Longitudinal variation in hepatitis C virus (HCV) viraemia and early course of HCV infection after liver transplantation for HCV cirrhosis: the role of different immunosuppressive regimens

G V Papatheodoridis, S G R G Barton, D Andrew, G Clewley, S Davies, A P Dhillon, G Dusheiko, B Davidson, K Rolles, A K Burroughs

Abstract

Background—The role of the type of immunosuppression in the natural history of post-transplant hepatitis C virus (HCV) infection is unclear.

Aims—To evaluate the fluctuation of HCV viraemia and the early course of infection, and their relation to the type of immunosuppression in HCV transplant patients.

Methods—In 47 HCV transplant patients, serum HCV RNA levels were determined pretransplant and at one and two weeks, and three and 12 months after transplant. Initial immunosuppression was triple (cyclosporin, azathioprine, prednisolone) in 31, double (cyclosporin, prednisolone) in five, and single (cyclosporin or tacrolimus) in 11 patients. Prednisolone was withdrawn at a median of six months.

Results—At three months, HCV RNA levels were higher in patients with single than with triple or double initial therapy. At 12 months, HCV RNA levels correlated only with duration of prednisolone treatment and were relatively higher in patients with triple compared with single initial immunosuppression. A higher necroinflammatory activity at 12 months post-transplant was found in patients with post-transplant acute hepatitis compared with those without. Extent of fibrosis at 12 months was associated with the 12 month HCV RNA level and occurrence of post-transplant acute hepatitis.

Conclusions—HCV RNA levels at three months after transplant are higher in patients treated with single initial immunosuppressive therapy, but at 12 months are higher in patients with longer duration of steroid treatment. HCV viraemia at 12 months seems to be particularly important, as its levels are strongly correlated with the severity of fibrosis.

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Keywords: HCV RNA; HCV genotype; grading score; staging score; immunosuppression; liver transplantation

Hepatitis C virus (HCV) infection recurs almost universally (90-98%) after orthotopic liver transplantation (OLT) in patients with HCV related cirrhosis.¹⁻⁴ Serum HCV RNA levels start to increase within the first two

weeks after transplant, reaching concentrations significantly higher than pretransplant levels in one to two months and peaking at the time of acute lobular hepatitis, usually between two and six months after transplant.⁵⁻⁸ Serum HCV RNA levels slightly decline thereafter but remain very high compared with pretransplant viraemia.⁵⁻⁷ Although the relation between levels of HCV viraemia and severity of histological lesions in the graft is controversial,^{4-7 9-11} it is now evident that HCV infection has a more aggressive course in liver transplant patients (a group with extremely high serum HCV RNA levels) than in immunocompetent patients.^{3 12 13} Furthermore, higher HCV RNA levels within the first two weeks have been associated with more severe disease in the long term,⁶ suggesting that the level of viraemia early after transplant may be crucial. Recently, pretransplant serum HCV RNA levels were found to be significantly associated with overall survival.¹⁴

Although it has been suggested that the degree of immune competence, in particular at the time of initial HCV infection, may influence disease progression,¹² the effect of different immunosuppressive regimens after OLT on HCV viraemia and the natural history of HCV recurrence remains unclear.¹⁵ Steroids have been shown to increase viraemia levels in immunocompetent patients,¹⁶ and short term courses of intense immunosuppression (for example, intravenous high dose methylprednisolone for cellular rejection) have been found to induce a significant but transient increase in serum HCV RNA levels.⁵ Moreover, intravenous courses of methylprednisolone or use of OKT3 as antirejection treatment have been associated with earlier and more severe HCV recurrence after OLT.^{17 18} Conversely, no association between the serum HCV RNA levels and the immunosuppressive load has been reported by others,¹⁹ while replacement of cyclosporin with tacrolimus has not been found to influence HCV recurrence rate.²⁰

In our centre, although initial immunosuppression with triple therapy (cyclosporin, azathioprine, and prednisolone) has most frequently been used, it has always been our policy to withdraw steroids early post-OLT.²¹

Abbreviations used in this paper: CMV, cytomegalovirus; HCV, hepatitis C virus; OLT, orthotopic liver transplantation; PCR, polymerase chain reaction.

