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Idiopathic post-transplantation hepatitis following living donor liver transplantation, and significance of autoantibody titre for outcome

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Summary

Idiopathic post-transplantation hepatitis (IPTH) is a common histology occurring late after liver transplantation. Its natural history and the effect of treatment have not been determined. This study is a matched case–control study that evaluates predictors, outcome and response to treatment for IPTH. Patients were divided by autoantibodies into high-titre (≥1:160) and low-titre (<1:160) groups, so as to evaluate clinicopathological differences between the two groups. IPTH was identified in 42 of 944 recipients (4.4%) with tacrolimus-based immunosuppression. They comprised 10 males and 32 females, having median age 6.0 (0–50) years. IPTH presented at a median duration of 5.2 (0.7–10.8) years after transplantation. Particular risk of IPTH was associated with acute rejection, late-onset acute rejection occurring later than 6 month post-transplant, and autoantibody positivity. IPTH was associated with dependence on steroids and frequent adverse outcomes: retransplantation in five (12%); relapse in four (9.5%); and progression of fibrosis in eight (19%). The high-titre group and low-titre group did not differ in their clinicopathological features, response to treatment or outcome. To prevent the development of IPTH, appropriate adjustment of immunosuppression and close follow-up is necessary for patients who suffer repeated episodes of rejection.

Introduction

Late graft dysfunction poses a significant risk for graft loss in long-term survivors of liver transplantation (LT). Histological features of chronic hepatitis (CH), which is interface hepatitis with portal lymphoplasmacytic infiltrates, are common after LT [1]. Post-transplantation CH of unknown aetiology (idiopathic post-transplantation hepatitis, IPTH) is a common late complication after LT. It is found in 64% of transplantations after 10 years in an asymptomatic paediatric population [2] and 32% in adults [3].

According to several studies, the classical biochemical, serological and histological features of autoimmune hepatitis (AIH) can develop in patients transplanted for diseases other than AIH. The condition has been referred to as *de novo* AIH, post-transplantation immune hepatitis, or hepatitis mimicking AIH [4–12]. As with non-LT patients, diagnosis of *de novo* AIH involves biochemical and histopathological criteria conforming to the international AIH group diagnostic criteria [4,13]. Typical histological abnormalities, such as CH, are insufficient for diagnosis of *de novo* AIH in the absence of autoantibodies and elevation of immunoglobulin G (IgG) [1,13]. It is uncertain whether these CH patients develop the full clinical scenario of *de novo* AIH. As positive autoantibodies are common in stable children after LT [10,14] and the prevalence of positive-autoantibody titres increases with

time post-transplant [14], the issue of whether the same criteria should be applied to non-LT and post-LT patients remains unsettled. In the subgroup of patients with histological CH, the outcome and response to treatment are poorly understood. In this study, patients histologically exhibiting CH of unknown cause were sought and their clinico-pathological features were assessed along with predisposing factors and the long-term outcome of CH. The presence of autoantibodies is strongly associated with unexplained CH after LT [1,2]. Significant autoantibody titres are required for diagnosis of *de novo* AIH. We therefore divided the CH patients into two groups as significant autoantibody titres (≥1:160) and less significant titres (<1:160); the threshold is chosen because autoantibody titres ≥1:160 are unlikely to be nonspecific background reactivities [1]. The high-titre group may represent de novo AIH. To characterize the natural history, predictors and response to treatment of CH, we also determined the differences in clinicopathological features between the two groups.

Patients and methods

Study population

From 1990 to July 2006, 1185 living donor liver transplantations (LDLT) were performed at our institution. Of these, 944 Japanese patients (425 men and 519 women) survived for at least 6 months following LDLT. Table 1 shows their indications for LDLT. The mean age at LDLT was 20.1 years (range 0–69; median 9). Left lobe grafts were used in 140 patients, lateral segment in 411, monosegment in 32 and right lobe in 361 patients. The graft was ABO identical in 642 patients, ABO compatible in 180 and ABO incompatible in 122. The mean duration of post-transplantation follow-up was 6.0 years (range 0.5–16.1; median 5.4) and exceeded 5 years in 427 patients.

Table 1. Indications for transplantation in 944 patients.

Biliary atresia	421
Liver cirrhosis	221
Hepatitis C	100
Hepatitis B	93
Alcoholic	9
Unknown	19
Primary biliary cirrhosis	44
Fulminant hepatic failure unknown aetiology	37
Congenital metabolic disease	30
Wilson's disease	29
Primary sclerosing cholangitis	25
Alagille syndrome	20
Hepatoblastoma	18
Progressive intrahepatic cholestasis	12
Autoimmune hepatitis	4
Retransplantation	31
Others	52

Laboratory data

Postoperative clinical data were collected retrospectively for each transplant patient. Laboratory data at the time of diagnosis of CH after LDLT and during follow-up include the following variables: serum aspartate aminotransferase (AST, normal 13–29 IU/l), alanine aminotransferase (ALT, 8–28 IU/l), gamma-glutamyltranspeptidase (GGT, 9–54 IU/l), alkaline phosphatase (ALP, 118–335 IU/l), total bilirubin (T-Bil, 0.2–1.0 mg/dl) and IgG (788–1841 mg/dl). Serum autoantibodies were analysed by indirect immunofluorescence. A positive autoantibody was defined as titres ≥1:40 in adults and ≥1:20 for antinuclear antibody/anti-smooth muscle antibody, or ≥1:10 for anti-liver-kidney microsomal antibody in children, based on the diagnostic criteria for AIH given by the International Autoimmune Hepatitis Group [13].

