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ORIGINAL ARTICLE

Clinical outcome of HCV-related graft cirrhosis and prognostic value of hepatic venous pressure gradient

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Summary

Hepatitis C virus (HCV) allograft cirrhosis may progress rapidly requiring re-transplantation but its course is little studied. We evaluated serially biopsied patients who developed HCV-related allograft cirrhosis. We assessed outcome of graft cirrhosis in 55 out of 234 consecutive patients and predictors of decompensation and mortality, including hepatic venous pressure gradient (HVPG) in 38. Allograft cirrhosis (Ishak stage 6, 60%; stage 5, 40%) was diagnosed between 12 and 172 months (median, 52) from transplantation; subsequent follow up was 22 (1–78) months. Faster development (≤48 months) was associated with tacrolimus and nonuse of azathioprine and prednisolone. Decompensation occurred in 22% with a probability of not developing decompensation reaching 60% at 5 years. Survival among compensated patients was 77% at 5 years, but fell rapidly after decompensation (12% at 1 year). Decompensation and mortality were independently associated with HVPG ≥ 10 mmHg, Child-Pugh score ≥ 7, and albumin levels ≤ 32 g/dl but not with fibrosis stage 5 or 6, HCV genotype (1b, 34%) or immunosuppression used after diagnosis of cirrhosis. In conclusion, Ishak stage 5 and 6 HCV-related cirrhosis have similar prognosis after liver transplantation. An HVPG ≥ 10 mmHg, in addition to liver dysfunction, gives independent prognostic information prior to decompensation, allowing early relisting before prognosis becomes extremely poor.

Introduction

Hepatitis C virus (HCV) cirrhosis is the leading cause for liver transplantation (LT) in USA and Europe [1]; HCV reinfection occurs almost universally [2,3] in those following a more rapid and aggressive course [1,4] than in immunocompetent patients [5–7], with allograft cirrhosis developing in up to 30% after 5 years [4,8]. Type of immunosuppression [9–11], viral genotype [2,12–14], older donors [15], and more recent transplantation [4] have been associated with a worse outcome.

Numerous studies have evaluated predictors of severity of allograft HCV infection [16], but only two assessed either outcome [17] or mortality predictors [18] once cirrhosis had developed.

Hepatic venous pressure gradient (HVPG), correlates with survival [19,20]; ascites and esophageal varices develop once HVPG reaches 10 mmHg [20–23], defined as clinically significant portal hypertension (CSPH) [24,25]. According to a recent study, HCV liver transplant recipients, with an HVPG ≥ 6 mmHg 1 year after LT, were at high risk of developing clinical decompensation [26], suggesting that HVPG could be used to evaluate progression of precirrhotic disease, as in immunocompetent HCV patients [27]. In cirrhotic patients some with HCV-related cirrhosis, initially without varices, an HVPG > 10 mmHg predicted decompensation [20].

Our aims were (i) to evaluate clinical outcome of HCV-related allograft cirrhosis after LT for HCV cirrhosis

diagnosed histologically during protocol follow-up biopsies, together with the impact of CSPH, immunosuppression, and viral genotype and (ii) to assess clinical and laboratory variables in predicting prognosis after developing allograft cirrhosis.

Materials and methods

Patients

Between April 1989 and January 2008, 234 consecutive patients with end-stage HCV-related cirrhosis underwent 247 cadaveric liver transplants at our center. Before LT, all patients had a positive anti-HCV antibody by recombinant immunoblot assay, and later, by positive HCV-RNA. Most patients consented to yearly protocol biopsies. Additional biopsies were performed when clinically indicated. Allograft cirrhosis was diagnosed histologically using the Ishak's *et al.* [28] classification, with concurrent positive HCV-RNA, and absence of biliary or vascular complications. In all cases, allograft cirrhosis was diagnosed on protocol biopsies. Both percutaneous and transjugular biopsy techniques were used, which have significant diagnostic agreement [26]; quality of specimens is similar in our center [29].

Among 58 patients with allograft cirrhosis, two were not evaluated because of hepatitis B virus co-infection, and another one because of ascites developing 20 days before diagnostic liver biopsy (33 months after LT – died with hepatorenal syndrome 2 months later). Therefore, 55 patients with clinically compensated allograft cirrhosis, 23 with fibrosis stage 5 and 32 with fibrosis stage 6 were evaluated. We compared stage 6 with stage 5, as the latter stage represents early cirrhosis. We also evaluated 16 patients who developed stage 4 and remained so till last follow up, to establish if there was a consistently different outcome from stage 5 or 6. These patients had not received any antiviral therapy prior to reaching stage 4.

Follow up

Clinical and laboratory evaluation was performed every 3 months. Patients with hepatocellular carcinoma or with incidental hepatocellular carcinoma who had undergone LT, had ultrasound performed every 6 months, and computed tomography of chest and abdomen every 12 months. Follow up stopped at death, re-transplantation, or end of the observation period (January 2008). Endpoints were clinical decompensation and death.

Clinical decompensation was defined as the first occurrence of ascites, hydrothorax, variceal bleeding, spontaneous bacterial peritonitis, or encephalopathy. The cause of death was categorized as related or unrelated to decompensation.

