LIVER DISEASE

Histology predicts cirrhotic evolution of post transplant hepatitis C

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Background: Hepatitis C recurring after orthotopic liver transplantation varies in severity and some patients rapidly develop fully established liver cirrhosis. Neither clinical nor biological markers of such rapid cirrhotic evolution are available.

Aim: To assess the value of histology in identifying patients who will develop cirrhosis shortly after liver transplantation.

Patients and methods: Only cases of recurrent hepatitis C diagnosed by both hepatitis C virus-RNA positive serum and liver changes consistent with hepatitis, with no other causes of allograft injury, were considered. A total of 128 liver biopsies were scored from 29 consecutive patients with a mean follow up of 48 (13.97) months. The histological activity index was evaluated according to Ishak *et al*. The time of the first histological diagnosis of recurrent hepatitis C in the absence of rejection was defined as time of histological recurrence (RHC-T).

Results: First histological diagnosis of recurrent hepatitis with no features of rejection was obtained at the six month biopsy in 23 of 29 cases. By the end of follow up, nine patients had developed cirrhosis (mean follow up 38 (14.39) months (range 18–60)). The remainder (mean follow up 46 (13.40) months (24–72)) showed a spectrum of fibrosis but no cirrhosis. Severe necroinflammatory lesions at RHC-T significantly correlated with rapid development of cirrhosis. At the RHC-T biopsy, only cases evolving into cirrhosis showed confluent necrosis. The median value of the histological activity index was 11 (mean 11.11 (1.76) (range 9–14)) in patients who developed cirrhosis and four (mean 4 (1.78) (range 1–8)) in the others (p<0.0001). A histological activity index $\geqslant 9$ was associated with rapid development of cirrhosis in 100% of cases.

Conclusions: After liver transplantation, the histological activity of recurrent hepatitis C predicts the risk of development of cirrhosis. By adopting Ishak's scoring system, a histological activity index $\geqslant 9$ was 100% sensitive/specific in identifying subjects who rapidly developed cirrhosis.

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In Western countries, cirrhosis related to hepatitis C virus (HCV) infection is a major indication for orthotopic liver transplantation (OLT). HCV infection (established by molecular techniques) recurs in virtually all patients and up to 80% of recipients show histological evidence of hepatitis.[1]

Post-OLT recurrent hepatitis C (RHC) is not as benign as earlier studies suggested and recent data indicate that the five year actuarial rate of cirrhosis ranges from 10% to 30%.[2–4] HCV genotype, viral titre, rejection, and type of immunosuppressive treatment have been related to the outcome of post-OLT RHC.[5–9] Reliable identification of features associated with rapid cirrhotic evolution has practical implications in selecting who to treat and would also enable patients to be submitted for retransplantation, before the onset of severe complications.[10]

In both transplanted and non-transplanted subjects, liver histology of chronic hepatitis C features a wide spectrum of lesions, ranging from minimal changes to severe necroinflammatory activity and fibrosis. In non-transplanted patients, both the grade of the histological activity and the stage of fibrosis at presentation correlate with clinical outcome.[11] In post-OLT recurrent disease, it has been suggested that necroinflammatory liver lesions are more severe in cases that evolve into cirrhosis.[12 13]

The aim of this study was to assess the sensitivity and specificity of liver histology in identifying subjects who rapidly develop post-OLT HCV related cirrhosis.

PATIENTS AND METHODS

Patients

Fifty five patients who underwent OLT between 1991 and 1996 for end stage HCV related liver disease were considered eligible for the study. Patients were included if they had RHC defined as post-OLT HCV infection (documented by HCV-RNA detection in sera using polymerase chain reaction based methods) and liver histology consistent with hepatitis. Other inclusion criteria included: (1) no other viral infections (hepatitis B virus, cytomegalovirus, Epstein-Barr virus) or causes of graft disease; (2) no alcohol intake after OLT; (3) no histological diagnosis of fibrosing cholestatic hepatitis (whose cirrhotic potential is well known) or chronic rejection; and (4) a post-OLT histological follow up lasting not less than 12 months. Patients with known or incidental hepatocellular carcinoma were also excluded. Twenty nine patients satisfied all of the inclusion criteria. There were 23 males and six females with a mean age of 49.9 years (range 25–60) and a follow up ranging from 18 to 72 months (mean 48 (13.97)). None of the patients was treated with antiviral therapy before or after OLT. HCV genotype 1 was prevalent (1a, 10 cases; 1b, 14 cases; other, five cases). Age, sex, HCV genotype, and immunosuppressive therapy did not differ between the excluded patients and the study group.