Human leucocyte antigen (HLA) typing for recipients and donors was undertaken by serological techniques for both class I and class II antigens, as reported previously [9].

Treatment

Full details of the standard immunosuppression used have been described previously [9]. The primary immunosuppressive regimen was a combination of tacrolimus (Tac) and prednisolone (PSL). The maintenance immunosuppressive regimen consisted of Tac monotherapy. PSL was restarted and was continued for longer in patients with CH including those who fulfilled the criteria for AIH, until normal liver function levels were attained [13]. Patients who did not respond to the treatment received mycophenolate mofetil (MMF), azathioprine (AZP) or mizoribine (MZR). Therapy was continued until remission took place, specified by improved serum transaminase levels and disappearance of interface hepatitis from liver tissue [15]. Patients had their PSL dose slowly reduced, while serum transaminase levels remained normal. Relapse was defined as recrudescence of histological inflammatory activity after induction of remission and withdrawal of medication; it warranted the resumption of steroids [16].

Histopathological evaluation

A retrospective review was undertaken of all liver post-transplant biopsies with morphological CH. Liver biopsies were performed when indicated clinically. Morphological characteristics were assessed using H&E and Masson's trichrome. All liver biopsy specimens were interpreted by pathologists (AM-H, and HH).

Definition of idiopathic post-transplantation hepatitis

Idiopathic post-transplantation hepatitis was defined as CH that cannot be ascribed to a specific cause. The features used in diagnosing CH were the presence of predominantly mononuclear portal inflammatory infiltrate associated with inflammatory spillover into periportal zones and/or parenchymal inflammation (Fig. 1a and b). Varying degrees of lobular inflammation, with hepatocyte necrosis or apoptosis, were often present (Fig. 1c). Other histological features included portal expansion with ductular reaction and perivenular inflammation (Fig. 1d); however, lymphocytic bile duct damage and endothelitis, which are features of acute cellular rejection (ACR), should be absent [1]. Virus-induced or drug-related hepatitis was excluded. Viral hepatitis was excluded by performing serology for hepatitis B, C, cytomegalovirus and Epstein–Barr virus. Any post-transplant complications including biliary stricture and vascular complications should be resolved by surgical repair at the diagnosis of CH. Recipients undergoing LDLT were excluded for which the indications are potentially recurrent diseases including hepatitis B, C, AIH, primary biliary cirrhosis, primary sclerosing cholangitis and non-alcoholic steatohepatitis [1].

Histological scoring for idiopathic post-transplantation hepatitis

Index diagnostic biopsies and all subsequent biopsies were reviewed for the presence and degree of necroinflammatory activity, character of fibrosis and infiltrate. The severity of

