

Anti-hepatitis C virus core IgM antibodies correlate with hepatitis C recurrence and its severity in liver transplant patients

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Abstract

Background—The significance of immunoglobulin (Ig) M antibody to hepatitis C virus (HCV) core antigen was studied in 60 patients with HCV infection after orthotopic liver transplantation (OLT) diagnosed by polymerase chain reaction.

Methods—Patients were followed up for a mean of 28 months after transplantation. Sera collected three months before transplantation, and one and 12 months after transplantation were analysed for anti-HCV core IgM (HCV-IgM EIA 2.0 assay). After OLT protocol biopsies, procedures were performed routinely every six months. Semiquantitative histopathological assessment of allograft hepatitis was performed using Knodell's score. The results were correlated with clinical features, liver histology findings, and virological features, such as genotype and viraemic levels assessed by a branched DNA assay.

Results—One year after liver transplantation, 29/60 (48%) patients had chronic hepatitis on graft biopsy. The presence of anti-HCV core IgM one month ($p=0.004$) and 12 months ($p=0.003$) after OLT was positively correlated with recurrence of chronic hepatitis. The positive predictive value of anti-HCV core IgM detected one month after transplantation was 0.88. A significant relationship was observed between severity of graft disease and presence of anti-HCV core IgM 12 months after transplantation. The mean Knodell score was 8.9 in anti-HCV core IgM positive patients compared with 3.6 in those who were anti-HCV core IgM negative ($p=0.001$). The presence of IgM anti-HCV did not correlate with serum HCV RNA level or HCV genotype.

Conclusion—We confirm that the presence of anti-HCV core IgM after OLT is a marker of HCV induced graft damage. The recurrence and severity of HCV hepatitis in patients undergoing OLT for HCV cirrhosis is related to the presence of anti-HCV core IgM after liver transplantation. These findings have diagnostic relevance and confirm that measurement of IgM anti-HCV core may help to better monitor the treatment of HCV recurrence after transplantation.

(Gut 2000;47:698-702)

Keywords: hepatitis C; orthotopic liver transplantation; anti-HCV core IgM; immunopathogenesis

End stage liver disease due to hepatitis C virus (HCV) has emerged as a leading indication for liver transplantation.¹⁻³ Recurrent HCV infection after transplantation is almost universal but for unknown reasons, only 50% of patients develop clinically overt liver disease due to HCV, usually in the first year after transplantation.^{4,5} Early histological recurrence is associated with a higher risk of disease progression after orthotopic liver transplantation (OLT)⁶ requiring detection of patients at high risk. Furthermore, in the transplant setting, liver damage may result from several causes (viral infection, graft rejection, ischaemia, drug hepatotoxicity) and a diagnosis of recurrent hepatitis remains difficult because of the lack of suitable markers of HCV induced liver damage.

Immunoglobulin M (IgM) antibodies to viral antigens are frequently detected in chronic infections. Persistence of IgM antibodies has been shown in both chronic type B and chronic delta hepatitis infections and in several other chronic viral diseases.⁷⁻⁹ Detection of IgM antibodies to these viral antigens is, in general, correlated with ongoing viral replication, liver disease activity, and response to antiviral treatment.¹⁰⁻¹³ During infection with HCV, the humoral immune response develops against a broad spectrum of structural and non-structural antigens, and the core, with NS4 regions being the most immunogenic.¹⁴ It has been shown that the IgM response directed against HCV core antigen is detected both early in the course of HCV infection and during the chronic phase. However, in the transplant setting, the frequency and clinical significance of anti-HCV core IgM is not well defined.¹⁵

To clarify these issues, we investigated the core IgM antibody response in patients who underwent transplantation for HCV cirrhosis. To assess the correlation between detection of anti-HCV core IgM with histological recurrence (defined by the presence of chronic hepatitis on the graft biopsy one year after OLT), HCV replication, and hepatitis C sever-

Abbreviations used in this paper: IgM, immunoglobulin M; HCV, hepatitis C virus; anti-HCV core IgM, immunoglobulin M antibody to hepatitis C virus core antigen; PCR, polymerase chain reaction; ALT, alanine transaminase; OLT, orthotopic liver transplantation.

