

Idiopathic Post Liver-Transplant Hepatitis Remains Obscure with Respect to Its Etiology and Relationship with Immunosuppression

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ABSTRACT

AIM: Idiopathic post-liver transplant hepatitis (IPTH) can lead to late graft fibrosis. The treatment for IPTH is not clearly delineated. We aimed to approach its pathophysiology and predictive factors for fibrosis progression (FP).

METHODS: Patients whose IPTH was diagnosed in 2006 and had undergone at least one subsequent liver biopsy (LB) were included.

The index LBs were reviewed and correlated with clinical, laboratory, C4d immunostaining retrospectively performed, and histological data on the most recent LBs.

RESULTS: Among the 299 post-transplant LBs, 37 presented IPTH. The index LBs mostly displayed mild fibrosis (65%) and activity (67.5%), and non-significant C4d immunostaining (i.e., weak, focal and/or portal stroma staining, 21.6%). Liver tests were normal in 46% of patients. Virological markers including Hepatitis E were negative. Antinuclear auto-antibodies were present concurrently in four patients and appeared later in two patients, together with features of autoimmune hepatitis (AIH) in one. Isolated AIH features appeared later in one patient. One patient displayed AIH features on the index LB, with negative auto-antibodies and elevated serum IgG. Fibrosis was stable, increased or decreased in 23, 11, and three patients, respectively. FP was less frequent in tacrolimus-treated patients ($p = 0.022$), more frequent in cyclosporine- ($p = 0.035$) and MMF-treated patients ($p = 0.023$), and not influenced by steroid-based treatment or increased overall immunosuppression or steroids. Under multivariate analysis, MMF remained an independent predictor of FP ($p = 0.048$).

CONCLUSION: The pathophysiology of IPTH remained unclear. Increasing steroid doses or overall immunosuppression did not prevent FP. The potential impact of MMF on FP will require prospective studies.

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Key words: Idiopathic post-liver transplant hepatitis; *de novo* autoimmune hepatitis; Fibrosis progression; Immunosuppression; HEV infection

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Abbreviations:

CH: chronic hepatitis;
 LT: liver transplantation;
 HBV: hepatitis B virus;
 HEV: hepatitis E virus;
 HCV: hepatitis C virus;
 AIH: autoimmune hepatitis;
 IPTH: idiopathic post-liver transplant hepatitis;
 FP: fibrosis progression,
 LTs: liver tests;
 IgG: immunoglobulin G;
 LB: liver biopsy;
 AR: acute rejection;
 PBC: primary biliary cirrhosis;
 PSC: primary sclerosing cholangitis;
 CNI: calcineurin inhibitor;
 TBC: trough blood concentration;
 MMF: Mycophenolate Mofetil;
 AMR: antibody-mediated rejection;
 DSA: donor-specific human leukocyte antigen antibodies.

INTRODUCTION

A histological pattern of chronic hepatitis (CH) characterized by lymphocytic inflammation with necro-inflammatory activity is often observed after liver transplantation (LT) and is linked to various causes such as viral infections and autoimmune disorders^[1-4]. Some histological features may indicate particular causes. The presence of ground-glass hepatocytes and positive immunostaining for Hepatitis B virus (HBV) antigens are pathognomonic of HBV infection. Lymphocytic cholangitis may be observed in Hepatitis E (HEV)^[5] and Hepatitis C (HCV) infection^[1-3]. Severe centrilobular necro-inflammatory activity and plasma cell-rich infiltrate best correlated with the diagnosis of *de novo* autoimmune hepatitis (AIH)^[6]. However, in some cases, the etiology of the "hepatic" pattern of injury cannot be suggested on histological grounds alone. In any case, a correlation with clinical findings is necessary, and autoimmune and viral testing form an integral part of the evaluation to supplement histological findings.

Once these clinical explorations have been completed, there remain a number of CH cases whose histological features are not obviously related to any recognized cause. This entity, referred to as idiopathic post-transplant hepatitis (IPTH), is mostly characterized by mild fibrosis, necro-inflammatory activity and near-normal liver tests (LT). In most long-term follow-up studies, it has been seen that IPTH usually has a benign outcome^[1,2,7,8], although some studies have produced controversial evidence, including the development of late graft fibrosis^[9-12]. IPTH is therefore considered as a CH form of rejection^[10,11,13,14], an atypical form of *de novo* AIH, a recurrent disease which lacks classic histological abnormalities or CH due to a possibly unknown viral agent^[2,7,8,15]. Probably because of this unclear pathophysiology and the lack of predictive factors for fibrosis progression (FP), the treatment for IPTH is not clearly delineated. The possibility that IPTH represents a form of rejection may indicate a need for increased immunosuppression that should decrease the activity and prevent the development of fibrosis^[10,11,13,14].

Because of these poorly defined attitudes towards IPTH, we conducted a retrospective analysis to evaluate the prevalence and pathophysiology of IPTH in liver transplant patients whose diagnosis had been based on histological findings, and the predictive factors for FP in these patients.

METHODS

Study population

Patients were initially selected from the Pathology Department database (Paul Brousse Hospital, Villejuif, France) using search criteria prospectively coded for a histological diagnosis of IPTH made in 2006. That year was chosen in order to ensure sufficient clinical and histological follow-up. IPTH was histologically defined as a pattern of CH characterized by lymphocytic inflammation and necro-inflammatory activity, without an apparent etiology (Figure 1). Liver biopsies (LB) were performed either for clinical reasons or as part of the systematic post-transplantation protocol (at 1, 2, 5, 10, 15 and 20 years after LT). The specimens were frozen if the LB was > 2.5 cm long, and routinely formalin-fixed paraffin-embedded and stained with hematein-eosin-safran and picosirius.

Among the LBs performed during the study period but excluded from the analysis were those with diagnostic categories including normal, mild non-specific changes, hepatic structural abnormalities, fatty liver disease, acute rejection (AR) and chronic rejection, biliary obstruction, and recurrent diseases (primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and AIH). LBs presenting with a pattern of CH related to a proven recurrence of HCV or HBV infection were also excluded, as were patients without any subsequent LB (Figure 2).