Hemodynamic studies

Hepatic venous pressure gradient has been routinely measured in HCV transplanted patients in the last 6 years. HVPG was measured in the interventional radiology suite using standard techniques [30], and always associated with transjugular biopsy. Thirty-eight patients had HVPG measurements at the time of diagnosis of allograft cirrhosis.

Immunosuppressive regimens

Early in our program a cyclosporine-based regimen (10 mg/kg/day in two divided doses) was used in 23 patients; the rest received tacrolimus-based regimens (0.1 mg/kg/day in two divided doses). Cyclosporine or tacrolimus were used as monotherapy in 2 and 19, respectively, or combined with azathioprine (≤1 mg/kg) and prednisolone (20 mg/day reducing over 3 months and stopping between 3 and 6 months) in 23 and 11 patients, respectively. Tacrolimus as part of triple therapy [31,32] or monotherapy [32] was used in randomized trials. Immunosuppressive doses were adjusted as described previously [11]. No patient received azathioprine or prednisolone at diagnosis of allograft cirrhosis or thereafter.

Virologic tests

Anti-HCV (second or third generation enzyme immunoassay), HCV RNA (reverse transcription polymerase chain reaction assay), and HCV genotype (reverse transcription polymerase chain reaction assay and reverse hybridization assay of the amplified sequence) were evaluated as previously described [11].

Histologic assessment

The liver biopsy samples were processed as described elsewhere [11] and reviewed blindly by two pathologists (APD, FG), having standardized their intra- and inter-observer reporting; the final score was consensual using the Ishak scoring system [28].

Statistical analysis

Results are expressed as median and ranges. Categorical variables were compared using the chi-squared or Fisher's exact tests. Continuous variables were compared by Student's *t*-test, or if not normally distributed, (determined by the Kolmogorov–Smirnov test), by the Mann–Whitney test. Continuous variables, if significant univariately, were used in a multivariate logistic regression analysis (forward stepwise method) to find independent variables associated

with development of decompensation or death. Categorical variables significantly associated with time from LT to graft cirrhosis univariately were also used in a multivariate linear-regression analysis. The cumulative probabilities for decompensation and survival were calculated using the Kaplan–Meier method and differences assessed by the log-rank test. Survival rates were evaluated in the whole population (total survival), among patients who remained compensated, after decompensation, and in a subgroup after excluding deaths not related to decompensation (liver-related survival). A P-value < 0.05 was considered significant.

Results

Clinical and laboratory features

Indications for LT were end-stage disease in 42 and hepatocellular carcinoma in 13. Alcohol abuse contributed to pretransplant liver injury in 11. Median age was 51 (28–65) years when transplanted and 55 (31–74) years at diagnosis of graft cirrhosis. There were 44 males and 11 females; 35 were caucasian. Allograft cirrhosis was diagnosed at a median of 52 (12–172) months after LT with 22 (1–78) months of follow up thereafter. Median donor age was 41 (16–65) years; 77% were male. Seven patients received antiviral treatment with interferon and ribavirin after LT before allograft cirrhosis was diagnosed but none responded. Patients had been treated at stage 4 and had progressed to stage 5 ($n = 6$) and stage 6 ($n = 1$). Hemodynamic, laboratory, and treatment characteristics are shown in Table 1. Median and range of HVPG in patients who had received antiviral therapy was 7 (4–14) mmHg pretreatment and 9 (6–18) mmHg post-treatment. Patients with stage 5 or 6 had similar characteristics, except for the international normalized ratio (INR) [1 (0.9–1.6) vs. 1.2 (0.9–1.6), respectively; $P = 0.01$]. The HVPG was measured at first diagnosis of stage 5 in 18 patients and stage 6 in 20 patients with similar median

Table 1. Hemodynamic, laboratory, and treatment characteristics of liver transplant recipients at histologic diagnosis of HCV-related allograft cirrhosis ($n = 55$).

	Total	HVPG measured	HVPG not measured
HVPG (mmHg)	$N = 55$ 8 (4–18)	$n = 38$ 8 (4–18)	$n = 17$
HCV genotypes (%)			
1b	34	36	36
1a	26	30	26
2	10	10	10
3	18	16	18
4	10	8	10
5	2	0	2
Pretransplantation viral load ($\log_{10}$ IU/ml)	1.6 (1–8.8)	1.9 (1.2–8.8)	1.4 (1.0–6.6)
Viral load at diagnosis of cirrhosis ($\log_{10}$ IU/ml)	1 .8 (0.5–5.0)	1.8 (1.0–5.0)	1.6 (0.5–4.0)
Child-Pugh score A/B (%)	79/21	79/21	79/21
Child-Pugh score	5 (5–8)	5 (5–8)	5 (5–7)
Alanine aminotransferase (U/l)	75.5 (14–250)	80 (22–250)	72 (14–220)
Aspartate aminotransferase (U/l)	91 (22–450)	92 (30–450)	90 (22–450)
Bilirubin (mg/dl)	0.8 (0.3–3.7)	1 (0.6–3.7)	0.7 (0.3–3.0)
Albumin (g/dl)	3.8 (2.2–5.2)	4.0 (2.2–5.2)	3.7 (2.2–4.8)
INR	1.1 (0.9–1.6)	1.0 (0.9–1.6)	1.1 (0.9–1.6)
Ishak grade	6 (3–9)	6 (3–9)	6 (3–9)
Piecemeal necrosis	2 (1–3)	2 (1–3)	2 (1–3)
Confluent necrosis	0 (0–1)	0 (0–1)	0 (0–1)
Focal necrosis	2 (0–3)	2 (0–3)	2 (0–3)
Portal inflammation	2 (1–4)	2 (1–4)	2 (1–3)
Initial cyclosporine-based immunosuppression after LT (%)	45	50	40
Cyclosporine at diagnosis of cirrhosis (%)	35	35	33
Cyclosporine dose at diagnosis of cirrhosis (mg/day)	100 (75–200)	100 (75–200)	100 (75–200)
Initial tacrolimus-based immunosuppression after LT (%)	55	58	51
Tacrolimus at diagnosis of cirrhosis (%)	52	54	48
Tacrolimus dose at diagnosis of cirrhosis (mg/day)	2 (0.5–5)	3 (1.0–5.5)	1.5 (0.5–4)