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Accepted for publication
7 March 2000

ity, we analysed retrospectively serum biochemical, virological profiles, and liver histology in a cohort of 60 patients transplanted for HCV cirrhosis.

Patients and methods

STUDY POPULATION

Between January 1992 and September 1996, 81 adult patients undergoing 83 transplantations had cirrhosis secondary to chronic HCV infection, defined as the presence of circulating HCV RNA before transplantation. The indication for OLT was either end stage cirrhosis (n=76) or cirrhosis with hepatocellular carcinoma (n=5). Patients were included if they fulfilled the following four criteria: (1) HCV RNA detection after OLT; (2) survival for more than six months after OLT; (3) liver histological monitoring after OLT; and (4) no antiviral therapy after OLT. Thus we excluded patients with vascular and biliary complications, drug induced hepatotoxicity, or hepatitis B virus graft infection.

A total of 60 patients were consecutively included in the study (39 men and 21 women) (table 1). Mean age at the time of transplantation was 56 years (range 28–67). HCV RNA level before OLT was 3.7 Meq/ml (range 2–6). All patients were followed up for a mean of 28 months (range 18–36). Only seven patients received ribavirin monotherapy over three months before OLT. Serum samples were obtained immediately, three months before OLT, and subsequently at one and 12 months, and stored at -80°C until use.

IMMUNOSUPPRESSION

The immunosuppressive regimen was conventional double therapy using cyclosporin (Novartis Pharma, Rueil-Malmaison, France) and prednisone in 45 patients. Tacrolimus (Fujisawa, Nanterre, France) and prednisone were used in 15 patients. In cases where acute rejection was diagnosed, a bolus dose of methylprednisolone was administered to a maximum of three doses. Steroid resistant rejection was treated with OKT3 (Janssen-Cilag SA, Issy les Moulineaux, France). Immunosuppression was not adjusted in liver transplant recipients for HCV or patients with histologically documented recurrent hepatitis C after liver transplantation.

SEROLOGICAL ASSAYS

IgM class antibodies to HCV core protein were detected using a commercially available enzyme immunoassay (HCV-IgM EIA; Abbot

Diagnostics Division, Wiesbaden, Delkenheim, Germany) based on a recombinant HCV core protein (amino acids 1–150) expressed in *Escherichia coli* and adsorbed onto polystyrene beads to capture anti-HCV core antibodies of the IgM class which are then detected by horseradish conjugated goat antiserum to human IgM antibodies (μ specific). Samples with a sample to cut off (S/CO) ratio greater than or equal to 1.0 were considered positive. Serum samples from each patient included in this study were tested three months before, and one and 12 months after OLT.

HCV DETECTION

Qualitative HCV RNA was determined by polymerase chain reaction (PCR) using primers from the 5' non-coding region, as described previously.¹⁶

HCV VIRAL QUANTITATION

The level of viraemia was measured before, and one and 12 months after OLT in 60 patients using a branched DNA (bDNA) assay (Quantiplex HCV 2.0; Chiron Corp, Emeryville, California, USA). The lower limit of sensitivity of this assay is 0.2 million viral equivalents per millilitre.

GENOTYPING METHODS

HCV genotyping was determined after transplantation on post-transplant samples. Serum was available for HCV genotyping after transplantation in 58 patients classified according to Simmonds.¹⁷ The following genotypes were identified: 1b (n=52), 1a (n=4), and 3a (n=2).

HISTOPATHOLOGY

Liver biopsy specimens from each patient were evaluated by the same liver transplantation pathologist. After OLT protocol biopsies, procedures were performed routinely every six months and all patients had a graft biopsy one year after liver transplantation. Additional liver biopsies were performed as clinically indicated for abnormal liver function tests. The primary end point in the study was the presence of chronic hepatitis on the graft biopsy one year after OLT. Biopsy specimens were stained with haematoxylin and eosin. A mean of nine biopsy specimens (range 3–12) were available per patient and mean time of histological follow up was 27 months (range 18–36 months). The diagnosis of recurrent hepatitis was based on the presence of portal, periportal, and lobular inflammation with lobular acidophilic bodies and/or lobular hepatocytolysis, all in the absence of endothelitis. Biopsy specimens were semiquantitatively evaluated using the histological activity index described by Knodell and colleagues.¹⁸ Knodell's score includes four items (periportal necrosis with or without bridging necrosis, intralobular degeneration and focal necrosis, portal inflammation, and fibrosis).

