Transplant Int (1992) 5 [Suppl 1]: S 179–S 184

TRANSPLANT
International

Seroprevalence and outcome of hepatitis C in liver transplantation

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Abstract. A recombinant enzyme-linked immunosorbent assay (ELISA) followed by a neutralization test (NT) and recombinant immunoblot assay (RIBA) were used for the detection of antibody to hepatitis C virus (anti-HCV) in 71 patients receiving 84 orthotopic liver grafts between 1984 and 1990. Before the liver transplantation (LTX) anti-HCV was present in six of the 71 recipients (8.5%) who were accepted for LTX because of acute or chronic liver failure. After LTX anti-HCV could not be detected in one of the patients, but it was continuously present in the others for more than 12 months. Detectable HCV antibodies were not present in the three patients who underwent LTX because of clinical evidence of fulminant NANB hepatitis. Two of 48 (4.2%) previously HCV seronegative recipients, who survived more than 3 months, seroconverted 9 and 16 months, respectively, after transplantation. The postoperative seroconversion was probably due to the transfer of virus via perioperative blood transfusions. Thus, these liver recipients may be able to respond by producing anti-HCV despite immunosuppressive therapy. None of the seven post-transplant HCV-seropositive patients developed symptoms such as icterus or fatigue, which would suggest the presence of liver insufficiency due to HCV infection. However, two of them had increased transaminase levels and histological signs of mild hepatitis. No significant difference was found in 1-year survival, prothrombin complex, albumin levels or the risk for retransplantation in post-transplant anti-HCV-seropositive patients, compared with those without detectable HCV antibodies (71% vs 69%, respectively). Thus, during the study period of 1–5 years, the clinical course of HCV infection was milder than that reported for hepatitis B infection in liver recipients.

Key words: Hepatitis C virus – Liver transplantation

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Hepatitis C virus (HCV) is the predominant cause of transfusion-associated non-A, non-B (NANB) hepatitis [3] and may occur concomitantly with hepatitis B virus (HBV) infection [11, 14]. Approximately 50% of NANB virus-infected patients have biochemical evidence of chronic hepatitis and about 20% of these cases develop histological evidence of cirrhosis [1]. In addition, HCV may be involved in the multifactorial genesis of hepatocellular carcinoma [6, 10, 24].

Fulminant or subacute NANB hepatitis may be an indication for performing an acute liver transplantation (LTX) [7]. In the USA chronic NANB hepatitis is the fourth commonest reason for LTX (Starzl, CDC 1989). It is also conceivable that liver recipients may contract an HCV infection after LTX, which has to be taken into account when analysing pathological liver tests after transplantation. In this study recombinant enzyme-linked immunosorbent assays (ELISA) [18] and a neutralization test (NT) as well as a recombinant immuno-blot assay (RIBA) were used to detect antibodies to hepatitis C virus (anti-HCV). The aim of the study was to determine the occurrence of anti-HCV and to explore the role of HCV as a possible cause of the underlying liver disease as well as a cause of postoperative complications.

Materials and methods

Patients

Between November 1984 and July 1990 61 adults (18 to 61 years) and ten children (9 months to 14 years) received a total of 84 liver grafts and were followed for 1–5 years after LTX. The indication for LTX was chronic liver disease in 50 cases, acute and subacute liver failure in eight cases and primary liver malignancy in 13 cases (Table 1). Liver transplantations were performed orthotopically according to the technique described by Starzl et al [25]. The standard immunosuppressive therapy, as previously described [12], consisted of cyclosporin A (Sandoz Ltd, Basel, Switzerland) and prednisolone, until June 1988, when azathioprine (Wellcome, London, UK) was added to the prophylactic regimen. Acute rejection episodes were initially treated with steroids, but if ineffective, rabbit anti-thymocyte globulin (ATG, Fresenius, FRG) or mouse monoclonal antibody (OKT3, Ortho Pharmaceuticals, Raritan, N.J., USA) [12] were given as well.

