

# What is the long-term outcome of the liver allograft?

Stefan G. Hübscher\*

School of Cancer Sciences, University of Birmingham and Department of Cellular Pathology, Queen Elizabeth Hospital, Birmingham, United Kingdom

## Summary

With improved long-term survival following liver transplantation (LT), issues relating to the assessment of the liver allograft in long-term survivors are becoming increasingly relevant. Histological abnormalities are commonly present in late post-transplant biopsies, including protocol biopsies from patients who appear to be well with good graft function. Recurrent disease is the commonest recognised cause of abnormal graft histology, but may be modified by the effects of immunosuppression or interactions with other graft complications, resulting in complex or atypical changes. Other abnormalities seen in late post-transplant biopsies include rejection (which often has different appearances to those seen in the post-transplant period), *de novo* disease, “idiopathic” post-transplant hepatitis (IPTH) and nodular regenerative hyperplasia. In many cases graft dysfunction has more than one cause and liver biopsy may help to identify the predominant cause of graft damage. Problems exist with the terminology used to describe less well understood patterns of graft injury, but there is emerging evidence to suggest that late rejection, *de novo* autoimmune hepatitis and IPTH may all be part of an overlapping spectrum of immune-mediated injury occurring in the late post-transplant liver allograft. Careful clinico-pathological correlation is very important and the wording of the biopsy report should take into account therapeutic implications, particularly whether changes in immunosuppression may be indicated. This article will provide an overview of the main histological changes occurring in long-term survivors post-LT, focusing on areas where the assessment of late post-transplant biopsies is most relevant clinically.

**Keywords:** Liver transplantation; Liver biopsy; Late rejection; Recurrent disease; *de novo* disease; Idiopathic post-transplant hepatitis; Tolerance.

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\*Address: Department of Cellular Pathology, Level-1, Queen Elizabeth Hospital Birmingham, Mindelsohn Way, Edgbaston Birmingham B15 2WB, United Kingdom. Tel.: +44 (0) 121 371 3345.

E-mail addresses: s.g.hubscher@bham.ac.uk, stefan.hubscher@uhb.nhs.uk

**Abbreviations:** LT, liver transplantation; IPTH, “idiopathic” post-transplant hepatitis; HCV, hepatitis C virus; AR, acute rejection; CP, central perivenulitis; CR, chronic rejection; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis; FCH, fibrosing cholestatic hepatitis; IFN, interferon; PCH, plasma cell hepatitis; NAFLD, non-alcoholic fatty liver disease; GSTT-1, glutathione-S-transferase T1; BSEP, bile salt export pump; LFT, liver function test; ALT, alanine transaminase; HEV, hepatitis E virus.

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## Introduction

Most of the main complications that occur during the early post-transplant period can also be seen in late post-transplant biopsies (>1 year post-LT) [1] (Table 1). However, there are important differences in their relative frequency. Recurrent disease is the commonest recognised cause of late graft dysfunction [2]. By contrast, acute and chronic rejection are uncommon at this time and may have different histological features to those seen in the early post-transplant period. A substantial proportion of biopsies obtained >1 year post-LT show changes of uncertain aetiology – examples include non-specific portal and/or lobular inflammation, unexplained (“idiopathic”) chronic hepatitis, and a range of architectural and vascular changes, sometimes referred to as “hepatic structural abnormalities”. Changes seen in late post-transplant biopsies are often complex and reflect more than one pathological process – histology may help to identify the dominant cause of graft damage in such cases.

The prevalence and spectrum of histological changes that have been reported in late post-transplant biopsies vary considerably from centre to centre (Tables 2 and 3) [3–15]. The reason for this variation is uncertain, but some of the likely factors are summarised in Table 4. The original indication for transplantation is clearly important. In centres where hepatitis C virus (HCV) cirrhosis is the main indication for transplantation, recurrent HCV hepatitis is the commonest diagnosis in late post-transplant biopsies. By contrast, in the paediatric population, where the great majority of transplants are carried out for non-recurring diseases, changes seen in late biopsies have to be attributed to another cause. Not surprisingly, histological abnormalities are more frequently seen when biopsies are taken to investigate the cause of abnormal graft function. However, abnormal graft histology has also been observed in long-term protocol biopsies from 5% to 85% of adults (Table 2) and 32% to 97% of children (Table 3) who are clinically well with normal liver biochemistry. The prevalence of histological abnormalities increases with time. Different approaches to immunosuppression may also account for centre-specific differences – for example, it has been



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**Table 1. Histological diagnoses in 1045 liver biopsies obtained >1 year post-transplant during a 5 year period (2004–2009) at the Liver Unit, Queen Elizabeth Hospital, Birmingham.** In biopsies where more than one pathological process appeared to be present, the biopsy was classified according to the main histological diagnosis. Seventy two percent of the biopsies were obtained on a protocol basis, the other 28% were clinically indicated.