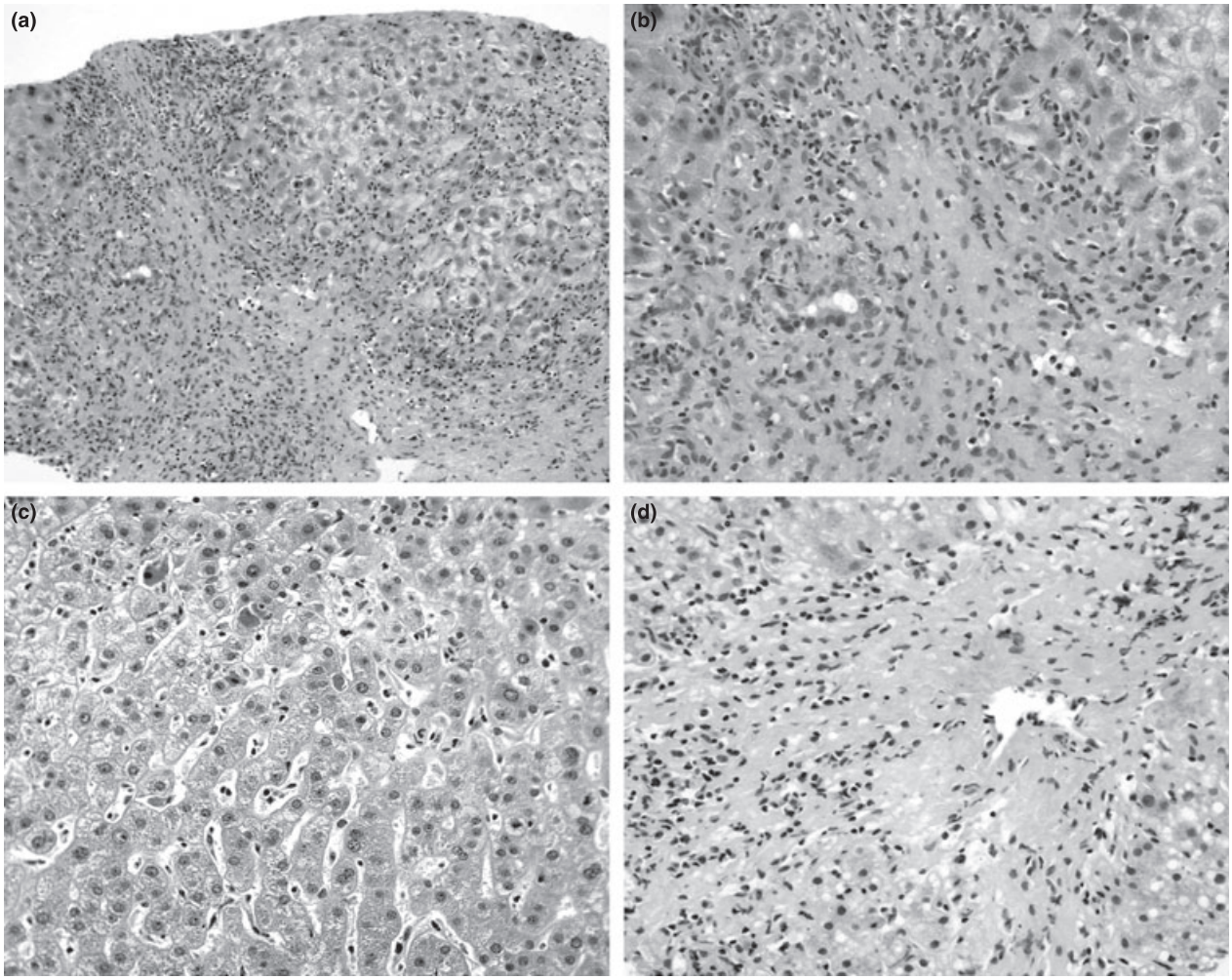

Figure 1 Liver allograft biopsy 5.4 years after transplant from a recipient who underwent liver transplantation at age 26 years for biliary atresia. (a) Chronic hepatitis with interface activity (H&E × 100). (b) Irregular interface between parenchyma and portal tract (×200). (c) The hepatic lobule is infiltrated by lymphocytes. Acidophil bodies are visible (×200). Liver allograft biopsy 1.9 years after transplantation from a 0-year-old girl with biliary atresia. (d) Perivenular mononuclear inflammation is present with lack of endothelitis (×200).

interface activity and lobular necroinflammation were graded semiquantitatively on a scale of 0–3 according to the METAVIR scoring system. Fibrosis was also quantified on a scale of 0–4: 0 = no fibrosis, 1 = periportal fibrosis without bridging, 2 = bridging fibrosis, 3 = numerous bridges and septa, 4 = cirrhosis [17]. The distribution of histological activity and fibrosis stages was assessed at presentation and at approximately 1, 3, 5, 7 and 10 years after onset of CH.

We took into account demographic data, interval after transplant, clinical data at the time of graft dysfunction and at last follow-up, history of ACR, chronic rejection, viral status, biliary and vascular complications, response to treatment and outcome. For patients who required retransplantation for graft failure secondary to CH, the day for retransplantation was taken as the end of follow-up. ACR was diagnosed according to the Banff classification [18]. Early ACR was defined as graft dysfunction accompanied by histology consistent with ACR within 6 months of transplantation. Late-onset ACR (LAR) was defined as ACR showing later than 6 months after transplantation. LAR may be present as isolated perivenular inflammation and hepatocyte dropout [19–21].

Analysis of predictors for CH

Each recipient in the chronic idiopathic hepatitis group was matched, for age (±5 years), gender, original disease and follow-up period (±2 years) after LDLT, with two recipients from the nonidiopathic CH group. Two controls for each patient were available in 38 of 42 patients; the remaining four patients were excluded from this analysis because of our inability to match the original disease and age. As a result, 76 controls were chosen for comparison from the database in Kyoto University Hospital between 1990 and 2006. In comparison with the matched controls, we determined which of the following factors are predictive of the development of CH: donor age, donor gender, ACR, the number of ACR episodes, LAR, gender mismatch, ABO blood type mismatch, mismatches at the HLA-A, B and DR loci, proportion of cases matched for HLA-DR4 in the donor and recipient, autoantibody positivity, IgG level, presence of bile duct stenosis and vascular complications.

High-titre group versus low-titre group

The CH patients were divided into two groups, as significant autoantibody titres (≥1:160), and less significant titres (<1:160). We compared demographic data, interval after transplant, laboratory data at the time of graft dysfunction and at last follow-up, response to treatment and outcome.