STATISTICAL ANALYSIS

Values are expressed as median or mean (range). A Student's *t* test was used to compare means of quantitative variables. Qualitative

Table 1 Characteristics of the study population

Age (y)	56 (28–67)
Sex (M/F)	39/21
HCC on liver explant	5/60
HCV RNA positive (PCR)	60/60
HCV RNA level before OLT (Meq/ml)	3.7 (2–6)
HCV genotype	
1b	52/60
1a	4/60
3a	2/60
4	0/60
Unclassified	2/60

HCV, hepatitis C virus; HCC, hepatocellular carcinoma; OLT, orthotopic liver transplantation.

variables were compared using the χ^2 test or Fisher's exact test. Correlations between variables were calculated using Spearman rank order correlations. *p* values less than 0.05 were considered statistically significant. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated by exact values.

Results

POST-TRANSPLANTATION OUTCOME

All 60 patients had recurrent HCV infection, as shown by detection of HCV RNA in serum by qualitative PCR. Patients underwent repeated HCV RNA testing during follow up, revealing persistent viraemia in all patients at 12 months post-transplantation. Mean HCV RNA level was 3 (0.5–4.4) Meq/ml before OLT, 11.5 (5–14.2) Meq/ml at the time of OLT, and 12.6 (4–14.9) Meq/ml 12 months after OLT.

A total of 29/60 patients (48%) developed recurrence of hepatitis on the graft biopsy at one year after OLT; only 6/29 had the same diagnosis on the six month graft biopsy. Acute lobular hepatitis developed in 12/29 patients (41%) at a mean of four months (range 2–6) after OLT and in no patient did hepatitis resolve. Acute lobular hepatitis was not present on the 12 month biopsy; 44/60 patients (73%) had chronic hepatitis after a mean follow up of 28 months. Mean histological score in patients with histological recurrence at one year was 7.3 (3–12). Mean alanine transaminase (ALT) value in patients at one year after transplantation was 104 IU/l (range 23–345) and mean bilirubin value was 45 μ mol/l (range 12–65). Mean HCV RNA level was 2.8 Meq/ml before OLT, 10.9 Meq/ml at the time of OLT, and 12 Meq/ml 12 months after OLT.

Acute rejection occurred in 20 patients (33%). Ten patients were treated with OKT3 for steroid resistant rejection. Two patients developed chronic rejection, diagnosed on liver biopsy at one year. There were no significant differences in the rejection rate in HCV infected patients with and without histological recurrence. Cytomegalovirus infection was documented in 13 patients (14%) and was treated with ganciclovir.

IgM ANTI-HCV CORE

Serological data

IgM anti-HCV core was present in 81 of 180 (45%) sera tested. In the group of patients with hepatitis C recurrence on the one year biopsy (*n*=29), anti-HCV IgM core was detected in 10/29 before OLT, in 23/29 at one month, and in 22/29 patients at 12 months of follow up after OLT. In patients without histological recurrence, anti-HCV IgM core was detected in 11/31 before OLT, in 4/31 at one month after OLT, and in 5/31 patients at 12 months after OLT. The positivity of anti-HCV IgM at one month after OLT and 12 months after transplantation correlated significantly with recurrence of hepatitis on the liver graft (*p*=0.004, *p*=0.003) with a high specificity (0.87 at 1 month and 0.83 at 12 months). The positive predictive value of IgM anti-HCV core was 0.88 at one month after OLT and 0.87 at

12 months after OLT. The positivity or negativity of anti-HCV core IgM was not related to the existence of acute lobular hepatitis.

Biochemical abnormalities

ALT levels measured one year after OLT were significantly higher for patients with positive anti-HCV core IgM than for those who were negative. Median levels were 199 IU/l versus 59 IU/l, respectively (*p*=0.002). Total bilirubin and aspartate aminotransferase levels did not differ significantly between groups.

