Diagnostic Value of Anti-Hepatitis C Virus (HCV) Core Immunoglobulin M in Recurrence of HCV Infection after Orthotopic Liver Transplantation†‡

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The significance of anti-hepatitis C virus (HCV) core immunoglobulin M (IgM) and its relationship with genotypes, alanine aminotransferase abnormality, and histological data were studied for 18 patients who had undergone orthotopic liver transplantation due to HCV-related end-stage disease. During follow-up, IgM response seemed to be associated with the recurrence of HCV infection but did not correlate with abnormal alanine aminotransferase levels and histological data. In addition, the results of this study indicated that the detection of HCV RNA is critical for diagnosis of reinfection in liver transplantation.

Several recently published papers have addressed the role of specific immunoglobulin M (IgM) in patients with chronic hepatitis C virus (HCV) infection (4, 9, 10, 12, 14, 16, 17, 19, 22, 23). This class of antibodies is apparently a marker of active viral replication in immunocompetent patients. IgM levels, HCV genotype, and alanine aminotransferase (ALT) levels have been correlated in the development of chronic HCV infection (12). Other authors have reported that the secretion of anti-HCV core IgM seemed to be associated with the recurrence of HCV infection after orthotopic liver transplantation (OLT) (11).

The aim of this study was to evaluate the diagnostic value of anti-HCV core IgM in HCV-positive liver transplant patients and its relationship with the genotype, the presence of abnormal ALT levels, and histological data.

Eighteen Italian HCV RNA-positive patients (mean age, 49.9 ± 10.9 years) who received OLT at the Transplantation Unit of Policlinico Umberto I in Rome between October 1993 and January 1997 were studied. The underlying diseases were HCV-related end-stage cirrhosis (n = 9) and hepatocellular carcinoma (n = 9). All patients but one were negative for hepatitis B surface antigen (HBsAg); they received organs from HCV-negative donors. The follow-up interval ranged from 1 to 39 months (mean, 14 months).

Patients received quadruple immunosuppressive therapy with cyclosporine, azathioprine, methylprednisolone, and antilymphocyte globulin. The dosages of immunosuppressants and the treatment of rejection episodes were established according to specific protocols that took the patients’ clinical conditions into consideration.

Sera were collected before transplantation, 1 week after OLT, and monthly thereafter. HCV antibody testing was performed by a third-generation recombinant immunoblot assay (Ortho Diagnostic Systems-Chiron Corporation). Anti-HCV core IgM levels were determined by a semiquantitative enzyme immunoassay (Abbott Laboratories, Wiesbaden, Germany) and expressed according to the manufacturer’s instructions as index values. Samples with an index value of >15 were considered positive (12). The significance of differences between index values at the different time points was tested by Student’s t test. P values of <0.05 were considered significant.

HCV RNA testing was performed, on all 18 series of sera, before OLT and monthly from the 1st week after surgery. HCV RNA was extracted from 100 µl of serum by an acid guanidinium thiocyanate-phenol-chloroform method described by Chomczynski and Sacchi (2). HCV RNA was reverse transcribed and amplified with primers from the 5’ noncoding region of the HCV genome (6) by nested PCR (21). As internal controls we used RNA transcripts with a 27-nucleotide deletion compared with HCV isolates. The sensitivity of our assay was 1.5 × 102 HCV RNA copies per 100 µl of serum.

Genotyping was performed by hybridization of PCR-amplified products of the 5’ untranslated region of HCV and by cleavage with a restriction enzyme to distinguish genotype 1a from 1b (20).

Biopsies were performed whenever clinically indicated and at yearly intervals. Specimens were formalin fixed and embedded in paraffin, and 5-nm sections were stained with hematoxylin and cosin, periodic acid-Schiff stain (with and without diastase digestion), and Gomori’s silver nitrate for reticulin. Hepatitis occurring after OLT was classified according to the system of Gane (5).

All the patients analyzed remained HCV IgG positive soon after the OLT and until the end of follow-up. Before the OLT (T0), all patients but 1 showed secretion of anti-HCV core IgM (mean index value, 117.82 ± 71.99); in the 1st week after OLT (T1), 11 patients were found positive (mean index value, 68.84 ± 57.36), and 6 of them were HCV RNA positive. One patient’s serum was negative for anti-HCV core IgM but showed the presence of HCV RNA. During follow-up (T2), four additional patients became HCV IgM positive, and at the same time the IgM index values of eight already positive pa-
TABLE 1. Clinical course, serology, and viremia of the patients analyzed

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Anti-HCV core IgM index value*</th>
<th>HCV RNA*</th>
<th>Genotype*</th>
<th>ALT level*</th>
<th>Histological result*</th>
<th>Follow-up (mo)</th>
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<tr>
<td>1</td>
<td>49 Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>4 RC</td>
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<td></td>
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<tr>
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<td>1 NT</td>
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<td>1 NT</td>
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<td>Pos</td>
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<td></td>
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<tr>
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<tr>
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<td>Pos</td>
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<td>9</td>
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<td>Pos</td>
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<td>1 NT</td>
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<tr>
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<tr>
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<td>Pos</td>
<td>3a 1 MiCH</td>
<td>36</td>
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</tr>
<tr>
<td>13</td>
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<td>Neg</td>
<td>4 RC</td>
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</tr>
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<td>14</td>
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<td>1b 2 AH</td>
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<tr>
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<td>Pos</td>
<td>2a/2c 1</td>
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<tr>
<td>17</td>
<td>158.1 16.9 253.3</td>
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<td>1b 5 AH</td>
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<tr>
<td>18</td>
<td>113.5 23.7 160.6</td>
<td>Neg</td>
<td>Pos</td>
<td>1b 1 MiCH</td>
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</tbody>
</table>

* Neg, negative.  Pos, positive.
* NT, not tested; ND, not determined.
* Expressed as a multiple of the normal range (<45 U/dl).
* RC, rejection crisis; MoCH, moderate chronic hepatitis; MiCH, mild chronic hepatitis; AH, acute hepatitis.
* HBSAg positive.
* Death.

The present study correlated anti-HCV core IgM with HCV RNA in OLT. Thirteen of 15 anti-HCV core IgM-positive patients had detectable HCV RNA in their sera. In agreement with a previous study (11), these findings indicate that detection of anti-HCV core IgM may be of diagnostic value in the follow-up of liver transplant recipients. In fact, during follow-up, in cases of HCV recurrence, IgM levels rose significantly (P = 0.03), despite the immunosuppression (18). These findings parallel those reported for immunocompetent patients with HCV chronic hepatitis (8, 9, 14). Therefore, it seems that a rise in the index value of anti-HCV core IgM may be considered a marker of active viral replication, even when serum ALT levels are normal. Consequently, its possible role in the differential diagnosis of recurrent HCV infection versus rejection following OLT should be considered (10). Nevertheless, the discrepancy observed in three patients between anti-HCV IgM and HCV RNA (one patient was IgM negative and HCV RNA positive, and two were IgM positive and HCV RNA negative) suggests that the detection of HCV RNA is critical for the diagnosis of reinfection after OLT.

Analysis of HCV IgM did not reveal any significant correlation with ALT levels and histological data, in agreement with a recent report (3). Nevertheless, further studies will be necessary for a better understanding of the IgM response in HCV infection.

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REFERENCES
3. Crespo, J., B. Carte, J. L. Lozano, F. Casafont, M. Rivero, F. de la Cruz, and F. Pons-Romero. 1997. Hepatitis C virus recurrence after liver transplanta-