Statistical analyses

The unpaired Student *t*-test or Mann–Whitney's *U*-test were used to compare continuous variables; summary data are presented as mean ± SD in the tables and text. Repeated measure analysis of variance (ANOVA) was used to analyse the means of liver function tests (LFTs) and autoantibody titre over time. The chi-squared test or Fisher's exact test were used to compare frequencies of categorical variables. Univariate logistic regression modelling was used to investigate factors associated with CH. Results are presented as a point estimate of the odds ratio, with 95% confidence intervals. Statistical analysis was performed using STATASE 9.0 (Stata corporation, Texas, TX, USA). A *P*-value smaller than 0.05 was taken as statistically significant.

Results

Histological CH with aetiology other than viral hepatitis was found in 45 patients. Three patients suffered recurrence of the original AIH for which LDLT was performed. These three were excluded from the study. CH was found in at least one biopsy specimen in 42 patients out of 944 recipients (4.4%). These 42 comprised 10 males and 32 females, with a mean age of 8.8 years (range 0–50; median 6.0) at LDLT. CH presented at a mean of 5.2 years (range 0.7–10.8; median 5.2) after LDLT. These patients were transplanted for biliary atresia (BA) ($n = 33$), Wilson's disease ($n = 2$), fulminant hepatic failure of unknown aetiology ($n = 2$) and one each for tyrosinemia, progressive familial intrahepatic cholestasis type 1, congenital biliary dilatation, a second graft for hepatic vein stenosis and a third graft for chronic rejection (both original disease BA). All were detected on the basis of abnormal LFTs.

Of these 42 patients, five (12%) were at least 18 years old at LDLT. There were four females and one male, with a mean age at LDLT of 33 (range 22–50; median 26). Their original diseases were fulminant hepatic failure of unknown aetiology ($n = 2$), BA ($n = 2$), and congenital biliary dilatation. CH was seen in five of 371 (1.3%) liver transplant recipients over 18 years old compared to 37 of 573 (6.5%) aged 18 or less ($P = 0.0002$).

Clinical and laboratory findings

At presentation, we found elevated AST (294 ± 323), ALT (294 ± 316), GGT (182 ± 183), ALP (1060 ± 615) and T-Bil (1.8 ± 2.4). Autoantibodies were detectable in 29 of 40 patients (73%) at the diagnosis of CH (antinuclear antibody positive in 28 and anti-smooth muscle antibody positive in 1; range 1:40–1:640). The patients had mean

raised IgG levels of 1988 ± 790 mg/dl. The mean interval from onset of CH to last follow-up was 4.5 years (range 0.1–13; median 3.7). There were significant differences in laboratory features at presentation and at last follow-up (AST 54 ± 53, ALT 70 ± 122, ALP 553 ± 294; all $P < 0.0001$), except for GGT (127 ± 166, $P = 0.1720$) and T-Bil (4.0 ± 10.5, $P = 0.2714$). When last tested, autoantibodies remained positive in 12 of 29 patients (41%) after treatment for CH (range 1:40–1:320) ($P = 0.091$). The mean IgG levels fell significantly to 1468 ± 476 ($P < 0.0001$).

Outcome and treatment

Table 2 shows the histological outcome for CH patients. Five developed cirrhosis and required retransplantation, at a mean interval of 4.9 years (range 1.4–7.9; median 5.0) after the diagnosis of CH. Four patients who entered remission had a relapse after drug withdrawal. The follow-up period after CH was significantly longer in patients who had relapsed (mean 9.5 years; range 7.8–13.0; median 8.6 years vs. others, $P = 0.0003$). Four patients without subsequent biopsies were followed up for a mean of 1.5 years (range 0.1–3.5; median 1.3) after onset; they showed an improvement with normalization of LFTs. Twenty-nine had serial biopsies, with progression of fibrosis in eight and improvement of fibrosis in 21.

Six patients had either a low maintenance dose of Tac or had discontinued Tac at the time of CH. Immunosuppression was based on Tac in all patients, except for one on a cyclosporine (CsA)-based regimen because of concomitant administration of anticonvulsant. Triple immunosuppression was used after the diagnosis of CH in 25 patients. Fourteen patients were treated with PSL in combination with Tac. Three were given a higher dose of Tac alone after graft dysfunction. After the mean of 4.5 (range 0.1–13.0; median 3.7) years after CH, 3 remained on Tac monotherapy. The mean follow-up period for these three was 1.9 years (range 1.4–2.3; median 2.0). Four others had discontinued PSL, of which three had been on Tac alone and one on Tac/MMF combination, after a mean interval of 5.9 years (range 3.5–7.8; median 6.2). The remaining 29 patients had been on PSL for a mean of 4.5 years (range 0.1–13.0; median 3.4); see Table 3. Of the five patients who required retransplantation, three were on PSL/Tac combination and two on triple therapy (PSL/Tac/AZP and PSL/Tac/MZR). Figure 2 shows the

Table 2. Outcome of CH patients ($n = 42$).