Table 1. Indications for liver transplantation in 71 patients

Indication	Patients (*n*)
Chronic liver disease	50
Primary biliary cirrhosis	15
Sclerosing cholangitis	10
Posthepatic cirrhosis	6
Autoimmune chronic active hepatitis	5
Cryptogenic cirrhosis	4
Metabolic liver disease	6
Primary biliary atresia	4
Acute and subacute liver failure	8
NANB hepatitis	5
acute	3
subacute	2
Budd-Chiari	2
Toxic hepatic failure	1
Primary liver malignancy	13
Total	71

Serum samples

Serum samples were obtained from each patient before LTX, at 1 week, and 3, 6 and 12 months, and yearly, if possible, after LTX. Additional specimens were analysed if an unexplained elevation of transaminases occurred. Samples from all liver donors to recipients who seroconverted to HCV were available for testing and were kept frozen at −20°C.

A recombinant enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Raritan, N.J., USA, or Abbott Laboratories, North Chicago, Ill., USA) was used to detect antibodies to HCV. In addition, reactive samples were tested by a neutralization test, NT (Abbott), and by a recombinant immunoblot assay (RIBA) (Ortho Diagnostic Systems) which utilized four different recombinant HCV antigens (5-1-1, C 100-3, C 22, C 33), representing different structural and non-structural parts of the virus genome. The procedures were carried out according to the manufacturer's instructions. Reactive samples were retested and regarded as positive only if the duplicate test result was above the cut-off value recommended by the manufacturer and if the positive result could be confirmed by NT or RIBA. Information concerning blood transfusions and liver tests were collected from the patients' charts.

Liver biopsies

Liver biopsies were performed when the liver tests were abnormal, but usually to rule out a possible acute rejection. In addition, they were routinely obtained 1 year after LTX. The biopsies were fixed in 4% buffered formaldehyde for 3 h and embedded in paraffin. The sections were stained with haematoxylin-eosin, Ladewig's trichrome, PAS, Russian blue and reticulin.

Results

Prevalence of anti-HCV antibodies prior to liver transplantation

The occurrence of HCV antibodies before LTX and the data about blood transfusions in these liver recipients are shown in Table 2. Before LTX, anti-HCV was present in six of 71 recipients (8.5%). In addition, ELISA reactivity, which could not be confirmed by significant blocking in the neutralization assay, was detected in 10 patients with underlying liver diseases such as autoimmune chronic active hepatitis (CAH) ($n=3$), primary biliary cirrhosis (PBC) ($n=3$), post-hepatic cirrhosis (PHC) ($n=2$), sclerosing cholangitis (SCA) ($n=1$) or cholangiocarcinoma ($n=1$). None of the three patients who received transplants because of clinical evidence of acute NANB hepatitis had detectable HCV antibodies.

Incidence of anti-HCV antibodies after liver engraftment

Among the six recipients who were HCV seropositive prior to LTX, two died of septicaemia within the first month. The patients with SCA, primary hyperoxalosis (PHO) and hepatoma (patients no. 2, 3 and 6) were repeatedly HCV seropositive during the follow-up of 1.5–2 years (Table 2). Only one of the six HCV-seropositive recipients had no detectable anti-HCV during the 12-month follow-up. Two liver recipients, one who received a transplant for SCA (no. 7) and the other for toxic liver

Table 2. The occurrence of anti-HCV antibodies in patients undergoing liver transplantation (LTX)

Patient no.	Diagnosis	Transfusions (units)		Anti-HCV antibodies					
		Pre-LTX	During LTX	Pre-LTX	Post-LTX				
					1 week	3 months	6 months	12 month	2 years
1	CAH	No	21	+	+	*			
2	SCA	Yes	12	+	+	+	+	+	+
3	PHO	Yes	9	+	+	+	+	+	+
4	NANB	Yes	I 8	+	+	re-tx			
			II 46	+	+	*			
5	PBC	No	13	+	−	−	−	−	
6	Hepatoma	Yes	12	+	+	+	+	+	
7	SCA	Yes	I 11	−	−	re-tx			
			II 15	−	−	−	−	+[a]	+
8	Tox. hep.	Yes	32	−	−	−	−	−	+[b]