Immunosuppression

The protocol of immunosuppression was based on steroids associated with a calcineurin inhibitor (CNI). Cyclosporine was systematically used until 2000; thereafter tacrolimus was preferentially used. CNI doses were gradually reduced and adapted according to a close monitoring of trough blood concentration (TBC). Regarding tacrolimus, the TBC were 8 to 12 ng/mL, 6 to 10 ng/ml and 3 to 8 ng/mL during the first month, first year and the long-term period post-

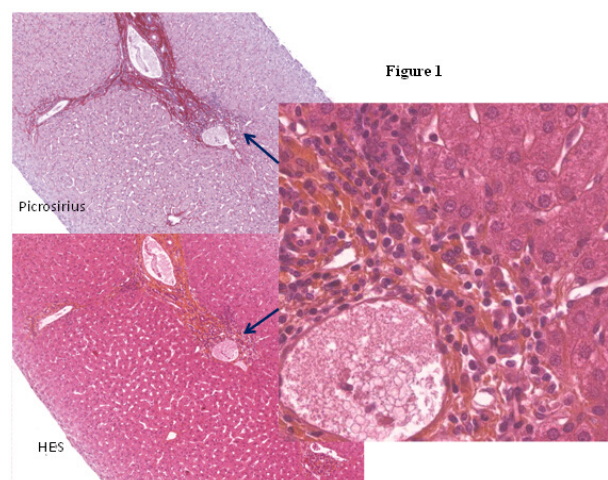


Figure 1 Index liver biopsy (stained with HES and picosirius) from a study patient with IPTH showing a porto-portal fibrosis, a predominantly lymphocytic portal infiltrate and a mild interface activity. Other histological features such as rosetting of liver cells, granulomatous cholangitis, emperipolesis and lobular granulomas are absent along the specimen.

LT, respectively. Regarding cyclosporine, the TBC were 250 to 350 ng/mL, 150 to 300 ng/mL and 50 to 200 ng/mL ml during the same periods. Steroids were tapered and withdrawn between 3 to 6 months post-LT except in patients transplanted for autoimmune disorders. Mycophenolate Mofetil (MMF) has been used in combination with CNi in patients with renal impairment since 2004 and extensively used in all patients whatever their renal function since 2007.

Histology

The presence of AR was determined by reviewing the pathological records concerning the previous LBs.

Index LBs with IPTH were all reviewed by the same pathologist blinded to the clinical and serological findings. Because IPTH resembles the pattern of HCV-related chronic hepatitis, the Metavir scoring system was chosen to grade the severity of fibrosis and the necro-inflammatory activity^[6]. In order to help the distinction between IPTH and *de novo* AIH, activity as well as plasma cell-rich infiltrates were also assessed separately in the periportal, lobular and centrilobular areas as previously described^[6]. "Tissue injury pattern consistent with acute antibody-mediated rejection (AMR)" including histological evidence of biliary tract obstruction in conjunction with the imaging studies and diffuse microvascular endothelial cell injury/microvasculitis were recorded.

The histological review also included all the most recent post-transplant LBs (or explants) in order to follow the evolution of fibrosis and necro-inflammatory activity.

Immunostaining for C4d

Immunohistochemistry was retrospectively performed on formalin-fixed paraffin-embedded tissue sections following standard protocols with a 1:100 dilution of rabbit polyclonal anti-human C4d antibody (DB Biotech, Popradská, Slovak republic). Focal intimal staining of portal arteries helped as an internal indicator of staining adequacy. Specimens from a C4d-immunopositive allograft ABO-incompatible explanted for hyperacute humoral rejection were positive controls (Figure 3A).

Semiquantitative evaluation of C4d immunolabelling was performed by assessing staining of portal stroma, portal capillaries, portal vein endothelium, sinusoidal endothelium and centrilobular vein endothelium. C4d deposition in portal structures of > 50% and <50% of the tracts was considered diffuse and focal staining, respectively. Staining of the sinusoids was graded as diffuse if > 50% of the sinusoidal endothelial bed was stained and focal if lesser degree of staining.

Clinical and biological correlations

Regular evaluations comprised a clinical assessment and biochemical and serological screening at least every 4 to 6 months. Data on patients with histological IPTH included changes in immunosuppression before the index LBs and two months after receiving the IPTH diagnosis, liver tests (LTs) including total bilirubin ($N < 17 \mu\text{mol/L}$), GGT ($N < 50 \text{ IU/L}$) and ALT ($N < 36 \text{ IU/L}$), serological studies (serum IgG levels and tissue auto-antibodies) and virological markers (HBV, HCV, HAV, CMV, and HHV6) based on local laboratory standards. For the purpose of this study, HEV RNA was determined retrospectively from the index LB because of a lack of stored sera. After total nucleic acid extraction (RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE, Ambion®), HEV RNA was detected using the hepatitisE@ceeramTools assay (Ceeram, La Chapelle sur Erdre, France).

Donor-specific human leukocyte antigen antibodies (DSA) were

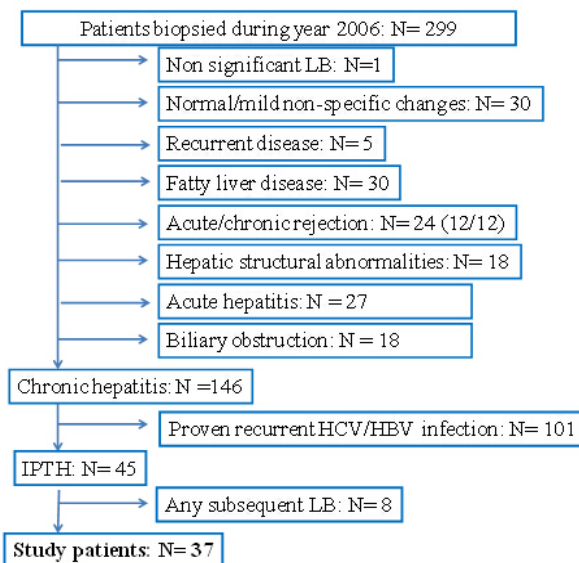


Figure 2 Flow chart of selection of study patients.

not routinely tested in our centre and the lack of stored sera did not allow their retrospective analysis.