	No. of patients	Median follow-up years after CH (range)
Retransplantation	5 (12)	5.0 (1.4–7.9)
Relapse	4 (9.5)	8.6 (7.8–13.0)
No follow-up histology	4 (all biochemical response)	1.3 (0.1–3.5)
With follow-up histology	29	
Progression of fibrosis	8 (19)	3.1 (0.3–6.2)
Improvement of fibrosis	21 (50)*	3.2 (0.1–7.8)

Values in parentheses are percentages.
*Three died of causes other from chronic hepatitis (CH).

Table 3. Immunosuppressive regimen at diagnosis and at last follow-up.

	At diagnosis of CH ($n = 42$)	At last follow-up ($n = 36$)
Tac	3 (7)	6 (17)
Tac, PSL	14 (33)	9 (25)
Tac, PSL, MMF	9 (21)	6 (17)
Tac, PSL, AZP	9 (21)	9 (25)
Tac, PSL, MZR	6 (14)	4 (11)
CsA, PSL, MMF	1 (2)	1 (3)
Tac, MMF	0	1 (3)

Five patients who resulted in retransplantation for CH were excluded from those at last follow-up. Medication unknown in one at last follow-up.
Values in parentheses are percentages.
AZP, azathioprine; CsA, cyclosporine; MMF, mycophenolate mofetil; MZR, mizoribine; PSL, prednisolone; Tac, tacrolimus.

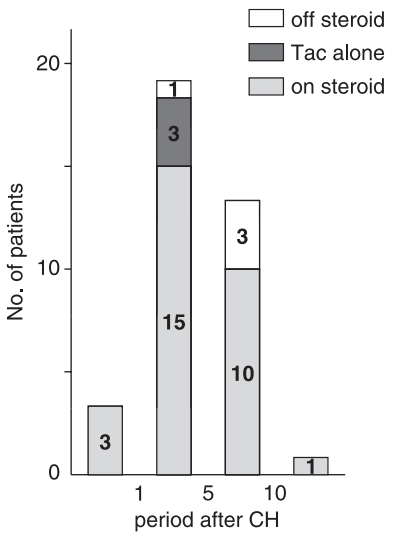

Figure 2 Last treatment involving steroids for chronic hepatitis (CH) patients at different follow-up periods after diagnosis of CH. Off steroid indicates patients who were weaned off of steroids at last follow-up. Tacrolimus (Tac) alone indicates patients who had been on Tac monotherapy and never used steroids during the course.

last treatment at differing follow-up times after CH (<1 year, 1–5, 5–10, >10 years). Long-term administration of PSL was needed in CH patients.

Histological findings

Figure 3 shows the distribution of histological activity and fibrosis stages at presentation and at approximately 1, 3, 5, 7 and 10 years after onset of CH, quoted as mean ± SD. The severity of activity decreased significantly between onset and 1 year after diagnosis with treatment (at onset 1.9 ± 0.7 vs. 1 year 1.3 ± 1.0, $P = 0.014$). There was also a decrease in activity scores between onset and at each follow-up point (at onset vs. 3 years 1.4 ± 0.9 years, $P = 0.063$; vs. 5 years 1.2 ± 0.6, $P = 0.060$; vs. 7 years 1.1 ± 0.6, $P = 0.049$; 10 years 1.1 ± 1.1, $P = 0.038$). The mean histological activity scores remained low in each interval after 1 year.

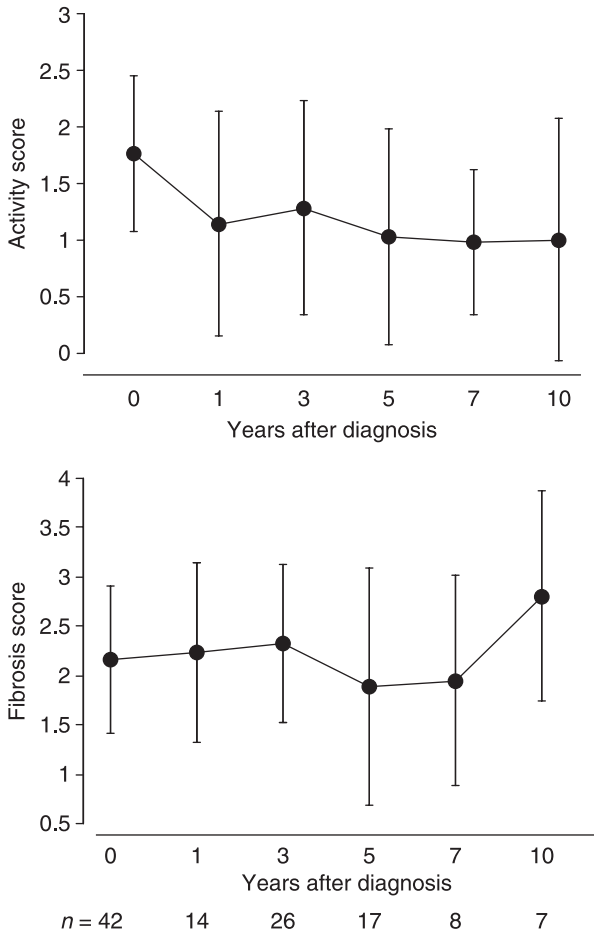

Figure 3 Evolution of activity and fibrosis scores at diagnosis of chronic hepatitis and at subsequent time points (mean ± SD). The number of patients in each point is indicated. There was a decrease in activity scores between onset and at each follow-up point. There is no significant difference in fibrosis scores at onset and at each time point.