[a, b] Seroconversion 9 and 16 months post-transplant, respectively
CAH, chronic active hepatitis
SCA, sclerosing cholangitis
PHO, primary hyperoxalosis
NANB, NANB hepatitis with cirrhosis
PBC, primary biliary cirrhosis
Tox. hep., toxic hepatic failure
*, patient with fatal outcome
re-tx, liver retransplantation

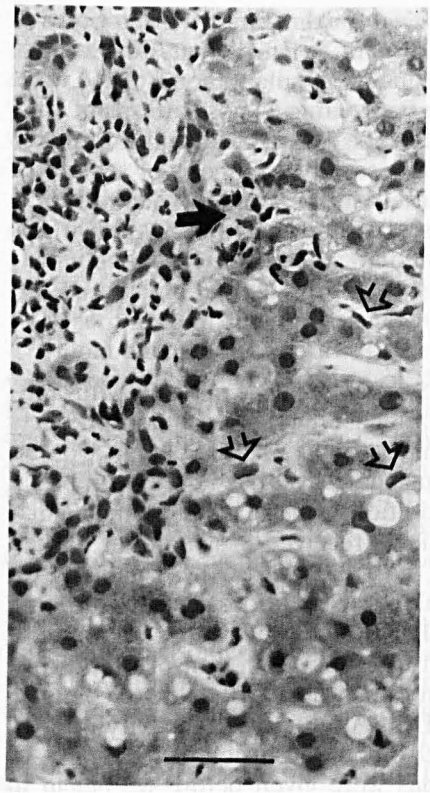

Fig. 1. Inflammatory infiltrate in the portal tract (left) and lymphocyte infiltration in the lobulus *(filled arrow)*. Note fatty changes in hepatocytes and hypertrophic Kupffer cells *(open arrows)*. Haematoxylin–eosin staining. Bar = 50 μm

failure (no. 8) seroconverted to HCV 9 and 16 months after LTX. They continued to be seropositive during the follow-up period of 4.5 and 2.5 years after LTX, respectively.

Clinical outcome in anti-HCV seropositive patients

Long-term follow-up was possible in four of six patients who were anti-HCV positive prior to LTX (Table 3). The patient with a hepatoma (no. 6) suffered from two early episodes of acute rejection requiring treatment with OKT3. His cholestatic liver tests (total bilirubin 50–70 μmol/l, ALP 6–17 μkat/l) did not normalize, nor did his increased transaminase levels (ALAT 3–6 μkat/l, GGT 3–25 μkat/l). Radiological investigations ruled out vascular or biliary complications. His liver biopsies showed neither rejection nor recurrence of the malignancy after 6 months. Nevertheless, we found a moderate lymphocytic infiltrate within the slightly widened portal tracts. Lymphocytes were also infiltrating the periportal areas of the lobuli, resembling piecemeal necrosis. Furthermore, there was a mild fatty change in the hepatocytes which was accompanied by Kupffer cell hypertrophy (Fig. 1). These changes are consistent with or indicative of NANB hepatitis. Subjectively, he felt well 18 months after LTX. The patients with SCA. PHO and PBC (Nos. 2, 3 and 5) had an uncomplicated postoperative period. They did not receive blood transfusions after the perioperative period. They had normal liver tests and were doing well 1.5–2 years post-transplantation (Table 3).