HCV, hepatitis C virus; HVPG, hepatic venous portal gradient; INR, international normalized ratio; LT, liver transplantation.
Results are expressed as medians (range) or % of patients.

values [8 (4–18) vs. 8 (4–15) mmHg, respectively], and proportions of HVPG ≥ 10 mmHg (36% vs. 33%, respectively). Baseline characteristics at the first diagnosis of cirrhosis histologically were similar in those in whom HVPG was measured (later in the consecutive cohort) when compared with those in whom HVPG was not measured (early in the cohort). Only one patient was re-transplanted, 7 months after the diagnosis of cirrhosis.

Decompensation

This was diagnosed after the diagnosis of Ishak stage 5 or 6 in all patients: 12 (22%) decompensated at a median of 9 (1–49) months after diagnosis of stage 5/6 (75% in whom HVPG was measured and 3−25% in whom it was not). The median interval between LT and diagnosis of cirrhosis was 34 (12–135) months in patients who decompensated and 52 (12–172) months in those who did not decompensate ($P = 0.06$). The follow up after diagnosis of stage 5 [15 (2–64) months] was comparable to stage 6 [21 (1–76) months]. The cumulative probability of not developing decompensation was 83%, 72%, and 60% at 1, 3, and 5 years, respectively after developing cirrhosis (Fig. 1). The first episode of decompensation was manifested by ascites in 10 patients (83%), hydrothorax in one; spontaneous bacterial peritonitis in three, variceal bleeding in one, and encephalopathy in one patient.

Survival

Sixteen (29%) patients died at a median of 9 (1–64) months from the diagnosis of allograft cirrhosis. Decompensation accounted for 50% of deaths (eight cases), infection for 25% (four cases; pneumonia, in one compensated and two decompensated patients; sepsis of unknown origin in one decompensated patient), cardiovascular events for 12.5% (stroke and myocardial infarction in two compensated patients), and other causes in 12.5% (recurrent hepatocellular carcinoma in one compensated patient, and bone marrow failure in one decompensated patient). In the 43 compensated patients, eight (18.6%) died at a median of 4 (1–43) months whereas 92% of decompensated patients ($P < 0.001$) died within 4.5 (1–15) months, after diagnosis of allograft cirrhosis. From diagnosis of allograft cirrhosis to death, the median interval was relatively longer for those dying as a result of allograft cirrhosis-related complications when compared with other causes [23 (2–64) vs. 6 (1–43) months; $P = 0.07$]. Survival of compensated patients was 91%, 86%, and 74% at 1, 3, and 5 years after the diagnosis of cirrhosis, respectively. Following decompensation, survival fell to 36% and 12% at 6 and 12 months, respectively (Fig. 2). Total and liver-related survival ($n = 41$) at 1, 3, and 5 years was 85%, 62%, 55%, and 94%, 74%, 74%, respectively (Fig. 3).

MELD score immediately post-LT and 1-year post-LT did not influence survival of patients either by Kaplan–Meier or Cox regression analysis: median and range post-LT was 14 (8–19), while 1-year post-LT was 16 (10–24).

Outcome of Stage 5 versus stage 6 – outcome of stage 4

Median time from LT to reaching stage 5 and 6 was 60 (12–157) months and 48 (12–112) months respectively ($P = 0.14$). There was no difference in comparing survival between the two groups at the same time intervals, i.e. 80% vs. 76% at 2 years for stage 5 and 6 respectively.

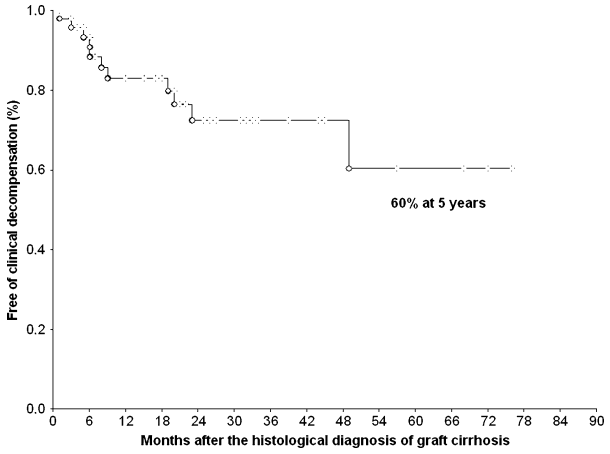

Figure 1 Cumulative probability of being free of decompensation among all patients who were included in the study ($n = 55$) after the histologic diagnosis of allograft cirrhosis.