Fibrosis persisted for years; there was no significant difference in the distribution of fibrosis stages between the time of diagnosis and at 1, 3, 5, 7 and 10 years in follow-up (2.2 ± 0.8, 2.3 ± 0.9, 2.4 ± 0.8, 1.9 ± 1.2, 2.0 ± 1.1, 2.9 ± 1.1, respectively; $P > 0.05$).

Factors predicting the development of CH

Table 4 shows the clinical characteristics of cases and controls. No differences were observed with regard to age and gender of the donors.

Table 5 shows the statistical analysis for factors predicting the occurrence of CH. The following were all more frequent in CH group than in controls: ACR episodes, the number of ACR episodes, early ACR episode, LAR episode, autoantibody positivity and high IgG level. ACR was identified in 31 of 38 (82%) CH patients and LAR in 21 of 38 (55%). The result shows that the risk of CH was nearly 2.8 times higher in patients with episodes of ACR [OR 5.5 (95% CI 2.14–13.96), $P < 0.0001$], and four times higher with episodes of LAR [OR 4.0 (95% CI 1.74–9.13), $P = 0.001$], than in those with no episodes of ACR. We found a higher risk of CH in patients who had positive autoantibody with an odds ratio of 5.2 [(95% CI 1.94–13.96), $P = 0.001$]. An IgG level above 2037 mg/dl gives a 13 times greater risk of developing CH.

There were no significant differences between the CH group and controls for blood type compatibility, gender mismatch, total number of HLA-mismatches (HLA-A, -B,

Table 4. Characteristics of recipients and donors with CH and of controls.

	CH group ($n = 38$)	Controls ($n = 76$)	*P*
Donor parameters			
Age (mean ± SD, year)	35.5 ± 7.5	36.7 ± 8.0	0.467
Male:female	21:17	33:43	0.233
Recipient parameters			
Age (mean ± SD, year)*	7.61 ± 1.5	7.63 ± 0.99	–
Male:female*	8:30	16:60	–
Cause of LDLT*			
Biliary atresia	33 (87)	66	
FHF unknown	1 (2.5)	2	
PFIC type1	1	2	
Tyrosinemia	1	2	
Wilson's disease	2 (5)	4	

Values in parentheses are percentages.
CH, chronic hepatitis; FHF, fulminant hepatic failure; LDLT, living donor liver transplantation; PFIC, progressive familial intrahepatic cholestasis.
*Matching factor.

Table 5. Factors predicting the development of CH.

	CH (n = 38)	Control (n = 76)	Odds ratio (95% CI)	P
Acute cellular rejection	31 (81.6)	34 (44.7)	5.5 (2.14–13.96)	<0.0001
Number of ACR episode	1.47 ± 1.08	0.67 ± 0.89		
0	7 (18.4)	42 (55.3)	1 (reference)	–
1	14 (36.8)	21 (27.6)	4.0 (1.40–11.40)	0.009
2	11 (29.0)	9 (11.8)	7.3 (2.23–24.11)	0.001
≥3	6 (15.8)	4 (5.3)	9.0 (2.01–40.21)	0.004
Early ACR	21 (55.3)	24 (31.6)	2.7 (1.20–5.97)	0.016
LAR	21 (55.3)	18 (23.7)	4.0 (1.74–9.13)	0.001
Gender mismatches	20 (52.6)	32 (42.1)	1.5 (0.70–3.34)	0.289
Blood type compatibility				
Identical/Compatible	36 (94.7)	68 (89.5)	1 (reference)	–
Incompatible	2 (5.3)	8 (10.5)	0.47 (0.09–2.34)	0.358
HLA A, B, DR mismatches				0.384
0–1	4 (10.5)	5 (6.6)	1 (reference)	–
>1	33 (86.9)	65 (85.5)	1.6 (0.29–7.84)	0.42
HLA DR mismatches				
0	6 (15.8)	10 (13.2)	1 (reference)	–
1–2	31 (81.6)	60 (78.9)	0.86 (0.29–2.59)	0.79
Autoantibody				
Negative	10 (26.3)	26 (34.2)	1 (reference)	–
Positive	26 (68.4)	13 (17.1)	5.2 (1.94–13.96)	0.001
Bile duct stenosis	10 (26.3)	14 (18.4)	1.58 (0.63–3.99)	0.332
Vascular complication	5 (13.2)	13 (17.1)	0.73 (0.24–2.24)	0.587
IgG (mean, range) mg/dl	1968 ± 791	1455 ± 515		0.002
988 (368–1208)	5 (13.2)	13 (17.1)	1 (reference)	–
1575 (1216–1995)	16 (42.2)	20 (26.4)	2.1 (0.61–7.07)	0.241
2706 (2037–3740)	15 (39.5)	3 (4.0)	13 (2.59–65.2)	0.002

Values in parentheses are percentages.
ACR, acute cellular rejection; LAR, late-onset acute cellular rejection.