Clinical outcome in patients with primary HCV infection after transplantation

The two patients who seroconverted to HCV after LTX had been followed at the time of writing for 4.5 and 2.5 years. The patient with SCA (no. 7) received another transplant on day 11 after severe, ATG-resistant rejection and a thrombosed portal vein. His transaminase levels fluctuated (ALAT 2–10 μkat/l, ALP 2.5–4.7 μkat/l) since the time of HCV seroconversion. No signs of rejection were noted in repeated liver biopsies, but mononuclear cells were seen in the sinusoids (Fig. 2 A). Focal cobblestone appearance, mild fatty changes in hepatocytes and single hepatocyte necrosis were also observed, as well as slight cholestasis and multinucleated hepatocytes (Fig. 2 B).

The second patient who received a transplant for toxic liver failure (no. 8) had an uneventful follow-up until 9 months after LTX. Elevated liver tests (ALAT 10 μkat/l, ALP 13 μkat/l) and a low cyclosporin A concentration

Table 3. Influence of HCV on liver function tests in HCV-infected liver transplant patients

Patient no.	Diagnosis	HCV antibodies			Liver test[a]			
		Pre-LTX	Post-LTX		Bil	ALAT	PK	ALB
			6–12 months	> 12 months				
1	CAH	+	*					
2	SCA	+	+	+	21	0.3	99	31
3	PHO	+	+	+	7	0.2	130	22
4	NANB I, II	+	*					
5	PBC	+	−	−	7	0.2	130	39
6	Hepatoma	+	+	+	50	3	130	37
7	SCA I, II	−	+	+	47	10	130	42
8	Tox. hep.	−	−	+	6	20	130	38

[a] At the time of seroconversion or 1 yr post-LTX
Normal values: bil, < 26 μmol/l; ALAT, < 0.7 μkat/l; PK, 70–130%; ALB 23–42 g/l
CAH, chronic active hepatitis
SCA, sclerosing cholangitis
PHO, primary hyperoxalosis
NANB, non-A, non-B hepatitis
PBC, primary biliary cirrhosis
Tox. hep., toxic hepatic failure
*, patient with fatal outcome

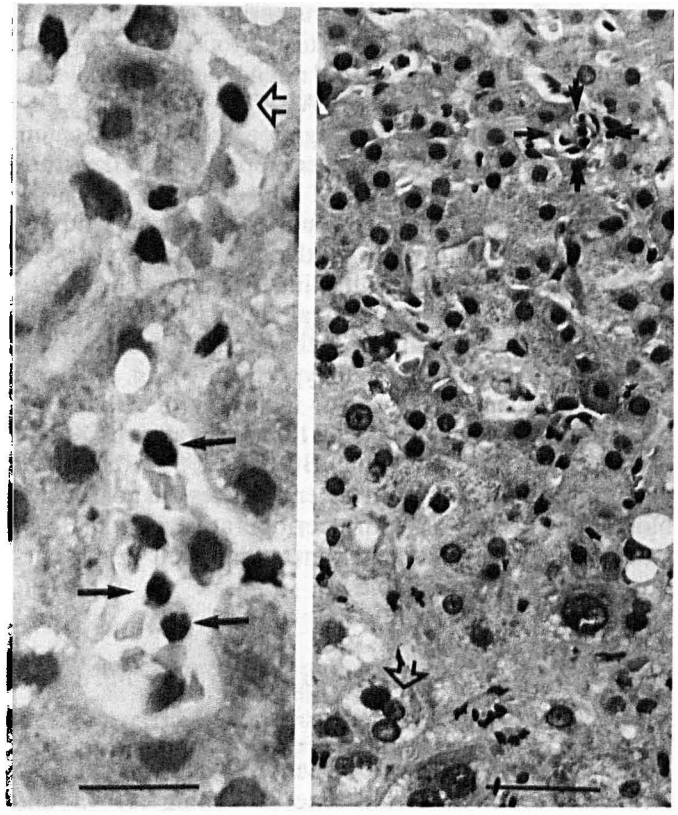

Fig. 2. **A** Lymphocytes within sinus *(thin arrows)* and hypertrophic Kupffer cell *(open arrow)*. Haematoxylin–eosin staining. Bar = 20 μm. **B** Hepatocyte necrosis *(filled arrows)*, fatty changes and multinucleated hepatocyte *(open arrow)*. Haematoxylin–eosin staining. Bar = 50 μm