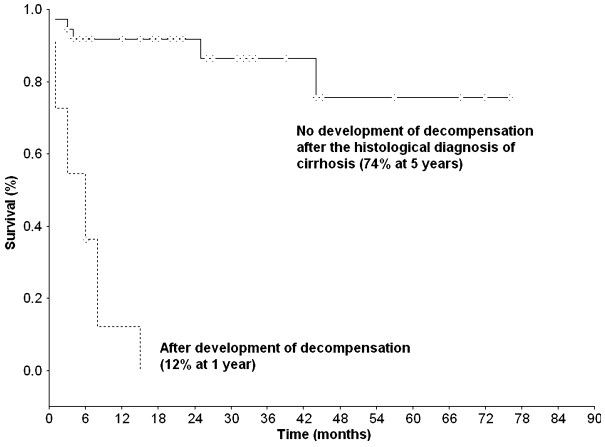

Figure 2 Patient survival rates after the histological diagnosis of allograft cirrhosis among patients who remained compensated, and after the development of decompensation.

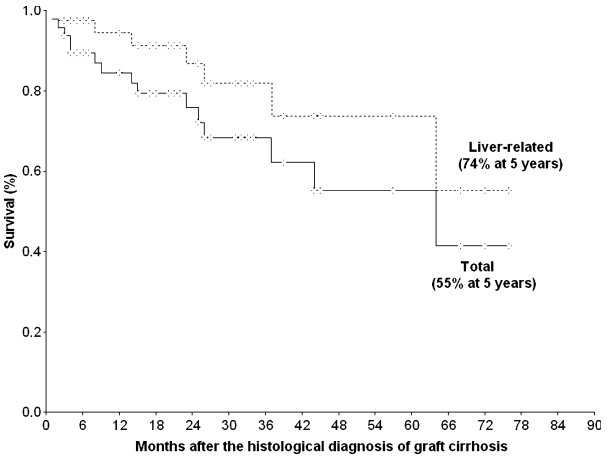

Figure 3 Total survival rates (n = 55) and liver-related survival rates (n = 41) after the histologic diagnosis of allograft cirrhosis.

Stage-5 and stage-6 patients showed comparable cumulative probability of not developing decompensation (73% vs. 67% at 3 years). Median interval between diagnosis of allograft cirrhosis and decompensation was also similar [12 (1–31) vs. 9 (5–49) months]. Additionally, no significant difference was noted in the proportion of deaths at 3 years between stage 5 and 6, either related (15% vs. 17%) or unrelated to decompensation (30% vs. 31%), time from diagnosis of allograft cirrhosis to death [14 (2–43) vs. 9 (1–64) months], total survival (75% vs. 63% at 3 years), or liver-related survival (87% vs. 80% at 3 years).

In the cohort of 16 patients who developed fibrosis stage 4 and remained so (median follow up: 70 (12–150) months after LT), there was no episode of decompensation or liver-related death. More of these patients had received low dose prednisolone, azathioprine long term and had less histologically proven acute hepatitis caused by recurrent HCV [11].

Impact of CSPH

Among the 38 patients with HVPG measurements at diagnosis of allograft cirrhosis, 15 (39%) had an HVPG ≥ 10 mmHg (CSPH). Hemodynamic, laboratory, and treatment characteristics (Table 1), at the time of HVPG measurement, were similar between patients with and without CSPH. Those with CSPH, when compared with those without CSPH, developed decompensation more frequently [5 (45%) vs. 1 (5%), P = 0.01] and had a lower probability of remaining free of decompensation (34% vs. 94% at 3 years, P = 0.02) (Fig. 4a). In patients with CSPH, decompensation occurred at a median of 19 (1–31) months and in the single patient without CSPH at

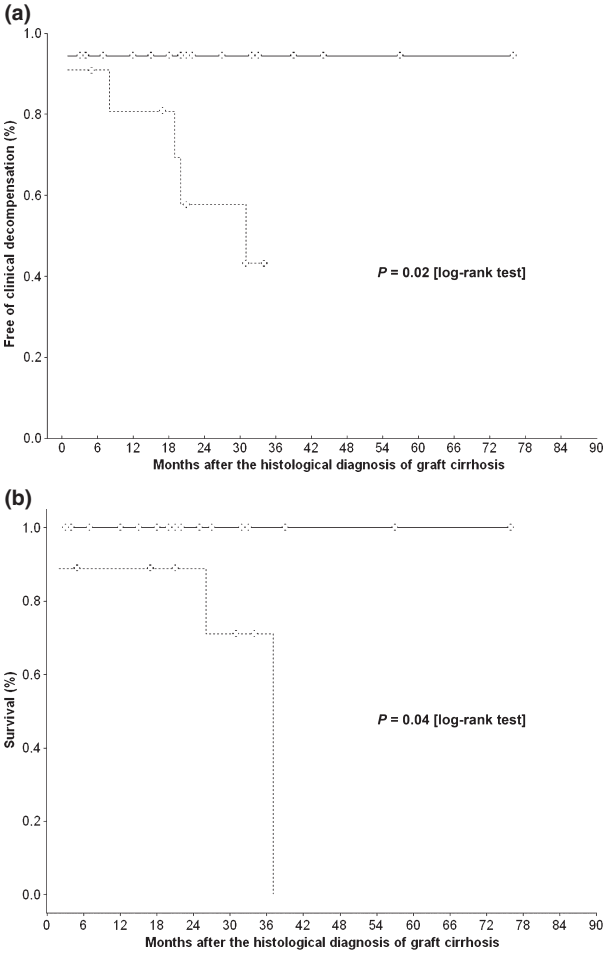

Figure 4 (a) Cumulative probability of being free of decompensation in patients with allograft cirrhosis with hepatic venous pressure gradient (HVPG) ≥ 10 mmHg (n = 15) and those with HVPG < 10 mmHg (n = 23). (b) Liver-related patient survival rates among patients with at histologic diagnosis of allograft cirrhosis in patients with HVPG ≥ 10 mmHg and those with HVPG < 10 mmHg.

