Transpl Int (1994) 7 [Suppl 1]: S216–S220

V. Vargas
P. Comas
Ll. Castells
J. Quer
J. I. Esteban
E. Allende
R. Esteban
J. Guardia
C. Margarit

Incidence and outcome of hepatitis C virus infection after liver transplantation

V. Vargas (✉)
C/Ganduxer 14, 4° 2ª,
E-08021 Barcelona, Spain

P. Comas · Ll. Castells · J. Quer
J. I. Esteban · E. Allende · R. Esteban
J. Guardia · C. Margarit
Liver Transplant Unit,
Liver Unit and Department of Pathology,
Hospital Vall d'Hebrón,
Universidad Autónoma de Barcelona,
Barcelona, Spain

Abstract The objective of this study was to determine the incidence and outcome of hepatitis C virus (HCV) infection after liver transplantation (OLT). Fifty-two transplanted patients were studied. Serum samples were examined for antibodies to HCV (anti-HCV) and HCV-RNA by PCR, before and after OLT. Patients were distributed into two groups: group 1 consisted of 24 patients (pretransplant anti-HCV positive) and group 2 consisted of 28 patients (pretransplant anti-HCV negative). One year after OLT, HCV-infected patients were evaluated by liver biopsy. HCV-RNA was detected in 28 of the 52 (53.9%) patients after OLT. Twenty-two patients in group 1 (96%) were reinfected. In group 2, acquired HCV infection was detected in six (21.4%) patients. At 6 and 12 months, one and five of six patients had seroconverted, respectively. Liver biopsy in 23 HCV-infected patients showed chronic hepatitis in 18 (78%) cases (2, chronic persistent hepatitis; 3, chronic lobular hepatitis and 13, chronic active hepatitis). Fourteen of the 23 (60.8%) patients were asymptomatic. Most symptomatic patients had chronic hepatitis with cholestasis. Overall, 18 of 20 cases of chronic hepatitis diagnosed in OLT recipients were HCV related. Mortality beyond 6 months after OLT was slightly higher in the HCV-infected group ($P = 0.055$). In conclusion, HCV reinfection is almost universal. Acquired HCV infection post-OLT is frequent. HCV-infected patients frequently develop chronic hepatitis. Most chronic hepatitis after transplantation are HCV related.

Key words Hepatitis C virus
Orthotopic liver transplantation
Chronic hepatitis

Introduction

Hepatitis C virus (HCV) is an important aetiological factor in liver disease in patients undergoing liver transplantation [12]. The incidence and repercussion of HCV infection in the allograft is not well known. Several groups have reported their experience on the acquisition and recurrence of HCV infection [8, 11, 16], but there are few data on the clinical and histological follow-up in these patients. The present study was designed to study the incidence, clinical and histological course of HCV infection in liver transplant recipients.

Materials and methods

Fifty-two patients who received a single orthotopic liver transplant (OLT) at our hospital between January 1989 and July 1992 were studied. The mean age of the study patients was 51 years (range, 24–62 years). Thirty-one patients were male and 21 were female. All patients received cyclosporin A and methylprednisolone as the primary immunosuppressive agents. Rejection episodes were treated with a bolus (1 g doses) of methylprednisolone. Steroid-resistant rejection was treated with a 5–10 day course of monoclonal antibody, OKT3.

Donor and recipient serum samples were obtained immediately before liver transplantation. Recipient samples were also obtained after transplantation during hospitalization and at outpatient visits. Serum samples were rapidly frozen and stored at −40 °C until analysis.

Donor and recipient pretransplant and selected posttransplant samples were tested for anti-HCV by second-generation enzyme-linked immunoassay (ELISA II) (Abbott Laboratories, Chicago, Illinois). All positive samples were confirmed by second-generation recombinant immunoblot assay (RIBA 2; Chiron Corp. Emeryville, California). Eight pretransplant serum samples and samples of liver recipients were tested for HCV-RNA. In patients with more than one serum samples available, the later sample, at least, was determined for anti-HCV and serum HCV-RNA.

HCV-RNA was tested by polymerase chain reaction using primers derived from the 5′ non-coding region. HCV-RNA extracted from 75 µl of plasma by the acid guanidinium thiocyanate-phenol-chloroform method [2] was reverse transcribed into cDNA and PCR amplified in a single tube reaction (RT-PCR; kit N 808-007, Perkin Elmer) for 35 cycles using specific oligonucleotide primers of the 5′ UTR [7]. The amplified product was 354 bp long and the specificity was confirmed by Southern hybridization with ^{32}P probes.

Liver biopsies were obtained in HCV-infected patients between 6 and 15 months after transplantation and were evaluated by a single staff pathologist for chronic hepatitis. If more than one liver biopsy was obtained during this period, the later one was evaluated. ALT levels and clinical evaluation were performed at regular intervals.