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Table 1 Necroinflammatory lesions at the time of histological recurrence in patients who did (group A) or did not (group B) develop cirrhosis

	Necroinflammatory lesions at RHC-T biopsy							
	Interface hepatitis**		Confluent necrosis***		Focal necrosis*		Portal inflammation**	
	Group A (n (%))	Group B (n (%))	Group A (n (%))	Group B (n (%))	Group A (n (%))	Group B (n (%))	Group A (n (%))	Group B (n (%))
Score								
0	0	5 (25)	2 (22)	0	0	0	0	3 (15)
1	1 (11)	10 (50)	0	0	0	8 (40)	1 (11)	11 (55)
2	1 (11)	4 (20)	2 (22)	0	4 (44)	10 (50)	1 (11)	5 (25)
3	4 (44)	1 (5)	3 (33)	0	3 (33)	2 (10)	4 (44)	0
4	3 (33)	0	2 (22)	0	2 (22)	0	3 (33)	0

RHC-T, time of first histological diagnosis of recurrent hepatitis C in the absence of rejection (see text). *p<0.01, **p<0.001; ***p<0.0001.

Immunosuppression

Cyclosporin and steroids formed the routine initial immunosuppressive regimen. Azathioprine was used instead of cyclosporin in renal failure. Histologically confirmed acute rejection (moderate or severe) was treated with 1 g of prednisolone for three days and the cycle was repeated in non-responders. Patients failing to respond to two cycles of steroids were treated with OKT3 until 1996 (5 mg/kg/day for 10 days) and changed to tacrolimus when no response was obtained.

Virological study

Anti-HCV antibodies were sought using a commercial second generation enzyme linked immunosorbent assay (ELISA 2.0, Ortho Diagnostic System, Raritan New Jersey, USA).

Qualitative HCV-RNA was evaluated by a nested reverse transcription-polymerase chain reaction using primers from the 5' untranslated region of the HCV genome.[14] HCV genotype was assessed by a first generation line probe assay (Inno-Lipa; Innogenetics, Zwijndrecht, Belgium) according to the manufacturer's instructions.

Histological study

A total of 128 liver biopsies (median 4 (range 3–6)) were analysed, after excluding post perfusion biopsies, specimens showing acute rejection of any grade, and inadequate biopsy samples (that is, with less than 10 portal tracts). Protocol biopsies at six and 12 months were available for all patients. Protocol biopsies at 24 and 36 months were available for 24 and 15 patients, respectively. The other biopsies were obtained when clinically indicated by unexplained raised liver test findings.

Liver specimens were formalin fixed, paraffin embedded, and routinely stained with haematoxylin-eosin, periodic acid-Schiff after diastase digestion, van Gieson for collagen and Perls' for iron. All biopsies were simultaneously reviewed by two pathologists in a blinded fashion, obtaining a consensus diagnosis and histological score.

The histological diagnosis of rejection and recurrent hepatitis was based on accepted criteria.[15] The histological activity index (HAI) and fibrosis were evaluated according to Ishak and colleagues.[16] Briefly, HAI represented the sum of the scores attributed to the necroinflammatory lesions—that is, interface hepatitis (0–4), confluent necrosis (0–6), focal necrosis (0–4), and portal hepatitis (0–4). The score for fibrosis ranged from 0 to 6 (from absent to cirrhosis). In addition, fibrosis was also evaluated by the METAVIR score (from 0=absent to 4=cirrhosis).[17][18] The fibrosis progression rate was defined as the ratio of the fibrosis score to time since transplantation.[19]

The time of first histological diagnosis of RHC—with no signs of rejection—was defined as RHC time (RHC-T)

Statistical analysis

The statistical study was performed using the χ^2 test for categorical data and the Mann-Whitney test for parametric variables. The number of patients included in the study did not allow for multivariate analysis.