were the indications for a liver biopsy which demonstrated signs of low-grade chronic rejection. After the administration of only 1 g of methylprednisolone as anti-rejection treatment the liver tests normalized. HCV seroconversion was detected 16 months after LTX. The patient had a second episode of increased transaminase levels (ALAT 20 μkat/l, ALP 26 μkat/l) 2 months previously. No biopsy was performed, as the ALAT decreased spontaneously within 3 days. His liver biopsy showed multiple foci of malignant changes 2 years after LTX, secondary to a previously diagnosed adenocarcinoma of the rectum, which subsequently led to his death (Table 3).

Patient survival

The blood levels of prothrombin complex (PK) and albumin were used to evaluate graft function. The albumin and PK levels in HCV-seropositive patients 1 year post-transplant, were 34.0 ± 7.8 g/l and 113 ± 27 %, respectively. In the anti-HCV-negative patient group, albumin was 34.2 ± 7.9 g/l and PK was 90 ± 39 %. Two of the seven patients (29 %) with HCV antibodies after LTX received second transplants within the first year. One of them died of multiple organ failure and septicaemia. One additional patient died shortly after surgery because of septicaemia. Thus, the patient survival for the anti-HCV-seropositive recipients was 71 % (five of seven patients) in the first year. In comparison, nine retransplantations (14 %) were performed during the first year in 64 post-transplant anti-HCV-seronegative patients, for reasons such as primary non-functioning graft, vascular thrombosis or severe rejection. The 1-year patient survival in this group was 69 % (40/64 patients). These differences are not significant.

Epidemiology

Blood transfusions. Six of the eight patients with anti-HCV seropositivity after LTX received blood products before liver replacement (Table 2). One patient worked as a supervisor of drug addicts, and had even been bitten by one of them. In addition to plasma and other coagulation factors, the patients received 8–46 units of packed erythrocytes during the LTX. All of these patients received additional blood transfusions during the first 8 weeks after LTX. The two patients who were found to seroconvert 9 and 16 months after transplantation received no subsequent transfusions. All of the ten patients with a non-specific ELISA anti-HCV reactivity prior to LTX had also received blood transfusions before LTX. All the blood transfusions were given before we began to screen the blood units for anti-HCV.

Donors. Serum samples from three of seven liver donors to the six patients who were anti-HCV positive before LTX and from all three donors to the two recipients who seroconverted were available (two patients had second transplants). They were all anti-HCV negative.

Discussion

The previously obscure condition called NANB hepatitis has now been elucidated, first by the specific cloning of HCV [9] and then by the development of an ELISA to detect antibodies against a major gene product (C100-3) of this virus. In addition, recombinant immunoassays now utilize four antigens [27]. HCV-RNA can also be detected by the polymerase chain reaction [16], a technique not yet routinely available.

SCA, PBC and CAH are chronic liver diseases which are frequently found among candidates for LTX [26]. In these diseases blood transfusions are often required. Patients with SCA and PBC develop gastrointestinal varices which, in the long run, often bleed. The majority of our liver transplant recipients with diagnoses of SCA or PBC had a history of blood transfusions, but HCV antibodies were found in only two of them prior to LTX. Blood transfusions are also assumed to be the primary route of infection for anti-HCV-seropositive patients with CAH, especially when associated with HBV infection [8], but sexual transmission may also occur [2]. In this study, the only one of the six recipients with post-hepatic cirrhosis who was HCV seropositive before LTX had a history of possible blood contact after being bitten by a drug addict.

The patient with subacute NANB hepatitis and a positive anti-HCV prior to LTX had received blood transfusions 20 years before when she delivered a baby. The patient with PHO had also been given several blood transfusions when undergoing haemodialysis 5 years pre-LTX.

