagnosis of occult HCV infection.

The search for a less invasive test for occult HCV infection has important clinical implications because of the risks associated with liver biopsy. Thus, it may be important to introduce more sensitive serological assays to test for HCV exposure in at-risk populations, such as HCV-seronegative individuals with liver disease, hemodialysis patients (1, 4), or blood donors (5).

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REFERENCES


### Table 1. Characteristics of patients reactive by Monolisa HCV Ag-Ab Ultra assay

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)/sex</th>
<th>ALT/AST/GGTP concn* (IU/liter)</th>
<th>HCV genotype</th>
<th>HCV-positive hepatocytes (%)</th>
<th>Liver histology</th>
<th>Monolisa HCV Ag-Ab Ultra result (SCO ratio)</th>
<th>Deciscan immunoblot result (protein reactive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62/F</td>
<td>34/38/93</td>
<td>1b</td>
<td>2</td>
<td>Chronic hepatitis</td>
<td>Positive (1.57)</td>
<td>Indeterminate (NS3)</td>
</tr>
<tr>
<td>2</td>
<td>64/F</td>
<td>85/55/17</td>
<td>1b</td>
<td>4</td>
<td>Cirrhosis</td>
<td>Negative (0.84)</td>
<td>Indeterminate (NS3)</td>
</tr>
<tr>
<td>3</td>
<td>33/M</td>
<td>106/43/35</td>
<td>1b</td>
<td>16</td>
<td>Minimal changes</td>
<td>Negative (0.62)</td>
<td>Indeterminate (NS4)</td>
</tr>
<tr>
<td>4</td>
<td>44/M</td>
<td>83/31/46</td>
<td>1b</td>
<td>10</td>
<td>Steatohepatitis</td>
<td>Negative (0.54)</td>
<td>Indeterminate (NS3)</td>
</tr>
</tbody>
</table>

* ALT/AST/GGTP, alanine aminotransferase/aspartic aminotransferase/gamma-glutamyl transpeptidase (normal values for ALT and AST, ≤43 IU/liter; normal values for GGTP, ≤45 IU/liter).