Pretransplant HCV infection was defined by anti-HCV positivity confirmed by RIBA 2. Posttransplant HCV infection was defined by serum HCV-RNA positivity in at least two samples. Acute hepatitis C was defined by a five-fold increase in ALT, HCV-RNA positivity, acute hepatitis histology and no evidence of acute rejection, CMV or EBV infection.

Statistical analysis

Data are expressed as mean values ±1 SD. The significance of differences between means was assessed by the 95% confidence interval. Comparisons of proportions are based on the chi-square test. For survival analysis, the Kaplan-Meier method was used.

Results

Pretransplant HCV infection

Twenty-four patients (46%) were infected by HCV (group 1). Their diagnoses were: HCV cirrhosis in 18 patients (3 with hepatocellular carcinoma), HBV cirrhosis in 2, alcoholic cirrhosis in 3 and Caroli's disease with HCV chronic active hepatitis in 1. Twenty-eight patients (54%) were not infected by HCV (group 2). Their diagnoses were: alcoholic cirrhosis in 13 patients, HBV cirrhosis in 2, primary biliary cirrhosis in 5, other cirrhosis in 3, Caroli's disease in 2, and giant haemangioma in 1.

One of the organ donors was positive for anti-HCV (ELISA II). The recipient was also anti-HCV positive pre-OLT.

Posttransplant HCV infection

Overall, HCV infection (serum HCV-RNA positive) was detected in 28 patients (54%).

Group 1

Of 24 patients with pretransplant HCV infection (anti-HCV positive confirmed by RIBA 2), 22 (92%) were viraemic posttransplantation. Of two patients that became HCV-RNA negative after liver transplantation, one also lost anti-HCV.

Group 2

Of 28 patients without pretransplant HCV infection, 6 (21%) acquired hepatitis C infection (HCV-RNA positive). Only one of these patients developed anti-HCV in the first 6 months post-OLT. After 6 months, anti-HCV seroconversion was demonstrated in five out of six cases.

Histological findings

Of 28 patients with posttransplant HCV infection, 2 died before 6 months, 23 had a liver biopsy between 6 and 15 months after OLT and liver biopsy was not available in 3 patients at this time. When the 23 liver specimens were evaluated, we found chronic hepatitis in 18 (78%) cases: 13 (56%) had chronic active hepatitis, 2 (9%) had chronic persistent hepatitis and 3 (13%) had chronic lobular hepatitis (Table 1). In the chronic active hepatitis group, two patients had HBV and HCV coinfection. Five patients had only "minimal changes" or non-specific changes on liver biopsy. Five out of six patients with "de novo" HCV infection developed chronic hepatitis. Seven of 13 cases of chronic active hepatitis were qualified as

Table 1 Characteristics of 23 patients with hepatic C virus (HCV) infection and liver biopsy (*OLT* orthotopic liver transplantation, *CAH* chronic active hepatitis, *CPH* chronic persistent hepatitis, *MC* minimal changes, *CLH* chronic lobular hepatitis)

Case	Months after OLT	Histological diagnosis	ALT	Evolution
1	15	CAH (+HBV)	86	Asymptomatic
2	15	CAH	57	Died (24 months)
3	6	CAH	131	Died (18 months)
4	6	CPH	103	Asymptomatic
5	15	MC	19	Asymptomatic
6	6	CAH	184	Cholestasis-retransplant
7	15	CLH	59	Asymptomatic
8	12	CLH	65	Asymptomatic
9	14	CAH	277	Cholestasis
10	11	CAH	215	Asymptomatic
11	6	CAH	401	Cholestasis-died (8 months)
12	6	MC	49	Asymptomatic
13	14	CPH	156	Asymptomatic
14	14	CAH	37	Asymptomatic
15	11	MC	58	Asymptomatic
16	6	MC	66	Asymptomatic
17	7	CAH	142	Cholestasis-died (20 months)
18	11	CLH	206	Cholestasis
19	11	CAH	315	Cholestasis-died (14 months)
20	6	CAH	158	Cholestasis-died (9 months)
21	6	CAH	298	Cholestasis-died (11 months)
22	15	MC	66	Asymptomatic
23	7	CAH (+HBV)	59	Died (22 months)

"cholestasic" and they were characterized by marked cholangitis with ductular proliferation and canalicular cholestasis.

In the group of HCV-negative patients we only demonstrated two with chronic hepatitis, both of them in relation to HBV infection. Overall, 18 out of 20 (90%) cases of chronic hepatitis were HCV related.

Clinical outcome

Mean follow-up after transplantation was 15 ± 7.5 months. In patients with posttransplant HCV infection and chronic hepatitis, nine were asymptomatic and nine developed malaise and jaundice. The subgroup of patients with "cholestatic" chronic active hepatitis (seven cases) were markedly symptomatic.