RESULTS

By the end of the study, nine patients (five males and four females; mean age 51 years) developed histologically confirmed cirrhosis (group A). In the remaining 20 cases (group B: 18 males and two females; mean age 49 years), various degrees of fibrosis were observed in the last follow up biopsy, with no signs of cirrhosis. There was no significant difference in follow up time between patients in group A (mean 38 (14.39) months (range 18–60)) and those in group B (mean 46 (3.40) months (range 24–72)).

In 25/29 (86%) patients, the first histological diagnosis of RHC (that is, RHC-T) was made at the six month protocol biopsy. In the remaining four cases, RHC-T was histologically documented at the fourth (one case), eight (two cases), and 12th (one case) month.

At RHC-T, both alanine aminotransferase and aspartate aminotransferase levels were higher in cases that evolved into cirrhosis but the difference between the two groups was only significant for aspartate aminotransferase (102.11 (76.07) (range 32–246) v 45.5 (21.01) (range 11–95); p<0.02).

With univariate statistical analysis, the patient's age, sex, and HCV genotype were found to be equivalent in groups A and B.

Rejection

Within four months of OLT, 10 patients experienced one and three patients had two episodes of moderate acute cellular rejection. OKT3 therapy was administered in four cases. Acute rejection (number of episodes and severity) and type of treatment did not correlate with development of cirrhosis. In particular, of the four patients who received OKT3, two developed cirrhosis and two did not.

Necroinflammatory activity

The prevalence of necroinflammatory lesions at RHC-T is shown in table 1. All lesions were significantly more severe in patients who developed cirrhosis. In particular, confluent necrosis was found in 78% of cases in group A but in none of those in group B. The odds ratio associated with early onset of cirrhosis was: 123 (95% confidence interval (CI) 5.3–2869) for confluent necrosis; 39 (95% CI 95% 4.4–349) for both moderate or severe portal inflammation and interface hepatitis; and 11.1 (95% CI 1.8–67.63) for moderate or severe focal necrosis.

The median value of HAI was 11 (mean 11.11 (1.76) (range 9–14)) in the group who developed cirrhosis and 4 (mean 4 (1.78) (range 1–8)) in the other group. The difference between the two groups was highly significant (p<0.0001). As the lowest HAI in group A was 9 and the highest in group B was 8, HAI ≥9 had 100% sensitivity and specificity in predicting the development of cirrhosis.

Table 2 Fibrosis at the time of initial histological diagnosis of recurrent hepatitis C (RHC-T) and at the last biopsy in 20 patients who did not develop cirrhosis at the end of follow up (Ishak's score)

	Fibrosis score	
Patient No	RHC-T biopsy	Last biopsy
1	0	1
2	0	1
3	0	1
4	0	1
5	0	2
6	0	2
7	0	3
8	0	3
9	1	1
10	1	1
11	1	1
12	1	1
13	1	4
14	1	3
15	2	1
16	2	1
17	2	2
18	2	2
19	2	2
20	3	3

In follow up biopsies, all patients in group A maintained a HAI higher than 9 while in group B, HAI remained lower than 9 in all but one case (in which a value of 11 was found in the biopsy obtained 18 months after histological recurrence).

Fibrosis

At RHC-T, fibrosis was observed in 21/29 patients (72.4%). It was grade 1 or 2 in 15 cases (fibrosis of some or all portal tracts, with or without short fibrous septa) and grade 3 in six (occasional portal-portal fibrous septa). More patients scored 3 in group A than in group B (p<0.01).

Acute rejection (number of episodes and severity) and type of treatment did not correlate with stage of fibrosis observed at the time of histological recurrence.