Statistical analysis

Continuous data were expressed as a median (range) and/or mean (\pm standard deviation) whereas categorical data were expressed as percentages. Categorical data were compared using Fisher's exact test. The study endpoints were: (i) any increase in fibrosis and (ii) any decrease in activity between the index and last LB. Variables with a p value ≤ 0.15 under univariate analysis were included in a logistic regression model for multivariate analysis. Statistical significance was indicated by a p value < 0.05 . Calculations were performed using R 2.14.1 (www.cran-project.org).

RESULTS

Characteristics of the study population (Table 1)

In 2006, 299 post-transplant LB were performed in 272 patients. A histological diagnosis of IPTH was made in 45 patients (15%), eight of whom were excluded because of a lack of subsequent LBs. The remaining 37 patients with at least one follow-up LB were therefore eligible for the study. This cohort included 17 men and 20 women with a median age of 37 years (4-61 years) at the time of the initial LT. IPTH developed on the first allograft in 32 patients, the second in three patients, the third in one patient, and the fifth in one patient. The diagnosis was made from an indicated ($N=18$) or protocol ($N=19$) LB obtained after a median period of 3.1 years (range: 0.6-17.3 years) post-LT. At the time of the index LB, the mean values for total bilirubin, GGT and ALT were $26.6 \mu\text{mol/L}$, 135 IU/L , and 58.9 IU/L , respectively. LTs were strictly normal in 17 patients (46%). Abnormal LTs were present in 20 patients (54%) in whom two have undergone a protocol LB.

Initial immunosuppression included steroids in all patients, a CNi (either cyclosporine in 11 or tacrolimus in 26 patients) and MMF in 13 patients. During follow-up, cyclosporine was switched to tacrolimus in two patients and to sirolimus in one patient; tacrolimus was withdrawn in one patient; MMF was added in four patients and withdrawn in three; and steroids were withdrawn in 20 patients.

Before the time of the index LB, overall immunosuppression was decreased in 24, was stable in 11 and increased in two patients.

Table 1 Table 1: Baseline characteristics and therapeutic management of patients with Idiopathic Post-liver Transplant Hepatitis.

Median age at the first liver transplantation	37 years (4-61)					
Sex (Female/Male)	17/20					
Indication for liver transplantation						
Alcohol cirrhosis	5					
Amyloidosis	8					
Primary biliary cirrhosis	1					
Primary sclerosing cholangitis	4					
Fulminant hepatitis failure	7 (1 toxic, 3 undetermined, 2 Hepatitis B Virus, 1 autoimmune hepatitis)					
Hepatitis B Virus-cirrhosis	4 (1 cholangiocarcinoma, 1 hepatocellular carcinoma)					
Wilson disease	2					
Biliary atresia	3					
Hepatic congenital fibrosis	1					
Liver trauma	1					
Biliary cirrhosis	1					
Immunosuppression (IS)	Corticosteroids	Tacrolimus	Cyclosporine	MMF	Sirolimus	
Initial IS	37	26	11	13 (TAC: 9; CSA: 4)	0	
From liver transplantation to 2005						
Introduction	0	2 (switch*)	0	4	1 (switch*)	
Withdrawal	20	1	3 (switch*)	3	0	
Total	17	27	8	14	1	
Just prior to index liver biopsy	Corticosteroids	Tacrolimus	Cyclosporine	MMF	Sirolimus	Overall IS
Introduction	1	0	0	0	0	-
Withdrawal	5	0	0	2	0	-
Decreased doses	6	14	4	3	0	24
Stable doses	6	12	4	9	1	11
Increased doses	0	1	0	0	0	2
Total	13	27	8	12	1	37
Just after index liver biopsy	Corticosteroids	Tacrolimus	Cyclosporine	MMF	Sirolimus	Overall IS
Introduction	4	0	0	4	0	-
Withdrawal	1	0	0	0	0	-
Decreased doses	2	7	4	0	0	10
Stable doses	5	14	4	9	1	13
Increased doses	5	6	0	3	0	14
Total	16	27	8	16	1	37

* Cyclosporine was switched to tacrolimus (because of chronic rejection) in two patients and to sirolimus (because of renal insufficiency).

In the two months after receiving the diagnosis of IPTH, overall immunosuppression was decreased in 10 patients, was stable in 13 and increased in 14 patients.

Histological characteristics of IPTH

The median number of LBs performed before the index LB was 2 (range: 0-9). Before the onset of IPTH, 10 patients experienced AR (graded as moderate in seven and mild in three) that all resolved.

All index LBs displayed increased fibrosis: F1 ($N=24$), F2 ($N=8$), F3 ($N=2$) and F4 ($N=3$). Activity graded by the Metavir scoring system was as follows: A1 ($N=25$), A2 ($N=9$), and A3 ($N=3$). More specifically, interface activity was graded as absent in 6/37, mild in 23/37, moderate in 6/37 and severe in 2/37. The portal infiltrate was predominantly lymphocytic in 27 LB, including occasional plasma cells (< 30%) in six, and between 30% and < 50% in four. Non-zonal lobular activity was present in 18/37 of cases, the great majority (16/18) being graded as mild. Lobular infiltrates were composed of lymphocytes. Centrilobular areas could be analysed in 34 of the 37 biopsies, cirrhosis preventing this in three patients. Centrilobular activity was present in 44% (15/34) of the cohort and graded as mild (9/34), moderate (2/34) or severe (4/34). The centrilobular infiltrate, present in 7/34 of biopsies, was made up of occasional plasma cells (< 30%) in six, and between 30% and 50% in four. One patient fulfilled the histological features of *de novo* AIH. "Tissue injury pattern consistent with acute AMR" was not observed in the index LBs, especially the three earliest LBs performed at < 1 year post-LT.