Relationship to HCV viral factors

HCV RNA was measured before, and one and 12 months after transplantation. Mean HCV RNA level was not significantly different before, and one and 12 months after OLT based on anti-HCV core IgM status (before OLT: 4 Meq/ml in positive patients *v* 3.5 Meq/ml in negative patients; one month after OLT: 12.2 Meq/ml in positive patients *v* 11 Meq/ml; 12 months after OLT: 12.1 Meq/ml in positive patients *v* 11.2 Meq/ml) (figs 1–3).

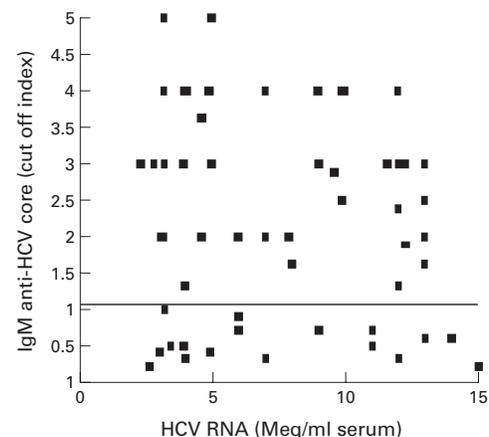


Figure 1 Absence of a relationship between serum hepatitis C virus (HCV) RNA titres and levels of immunoglobulin M antibody to hepatitis C virus core antigen (IgM anti-HCV core) in patients before orthotopic liver transplantation.

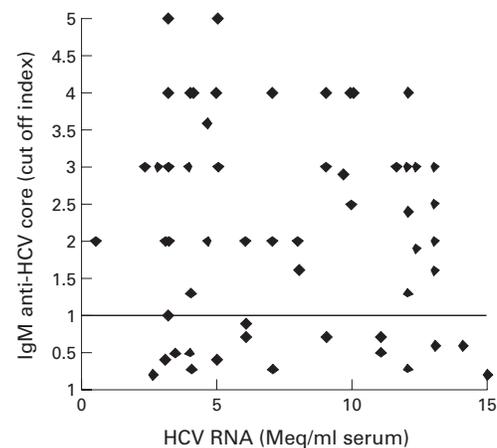


Figure 2 Absence of relationship between serum hepatitis C virus (HCV) RNA titres and levels of immunoglobulin M antibody to hepatitis C virus core antigen (IgM anti-HCV core) in patients one month after orthotopic liver transplantation.

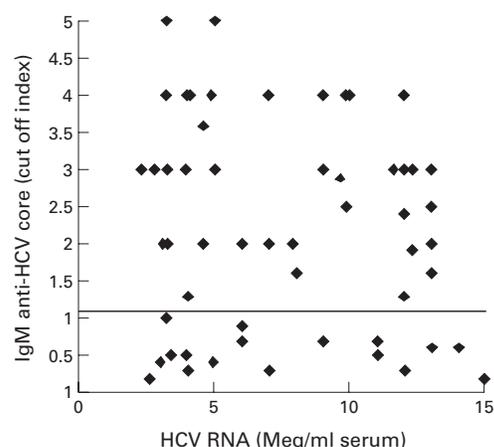


Figure 3 Absence of relationship between serum hepatitis C virus (HCV) RNA titres and levels of immunoglobulin M antibody to hepatitis C virus core antigen (IgM anti-HCV core) in patients 12 months after orthotopic liver transplantation.

The presence of anti-HCV core IgM was not related to HCV genotype determined after OLT. The genotype 1b was present in 87% of positive anti-HCV core IgM patients versus 89% in negative anti-HCV core IgM patients (NS).

VARIABLES WITH HISTOLOGICAL SEVERITY OF LIVER DISEASE

Of 60 patients tested for IgM anti-HCV after OLT, chronic hepatitis was present in 29 patients after one year. Two of 29 patients also had chronic rejection on biopsy at 12 months and were excluded from subsequent analysis. There was a significant association between severity of graft disease and presence of anti-HCV core IgM one month after liver transplantation. The mean Knodell score was 8.9 in positive anti-HCV core IgM patients versus 3.6 in negative anti-HCV core IgM patients ($p=0.001$).