Liver Transplantation Unit, Royal Free Hospital, London, UK

G V Papatheodoridis
S G R G Barton
G Dusheiko
B Davidson
K Rolles
A K Burroughs

Department of Virology, Royal Free Hospital, London, UK
D Andrew
G Clewley

Department of Histopathology, Royal Free Hospital, London, UK

S Davies
A P Dhillon

Correspondence to:
Dr A K Burroughs, Liver Transplantation and Hepatobiliary Medicine, Royal Free Hospital, Pond Street, London NW3 2QG, UK.

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Furthermore, initial immunosuppression with double (cyclosporin and prednisolone) or even single therapy (cyclosporin or tacrolimus) has been used during study periods (K Rolles, unpublished data). Thus, the aim of this study was to determine the relation of the immunosuppressive regimen to the type of fluctuation of HCV viraemia and the clinical course of HCV recurrence during the first year after OLT.

Methods

Patients who underwent OLT between June 1990 and May 1997 for HCV cirrhosis were considered for inclusion in this study if they: were positive for serum HCV RNA before transplant; survived for at least 12 months after OLT; and had stored serum samples available for quantitation of HCV RNA collected pretransplant and at three and 12 months post-transplant. Patients positive for HBsAg were excluded. No patient received any kind of antiviral treatment against HCV during the study or the pretransplant period.

Sixty three (77%) of the 82 HCV RNA positive/HBsAg negative patients who were transplanted in our centre during the above period survived for at least 12 months after OLT. Forty seven (74.6%) of them had suitable stored serial serum samples and were included in this study. Table 1 presents their main characteristics. There was no difference in any of the patient characteristics between the 47 patients who were included and the 16 patients who were excluded from the study. Initial immunosuppression was triple (cyclosporin, azathioprine, and prednisolone) in 31, double (cyclosporin and prednisolone) in five, and single therapy (cyclosporin or tacrolimus) in 11 patients.

Triple therapy was the routine initial immunosuppressive regimen, while double or single therapy was used during study periods on the basis of certain protocols including transplant patients irrespective of aetiology of liver disease. Cyclosporin was started at a dose of 5 mg/kg twice daily and for the first year doses were adjusted to maintain a plasma level of

100–150 ng/ml by high performance liquid chromatography or a whole blood level of 200–300 ng/ml by enzyme immunoassay (Emit 2000 Cyclosporin Specific Assay, Behring Diagnostics Inc., Cupertino, California, USA). Azathioprine was given at a dose of 1 mg/kg/day, but it was discontinued because of side effects in nine of the 31 patients who had triple therapy at a median of 1 (1–3) months after OLT. Prednisolone was started at a daily dose of 1 mg/kg, gradually reduced (5 mg every week, but increased or decreased in patients with more than one episode of acute cellular rejection or in patients with bacterial infection respectively) and completely withdrawn in a median of 6 (2–12) months. Tacrolimus was started at a dose of 0.05 mg/kg twice daily and for the first year doses were adjusted to maintain a whole blood level of 6–14 ng/ml by microparticle enzyme immunoassay (IMx Tacrolimus II, Abbott Labs, Illinois, USA).

A three day intravenous course of 1 g methylprednisolone was given to patients with acute cellular rejection. A second course of methylprednisolone was given if continued rejection was shown biochemically and histologically. Three patients were treated with OKT3, an anti-CD3 murine monoclonal antibody, because of continuing cellular rejection despite two courses of methylprednisolone. Hepatocellular cancer was diagnosed pretransplant (n=9) or found at explant (n=2) in 11 patients (23.4%), while seven patients (14.9%) admitted pretransplant alcohol abuse and had histological evidence of mixed HCV and alcohol related liver disease at explant (table 1). No patient admitted alcohol abuse after transplant. There was no change in the immunosuppressive treatment in the patients with pretransplant hepatocellular cancer or alcohol abuse.

All patients were evaluated twice weekly for cytomegalovirus (CMV) infection by a blood polymerase chain reaction assay (PCR)²² for the first month after transplant or, in patients who remained in hospital for more than one month, for the hospitalisation period. Pre-emptive treatment with ganciclovir was started

Table 1 Main characteristics of 47 patients transplanted for HCV cirrhosis according to their initial immunosuppressive regimen