-DR loci), HLA DR-mismatch, bile duct stenosis and vascular complication. No combinations of HLA-DR4 status between donor and recipient were associated with a statistically significant increased risk of CH.

Influence of autoantibody titre in the development of CH

Table 6 shows the clinical characteristics of the two groups. Two patients were excluded because their serum autoantibody data were not available. Seven of 40 patients were found to be in the high-titre group, and 33 in the low-titre group. No statistically significant difference was found in demographic data, the time of diagnosis of CH or the follow-up period.

The autoimmune titres at the time of diagnosis of CH and at last follow-up are shown for the two groups in Fig. 4. Autoantibody positivity in the low-titre group was 22 of 33 (67%) at the diagnosis of CH and six of 23 (26%) at last follow-up. Based on repeated measures ANOVA, the distribution of the autoantibody titre at diagnosis decreased significantly with treatment down to the last follow-up in both groups (high-titre group, $P = 0.0005$; low-titre group, $P = 0.0002$). Six patients in the high-titre group had positive autoantibodies at last follow-up, compared with six of 23 patients (26%) in the

Table 6. Clinical characteristics of CH patients with or without significant titres.

	CH with autoantibodies ≥1:160 (high-titre group, n = 7)	CH with autoantibodies <1:160 (low-titre group, n = 33)	P
F:M	5:2	25:8	0.81
Mean age at LT	5.7 ± 3.7	8.5 ± 9.8	0.47
Time from LT to CH (mean, year)	6.0 ± 3.0	4.9 ± 3.1	0.39
Follow-up after CH (mean, year)	3.5 ± 1.7	4.9 ± 3.1	0.24

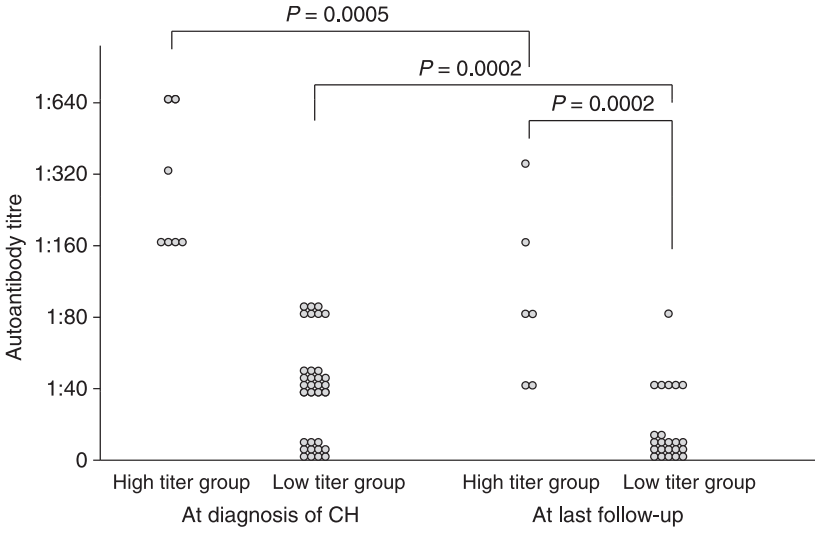

Figure 4 The distribution in autoantibody titres at diagnosis of chronic hepatitis (CH) and at last follow-up (mean follow-up: 4.6 years), in high-titre (autoantibody ≥ 1:160) and low-titre (autoantibody < 1:160) groups.

low-titre group (high-titre group vs. low-titre group, $P = 0.002$). At the last follow-up, the distribution in the titre was still significantly higher in the high-titre group than in the low-titre group ($P = 0.0002$).

There was no significant difference in LFTs, either at the time of diagnosis of CH or at last follow-up, between the high-titre and low-titre groups, except for GGT at last follow-up (high-titre group, 295 ± 362 vs. low-titre group, 100 ± 78; $P = 0.008$).

The mean IgG levels at diagnosis were 1693 ± 837 in the high-titre group and 2050 ± 778 in the low-titre group, with no significant difference between these ($P = 0.28$). No differences were apparent between the two groups for outcome, including relapse, development of cirrhosis and need for retransplantation or improvement or progression of fibrosis.

No association was found in the type of immunosuppression used for treatment at the onset of CH or at last follow-up.

Discussion

Most native diseases have the potential to recur with morphological features of CH [1,22]. No obvious cause for CH was identified in our study, since we clinically excluded drug toxicity and known acquired viral infection and also the potential recurrent causes of native disease. Whatever remains can be called IPTH [1,2].

This study had the limitation that protocol biopsy was not available. We identified CH in 4.4% of symptomatic recipients; a higher prevalence of CH has been reported in asymptomatic children who were on CsA-based immunosuppression and underwent protocol biopsies. Their frequency of CH is 20% at 1 year, rising to 60% at 10 years, suggesting that subclinical CH is present [2]. Consequently, protocol biopsies may be required to detect CH. The significance of treatment in the early stage of this dysfunction is not clear for asymptomatic patients [3], but CH leads to cirrhosis in some cases and adherence to medication may be important in maintaining good graft function.