Genotype and level of viraemia were also evaluated as determinants of disease severity. Genotype 1b versus other genotypes was not associated with disease severity ($p=0.67$, χ^2 test). HCV-RNA levels evaluated by bDNA before and after OLT were not significantly different in patients with and without recurrent hepatitis on the graft.

Discussion

In patients transplanted for cirrhosis caused by hepatitis C, HCV reinfection is universal, defined by detection of HCV RNA in serum or liver biopsy tissues.^{19, 20} HCV reinfection must be differentiated from recurrent HCV liver disease. This is difficult because of the lack of specific markers for HCV induced liver damage. In this study, we assessed the diagnostic and prognostic significance of detection of anti-HCV core IgM in patients with HCV related cirrhosis undergoing OLT. The presence of anti-HCV core IgM has been correlated with liver disease, viral replication, and response to treatment in non-immunocompromised patients with chronic hepatitis.¹⁰⁻¹³ However, the clinical significance of the detection of anti-

HCV core IgM in patients undergoing OLT for HCV associated liver cirrhosis has not been determined. In this setting, conflicting results have been reported with respect to the frequency of the detection of anti-HCV IgM, ranging from 20% to 90% of cases. Several explanations for these differences have been proposed, including different antigens used, geographical heterogeneity of HCV genotypes, and different technologies.

In our study, anti-HCV core IgM was present in 81 of 180 sera tested. The most interesting result of the study was that the presence of anti-HCV core IgM detected one month after OLT was significantly correlated with recurrence of HCV hepatitis in the graft. Specificity and positive predictive values were high (0.87, 0.88). In only four patients positive for anti-HCV core IgM did we fail to observe HCV recurrence on liver biopsy during follow up. However, as it was shown that the appearance of anti-HCV core IgM may precede by several months recurrence of HCV, we cannot exclude the fact that these patients may eventually develop histological signs of hepatitis during follow up. Two cases of anti-HCV core IgM positive at the time of transplantation were negative 12 months after transplantation. These two patients developed chronic rejection associated with recurrent hepatitis. The same evolution was also shown by Negro and colleagues.²¹ The persistent inability to detect anti-HCV core IgM in a proportion of HCV infected patients has been reported in other studies. This phenomenon may be accounted for by the early clearance of antibodies during the course of infection, by the synthesis of low titre antibodies, or by the absence of their synthesis. The mean number of rejection episodes was not significantly different in this group and we do not believe that the immunosuppressive therapy was responsible for the persistent disappearance of anti-HCV core IgM in these patients.

Similar data have been reported previously in a pilot study of only 25 patients.²² In our study involving 60 patients, we confirmed that measurement of anti-HCV core IgM after transplantation may be useful in the assessment of the risk of recurrent HCV hepatitis in patients who have undergone transplantation for HCV cirrhosis. Detection of anti-HCV core IgM could be used for selection of patients who should be treated prophylactically with antiviral therapy after transplantation if further studies confirm the efficacy of this strategy.

In this study we also examined the relationship between the severity of recurrent disease and positivity of anti-HCV core IgM after transplantation. We did not include the two patients with chronic rejection in the analysis. The Knodell score at the time of diagnosis of recurrent hepatitis was significantly higher in patients with anti-HCV core IgM (8.9 *v* 3.6 in those who were negative). These data have not been reported previously and must be confirmed in further studies.

Although it was suggested in previous studies that anti-HCV core IgM was an indirect indicator of HCV replication,^{23, 24} we

did not find higher levels of viraemia in patients with anti-HCV core IgM and detection of anti-HCV core IgM did not appear to correlate with HCV replication in the transplantation setting. On the other hand, we have shown that anti-HCV core IgM is a marker of recurrence of hepatitis and its severity. This discrepancy may further indicate that HCV lacks a direct cytopathic effect and suggests that hepatic lesions are mostly mediated by immune mechanisms.^{25 26} Genotype 1b was seen in more than 80% of patients in this study. Because of the predominance of genotype 1b in our transplanted population, we were unable to investigate the effect of genotype on the core IgM antibody response.

In conclusion, this study confirms that anti-HCV core IgM may be useful in the assessment and management of hepatitis recurrence in patients transplanted for HCV cirrhosis.

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