Patient characteristics	Total (n=47)	CYA+AZA+PRED (n=31)	CYA+PRED (n=5)	CYA/TACR* (n=11)
Sex (male/female)	40/7	28/3	4/1	8/3
Age (y), mean (SD)	51 (10)	51 (11)	53 (7)	50 (8)
HCC diagnosed pretransplant or at explant, n (%)	11 (23.4)	6 (19.4)	0	5 (45.5)
Pretransplant alcohol abuse, n (%)	7 (14.9)	3 (9.7)	1 (20.0)	3 (27.3)
HCV genotype, n (%)				
1a	8 (17.0)	5 (16.1)	1 (20.0)	2 (18.2)
1b	22 (46.9)	15 (48.4)	2 (40.0)	5 (45.4)
2	5 (10.6)	2 (6.5)	1 (20.0)	2 (18.2)
3	7 (14.9)	6 (19.3)	0	1 (9.1)
4	5 (10.6)	3 (9.7)	1 (20.0)	1 (9.1)
HLA mismatch, mean (SD) (in 29 patients)	6.6 (1.2)	6.5 (1.1)	6.5 (1.0)	6.6 (1.5)
Rejection episodes, mean (SD)	1.7 (1.0)	1.8 (1.1)	1.0 (0.7)	1.7 (1.0)
Methylprednisolone courses, mean (SD)	1.2 (0.9)	1.2 (0.8)	1.6 (1.1)	1.2 (1.2)
OKT3 for refractory rejection, n (%)	3 (6)	3 (10)	0	0
PRED duration (months), mean (SD)	4.7 (3.5)	6.5 (2.6)	4.4 (1.1)	NA
Discontinuation of AZA, n (%)	9 (19)	9 (29)	NA	NA
Ganciclovir treatment, n (%)	9 (19)	5 (16)	1 (20)	3 (27)
CMV hepatitis, n (%)	2 (4)	1 (3)	0	1 (9)
Acute hepatitis C, n (%)	23 (49)	12 (39)	4 (80)	7 (64)
Timing of acute hepatitis C† (months after transplant), median (range)	3 (0.5–11.0)	3.0 (1.0–11.0)	3.8 (1.5–7.0)	1.5 (0.5–4.5)

*3/11 patients treated with TACR monotherapy; †only in the 23 patients with acute hepatitis C.

CYA, cyclosporin; AZA, azathioprine; PRED, prednisolone; TACR, tacrolimus; HCC, hepatocellular cancer; NA, not applicable.

when two consecutive blood PCRs were found to be positive. CMV hepatitis as defined by immunohistochemical evidence of CMV in the liver tissue developed in only two patients.

Typing of donor and recipient HLA antigens was performed with a standard assay using lymphocytotoxic antibodies.²³ Total HLA mismatch score was available in 29 patients. HLA mismatch score (0–8) was defined as the sum of the number of mismatches for the four loci HLA-A, -B, -DR, -DQ. For each locus, the number of mismatches was scored as 0, 1, or 2.

SERUM HCV RNA AND HCV GENOTYPE

Serum samples were prospectively collected pretransplant and then at one week, two weeks, three months, and 12 months after transplant and stored at -20°C . Serum samples at one week were available in 35 and at two weeks in 38 of the 47 patients. All serum samples were separated and frozen within three hours after phlebotomy and not thawed and refrozen before analysis. Quantification of serum HCV RNA in these samples was performed by a second generation branched DNA assay (bDNA 2.0, Quantiplex, Chiron, Emeryville, California, USA) according to the manufacturer's instructions. The sensitivity of this assay is 0.2×10^6 Eq/ml. This assay quantifies HCV RNA of different genotypes with similar sensitivity. Serum preservation time for each sample was considered to be the interval (in days) between storage and HCV RNA quantitation date.

Serum samples negative by bDNA were also tested by a reverse transcription PCR assay (Amplicor HCV, Roche Diagnostics Systems Inc., Branchburg, New Jersey, USA) for detection of lower viraemia levels. The analytical sensitivity of the assay was 10^3 HCV RNA copies/ml.²⁴

Serum samples drawn within the first three months after transplant were tested for determination of HCV genotype by reverse transcription PCR and reverse hybridisation assay of the amplified sequence (InnoLipa HCV II, Innogenetics, Zwijnaarde, Belgium).²⁵ The nomenclature system proposed by Simmonds *et al* was used.²⁶