1 month. Decompensation-related deaths occurred in three (27%) patients with CSPH, within 2–37 months, when compared with no patients without CSPH (P = 0.01). Liver-related survival rates were higher in patients without CSPH (n = 16) than in those with CSPH (n = 10): 100% vs. 74% at 3 years, P = 0.04 (Fig. 4b).

Impact of immunosuppression

After November 1998, all patients were taking tacrolimus-based, single or triple initial immunosuppression; cyclosporine had been used previously. The time from LT to diagnosis of allograft cirrhosis was 62 (21–164) months in patients treated with cyclosporine-based immunosuppression and 40 (12–105) months in those treated with

Table 2. Univariate and multivariate analysis of variables associated with the median time from liver transplantation to histologic diagnosis of hepatitis C virus-related allograft cirrhosis.

	≤48 months (n = 28)	>48 months (n = 27)	P-value*	P-value†
Tacrolimus-based immunosuppression	79%	39%	0.02	0.02
No added azathioprine and/or prednisolone	78%	48%	0.03	0.04
Recipient age (years)				
≤50	40%	56%	NS	
>50	60%	44%	NS	
Recipient sex (male)	76%	87%	NS	
Donor age (years)				
<30	24%	30%	NS	
30–50	44%	39%	NS	
>50	32%	31%	NS	
Donor sex (male)	60%	44%	NS	
Genotype 1b	32%	35%	NS	

Results are expressed as % of patients.
*Univariate analysis.
†Multivariate analysis.

tacrolimus-based immunosuppression (P = 0.005). Using the median time from LT to histologic diagnosis of allograft cirrhosis (≤48 months, n = 26; >48 months, n = 24) as a dichotomous endpoint, and use of tacrolimus versus cyclosporine, initial use of azathioprine and prednisolone, recipient age (<50 and >50 years) and gender, donor age (<30, 30–50, and >50 years) and gender, and genotype (1b versus non-1b), as variables, the use of tacrolimus versus cyclosporine and single initial immunosuppression were significantly associated with earlier development of allograft cirrhosis in both univariate (P = 0.02 and P = 0.03, respectively) and multivariate analyses (P = 0.02 and P = 0.04, respectively) (Table 2).

Among 23 patients initially treated with cyclosporine-based regimens, three later changed to tacrolimus, while six, one starting with cyclosporine and five with tacrolimus, were taking sirolimus at the time of diagnosis of cirrhosis because of renal dysfunction. Thus, 17 (38%) and 25 (50%) patients who developed cirrhosis remained on cyclosporine and tacrolimus, respectively. At diagnosis of cirrhosis, hemodynamic, laboratory, and treatment characteristics (Table 1) and outcomes were similar between these two groups, except for the interval to death [14 (1–23) months with cyclosporine vs. 24 (2–64) months with tacrolimus; P = 0.05].

Impact of HCV genotypes

These were 1b in 34% and non-1b in 66% (Table 1). Baseline hemodynamic, laboratory, and treatment characteristics (Table 1) were similar between 1b and non-1b with similar outcomes. Despite differences in genotypes, the median time from LT to diagnosis of allograft cirrhosis was similar in caucasians and noncaucasians [49 (12–151) vs. 47 (27–164) months].

Risk factors for decompensation and mortality

The risk of decompensation was significantly higher in patients with high HVPG values (P = 0.004 for both median value ≥ 11 mmHg, and absolute value ≥ 10 mmHg), a Child-Pugh score ≥ 7 (P < 0.001) or Child-Pugh class B (P < 0.001), and with higher initial levels of AST (P = 0.007), bilirubin (P = 0.008), INR (P = 0.008), and lower initial levels of albumin (P = 0.001) (Table 3). In the multivariate model, a high HVPG (≥10 mmHg), a high Child-Pugh score (≥7), and low albumin had an independent association with developing decompensation (P = 0.04, P = 0.01, and P = 0.001, respectively); INR showed a trend towards being significant (P = 0.05).

A higher mortality rate was associated univariately with a high Child-Pugh score [7 (6–8) vs. 5 (5–8), P < 0.001], a higher INR [1.3 (1.2–1.6) vs. 1.1 (0.9–1.6), P = 0.005], and lower albumin concentrations [32 (27–34) vs. 39 (22–52) g/l, P = 0.003]. A high Child-Pugh score and low albumin levels remained significant in the multivariate analysis (P = 0.01 and P = 0.03, respectively). Six patients with elevated HVPG measurements died: three had CSPH and all died with decompensation. CSPH was also found in one other patient who died unrelated to decompensation.