Main Diagnosis	Number (%) of Cases	Comments
Normal/near normal	152 (15)	
Rejection	82 (8)	Many cases co-exist with other patterns of graft damage
Biliary obstruction/cholestasis	11 (1)	
Chronic hepatitis	294 (28)	59 (6%) cases related to recurrent disease 235 (22%) cases other/unknown cause
Recurrent disease	211 (20)	
Other findings	295 (28)	Fatty change, vascular/structural changes, fibrosis, siderosis

suggested that a higher prevalence of unexplained inflammatory changes in late biopsies may reflect low grade immune-mediated injury occurring in centres where immunosuppression is provided with a “light hand” [16]. There are also potential problems with the terminology used to describe changes of uncertain aeti-

ology in late biopsies. For example, late rejection (with hepatitis-like features), *de novo* autoimmune hepatitis and “idiopathic” post-transplant hepatitis are generally regarded as distinct entities. However, as will be discussed later, there is emerging evidence to suggest that these are probably all part of an

**Table 2. The frequency and spectrum of histological abnormalities in late post-transplant liver biopsies obtained as protocol biopsies or on a clinically-indicated basis.** The majority of patients in these studies are adults.

Centre	No of biopsies	Length of follow-up	Abnormal histology	Abnormal histology with:		Main histological diagnoses/other comments
				LFTs normal	LFTs Raised	
Pittsburgh, (Pappo 1995) [3]	65	>5 years	66%	36%	75%	Rejection (17%), cholangiopathy (8%), viral hepatitis (11%), recurrent disease (31%)
Kings College Hospital, London (Slapak 1997) [4]	116	>5 years	72%	46%	87%	Chronic hepatitis-HBV/HCV/AIH (12%), chronic hepatitis-cause unknown (11%), recurrent PBC (7%), structural abnormalities (22%), cholangitis (13%), rejection (3%), other (4%)
Valencia (Berenguer 2001) [5]	254	>1 year	42% at 1 year 56% at 5 years	-	-	Hepatitis (53%), chronic rejection (3%)-at 5 years
Paris (Sebagh 2003) [6]	143	>10 years	56% at 5 years 80% at 10 years	72%	90%	Chronic rejection (11%), chronic rejection and chronic viral hepatitis (12%), chronic viral hepatitis (38%), recurrent AIH (3%), recurrent PBC (4%), other (11%) - at 10 years
Mayo Clinic (Abraham 2008) [7]	165	3 months 5 years	27%	27%*	N/A	Fatty liver disease (11%), recurrent disease (10%), rejection/central perivenulitis (7%), other (2%)
Dallas (Vasani 2008) [8]	>4000	1-20 years	<5%	<5%*	N/A	Acute rejection (2-3%), recurrent hepatitis C (0.5-2%)
Birmingham (Mells 2009) [9]	237	1-10 years	76%	76%*	N/A	Unexplained chronic hepatitis (33%), recurrent disease (23%), fatty liver disease (14%), other (5%)
Cambridge (Gelson 2010) [10]	55	>3 years	85%	85%*	N/A	Non-specific hepatitis (45%), recurrent disease (25%), steatosis (11%), siderosis (4%)

\* All protocol biopsies from patients with normal liver function tests.  
HBV, hepatitis B virus; HCV, hepatitis C virus; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis.

# Frontiers in Liver Transplantation

**Table 3. Histological findings in children undergoing liver biopsy >1 year post-transplant.** All of these studies included biopsies that were obtained on a protocol basis, with >50% of children having normal liver biochemistry at the time of biopsy.

Centre	No of biopsies	Length of follow-up	Abnormal histology	Main histological diagnoses/other comments
Paris (Fouquet 2005) [11]	67	>10 years	73%	Chronic rejection (42%), centrilobular fibrosis (22%), biliary cirrhosis (4%), other (4%)
Birmingham (Evans 2006) [12]	113, 135, 64	1, 5, 10 years	32% at 1 year 55% at 5 years 69% at 10 years	Chronic hepatitis ± fibrosis (64%), biliary fibrosis (2%), recurrent PSC (2%), other (2%) - at 10 years
Kings College Hospital London (Bachina, 2008) [13]	13	>10 years	92%	Fibrosis (92%) - moderate/severe in 30% lymphocytic infiltration of variable severity (54%) All protocol biopsies from children with normal LFTs
Chicago (Ekong 2008) [14]	63	>3 years	97%	Fibrosis (97%), inflammation (70%)
Groningen (Scheenstra 2009) [15]	77, 64, 66, 55	1, 3, 5, 10 years	34% at 1 year 48% at 3 years 65% at 5 years 69% at 10 years	Fibrosis (69%) - at 10 years

overlapping spectrum of immune-mediated damage in the liver allograft [17].