LIVER HISTOLOGY

All patients had protocol liver biopsies on day 7–10 after transplant. Additional biopsies were performed if indicated by abnormal liver function tests and to confirm the histological resolution of rejection. Acute cellular rejection was diagnosed and graded according to the Royal Free Hospital grading system (total score: 0–12).²⁷ A diagnosis of acute cellular rejection was made in case of a rejection score of at least 4 and antirejection treatment was given in case of a rejection score of at least 6. Histological evidence of HCV recurrence was diagnosed based on features consistent with HCV, including portal lymphoid aggregates or lobular infiltration by lymphocytes, in the absence of any other causes. Hepatitis C was considered to be acute in patients with increased alanine aminotransferase levels (more than twice the

upper normal limit) together with histological evidence of lobular inflammation, with or without acidophilic degeneration of hepatocytes and without signs of cellular rejection or any other cause of liver injury. Acute hepatitis C was classified as mild, moderate, or severe according to internationally accepted histological criteria.²⁸

Forty two patients had a protocol liver biopsy at 12 months post-transplant. All specimens were reviewed blindly and histological changes of chronic hepatitis were classified according to the classification proposed by Ishak *et al*.²⁹ The chronic hepatitis grading score (0–18), which represents the necroinflammatory activity, was the sum of piecemeal necrosis score (0–4), confluent necrosis score (0–6), focal lytic necrosis, apoptosis, and focal inflammation score (0–6), and portal inflammation score (0–4). Grading scores ≤ 3 , 4–8, and ≥ 9 were respectively considered as absent or minimal, mild, and moderate necroinflammatory activity. The chronic hepatitis staging score (0–6), which is also referred to as fibrosis score, was based on the extent of fibrosis and the development of cirrhosis.

STATISTICAL ANALYSIS

All data were analysed using the statistical package SPSS 6.0. Serum HCV RNA in samples negative by bDNA but positive by PCR was considered equal to 0.1×10^6 Eq/ml and in samples negative by both bDNA and PCR equal to 0 Eq/ml. The relations of HCV RNA levels (in logarithmically transformed values) at all time points (pretransplant and at one week, two weeks, three months, and 12 months post-transplant) and sex, age, HCV genotype (1b versus non-1b or 1 versus non-1), HLA mismatch score, type of initial immunosuppression, duration of prednisolone treatment, discontinuation of azathioprine (only in patients with triple initial therapy), number of rejection episodes, courses of methylprednisolone, OKT3 therapy, ganciclovir treatment, CMV hepatitis, and occurrence and severity of acute hepatitis C were evaluated. The relations of chronic hepatitis grading and staging scores at 12 months post-transplant and HCV RNA levels at all time points as well as all the above mentioned variables were also evaluated. Both relations with $p \leq 0.10$ and some potentially clinically important associations are given in the results.

The Mann-Whitney test was used for comparisons of quantitative variables between groups, Wilcoxon matched pairs, signed ranks test for evaluation of changes of variables within the same group, Spearman correlation for evaluation of relations between two quantitative variables, and the corrected χ^2 method or two tailed Fisher's exact test for qualitative data. All variables associated with the dependent variable at a level of significance ≤ 0.10 were evaluated using multiple regression analysis. A two tailed $p < 0.05$ was considered significant.

Results

LONGITUDINAL VARIATION IN HCV RNA LEVEL AND TYPE OF IMMUNOSUPPRESSION

The median pretransplant HCV RNA level was $0.4 (<0.2-5.5) \times 10^6$ Eq/ml, at one week post-transplant $0.5 (<0.2-11.4)$, at two weeks $1.35 (<0.2-26.0)$, at three months $7.6 (<0.2-210.0)$, and at 12 months $8.7 (0-2000.0) \times 10^6$ Eq/ml. Only one serum sample at 12 months post-transplant was found to be negative by both bDNA and PCR, while all other bDNA negative samples were positive by PCR. Mean preservation time was 1307 (40) days (range 10-2555) and was not correlated with HCV RNA levels ($r=-0.03$, $p=0.69$).

The pretransplant HCV RNA level was not related to the post-transplant HCV RNA level at any time point. Serum HCV RNA at one or two weeks post-transplant was not related to the type of initial immunosuppression (fig 1) or to any other variable.