Discussion

Published data are scanty regarding outcomes of compensated HCV-related allograft cirrhosis, yet this information is very relevant to formulate prognosis and re-transplantation policy. A Spanish study evaluated allograft cirrhosis because of genotype 1b HCV re-infection [17] in a center experiencing a worse outcome of HCV-related allograft disease when compared with other centers, including our own [11,16], while a US study assessed survival predictors after the diagnosis of HCV allograft cirrhosis [18].

In our study, outcomes were evaluated following diagnosis of histologically proven stage-5 or -6 allograft cirrhosis. Except INR, these two groups did not differ at diagnosis with regard to clinical and laboratory characteristics, including HVPG, and outcomes were similar. This suggests that stages 5 and 6 after LT, have similar prognosis and thus similar clinical significance. In contrast to the Ishak system, the METAVIR system [33], used in the

Table 3. Univariate analysis of variables at histologic diagnosis of HCV-related allograft cirrhosis associated with the development of decompensation.

	No decompensation (n = 43)	Decompensation (n = 12)	P-value
Recipient age at LT (years)	50 (35–65)	53 (28–62)	NS
Recipient age at diagnosis of cirrhosis (years)	55 (37–74)	54 (31–73)	NS
Recipient sex (% male)	86	73	NS
Donor age (years)	39 (16–65)	43 (20–61)	NS
Donor sex (% male)	59	45	NS
HVPG (mmHg)	7 (4–14)	11 (6–18)	0.004
HVPG > 10 mmHg (%)	22	83	0.004
Genotype 1b (%)	38	27	NS
Pretransplantation viral load (log_{10} IU/ml)	1.1 (0.7–5.8)	2.2 (1–8.8)	NS
Viral load at diagnosis cirrhosis (log_{10} IU/ml)	1700 (10–50000)	2000 (5.7–39000)	NS
Child-Pugh class A/B (%)	90/10	36/64	<0.001
Child-Pugh score	5 (5–8)	7 (6–8)	<0.001
Alanine aminotransferase (U/l)	71 (14–250)	109 (27–230)	NS
Aspartate aminotransferase (U/l)	79 (22–340)	141 (45–450)	0.008
Bilirubin (mg/dl)	0.7 (0.3–3)	1 .8 (0.6–3.7)	0.008
Albumin (g/dl)	39 (22–52)	32 (22–38)	0.001
INR	1.1 (0.9–1.6)	1.3 (1.1–1.6)	0.007
Grade	6 (3–9)	5 (3–8)	NS
Piecemeal necrosis	2 (1–3)	2 (1–3)	NS
Confluent necrosis	0 (0–1)	0 (0–1)	NS
Focal necrosis	2 (0–3)	1 (0–3)	NS
Portal inflammation	2 (1–4)	2 (1–3)	NS
Initial cyclosporine-based immunosuppression after LT	38	64	NS
Cyclosporine at diagnosis of cirrhosis (%)	32	45	NS
Cyclosporine dose at diagnosis of cirrhosis (mg/day)	100 (75–200)	100 (75–175)	NS
Initial tacrolimus-based immunosuppression after LT (%)	62	55	NS
Tacrolimus at diagnosis of cirrhosis (%)	49	64	NS

Spanish study [17], does not separate patients with incomplete cirrhosis, classifying these as fibrosis 3 (bridging fibrosis) [34]. Thus some patients with METAVIR stage less than 4 could correspond to Ishak stage 5. This could explain why 20% of patients in the Spanish study had allograft cirrhosis diagnosed after decompensation, when compared with only one patient in this study (excluded from the analysis). We have extended previous findings from Berenguer *et al.* [17] by evaluating the impact of CSPH, immunosuppression, across a wider spectrum of HCV genotypes and with a longer follow up.

In addition, antiviral therapy response does not constitute a bias in our study as none responded. This failure of response is likely to be because of the previous use of standard interferon versus the current pegylated type, the nonsystematic use of erythropoietin and colony stimulating factors, and perhaps the late introduction of therapy in relation to disease severity.

A major difference, when compared with the Spanish cohort (possibly explained by its unusually severe progression), was a longer interval from LT to diagnosis of allograft cirrhosis in our patients (4 vs. 2 years) with longer median follow up after diagnosis of cirrhosis (21 vs. 11.3 months) [17,18]. Moreover, 79% with compensated allograft cirrhosis remained so, several years after the diagnosis when compared with 49% in the Spanish [17], and only 8% in the American study [18]. Clinical decompensation, most frequently ascites, developed in 24% (median of 19 months) in our cohort, when compared with 51% of Spanish patients (median of 8 months), and 92% of American patients (mean of approximately 21 months). The cumulative probability of not developing clinical decompensation was significantly lower in the Spanish cohort, reaching 58% after just 1 year, when compared with 60% at 5 years in the present study. Nevertheless, the overall survival rates of our cohort was similar to the Spanish one [17] but significantly higher than the US cohort (66% at 1 year) [18]. However, liver-related survival and survival among patients who remained compensated were reasonably high (both 74% at 5 years). Once clinical decompensation occurred, survival rates fell to 12% at 1 year when

compared with 41% at 1 year in the Spanish study [17]. This apparent difference, however, may be related to our smaller number of decompensated cirrhotics.