Optimal management of post-transplant CH is not settled. CH patients were difficult to maintain on Tac monotherapy and required other medication including PSL, AZP or MMF. Although the retrospective nature of the study did not allow clear recommendations regarding treatment, conventional steroid regimens were able to achieve resolution of the disease. CH patients remained dependent on steroid to maintain normal graft function for many years [12]. The follow-up period after CH was significantly longer in patients who had relapsed. Repeated relapse and retreatment might be associated with a poor long-term prognosis, as with non-LT patients [15]. Adjustment of immunosuppression and longer maintenance with steroid was necessary. Because of side effects of steroid, alternative treatment strategies should be considered, including MMF. Combination with rapamycin might reduce the dosage in CH patients who have been on steroid for a long period [23].

The relationship between de novo AIH and IPTH remains unclear, although they share similar clinical and histological features as well as responses to steroid therapy [1]. The minimal diagnostic criteria proposed by the Banff Working Group for *de novo* AIH in an allograft are: (i) histological CH; (ii) significant titres (≥1:160) of autoantibodies; (iii) hyper-gammaglobulinemia; and (iv) exclusion of virus-induced or drug-related hepatitis and rejection [1]. Progression to *de novo* AIH from IPTH has been reported in four of 158 children, and in two of 30 unexplained CH in adults [2,3]. High antinuclear

antibody titres (>1:1600) are associated with progressive fibrosis in CH [3], but we found no differences between the high-titre and low-titre autoantibody groups in any of the parameters investigated or in the response to treatment; steroids were important in both groups to maintain remission. The presence of autoantibodies and elevated IgG did not influence the medication regime. Even where it does not conform to the diagnosis of *de novo* AIH, the severity of fibrosis associated with CH increases with time [2,3]. CH and de novo AIH may simply be different parts of the spectrum of a single entity. Our study shows that the Banff criteria were not accurate for the diagnosis of *de novo* AIH. The autoantibody titre was irrelevant for the diagnosis of the disease, which must be suspected promptly when histologically interface hepatitis is present without waiting for the autoantibody titre to rise. It responds well to modified treatment, but if untreated, the prognosis can be severe.

The relationship between ACR, LAR, and CH and the nature of these processes remains uncertain. LAR may display slightly different features from typical ACR soon after transplantation. Increased interface activity, less venous subendothelial inflammation and greater lobular activity all cause LAR to resemble CH [1]. Approximately 80% of children who developed *de novo* AIH after LAR had autoantibodies at the time of LAR and five of 20 children with LAR progressed to de novo AIH at 9–48 months after LAR episodes [19]. As these graft dysfunctions share some biochemical and histological features, CH could be related to alloimmune response against graft antigens. This hypothesis is supported by the observation that previous ACR increases the risk of CH, together with the good response to additional immunosuppression with steroids. CH may involve a characteristic time course of rejection: from typical ACR, with endothelitis occurring early post-transplant, then LAR with more hepatitic features and less subendothelial inflammation, to hepatitic form in CH/*de novo* AIH occurring late after transplantation. The sinusoidal endothelial cells of the liver are the interface between donor and recipient cells and prevent recipient passenger leucocytes from coming into direct contact with hepatocytes. Endothelial cells in the blood vessels are believed to be a major target for graft rejection [24]. The repeated alloimmune response against endothelial cells may cause endothelial damage and render T cells accessible to hepatocytes [25]. A recent study has found antibodies against cytoplasmic filaments of hepatocytes, specifically cytokeratin 8/18, in a patient with *de novo* AIH [26]. Some *de novo* AIH has been attributed to transplantation of a glutathione *S*-transferase T1 positive graft into a negative recipient [27]. These studies suggest that the immune response observed in *de novo* AIH was directed against hepatocytes in the graft. The repeated alloimmune response against endothelial cells may accelerate replacement of allograft endothelial cells with cells of recipient type [28]. The proportion of endothelial cells of recipient origin increases with time in liver allografts [29]. These findings suggest that endothelial cells lose their antigenicity with time and that the target of rejection may shift from endothelial cells to donor hepatocytes in liver allografts.

In conclusion, our findings emphasize the existence of a clinically significant subset of patients with CH of unknown aetiology after LDLT. Distinction between CH and *de novo* AIH does not seem to be meaningful. CH resulted in progression to fibrosis with its associated graft loss. Repeated episodes of rejection, LAR and positive autoantibodies confer particular risk for CH. Appropriate adjustment of immunosuppression and close follow-up is necessary for patients who suffer repeated episodes of rejection to prevent the development of CH.

Authorship

AMH: designed and performed the study, collected data and wrote the paper. HH: designed study, collected data and contributed important reagents. HE: collected data and contributed important reagents. YH: analysed data. SU and TM: contributed important reagents.

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