Levels of HCV RNA at three months were significantly associated only with the type of initial immunosuppression. In particular, three month HCV RNA levels were higher in the patients with single (median, 18.0×10^6 Eq/ml) than in those with triple (median, 4.7×10^6 Eq/ml; $p=0.002$) or double initial immunosuppressive therapy (median, 4.4×10^6 Eq/ml; $p=0.054$) (table 2; fig 1). In the single therapy group, three month HCV RNA levels were relatively higher in the patients treated with cyclosporin (median, 43.5×10^6 Eq/ml; range, 8.8-210.0) than in those treated with tacrolimus (median, 10.6×10^6 Eq/ml; range, 8.7-25.0), but the difference was not statistically significant ($p=0.15$). Average doses and levels

Figure 1 Serum HCV RNA levels pretransplant and at one week (in 35 patients), two weeks (in 38 patients), and three and 12 months post-transplant in 47 patients transplanted for HCV cirrhosis in relation to the type of initial immunosuppressive therapy. Initial immunosuppressive therapy was triple (cyclosporin, azathioprine, prednisolone) in 31, double (cyclosporin and prednisolone) in five, and single (eight cyclosporin; three tacrolimus) in 11 patients. Box and whisker plots express medians, and interquartile and overall ranges. The outlying values are plotted individually. HCV RNA levels are presented in a logarithmic scale.

Table 2 Serum HCV RNA levels at three and 12 months after transplantation for HCV cirrhosis in relation to type of initial immunosuppressive therapy

Initial immunosuppression	Median (range) HCV RNA ($\times 10^6$ Eq/ml)		p Value
	3 months	12 months	
Triple (n=31)	4.7 (<0.2-98.0) ¹	10.5 (<0.2-2000.0) ⁴	0.08
Double (n=5)	4.4 (<0.2-18.0) ²	5.0 (0-61.5) ⁵	0.89
Single (n=11)	18.0 (8.7-210.0) ³	4.9 (0.5-12.8) ⁶	0.003

Triple therapy included cyclosporin, azathioprine, and prednisolone; double, cyclosporin and prednisolone; and single, cyclosporin (or tacrolimus in three patients) alone.

1 versus 3, $p=0.002$; 2 versus 3, $p=0.054$; 4 versus 5, $p=0.51$; 5 versus 6, $p=0.78$, 4 versus 6, $p=0.08$.

of cyclosporin were similar in the three groups at three and 12 months after transplant (data not shown). Tacrolimus was only used in the monotherapy group.

HCV RNA levels decreased between three and 12 months post-transplant in all patients with initial single immunosuppressive therapy (12 month median, 4.9×10^6 Eq/ml; $p=0.003$ compared with three month values). In contrast, it increased in 19 (61%) and decreased in only 11 (35%) patients with triple therapy (12 month median, 10.5×10^6 Eq/ml; $p=0.08$ compared with three month values) and did not change significantly (increased in two and decreased in three) in the patients with initial double therapy (12 month median, 5.0×10^6 Eq/ml; $p=0.89$ compared with three month values) (table 2; fig 1).

HCV RNA levels at 12 months were significantly correlated with previous duration of prednisolone treatment ($r=0.37$, $p=0.01$). They were relatively higher in patients with triple therapy compared with those with single initial immunosuppressive regimen ($p=0.058$) (table 2; fig 1). There was no significant difference in the 12 month HCV RNA levels between patients treated with cyclosporin (median, 7.4×10^6 Eq/ml) and tacrolimus (median, 2.7×10^6 Eq/ml) single therapy ($p=0.41$).

HCV genotype was not associated with serum HCV RNA levels at any time point (data not shown).

HCV RNA LEVELS AND ACUTE HEPATITIS C

Acute hepatitis C was diagnosed in 23 (49%) of the 47 patients at a median of 3 (0.5-11) months. In particular, it was diagnosed in 39% of the patients with triple, 80% of those with double, and 64% of those with single initial immunosuppressive therapy. No patient developed severe cholestatic or histologically severe acute hepatitis; seven patients had moderate and 16 mild acute hepatitis. In patients with initial triple therapy, acute hepatitis C was diagnosed in five (55%) of the nine with azathioprine discontinuation (in all cases within one to two months after azathioprine withdrawal) and in seven (32%) of the remaining patients. Acute hepatitis was diagnosed at a median of 3.0 (1.0-11.0) months in patients with triple therapy, 3.8 (1.5-7.0) months in those with double therapy, and 1.5 (0.5-4.5) months in those with single therapy as initial immunosuppression ($p=0.10$ for comparison between patients with single and patients with double or triple therapy) (table 1).