A HVPG ≥ 10 mmHg, a Child-Pugh score ≥ 7, and low albumin levels when allograft cirrhosis was diagnosed, were independent predictors of decompensation and survival, similar to those reported in immunocompetent patients [5,19–21,35,36], and recently, in HCV-infected liver transplant recipients [17], although in the latter case multivariate analysis was not performed. The value of measuring HVPG once allograft cirrhosis is diagnosed, may substantiate and extend the recent finding [26] as well as our own [37] that HVPG *per se* has prognostic value in monitoring HCV recurrent disease. Our study is the first to report the impact of CSPH on the outcome of clinically compensated allograft cirrhosis because of HCV re-infection. A HVPG threshold of about 10 mmHg defines CSPH [24,25] and predicts survival and decompensation in nontransplanted cirrhotic patients [20–23]. Indeed, patients with cirrhosis and an HVPG < 10 mmHg have a 90% probability of not developing clinical decompensation in a median follow up of 4 years [20]. In our cohort, decompensation occurred in only 5% of the patients without CSPH at the time of diagnosis of allograft cirrhosis but in 45% with CSPH, with a higher probability of developing decompensation over time when HVPG was ≥10 mmHg. Patient survival rates were significantly lower in patients with CSPH. Importantly, stage 5 or 6 were no different with respect to baseline HVPG or increased risk of decompensation, so that HVPG *per se* appears to contribute substantially in the assessment of severity in these patients. Additionally, selective deposition of collagen within liver sinusoids may occur in liver transplant recipients [26], which may render current scoring systems for liver fibrosis suboptimal. Thus, HVPG could be a better quantitative marker of progressive liver disease in HCV-related allograft cirrhosis.

Genotype 1b has been associated with worse outcomes following LT according to Spanish studies [8,15,17], similarly to some immunocompetent cohorts [38,39], but not according to other centers [13,14,16], including our cohort. A possible explanation for a worse outcome of genotype 1b could be the variable prevalence of particularly aggressive strains [40].

Although the evaluation of factors associated with the development of HCV allograft cirrhosis was not within the aims of the present study, it is interesting that patients treated with tacrolimus-based regimens developed graft cirrhosis significantly faster than those treated with cyclosporine-based regimens. This different rate of progression could be associated with the initial use of tacrolimus versus cyclosporine as cyclosporine has an *in vitro* antiviral effect (however this is at pharmacologic doses 0.5–1 μg/ml) [41,42] and a possible differential effect on fibrogenesis [43,44]. However, the use of calcineurin inhibitors in our cohort was also associated with different uses of azathioprine and prednisolone in the initial immunosuppression whose long-term use we have found 'protective' with regard to developing fibrosis [11]. In this regard, a recent review has found no difference between the calcineurin inhibitors [45].

In conclusion, patients with HCV-related cirrhosis transplanted in our center develop allograft cirrhosis or decompensation at a significantly longer interval than previously reported, while survival remains reasonable at 77% at 5 years among patients who remain compensated or do not die from causes unrelated to decompensation. There is no difference in prognosis between Ishak stage 5 and stage 6, but an HVPG ≥ 10 mmHg before decompensation as well as Child-Pugh B, and albumin concentrations ≤ 32 g/dl provide prognostic information about which patients are at a higher risk for future decompensation and death. These risk-factors could be taken into consideration for selective relisting even while still being clinically compensated, whilst continuing to observe patients without them.

Authorship

GK: the principal writer, performed final analysis of the data. PM: revised written manuscript, initiated data base and maintained it. DS: initiated data base and maintained it. FG and APD: the histopathologists who graded/staged liver biopsies. DP and JOB: performed transjugular biopsies and HVPG measurements. KR: the Director of Liver Transplantation and operated on most of the patients. AKB: the lead investigator, intellectual strategy, corrected/revised manuscript and ultimately responsible for data and its use.

References

1. Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C and liver transplantation. *J Hepatol* 2001; **35**: 666.
2. Gane EJ, Portmann BC, Naoumov NV, *et al.* Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; **334**: 815.
3. Wright TL, Donegan E, Hsu HH, *et al.* Recurrent and acquired hepatitis C viral infection in liver transplant recipients. *Gastroenterology* 1992; **103**: 317.
4. Berenguer M, Ferrell L, Watson J, *et al.* HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol* 2000; **32**: 673.
5. Fattovich G, Giustina G, Degos F, *et al.* Morbidity and mortality in compensated cirrhosis type C: a retrospective

follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463.
6. Feray C, Gigou M, Samuel D, *et al.* The course of hepatitis C virus infection after liver transplantation. *Hepatology* 1994; **20**: 1137.
7. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825.
8. Prieto M, Berenguer M, Rayon JM, *et al.* High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. *Hepatology* 1999; **29**: 250.
9. Papatheodoridis GV, Patch D, Dusheiko GM, Burroughs AK. The outcome of hepatitis C virus infection after liver transplantation – is it influenced by the type of immunosuppression? *J Hepatol* 1999; **30**: 731.
10. Papatheodoridis GV, Davies S, Dhillon AP, *et al.* The role of different immunosuppression in the long-term histological outcome of HCV reinfection after liver transplantation for HCV cirrhosis. *Transplantation* 2001; **72**: 412.
11. Samonakis DN, Triantos CK, Thalheimer U, *et al.* Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. *Liver Transpl* 2005; **11**: 386.
12. Feray C, Gigou M, Samuel D, *et al.* Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. *Gastroenterology* 1995; **108**: 1088.
13. Zhou S, Terrault NA, Ferrell L, *et al.* Severity of liver disease in liver transplantation recipients with hepatitis C virus infection: relationship to genotype and level of viremia. *Hepatology* 1996; **24**: 1041.
14. Vargas HE, Laskus T, Wang LF, *et al.* The influence of hepatitis C virus genotypes on the outcome of liver transplantation. *Liver Transpl Surg* 1998; **4**: 22.
15. Berenguer M, Prieto M, San Juan F, *et al.* Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 2002; **36**: 202.
16. Berenguer M, Crippin J, Gish R, *et al.* A model to predict severe HCV-related disease following liver transplantation. *Hepatology* 2003; **38**: 34.
17. Berenguer M, Prieto M, Rayon JM, *et al.* Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology* 2000; **32**: 852.
18. Saab S, Niho H, Comulada S, *et al.* Mortality predictors in liver transplant recipients with recurrent hepatitis C cirrhosis. *Liver Int* 2005; **25**: 940.
19. Abraldes JG, Tarantino I, Turnes J, Garcia-Pagan JC, Rodes J, Bosch J. Hemodynamic response to pharmacological treatment of portal hypertension and long-term prognosis of cirrhosis. *Hepatology* 2003; **37**: 902.
20. Ripoll C, Groszmann R, Garcia-Tsao G, *et al.* Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481.
21. Garcia-Tsao G, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 1985; **5**: 419.
22. Morali GA, Sniderman KW, Deitel KM, *et al.* Is sinusoidal portal hypertension a necessary factor for the development of hepatic ascites? *J Hepatol* 1992; **16**: 249.
23. Casado M, Bosch J, Garcia-Pagan JC, *et al.* Clinical events after transjugular intrahepatic portosystemic shunt: correlation with hemodynamic findings. *Gastroenterology* 1998; **114**: 1296.
24. Groszmann RJ, Garcia-Tsao G, Bosch J, *et al.* Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; **353**: 2254.
25. Bosch J, Abraldes JG, Groszmann R. Current management of portal hypertension. *J Hepatol* 2003; **38**(Suppl. 1): S54.
26. Blasco A, Forns X, Carrion JA, *et al.* Hepatic venous pressure gradient identifies patients at risk of severe hepatitis C recurrence after liver transplantation. *Hepatology* 2006; **43**: 492.
27. Burroughs AK, Groszmann R, Bosch J, *et al.* Assessment of therapeutic benefit of antiviral therapy in chronic hepatitis C: is hepatic venous pressure gradient a better end point? *Gut* 2002; **50**: 425.
28. Cholongitas E, Quaglia A, Samonakis D, *et al.* Transjugular liver biopsy: how good is it for accurate histological interpretation? *Gut* 2006; **55**: 1789.
29. Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696.
30. Groszmann RJ, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. *Hepatology* 2004; **39**: 280.
31. O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A. Tacrolimus versus microemulsified ciclosporin in liver transplantation: the TMC randomised controlled trial. *Lancet* 2002; **360**: 1119.
32. Samonakis DN, Mela M, Quaglia A, *et al.* Rejection rates in a randomised trial of tacrolimus monotherapy versus triple therapy in liver transplant recipients with hepatitis C virus cirrhosis. *Transpl Infect Dis* 2006; **8**: 3.
33. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289.
34. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. *J Hepatol* 2003; **38**: 223.
35. Serfaty L, Aumaitre H, Chazouilleres O, *et al.* Determinants of outcome of compensated hepatitis C virus-related cirrhosis. *Hepatology* 1998; **27**: 1435.
36. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217.

37. Samonakis DN, Cholongitas E, Thalheimer U, *et al.* Hepatic venous pressure gradient to assess fibrosis and its progression after liver transplantation for HCV cirrhosis. *Liver Transpl* 2007; **13**: 1305.
38. Dusheiko G, Schmilovitz-Weiss H, Brown D, *et al.* Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology* 1994; **19**: 13.
39. Pozzato G, Kaneko S, Moretti M, *et al.* Different genotypes of hepatitis C virus are associated with different severity of chronic liver disease. *J Med Virol* 1994; **43**: 291.
40. Gigou M, Roque-Afonso AM, Falissard B, Penin F, Dussaix E, Feray C. Genetic clustering of hepatitis C virus strains and severity of recurrent hepatitis after liver transplantation. *J Virol* 2001; **75**: 11292.
41. Nakagawa M, Sakamoto N, Enomoto N, *et al.* Specific inhibition of hepatitis C virus replication by cyclosporin A. *Biochem Biophys Res Commun* 2004; **313**: 42.
42. Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology* 2003; **38**: 1282.
43. Frizell E, Abraham A, Doolittle M, *et al.* FK506 enhances fibrogenesis in *in vitro* and *in vivo* models of liver fibrosis in rats. *Gastroenterology* 1994; **107**: 492.
44. Ikeda H, Fujiwara K. Cyclosporin A and FK-506 in inhibition of rat Ito cell activation *in vitro*. *Hepatology* 1995; **21**: 1161.
45. Berenguer M, Royuela A, Zamora J. Immunosuppression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. *Liver Transpl* 2007; **13**: 21.