C4d immunolabelling was negative in 14 LBs. C4d immunolabelling of the portal stroma was present in 15 LBs (diffuse in five and focal in 11) in whom three was associated with an endothelial pattern. An endothelial pattern (portal vein and/or sinusoids and/or centrilobular vein) was present in eight LBs (Figure

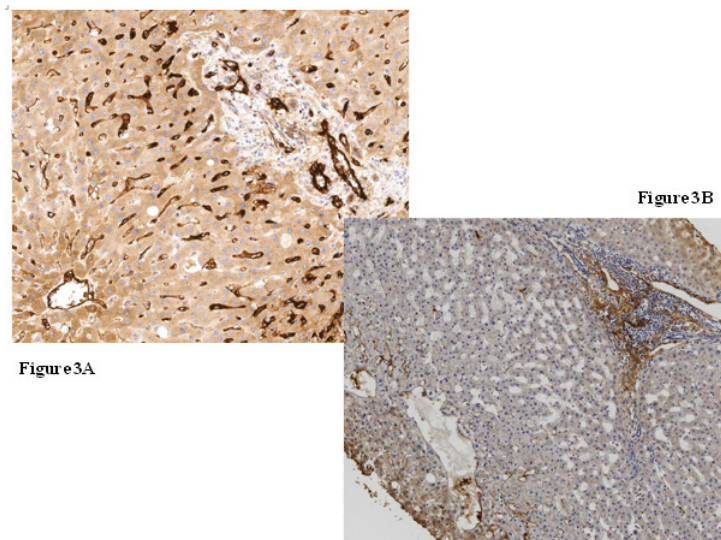


Figure 3 A. Specimen from an ABO-incompatible allograft explanted for hyperacute humoral rejection. The portal microvessels, centrilobular veins and sinusoids are diffusely and strongly C4d-positive. B. Index liver biopsy from a study patient with IPTH. The C4d-positivity was essentially portal stromal and focally endothelial (centrilobular vein and few sinusoids).

3B). More specifically, the "early" LBs showed no immunolabelling in one and a focal portal stroma staining in two. The LB fulfilling the histological features of *de novo* AIH showed a focal portal stroma staining.

The median interval between the index LB and last follow-up LB was 4.2 years (range: 0.1-7.3 years). Fibrosis and activity on the last LB were graded as follows: F1 ($N=19$), F2 ($N=10$), F3 ($N=2$)

Table 2 Analysis of factors associated with an increase in fibrosis between the index and last follow-up liver biopsies.

Univariate analysis							
Variables	Increase in fibrosis			P			
	No	Yes	Total				
IS increase	No	16 (61.5%)	7 (63.6%)	23 (62.2%)	0.603		
	Yes	10 (38.5%)	4 (36.4%)	14 (37.8%)			
Corticoids	No	15 (57.7%)	6 (54.5%)	21 (56.8%)	0.571		
	Yes	11 (42.3%)	5 (45.5%)	16 (43.2%)			
Corticoid increase	No	19 (73.1%)	9 (81.8%)	28 (75.7%)	0.454		
	Yes	7 (26.9%)	2 (18.2%)	9 (24.3%)			
Corticoid doses	> 10mg	2 (7.7%)	3 (27.3%)	5 (13.5%)	0.144		
	< 10 mg	24 (92.3%)	11 (72.7%)	32 (86.5%)			
TAC	No	4 (15.4%)	6 (54.5%)	10 (27.0%)	0.022		
	Yes	22 (84.6%)	5 (45.5%)	27 (73.0%)			
CsA	No	23 (88.5%)	6 (54.5%)	29 (78.4%)	0.035		
	Yes	3 (11.5%)	5 (45.5%)	8 (21.6%)			
MMF	No	18 (69.2%)	3 (27.3%)	21 (56.8%)	0.023		
	Yes	8 (30.8%)	8 (72.7%)	16 (43.2%)			
MMF doses	> 500mg	6 (23.1%)	7 (63.6%)	13 (35.1%)	0.025		
	< 500mg	20 (76.9%)	4 (36.4%)	24 (64.9%)			
MMF doses	> 1000mg	4 (15.4%)	5 (45.5%)	9 (24.3%)	0.066		
	< 1000mg	22 (84.6%)	6 (54.5%)	28 (75.7%)			
IS regimen	None	1* (3.8%)	1** (9.1%)	2 (5.4%)	0.200		
	CsA	1 (3.8%)	2 (18.2%)	3 (8.1%)			
	CsA+MMF	2 (7.7%)	3 (27.3%)	5 (13.5%)			
	TAC	5 (19.2%)	4 (36.4%)	9 (24.3%)			
	TAC+MMF	17(65.4%)	1 (9.1%)	18(48.6%)			
Age at first LT	> 20 years	24 (92.3%)	9 (81.8%)	33 (89.2%)	0.341		
	< 20 years	2 (7.7%)	2 (18.2%)	4 (10.8%)			
Sex	Female	14 (53.8%)	6 (54.5%)	20 (54.1%)	0.627		
	Male	12 (46.2%)	5 (45.5%)	17 (45.9%)			
Indication for LT	Non-fibrotic	12 (46.2%)	4 (36.4%)	16 (43.2%)	0.429		
	Cirrhosis	14 (53.8%)	7 (63.6%)	21 (56.8%)			
Autoantibodies	-	20 (76.9%)	11 (100.0%)	31 (83.8%)	0.099		
	+	6 (23.1%)	0 (0.0%)	6 (16.2%)			
Autoantibodies (at significant titers)	-	22 (84.6%)	11 (100.0%)	33 (89.2%)	0.226		
	+	4 (15.4%)	0 (0.0%)	4 (10.8%)			
Time to index biopsy	< 1year	6 (23.0%)	2 (18.1%)	8 (21.6%)	1		
	> 1year	20 (76.9%)	9 (81.8%)	29 (78.3%)			
	< 3.1 years	14 (53.8%)	5 (45.4%)	19 (51.3%)			
	> 3.1 years	12 (46.1%)	6 (54.5%)	18 (48.6%)			
Interval index/ last biopsy	< 4.2 years	12 (46.1%)	7 (63.6%)	19 (51.3%)	0.550		
	> 4.2 years	14 (53.8%)	4 (36.3%)	18 (48.6%)			
Liver tests	Normal	13 (50.0%)	4 (36.4%)	17 (45.9%)	0.347		
	Abnormal	13 (50.0%)	7 (63.6%)	20 (54.1%)			
First graft	No	2 (7.6%)	3 (27.2%)	5 (13.5%)	0.144		
	Yes	24 (92.3%)	8 (72.7%)	32 (86.4%)			
Previous AR	No	20 (76.9%)	7 (63.6%)	27 (72.9%)	0.328		
	Yes	6 (23.0%)	4 (36.3%)	10 (27.0%)			
Univariate analysis							
	A	E.S.	Wald	ddl	RR	95% IC	P.
MMF	1.671	0.844	3.919	1	5.316	1.017-27.792	0.048
CsA	1.702	0.929	3.354	1	5.483	0.887-33.880	0.067
Constante	-2.170	0.707	9.408	1	0.114		0.002

* Sirolimus alone; ** MMF alone; IS: immunosuppression; CsA: cyclosporine; TAC: tacrolimus; MMF: Mycophenolate Mofetil; LT: liver transplantation; Ab: autoantibodies; LFTs: liver function tests; AR: acute rejection.

and F4 (N=6) and A0 (N=17), A1 (N=15), A2 (N=4) and A3 (N=1), respectively.