Serum HCV RNA levels at three months post-transplant were significantly higher in the 23 patients with acute hepatitis C (median, 17.8×10^6 Eq/ml; range, $<0.2-98.0$) compared with the 24 patients without (median, 5.0×10^6 Eq/ml; range, $<0.2-210.0$; $p=0.03$). In contrast, HCV RNA levels at 12 months did not differ between the two groups (fig 2). In the 23 patients with acute hepatitis C, timing of acute hepatitis was not correlated with level of viraemia at three months ($r=0.05$, $p=0.83$) or 12 months ($r=0.19$, $p=0.38$). In particular, HCV RNA levels at three months were similar in the

Figure 2 Serum HCV RNA levels pretransplant and at one week (in 35 patients), two weeks (in 38 patients), and three and 12 months post-transplant in 47 patients transplanted for HCV cirrhosis in relation to the occurrence of acute hepatitis C. Box and whisker plots express medians, and interquartile and overall ranges. The outlying values are plotted individually. HCV RNA levels are presented in a logarithmic scale.

Figure 3 Serum HCV RNA levels pretransplant and at one week (in 35 patients), two weeks (in 38 patients), and three and 12 months post-transplant in 47 patients transplanted for HCV cirrhosis in relation to chronic hepatitis grading score. Box and whisker plots express medians, and interquartile and overall ranges. The outlying values are plotted individually. HCV RNA levels are presented in a logarithmic scale.

Figure 4 Serum HCV RNA levels pretransplant and at one week (in 35 patients), two weeks (in 38 patients), and three and 12 months post-transplant in 47 patients transplanted for HCV cirrhosis in relation to fibrosis or chronic hepatitis staging score. Box and whisker plots express medians, and interquartile and overall ranges. The outlying values are plotted individually. HCV RNA levels are presented in a logarithmic scale.

Table 3 Histological findings at 12 months post-transplant in 42 patients transplanted for HCV cirrhosis in relation to previous acute hepatitis C

Histological characteristics at 12 months post-transplant	Acute hepatitis C		p Value
	Yes (n=20)	No (n=22)	
Piecemeal necrosis	1.3 (0.9)	0.8 (0.9)	0.09
Confluent necrosis	0.3 (0.9)	0.05 (0.2)	0.48
Focal lytic necrosis/apoptosis/focal inflammation	1.7 (0.7)	1.3 (0.6)	0.03
Portal inflammation	1.9 (0.9)	1.5 (0.9)	0.25
Grading score	5.1 (2.6)	3.6 (2.0)	0.038
Fibrosis (staging score)	2.4 (1.1)	1.6 (0.7)	0.026

Results are expressed as mean (SD).

11 patients with acute hepatitis before three months (median, 17.7×10^6 Eq/ml; range, <0.2 – 90.0) and in the 12 patients with acute hepatitis after three months (median, 18.0×10^6 Eq/ml; range, 1.2 – 98.0 ; $p=0.93$).

Multivariate analysis showed that three month HCV RNA levels were significantly associated only with the type of initial immunosuppressive regimen (β (SE): 0.30 (0.12), $p=0.017$) and not with occurrence of acute hepatitis, while 12 month HCV RNA levels were significantly associated only with previous duration of steroid treatment (β (SE): 0.14 (0.05), $p=0.004$) and not with the type of initial immunosuppression.

HISTOLOGICAL LESIONS AT 12 MONTHS AFTER TRANSPLANT

At 12 months post-transplant, necroinflammatory activity was absent or minimal in 16 (38%) and mild or moderate in 26 (62%) (grading score: 4 in six, 5 in 12, 6–8 in seven, and 13 in one) of the 42 patients who had a liver biopsy. Fibrosis was present (fibrosis score >0) in all but one patient, but no patient had fully established cirrhosis (fibrosis score = 6). Fibrosis score was ≤ 2 in 32 (76%) and ≥ 3 in 10 (24%) (3 in seven and 4–5 in three) patients. Chronic rejection was not diagnosed in any of the 47 patients.

Serum HCV RNA levels at any time point were not associated with necroinflammatory activity at 12 months (fig 3). No association was also found between HCV RNA levels and piecemeal necrosis, confluent necrosis, focal lytic necrosis, apoptosis, and focal inflammation, or portal inflammation score (data not shown). In contrast, 12 month HCV RNA was significantly correlated with the fibrosis score ($r=0.56$, $p<0.001$); the fibrosis score was not associated with HCV RNA at any other time point (fig 4).

High necroinflammatory activity was significantly associated with the occurrence of post-transplant acute hepatitis C. Chronic hepatitis grading score was higher in 20 patients with previous acute hepatitis C (5.1 (2.6)) than in 22 patients without (3.6 (2.0); $p=0.038$) and this was mainly due to significantly higher score for focal lytic necrosis, apoptosis, and focal inflammation and in part due to higher score for piecemeal necrosis (table 3). There was no difference in necroinflammatory activity between patients with mild (5.0 (2.1)) and patients with moderate (5.1 (3.6)) previous acute hepatitis C ($p=0.44$).