Acute hepatitis C was detected in seven patients (25%) at a mean interval of 3.1 ± 0.6 months after OLT. Five

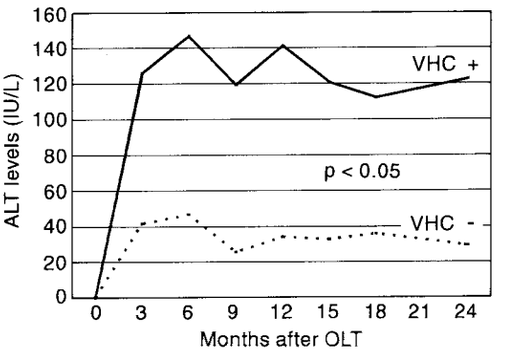

Fig. 1 Mean ALT levels according to the hepatitis C virus (HCV) status after orthotopic liver transplantation

cases were related to HCV-acquired ("de novo") infection. Only one patient was symptomatic and all cases evolved into chronic hepatitis. Mean ALT levels were higher in the HCV-infected group throughout the follow-up period ($P < 0.05$; Fig. 1).

Nine patients with HCV infection and three patients without HCV infection died during the follow-up period. This was not significant. Nevertheless, late mortality (beyond 6 months posttransplantation) was slightly higher in the HCV-infected group (seven patients) than in the non-HCV-infected group (two patients; $P = 0.055$). Patients with chronic active hepatitis with prominent cholestatic features had the worst outcome: two of seven were retransplanted because of severe hepatic dysfunction (one died shortly after retransplantation) and four patients died beyond 6 months posttransplantation due to opportunistic infections. No case with hepatic cirrhosis was detected during the follow-up.

Discussion

In our study, HCV reinfection of liver recipients was the rule. Reinfection was seen in 92% of patients with pretransplant HCV virus infection (anti-HCV positive by a second-generation assay). These results were similar to those of other authors [4, 9, 16]. It has been postulated that HCV remains in peripheral blood cells and perhaps in other tissues [6]. Viraemia at the time of surgery or at any time from extrahepatic sources may expose the liver allograft to infectious particles leading to recurrent hepatitis C. In spite of immunosuppression, anti-HCV levels remained detectable in all our reinfected patients, although other authors have reported a variable percentage of antibody loss [12, 14]. Only one patient lost

antibodies 4 months after becoming HCV-RNA negative, which suggests viral clearance, an exceptional event in the posttransplant setting.

Peritransplant acquired infection was high, with an incidence of 21%, similar to the results reported in other series that also used HCV-RNA as a marker of HCV infection [4, 16]. Presumably, the main source of HCV infection was the large amounts of blood products required during the surgical procedure, since in our series, only one patient received a liver graft from an anti-HCV-positive donor tested by ELISA-II (negative by ELISA-I). As has been pointed out by Poterucha et al. [11] and Pereira et al. [10], early diagnosis of "de novo" HCV infection requires HCV-RNA determination since anti-HCV appeared later than 6 months after OLT in most of our patients. Anti-HCV screening in blood and organ donors by more sensible methods and less blood product requirements during transplantation will probably decrease the incidence of acquired HCV infection.

In the present study, acute hepatitis C was usually subclinical and it was detected in only 25% of HCV infections. In some cases, it was probably unrecognized because mild increases in ALT levels are frequent in the early posttransplant period and can be attributed to multiple aetiologies (acute rejection, CMV hepatitis, ischaemic injury, etc). As reported by Müller et al., acute hepatitis evolves later (between 3 and 4 months after transplantation) in transplant recipients than in patients who develop posttransfusion hepatitis [9].

HCV-infected transplant recipients very often developed chronic hepatitis (78%), and chronic active hepatitis was the most frequent. Overall, the clinical course was silent with slight increases in ALT and preserved hepatic function during our follow-up. These findings were in agreement with those of König et al. [8] and Sallie et al.. [13], who have reported that HCV infection is associated with chronic inflammation in most allografts, but progression seems to be slow.

Although the majority of chronic hepatitis cases were asymptomatic, some cases developed cholestatic features with duct damage, jaundice and a high mortality or graft loss. Duct damage, as fatty change and lymphoid aggregation, is more frequently induced by HCV infection than by other causes of hepatitis [5] and, in our patients, difficulties arose in determining whether chronic rejection or hepatitis was responsible for the duct damage. Immunosuppression allows higher viral replication, and so, higher viraemia levels in the liver recipient [1]. We can speculate that this large amount of cytopathic virus and/or specific viral strains could produce a different and more aggressive chronic hepatitis with duct damage in certain transplant recipients. In fact, a different behaviour of hepatic viral infection in the immunosuppressed host, has been described in transplant recipients with chronic hepatitis B [3]. An alternative explanation could be that HCV infection, the CMV infection [15], has a role in the development of chronic rejection. This subgroup of patients is responsible for the trend towards a higher mortality observed in the HCV-infected patients.