Autoimmune features at the time of IPTH diagnosis and during follow-up

At the time of the index LB, 26/37 patients were tested for auto-antibodies. Four of them were positive for antinuclear auto-antibodies (a low titre in one patient and significant titres in three patients). Auto-antibodies remained positive over time in three and disappeared in one patient. None of the four seropositive patients concurrently displayed AIH features and none had elevated IgG.

Of the 22 seronegative patients, one displayed severe centrilobular activity associated with severe centrilobular plasma cell-rich

infiltrate fulfilling histological AIH criteria, and serum IgG at 25.5g/l. He remained seronegative and developed cirrhosis. The 21 other seronegative patients did not display any AIH features on their index LBs: two of them were not retested for auto-antibodies and did not subsequently display any AIH features. Nineteen patients were retested later: 17 remained seronegative concurrently with AIH features on the last LB, with IgG at 12.7g/L in one of them. Antinuclear auto-antibodies appeared in two patients at a significant titre concurrently with AIH features on the subsequent LB, with IgG at 13.8g/l in one patient, and at a low titre in the other patient without AIH features and concomitantly elevated serum IgG.

Of the 11 patients not tested at the time of the index LB, none concurrently displayed AIH features. Seven were tested subsequently and all were seronegative concurrently with the absence of AIH features. The remaining four patients were never tested for auto-antibodies and displayed no AIH features at any time.

Virological features

Virological markers at the time of the index LB revealed an absence of acute infection or reactivation (HAV, HBV, HCV, CMV, and HHV6) in all patients. HEV RNA, determined retrospectively from the index LB (20 frozen and 17 embedded paraffin), was also negative.

Outcomes in the study population

Clinical outcomes: The time elapsing between the index LB and the last follow-up ranged from 0.1 to 7.9 years (median=7.2 years). Eight patients died, three of infections, two of cerebral haemorrhage and brain stroke, one with amyloidosis-related cardiac insufficiency, and two with extra-hepatic malignancies. Two of three patients recognized as non compliant received a repeat transplant for IPTH-related non-active cirrhosis.

Increase in fibrosis and decrease in activity between the index and last LBs:

Fibrosis increased in 11 patients, decreased in 3 and was stable in 23. Under univariate analysis (Table 2), an increase in fibrosis was significantly less frequent in patients receiving tacrolimus than others (5/27 vs 6/10, $p = 0.022$), and more frequent in patients receiving cyclosporine than others (5/8 vs 6/29, $p=0.035$) and in patients receiving MMF than those who did not (8/16 vs 3/21, $p = 0.023$). There was no difference in patients treated with steroids (whatever the doses) versus those who were steroid-free. No differences were seen between patients whose steroids and overall immunosuppression were increased and those in whom they were not. Under multivariate analysis, MMF remained an independent predictor of FP ($p = 0.048$). Cyclosporine tended to remain independent ($p = 0.067$). The same results were obtained when the three patients with cirrhosis at the time of the index LB were excluded.

Activity decreased in 22 patients, increased in 5 and was stable in 10. The incidence of a decrease in activity was not statistically different between patients who concurrently showed an increase in fibrosis and those who did not (6/11 vs 16/26). Under univariate analysis (Table 3), a previous AR was significantly associated with a reduction in activity ($p = 0.005$), and MMF (at < 1,000mg) tended to be associated ($p = 0.075$). There were no differences between patients under steroids (whatever the doses) and those without, between patients whose steroid doses were increased and those in whom they remained stable, or between patients whose overall immunosuppression was increased and those in whom it was unchanged. Under multivariate analysis, a previous AR remained an independent factor for a decrease in activity ($p = 0.007$). The same results were obtained when the patient with AIH features was excluded.

DISCUSSION

This is the most important study that focused on IPTH. Cases were mostly characterized by mild histological changes and mild LT abnormalities. Some cases were associated with auto-antibodies, others evolved towards CH with AIH features. The evolutions of fibrosis and activity did not differ between patients whose baseline immunosuppression or steroids had been increased after receiving the IPTH diagnosis, and the others. Finally, tacrolimus use prevented FP, unlike MMF and cyclosporine. MMF was the only independent factor of FP.

The prevalence of IPTH is difficult to determine and varies considerably among transplant centres. The first reason is the lack of proper definition^[4]. Indeed, IPTH is most common in centres that perform protocol LBs and in those that traditionally run patients on low levels of immunosuppression^[4,8]. Overall, prevalence increases over time from 10% to 50% of protocol LB performed > 1 year post-transplant in adults, and in up to 60% of children at 10 years post-LT^[2]. In our centre where protocol LBs are routinely performed, the prevalence in 2006 was 15% (45/299) with a median period of 3.1 years (range: 0.6-17.3 years) after LT.

Differential diagnoses include rejection, *de novo* AIH, and infections. Some cases may constitute a form of late rejection, especially cases presenting as prominent centrilobular features more closely resembling viral CH or AIH. In our series, 10/37 (27%) patients had experienced episodes of AR previously to IPTH. Three patients were recognized as poorly compliant. IPTH occurred following a decrease in the overall immunosuppression in 24/37 patients (65%). A study of immunosuppression-free patients found more fibrosis (with mild portal activity but no signs of acute/chronic rejection) that regressed after the reinstatement of immunosuppression^[17].