The extent of fibrosis at 12 months was also significantly associated with the occurrence of acute hepatitis C. Fibrosis was more severe in patients with previous acute hepatitis C (table 3) and a fibrosis score ≥ 3 was found in 40% (8/20) of patients with and in only 9% (2/22) of patients without previous acute hepatitis C ($p=0.03$). There was no difference in the extent of fibrosis at 12 months between patients with mild (2.4 (1.0)) and patients with moderate (2.3 (1.5)) previous acute hepatitis C ($p=0.65$). HCV genotype was not found to be associated with chronic hepatitis grading or staging score.

Multiple regression analysis showed that the fibrosis score was significantly associated with the levels of 12 month HCV RNA (β (SE): 0.52 (0.16), $p=0.002$) and relatively with previous acute hepatitis (β (SE): 0.53 (0.27), $p=0.056$).

Discussion

Our study has the largest number of patients evaluated by repeated measurement of viraemia levels at certain time points post-transplant and the advantage of a blinded evaluation of liver histology based on an objective classification system. Although our patients were not randomised to the different immunosuppressive regimens, the type of treatment was not selected on the basis of patient characteristics but it changed according to study protocols. There was no significant difference in the pre-transplant patient or viral characteristics among the three treatment groups. We included a relatively small number of patients treated with double or single therapy, but our study is the first which included patients treated immediately after transplant with only a single immunosuppressive drug.

HCV recurrence after transplantation, defined as detectable serum HCV RNA by PCR, was observed in 100% of the patients in this study. Longitudinal variation of serum HCV RNA levels was similar overall to that reported by others in liver transplant patients.⁵⁻⁶ However, we found that patients initially treated with cyclosporin or tacrolimus monotherapy had significantly higher HCV RNA levels at three months post-transplant than the patients treated with combinations of immunosuppressive drugs. The explanation for this finding is not clear. The number of intravenous courses of methylprednisolone as antirejection treatment must not have had an impact on this difference in the three month viraemia levels, as the number of rejection episodes and methylprednisolone courses were similar among the patients treated with different immunosuppressive regimens. Occurrence of acute hepatitis C may have been a confounding factor for the difference of the three month viraemia levels between patients treated with different immunosuppressive regimens. Although we tried to adjust the HCV RNA levels for occurrence and timing of acute hepatitis C, occurrence of acute hepatitis C may have been underestimated. It has been suggested that the type of initial immunosuppression and courses for rejection may be associated with the timing and severity of HCV recurrence.³⁰ Another possibility could be that liver injury during the initial phase of HCV infection may be more severe in patients under less immunosuppression (or in patients treated without steroids) and that serum HCV RNA levels could reflect increased release of HCV RNA from destroyed hepatocytes. Liver HCV RNA levels have been shown to be particularly high during the acute hepatitis phase in HCV transplant patients and to decline thereafter, suggesting that a host's response may be involved in the pathogenesis of HCV infection.³¹ Earlier and more severe acute hepatitis in patients under less immunosuppression would further support the hypoth-

esis of immunologically mediated liver injury during the acute hepatitis phase. A more severe acute hepatitis phase in HCV transplant patients with less depressed immune systems may be associated with a more favourable long term course of HCV infection.¹⁵ In immunocompetent patients, an efficient T helper lymphocyte response during the acute phase of HCV infection has been suggested as a critical determinant of the course of the disease.³²⁻³³

HCV RNA levels at 12 months post-transplant were found to be significantly associated with previous duration of prednisolone treatment. The prednisolone start daily dose (1 mg/kg or 50–75 mg) was relatively high in our study, as some centres, including ours, now start with 20 mg of prednisolone daily. We do not know whether a lower start dose of prednisolone would have had a different effect on the viraemia levels. Steroids have been shown to increase HCV viraemia levels in other studies in transplant⁵ as well as in non-transplant patients.¹⁶ However, direct stimulation of viral replication through a steroid responsive element on the viral genome such as that found in hepatitis B virus infection³⁴ seems unlikely. HCV RNA levels did not differ at one or two weeks between patients treated with or without steroids and, at three months, they were significantly higher in patients treated without steroids. The association of HCV RNA levels at 12 months, when only one patient remained on steroids, and duration of previous steroid treatment also favour the involvement of immune mediated mechanisms in the pathogenesis of HCV related liver injury. Withdrawal of steroids may be associated with an immunological attack and increased lysis of infected hepatocytes with subsequent release of a significant number of virions into the circulation.