The increased incidence of HCV seropositivity in patients with primary liver malignancy has aroused the suspicion that HCV is one of the multifactorial causes [6, 10, 24]. This would be in accordance with the pretransplant HCV seropositivity in our patient with hepatoma.

Fulminant NANB hepatitis is a predominant indication for an acute LTX [7]. Because of the fulminant course (< 8 weeks) these patients may not have had time to produce specific antibodies against HCV prior to LTX. This would accord with the reported mean interval of 15 weeks from the onset of hepatitis to anti-HCV seroconversion [3] which might explain the seronegativity in our three patients with acute liver replacement for clinical evidence of NANB hepatitis. The same authors [7] also noted a mean delay of 22 weeks for seroconversion after blood transfusions and that some patients showed fluctuations in the anti-HCV titre and even became seronegative [3]. Moreover, a liver recipient's NANB hepatitis may have been caused by a second NANB agent [4,20,28] or may have represented a cryptic form of hepatitis B infection in which serological markers are absent [5]. It is also possible that the patient was misdiagnosed as having NANB hepatitis but in fact had a non-viral hepatocellular inflammation.

Seroconversion from HCV negativity prior to LTX to seropositivity may be caused by virus transmission by the transplanted organ or by perioperative blood transfusions. None of the donors to our patients who became seropositive after LTX had detectable HCV antibodies. Therefore, blood transfusions remain the most likely source. In Scandinavia around 0.5 % of blood donors have proved to be anti-HCV seropositive (Dr A. Lindholm, personal communication), which is still a considerable risk. The seroconversion in two patients, 9 and 16 months post-LTX, agrees with reports from other LTX centres [15, 21], but occurs later than in non-immunocompromised patients (12–22 weeks), according to studies of post-transfusion NANB hepatitis [3, 13]. Two patients continued to be seropositive after LTX and had normal liver tests (Tables 2 and 3). Another patient, who was also HCV seropositive before LTX, had a primary HCV infection in the graft as judged by changes in the liver biopsy and by persistent slightly pathological liver tests, at the time of a recurrence of seropositivity (Fig. 1). A similar low-grade effect of an HCV infection in LTX recipients was reported by Read et al [22]. The last pre-LTX seropositive patients who received transplants because of PHC and known NANB hepatitis had been followed for too short a time (1 month) to determine the effect on the graft. The patients who had a primary HCV infection concomitantly had increased transaminase levels at the time of seroconversion.

Unlike HBV infection in the liver graft, HCV infection did not cause severe liver degenerative disease, a finding also reported by others [17, 22]. Furthermore, HCV seemed not to influence graft function in the patients when liver function tests and the frequency of retransplantations were taken into account.

False positive reactivity in anti-HCV ELISA tests was found particularly among samples drawn before LTX. It has previously been shown that patients having autoimmune CAH with high serum immunoglobulin levels may react in a non-specific way in anti-HCV ELISA tests [23], a finding also made in this study. Furthermore, it has been demonstrated that disease activity and immunoglobulin levels in patients with autoimmune CAH are correlated to the reactivity in the anti-C-100-3 region of HCV [19]. Our patients were probably in such a condition when accepted as candidates for LTX.

We conclude that the introduction of anti-HCV testing of blood and organ donors should reduce the risk of hepatitis caused by HCV. In liver tansplant patients such testing should be helpful for distinguishing hepatic inflammation from rejection. In view of the limited size of our sample, seropositivity to HCV does not preclude LTX. However, this point should be re-evaluated by the addition of HCV-RNA assay, which should show the true incidence of HCV in this patient population.

Acknowledgements. Ms. Helene Norder, Berit Hammas, Ulla Magnusson and Pia Andersson are thanked for their skilful technical assistance and Dr. Zoe Walsh and F. P. Walsh for the revision of the language.

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