Although early cirrhosis development has been reported exceptionally, the course of chronic hepatitis C in the posttransplant setting is uncertain [5, 9]. In our series, we did not any case of early cirrhosis. Only a longer follow-up will show if the behaviour of HCV infection in liver transplant recipients is indolent or quickly progresses to advanced chronic liver disease and graft loss.

We found that HCV was the most frequent aetiology of chronic hepatitis after liver transplantation (90%). In the group of patients studied, the incidence of HCV in chronic hepatitis after liver transplantation was higher than in the series reported by Poterucha et al. [11], which demonstrated that 60% of patients with chronic hepatitis had HCV-RNA. This difference could be explained by the higher percentage of HCV-positive patients included in our transplant programme.

In summary, HCV infection is the most frequent aetiology of chronic hepatitis in the liver allograft. This is due to the constant reinfection of pretransplant-infected patients, the frequent peritransplant-acquired infection and the high rate of development of chronic hepatitis in the HCV-infected graft.

References

1. Chazouilleres O, Kim M, Ferrell L, Roberts J, Ascher N, Wilber J, Neuwald P et al (1992) Quantitative study of hepatitis C virus in liver transplant recipients. Hepatology 16:45A
2. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocynate-phenol-chloroform extraction. Anal Biochem 162:156–159
3. Davies SE, Portmann BC, O'Grady JG, Aldis PM, Chagger K, Alexander GJ, Williams R (1991) Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. Hepatology 13:150–157

4. Féray C, Samuel D, Thiers V, Gigou M, Pichnon F, Bismuth H, Reynes M et al (1992) Reinfection of liver graft by hepatitis C virus after liver transplantation. J Clin Invest 89:1361–1365
5. Ferrell LD, Wright TL, Roberts J, Ascher N, Lake J (1992) Hepatitis C viral infection in liver transplant recipients. Hepatology 16:865–876
6. Goeser T, Müller H, Padron G, Pfaff E, Hoffman WJ, Kommerell B, Theilmann L (1992) Hepatitis C virus replication (letter). N Engl J Med 326:65–66
7. Han JH, Syamala KH, Richman M, Brauer B, Irvine MS, Urdea P, Tekamp-Olson P, Kuo G, Choo QL, Houghton M (1991) Characterization of the terminal regions of hepatitis C viral RNA: identification of conserved sequences in the 5′ untransplanted region and poly(A) tails at the 3′ end. Proc Natl Acad Sci USA 88:1711–1715
8. König V, Bauditz J, Lobeck H, Lüsebrink R, Neuhaus P, Blumhardt G, Bechstein WO et al (1992) Hepatitis C virus reinfection in allografts after orthotopic liver transplantation. Hepatology 16:1137–1143
9. Müller H, Otto G, Goeser T, Arnold J, Pfaff E, Theilmann L (1992) Recurrence of hepatitis C virus infection after orthotopic liver transplantation. Transplantation 54:743–745
10. Pereira BJG, Milford EL, Kirkman RL, Quan S, Sayre KR, Johnson PJ, Wilber JC et al (1992) Prevalence of hepatitis C virus in organ donors positive for hepatitis C antibody and in the recipients of their organs. N Engl J Med 327:910–915
11. Poterucha JJ, Rakela J, Lumeng L, Lee CH, Taswell HF, Wiesner RH (1992) Diagnosis of chronic hepatitis C after liver transplantation by the detection of viral sequences with polymerase chain reaction. Hepatology 15:42–45
12. Read AE, Donegan E, Lake J, Ferrell L, Gaebraith C, Kuramoto IK, Zeldis JB et al (1991) Hepatitis C in patients undergoing liver transplantation. Ann Intern Med 114:282–284
13. Sallie R, Cohen A, Rayner A, O'Grady J, Tan KC, Portmann B, Williams R (1992) Hepatitis C recurrence following orthotopic liver transplantation: a PCR and histological study. Hepatology 16:48A
14. Shah G, Demetris AJ, Gavaler JS, Lewis JH, Todo S, Starzl TE, Van Thiel D (1992) Incidence, prevalence and clinical course of hepatitis C following liver transplantation. Gastroenterology 103:323–329
15. Wright T (1992) Cytomegalovirus infection and vanishing bile duct syndrome: culprit or innocent bystander. Hepatology 16:494–496
16. Wright TL, Donegan E, Hsu H, Ferrell L, Lake J, Kim M, Combs C et al (1992) Recurrent and acquired hepatitis C infection in liver transplant recipients. Gastroenterology 103:317–322