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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 enzyme-linked immunosorbent assay (Abbott Diagnostics). Positive results were confirmed 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 1.5×10^2 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 eosin, 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-

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TABLE 1. Clinical course, serology, and viremia of the patients analyzed

Patient no.	Anti-HCV core IgM index value[a]			HCV RNA[b]		Genotype[c]	ALT level[d]	Histological result[e]	Follow-up (mo)
	T0	T1	T2	T1	T2				
1	49	Neg	Neg	Neg	Neg		4	RC	9
2	18.7	Neg	21.9	Neg	Neg		1	NT	5
3	61.2	Neg	Neg	Neg	Neg		1	NT	1
4	50.5	16.4	21.5	Pos	Pos	1b	2	MoCH	21
5	58.3	Neg	Neg	Pos	Pos	1b	3	RC	16
6	160.6	73.9	60.3	Pos	Pos	NT	2	Cirrhosis	12[f]
7	152.9	111.5	253.3	Pos	Pos	1b	3	MiCH	21
8	62.8	19.3	132.2	Neg	Pos	1b	4	MiCH	39
9	181.3	57.1	22.8	Pos	Pos	1b	50	RC	0.5[g]
10	Neg	Neg	48.8	Neg	Pos	1a	1	NT	6
11	36.4	Neg	119.7	Neg	Pos	1b	1	NT	7
12	224.5	70	253.3	Pos	Pos	3a	1	MiCH	36
13	253.3	148.8	181.3	Neg	Neg		4	RC	1
14	60.3	Neg	66.7	Neg	Pos	1b	2	AH	3[g]
15	218.9	195	253.3	Pos	Pos	2a/2c	1	MoCH	13
16	142.7	24.6	253.3	Neg	Pos	ND	1	MoCH	36
17	158.1	16.9	253.3	Neg	Pos	1b	5	AH	15
18	113.5	23.7	160.6	Neg	Pos	1b	1	MiCH	13

[a] Neg, negative.
[b] Pos, positive.
[c] NT, not tested; ND, not determined.
[d] Expressed as a multiple of the normal range (<45 U/dl).
[e] RC, rejection crisis; MoCH, moderate chronic hepatitis; MiCH, mild chronic hepatitis; AH, acute hepatitis.
[f] HBsAg positive.
[g] Death.

tients rose significantly; all of these but one were HCV RNA positive (Table 1).

For five of the seven patients with recurrence of HCV viremia, the IgM index value rose concomitantly with the recurrence of HCV RNA, but for two patients it rose 1 month later.

Thirteen of the 15 patients who were anti-HCV core IgM positive (mean index value, 140.15 ± 92.58) at the end of follow-up (T2) were found HCV RNA positive, while 1 of the 3 anti-HCV core IgM-negative patients was HCV RNA positive. A significant difference ($P = 0.03$) was observed between IgM index values obtained 1 week after the OLT (T1) and those obtained during follow-up (T2). Overall, the study of HCV RNA after OLT (T2) revealed the recurrence of HCV infection in 14 patients (78%) (Table 1).

The HCV genotype was clearly identified for 12 of the 14 patients with recurrent infection. Two patients infected with subtype 1b developed acute liver failure and died (Table 1). Patients with abnormal ALT levels (2 to 5 times the upper limit of normal) had an anti-HCV core IgM mean index value of 123.3 ± 96.27, while patients with normal ALT levels had a mean index value of 158.7 ± 99.3. There were no significant differences between these two groups. No correlation with the histological data was observed (Table 1).

To date, the clinical and diagnostic significance of IgM response in HCV infection is still unclear (23). In fact, IgM antibodies to HCV have been found in a variable (54 to 91%) percentage of patients with chronic HCV infection (1, 8, 15). Furthermore, evidence on the detection of anti-HCV core IgM in chronic HCV infection has indicated an active immune response to persistent viral replication (9, 16). However, no significant relationship was found between anti-HCV core IgM antibodies and HCV RNA quasispecies heterogeneity (13), which has recently been suggested to be involved in viral persistence (7). Moreover, some investigators have associated the anti-HCV core IgM level with genotype 1b (12) and with the ALT level (9, 12).

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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