The hypothesis of a component of AMR may be discussed in the development of fibrosis/IPTH, as reported a paediatric series^[18]. O'Leary *et al*^[19] showed that the routine histological features could be used to screen patients with acute AMR. However, a definite diagnosis requires substantiation by DSA testing, diffuse C4d staining and the exclusion of other insults. Regarding "tissue injury pattern consistent with AMR", acute AMR resembles the histological findings for ischemic injury or biliary/vascular complications^[19-22], but little is known about AMR in patients with chronic allograft dysfunction. Regarding C4d positivity, its interpretation and practical utility in liver allografts is unclear and no consensus for the C4d pattern has been developed. In most studies, a strong, diffuse and endothelial staining were diagnostically useful, unlike focal, weak and portal stroma staining^[22]. To test the hypothesis that IPTH may represent a "tissue injury pattern consistent with late AMR", we retrospectively performed C4d staining on the index LBs. Our staining results (always weak, often focal and rarely endothelial) did not reasonably argue for this hypothesis but should be interpreted with caution in the absence of available DSA.

Emerging evidence suggests that IPTH and *de novo* AIH are part of an overlapping spectrum of immune-mediated damage. IPTH with negative auto-antibodies and AIH with positive auto-antibodies are common patterns, while IPTH with positive auto-antibodies and AIH with negative auto-antibodies pose theoretical problems: in the former case, a diagnosis of *de novo* AIH should be considered. However, many such patients have near-normal transaminase levels and therefore do not fulfil the clinical criteria for AIH. Some cases of auto-antibody-negative *de novo* AIH have been reported, mainly in children^[23-26] and rarely in adults^[6]. In the current study,

Table 3 Analysis of factors associated with a decrease in activity between the index and last follow-up liver biopsies.

Univariate analysis							
Variables	Increase in fibrosis			P			
	No	Yes	Total		A	E.S.	
IS increase	No	9 (60.0%)	14 (63.6%)	23 (62.2%)			
	Yes	6 (40.0%)	8 (36.4%)	14 (37.8%)			
Corticoids	No	8 (53.3%)	13 (59.1%)	21 (56.8%)			
	Yes	7 (46.7%)	9 (40.9%)	16 (43.2%)			
Corticoid increase	No	11 (73.3%)	17 (77.3%)	28 (75.7%)			
	Yes	4 (26.7%)	5 (22.7%)	9 (24.3%)			
Corticoid doses	> 10 mg	1 (6.7%)	4 (18.2%)	5 (13.5%)			
		14 (93.3%)	18 (81.8%)	32 (86.5%)			
TAC	No	4 (26.7%)	6 (27.3%)	10 (27.0%)			
	Yes	11 (73.3%)	16 (72.7%)	27 (73.0%)			
CsA	No	12 (80.0%)	17 (77.3%)	29 (78.4%)			
	Yes	3 (20.0%)	5 (22.7%)	8 (21.6%)			
MMF	No	7 (46.7%)	14 (63.6%)	21 (56.8%)			
	Yes	8 (53.3%)	8 (36.4%)	16 (43.2%)			
MMF doses	> 500 mg	7 (46.7%)	6 (27.3%)	13 (35.1%)			
	< 500 mg	8 (53.3%)	16 (72.7%)	24 (64.9%)			
MMF doses	> 1000 mg	6 (40.0%)	3 (13.6%)	9 (24.3%)			
	< 1000 mg	9 (60.0%)	19 (86.4%)	28 (73.7%)			
IS regimen	None	1*(6.7%)	1**(4.5%)	2 (5.4%)			
	CsA	0 (0.0%)	3 (13.6%)	3 (8.1%)			
	CsA + MMF	3 (20.0%)	2 (9.1%)	5 (13.5%)			
	TAC	7 (46.7%)	11 (50.0%)	18 (48.6%)			
	TAC + MMF	4 (26.7%)	5 (22.7%)	9 (24.3%)			
Age at first LT	> 20 years	14 (93.3%)	19 (86.4%)	33 (89.2%)			
	< 20 years	1 (6.7%)	3 (13.6%)	4 (10.8%)			
Sex	Female	6 (40.0%)	14 (63.6%)	20 (54.1%)			
	Male	9 (60.0%)	8 (36.4%)	17 (45.9%)			
Indication for LT	Non-fibrotic	7 (46.7%)	9 (40.9%)	16 (43.2%)			
	Cirrhosis	8 (53.3%)	13 (59.1%)	21 (56.8%)			
Ab	-	13 (86.7%)	18 (81.8%)	31 (83.8%)			
	+	2 (13.3%)	4 (18.2%)	6 (16.2%)			
Ab (significant titers)	-	15 (100.0%)	18 (81.8%)	33 (89.2%)			
	+	0 (0.0%)	4 (18.2%)	4 (10.8%)			
Time to index biopsy	< 1 year	3 (20.0%)	5 (22.7%)	8 (21.6%)			
	> 1 year	12 (80.0%)	17 (77.3%)	29 (78.4%)			
Interval index /last biopsy	< 3.1 year	6 (40.0%)	13 (59.0%)	19 (51.3%)			
	> 3.1 year	9 (60.0%)	9 (41.0%)	18 (48.6%)			
Liver tests	< 4.2 years	7 (46.6%)	12 (50.0%)	19 (51.3%)			
	> 4.2 years	8 (53.3%)	12 (50.0%)	18 (48.6%)			
First graft	Normal	7 (46.7%)	10 (45.5%)	17 (45.9%)			
	Abnormal	8 (53.3%)	12 (54.5%)	20 (54.1%)			
Previous AR	No	1 (6.6%)	4 (18.1%)	5 (13.5%)			
	Yes	14 (93.3%)	18 (81.8%)	32 (86.4%)			
Univariate analysis	No	7 (46.6%)	20 (90.9%)	27 (72.9%)			
	Yes	8 (53.3%)	2 (9.0%)	10 (27.0%)			
Univariate analysis							
	A	E.S.	Wald	ddl	RR	95% IC	P.
MMF <1000mg	1.634	0.925	0.077	1	5.127	0.836-31.443	0.077
AR	2.561	0.954	0.007	1	12.950	1.994-84.088	0.007
Constante	-2.689	1.151	0.019	1	0.068		0.019

* Sirolimus alone; ** MMF alone; IS: immunosuppression; CsA: cyclosporine; TAC: tacrolimus; MMF: Mycophenolate Mofetil; LT: liver transplantation; Ab: autoantibodies; LFTs: liver function tests; AR: acute rejection.

the patient with AIH features on his index LB, elevated serum IgG and transaminase levels but negative auto-antibodies was probably misdiagnosed and should be re-classified as *de novo* AIH.