In the group of patients who did not develop cirrhosis, the fibrosis score in subsequent biopsies was unchanged in eight cases while it increased in 10 and decreased in two patients (table 2). Among patients showing an increased score, one progressed to 4 (that is, presence of portal-portal and portal-central fibrous septa). It is worth noting that this was the same patient whose HAI increased to 11.

According to the method proposed by Poynard and colleagues,[19] the median rate of fibrosis progression per year was 1.5 in group A and 0.23 in group B (p<0.003). At this rate of progression, the time expected from OLT to cirrhosis for group B patients is 17.3 years (4 METAVIR units divided by 0.23).

DISCUSSION

In this study we only considered patients with HCV recurrent infection (assessed by polymerase chain reaction detection of HCV-RNA in serum) and liver histology consistent with viral hepatitis, in the absence of any adjunctive cause of graft damage. The findings of the present study confirm that RHC fibrosis progresses at an accelerated rate and cirrhosis develops rapidly in a subgroup of patients with post-OLT hepatitis C.[2 3 20] The main result of the study was that histological activity (expressed by a numerical index) may allow early identification of RHC which rapidly develops into cirrhosis. By adopting Ishak's score system, HAI ≥9 at the time of the first histological demonstration of RHC was associated with 100%

sensitivity and 100% specificity in predicting rapid cirrhotic evolution. In dealing with numerical scoring systems, the most important problem is inter-observer reproducibility. Reasonable intra- and interobserver consistency has previously been demonstrated for Ishak's scoring system.[21] In the present study, liver biopsies were reviewed simultaneously by two pathologists, which has been proved as a good method of reducing interobserver variability.[17]

The observation that necroinflammatory lesions were more severe in the liver biopsy of patients who developed cirrhosis is consistent with previous reports.[12 13] Confluent necrosis (which is rare in chronic hepatitis C outside the transplant setting) was evident in the majority of cases who rapidly developed into cirrhosis whereas it was consistently absent in patients who did not develop cirrhosis. It is worth noting that the HAI was always higher than 9 in all follow up biopsies obtained from patients who developed cirrhosis while it was consistently lower in all but one patient who had no cirrhosis at the end of the study. In this one exception, a significant progression of fibrosis was also recorded: it suggests that shifting from low to high grade necroinflammatory lesions—whenever occurring—is associated with an increasing risk of fibrosis.

Fibrosis was also more severe at RHC-T in patients who developed cirrhosis but the overlap between the two groups makes this parameter less reliable than HAI in predicting histology outcome.

It remains unclear (and is beyond the scope of this study) why some patients develop an aggressive necroinflammatory disease which rapidly progresses to cirrhosis. In our patients, necroinflammatory activity showed no significant relationship with either acute rejection (number of episodes and severity) or immunosuppressive treatment. No final conclusions can be drawn however because of the limited number of cases involved.

Whatever the viral and/or host factors responsible, the present data trace a "natural" clinicopathological history of post-OLT RHC, described by two divergent pathways: a "malignant" outcome characterised by high grade necroinflammatory activity followed by rapid cirrhotic evolution, and a "benign" course in which mild activity and slow fibrosis progression result in a delayed risk of cirrhosis. According to Poynard's model,[19] however, in our "benign RHC", the expected time from OLT to cirrhosis would be considerably shorter (nearly 10 years) than that calculated for non-transplanted HCV carriers.[19] This observation would support the fact that fibrogenetic mechanisms work more efficiently in the transplant than in the non-transplant setting. While Poynard's model presupposes a linear progression of fibrosis, in dealing with RHC (and we would say also in untransplanted patients) the variable host/virus interaction may notably affect the outcome of disease. In fact, one patient shifted from low to high grade liver disease activity and this was associated with progression of fibrosis. This finding suggests that liver biopsies should be performed regularly during follow up of transplanted patients with RHC to identify any change in the behaviour of the disease.

In conclusion, our study demonstrated that monitoring the histological grade of RHC may provide clinically useful information in identifying subjects who rapidly develop HCV associated post transplant cirrhosis.

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