At 12 months post-transplant, necroinflammatory activity of chronic hepatitis was absent or minimal (grading score 0–3) in 38% and mild (grading score 4–8) in 60%, but fibrosis was already present in all but one patient and it was significant (staging score ≥ 3) in 24%.

Necroinflammatory activity at 12 months was not associated with concentrations of serum HCV RNA at any time point in this study. Thus, we did not confirm the finding from a smaller study (18 patients) that serum HCV RNA levels within the first two weeks post-transplant are associated with the activity of disease at 12 months.⁶ The presence of similar viraemia levels in our patients with or without histologically active hepatitis further suggests involvement of immune mechanisms and is against a direct cytopathic effect in the pathogenesis of HCV related liver injury.

Higher necroinflammatory activity was strongly associated with an occurrence of previous post-transplant acute hepatitis C. A relation of previous acute lobular hepatitis and severity of HCV infection has also been described by others,⁹ although different definitions of disease severity have been used. Although the type of initial immunosuppression was not associated with disease activity at 12 months after transplant, its effect on disease

activity later on cannot be evaluated in the present study. Recently it has been shown that the number of methylprednisolone courses as antirejection treatment within the first year post-transplant was significantly associated with development of chronic active hepatitis at two years despite no association at one year post-transplant.¹⁸

We found a strong correlation between fibrosis and HCV RNA levels at 12 months post-transplant. Gane *et al* have also reported an association between the viraemia level and histological severity of HCV recurrence, although they used a different classification of histological lesions and did not score for fibrosis; there was also a variable follow up ranging from six to 69 months.⁵ Data from immunocompetent patients suggest that progression of fibrosis is the most important predictor of the outcome of chronic hepatitis C.¹⁴ If this is also the case in the post-transplant setting, then the association between the viraemia level and the extent of early fibrosis may be extremely important and higher 12 month HCV RNA levels may be associated with worse long term outcome of post-transplant HCV infection. Furthermore, the extent of fibrosis at 12 months post-transplant was also associated with the occurrence of previous acute hepatitis. More severe hepatocyte injury during the acute hepatitis phase in the patients with, compared with those without a diagnosis of post-transplant acute hepatitis C, might induce fibrogenesis. In the patients with a diagnosis of acute hepatitis C, however, we did not find an association between the severity of acute hepatitis and the extent of fibrosis at 12 months. It was recently reported that a more severe initial HCV recurrence may be associated with subsequent development of cirrhosis.^{35, 36} Although the results of the latter studies seem to be in contrast with some of our data, different definitions of initial HCV recurrence and longer follow up times may be responsible for the seemingly discrepant findings.

The role of HCV genotype in the course of post-transplant HCV infection has been controversial. Genotype 1b or 1 has been associated with worse disease progression in some studies,^{3, 7, 37, 38} but no association between HCV genotype and course of HCV infection has been reported by others.³⁹ Similarly, we did not find any association between HCV genotype and activity or stage of disease at 12 months post-transplant. However, a relation between HCV genotype and histological severity later on cannot be excluded. HCV genotype was also not found to be related to serum HCV RNA levels.

In conclusion, we found that HCV RNA levels at three months after transplant are higher in patients treated with single initial immunosuppressive therapy but HCV RNA levels at 12 months are higher in patients on steroids for longer. There was also a trend for an association of higher HCV RNA levels at 12 months with triple compared with single initial immunosuppressive therapy ($p=0.058$). HCV viraemia at 12 months seems to be particularly important, as its levels are strongly correlated

with the extent of fibrosis. If our findings are confirmed by other studies and particularly if an association between the type of immunosuppressive therapy and the long term histological outcome is established, then initial immunosuppression in patients transplanted for HCV cirrhosis may be modified towards less intense regimens. Furthermore, if an association between the occurrence of acute hepatitis C and the histological outcome of post-transplant HCV infection is confirmed, then early antiviral treatment at the time of acute hepatitis may be beneficial. Studies with longer follow up are certainly needed to evaluate whether different types of initial immunosuppressive regimens have a different effect on the histological outcome of post-transplant HCV infection.

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