Viral CH related to recurrent infection on the graft and newly acquired infection should always be considered. Because HCV reinfection was universal, HCV-patients were excluded from the study. HEV infection has been suggested to cause CH in immunocompromised patients^[27]. Some recipients with IPTH might be suffering from chronic HEV infection and could benefit from reduced immunosuppression and/or antiviral therapy^[28,29]. In our study, the retrospective determination of HEV was negative in all patients, including the transplanted ones for liver disease of unknown cause.

Few studies have reported any factors predisposing to the onset of IPTH. One study found an association with previous rejection and auto-antibody positivity^[13]. In another study, post-transplant cirrhosis as a complication of IPTH ($N = 10$) occurred more in patients transplanted for fulminant seronegative hepatitis than in those transplanted for other causes^[13]. Our work aimed to determine predictive factors for FP, as IPTH is a cause of late fibrosis. Assuming that most cases of IPTH have an underlying immune basis^[10,11,13,14], additional immunosuppression may be justified in cases where a viral aetiology can be clearly excluded. However, its optimum management has not yet been determined, and the role of steroids remains unproven. The study by Miyagawa *et al*^[13] found a decrease in activity but not in fibrosis scores over time. Without taking account of other changes in immunosuppression, the authors concluded however that treatment with steroids improved the biochemical abnormalities, causing a disappearance of activity. In the study by Seyam *et al*^[12], steroids were given to 8/10 patients who developed cirrhosis as a complication of IPTH, but changes in activity and fibrosis under steroids were not mentioned. In the study by Syn *et al*^[14], only four of their 30 patients were treated with additional immunosuppression, and all were receiving steroids. Complete resolution or a decrease in activity was observed in 15/30 patients, including 4 who received steroids. FP was observed in 13/30 patients, two receiving steroids. To our best knowledge, our study is the first to have demonstrated that steroid therapy, an increase in the steroid doses and an increase in overall immunosuppression did not prevent FP or improve activity. This result different from the usually preconized treatment for *de novo* AIH may support that *de novo* AIH and IPTH are two distinct entities. This is also supported by the different histological patterns. However, the frontier is limited since some cases of IPTH could be associated with autoantibodies and others could evolve towards CH with AIH features.

This study also demonstrated that treatment with tacrolimus prevented FP, while cyclosporine and MMF were significantly associated with FP. MMF remained an independent prognostic factor of FP. In the literature, the impact of MMF on FP of IPTH has never been assessed. It has mainly referred to HCV recurrence. In the review by Germani *et al*^[30] which evaluated 17 studies, only two studies found a reduction in the severity of HCV recurrence, nine studies documented similar severity and six increased severity.

The main limitations of this study include retrospective analysis and absence of DSA testing. Secondly the fact that the study population is small limits statistical analysis. However, regarding literature, this series is the most important cohort describing this uncommon pathological condition. The main strengths are the availability of paired liver biopsies, the histologic reviewing by a single pathologist, the complete autoimmune and virological evaluation and the relatively long follow-up period (median 7.2 years). This study is also the first to investigate prognostic factors of FP in IPTH.

In conclusion, we confirmed a pattern of CH that cannot be ascribed to any obvious cause. In particular, virological markers including Hepatitis E were negative. With respect to the C4d immunostaining findings, AMR component to injury was unlikely but results should be interpreted with caution in the absence of DSA testing. We could not determine the beneficial effects of the increase in overall immunosuppression and/or steroids. This finding may support that although close, *de novo* AIH and IPTH are two distinct entities. The beneficial impact of tacrolimus and detrimental impact of MMF and cyclosporine on FP should be prospectively investigated.

CONFLICT OF INTERESTS

The Authors state that they have no conflict of interest (COI).

REFERENCES

- 1 Adeyi O, Fischer SE, Guindi M. Liver allograft pathology: approach to interpretation of needle biopsies with clinicopathological correlation. *J Clin Pathol* 2010; **63**: 47-74
- 2 Hubscher S. What is the long-term outcome of the liver allograft? *J Hepatol* 2011; **55**: 702-17
- 3 Demetris AJ, Adeyi O, Bellamy CO, Clouston A, Charlotte F, Czaja A, Daskal I, El-Monayeri MS, Fontes P, Fung J, Gridelli B, Guido M, Haga H, Hart J, Honsova E, Hubscher S, Itoh T, Jhala N, Jungmann P, Khettry U, Lassman C, Ligato S, Lunz JG, 3rd, Marcos A, Minervini MI, Molne J, Nalesnik M, Nasser I, Neil D, Ochoa E, Pappo O, Randhawa P, Reinholt FP, Ruiz P, Sebagh M, Spada M, Sonzogni A, Tsamandas AC, Wernerson A, Wu T, Yilmaz F. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 2006; **44**: 489-501
- 4 Neil DA, Hubscher SG. Current views on rejection pathology in liver transplantation. *Transpl Int* 2010; **23**: 971-83
- 5 Selves J, Kamar N, Mansuy JM, Peron JM. Hepatitis E virus: A new entity. *Ann Pathol* 2010; **30**: 432-438
- 6 Sebagh M, Castillo-Rama M, Azoulay D, Coilly A, Delvart V, Allard MA, Dos Santos A, Johanet C, Roque-Afonso AM, Saliba F, Duclos-Vallee JC, Samuel D, Demetris AJ. Histologic findings predictive of a diagnosis of *de novo* autoimmune hepatitis after liver transplantation in adults. *Transplantation* 2013; **96**: 670-678
- 7 Banff Working Group on Liver Allograft Pathology. Importance of liver biopsy findings in immunosuppression management: biopsy monitoring and working criteria for patients with operational tolerance. *Liver Transpl* 2012; **18**: 1154-1170
- 8 Shaikh OS, Demetris AJ. Idiopathic posttransplantation hepatitis? *Liver Transpl* 2007; **13**: 943-946
- 9 Ekong UD, Melin-Aldana H, Seshadri R, Lokar J, Harris D, Whittington PF, Alonso EM. Graft histology characteristics in long-term survivors of pediatric liver transplantation. *Liver Transpl* 2008; **14**: 1582-1587
- 10 Evans HM, Kelly DA, Mckiernan PJ, Hubscher S. Progressive histological damage in liver allografts following pediatric liver transplantation. *Hepatology* 2006; **43**: 1109-1117
- 11 Herzog D, Soglio DB, Fournet JC, Martin S, Marleau D, Alvarez F. Interface hepatitis is associated with a high incidence of late graft fibrosis in a group of tightly monitored pediatric orthotopic liver transplantation patients. *Liver Transpl* 2008; **14**: 946-955
- 12 Seyam M, Neuberger JM, Gunson BK, Hubscher SG. Cirrhosis after orthotopic liver transplantation in the absence of primary disease recurrence. *Liver Transpl* 2007; **13**: 966-974
- 13 Miyagawa-Hayashino A, Haga H, Egawa H, Hayashino Y, Uemoto S, Manabe T. Idiopathic post-transplantation hepatitis following living donor liver transplantation, and significance of autoantibody titre for outcome. *Transpl Int* 2009; **22**: 303-312
- 14 Syn WK, Nightingale P, Gunson B, Hubscher SG, Neuberger JM. Natural history of unexplained chronic hepatitis after liver transplantation. *Liver Transpl* 2007; **13**: 984-989
- 15 Hubscher S. What does the long-term liver allograft look like for the pediatric recipient? *Liver Transpl* 2009; **15 Suppl 2**: S19-24
- 16 Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293
- 17 Yoshitomi M, Koshihata T, Haga H, Li Y, Zhao X, Cheng D, Miyagawa A, Sakashita H, Tsuruyama T, Ohe H, Ueda M, Okamoto S, Egawa H, Wood K, Sakaguchi S, Manabe T, Tanaka K, Uemoto S. Requirement of protocol biopsy before and after complete cessa-

- tion of immunosuppression after liver transplantation. *Transplantation* 2009; **87**: 606-614
- 18 Miyagawa-Hayashino A, Yoshizawa A, Uchida Y, Egawa H, Yurugi K, Masuda S, Minamiguchi S, Maekawa T, Uemoto S, Haga H. Progressive graft fibrosis and donor-specific human leukocyte antigen antibodies in pediatric late liver allografts. *Liver Transpl* 2012; **18**: 1333-1342
 - 19 O'leary JG, Demetris AJ, Friedman LS, Gebel HM, Halloran PF, Kirk AD, Knechtle SJ, McDiarmid SV, Shaked A, Terasaki PI, Tinckam KJ, Tomlanovich SJ, Wood KJ, Woodle ES, Zachary AA, Klintmalm GB. The role of donor-specific HLA alloantibodies in liver transplantation. *Am J Transplant* 2014; **14**: 779-787
 - 20 Lunz J, Ruppert KM, Cajaiba MM, Isse K, Bentlejewski CA, Minervini M, Nalesnik MA, Randhawa P, Rubin E, Sasatomi E, De Vera ME, Fontes P, Humar A, Zeevi A, Demetris AJ. Re-examination of the lymphocytotoxic crossmatch in liver transplantation: can C4d stains help in monitoring? *Am J Transplant* 2012; **12**: 171-182
 - 21 Kozlowski T, Andreoni K, Schmitz J, Hayashi PH, Nickeleit V. Sinusoidal C4d deposits in liver allografts indicate an antibody-mediated response: diagnostic considerations in the evaluation of liver allografts. *Liver Transpl* 2012; **18**: 641-658
 - 22 Bellamy CO. Complement C4d immunohistochemistry in the assessment of liver allograft biopsy samples: applications and pitfalls. *Liver Transpl* 2011; **17**: 747-750
 - 23 Andries S, Casamayou L, Sempoux C, Bulet M, Reding R, Bernard Otte J, Buts JP, Sokal E. Posttransplant immune hepatitis in pediatric liver transplant recipients: incidence and maintenance therapy with azathioprine. *Transplantation* 2001; **72**: 267-272
 - 24 Hernandez HM, Kovarik P, Whittington PF, Alonso EM. Autoimmune hepatitis as a late complication of liver transplantation. *J Pediatr Gastroenterol Nutr* 2001; **32**: 131-136
 - 25 Miyagawa-Hayashino A, Haga H, Sakurai T, Shirase T, Manabe T, Egawa H. De novo autoimmune hepatitis affecting allograft but not the native liver in auxiliary partial orthotopic liver transplantation. *Transplantation* 2003; **76**: 271-272
 - 26 Richter A, Grabhorn E, Helmke K, Manns MP, Ganschow R, Burdelski M. Clinical relevance of autoantibodies after pediatric liver transplantation. *Clin Transplant* 2007; **21**: 427-432
 - 27 Unzueta A, Rakela J. Hepatitis E infection in liver transplant recipients. *Liver Transpl* 2014; **20**: 15-24
 - 28 Halac U, Beland K, Lapierre P, Patey N, Ward P, Brassard J, Houde A, Alvarez F. Chronic hepatitis E infection in children with liver transplantation. *Gut* 2012; **61**: 597-603
 - 29 Kamar N, Garrouste C, Haagsma EB, Garrigue V, Pischke S, Chauvet C, Dumortier J, Cannesson A, Cassuto-Viguier E, Therivet E, Conti F, Lebray P, Dalton HR, Santella R, Kanaan N, Essig M, Mousson C, Radenne S, Roque-Afonso AM, Izopet J, Rostaing L. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* 2011; **140**: 1481-1489
 - 30 Germani G, Pleguezuelo M, Villamil F, Vaghjiani S, Tsochatzis E, Andreana L, Burroughs AK. Azathioprine in liver transplantation: a reevaluation of its use and a comparison with mycophenolate mofetil. *Am J Transplant* 2009; **9**: 1725-1731

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