The extent to which protocol biopsies are obtained as part of annual review has changed in recent years [18]. Whilst most centres still carry out protocol biopsies in HCV-positive patients, mainly to assess disease progression, the majority have discontinued this practice in other allograft recipients. However, recent studies have shown that histological abnormalities are not only frequently present in protocol biopsies from patients with normal liver function tests (Tables 2 and 3), but also that they include changes that are potentially significant clinically, such as progressive graft fibrosis [7,9,12,14]. Conversely, the finding of normal or near-normal graft histology in a protocol biopsy may be useful in supporting the decision to reduce immunosuppressive therapy, if this is clinically indicated (e.g. in a person with renal impairment) [9], and is also regarded as an important baseline assessment for patients who may be tolerant to immunosuppression weaning [19–23].

## Late rejection

### Acute rejection (AR)

Although the prevalence of acute rejection (AR) is declining, around 20–40% of patients still have one or more episodes requiring treatment with additional immunosuppression [24]. The majority of episodes still occur during the first few weeks of transplantation and typically present with predominantly portal-based inflammation, which is associated with inflammation of bile ducts and portal venules [1]. Studies dating back to the late 1980s have suggested that late AR may have different histological features to early acute rejection [3,25,26] –

these include a predominantly mononuclear portal inflammatory cell infiltrate (contrasting with the mixed population of cells more typically seen in early AR), less severe inflammation of bile ducts and portal venules, more prominent interface hepatitis (in some cases associated with periportal fibrosis), and more prominent lobular hepatitis [27,28]. Lobular inflammatory changes tend to be most prominent in centrilobular regions and are often associated with foci of centrilobular or bridging necrosis [29–37] – these changes are collectively referred to as “central perivenulitis” (CP) [30,37] and can occur in the absence of significant portal inflammation (“isolated central perivenulitis”) [29,30,37–39] (Fig. 1). For example, in two recent studies isolated CP was present in 22% of children biopsied >3 months post-LT [38] and 28% of adults undergoing protocol biopsy >3 years post-LT [39]. Hepatic venous endothelitis, which is typically seen in association with CP in early acute rejection, is rarely conspicuous in late rejection. Grading of late rejection with features of CP is often difficult according to the conventional Banff criteria, which require the presence of typical portal tract changes of AR [40]. However, a system proposed by the Banff Working Group for grading the severity of CP [30] appears to have some value in predicting adverse outcomes [39].

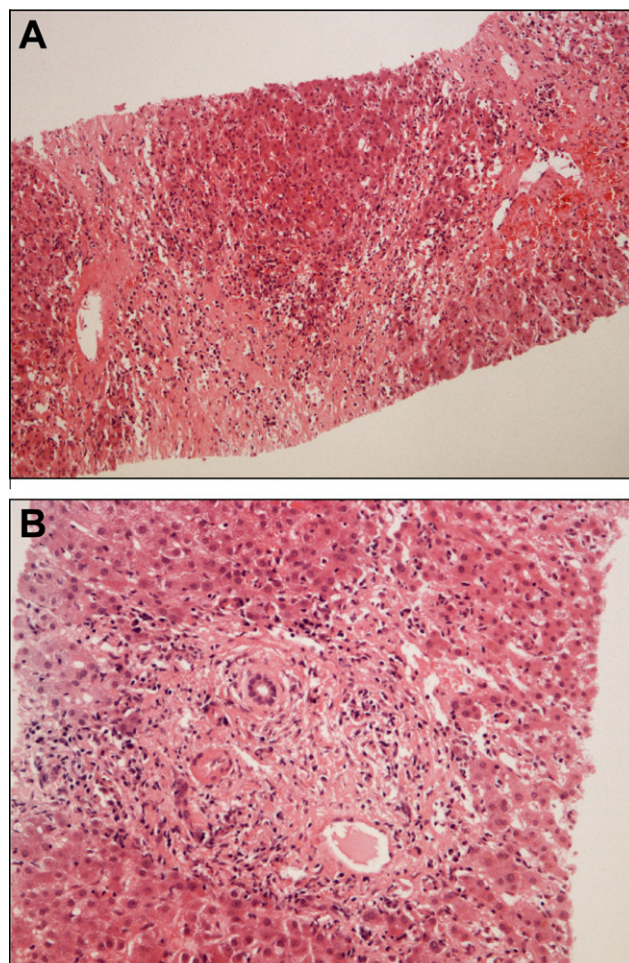
**Table 4. Factors influencing the histological assessment of late post-transplant biopsies.**

Nature of original liver disease
Indication for liver biopsy (protocol or clinically indicated)
Duration of follow up
Type/amount of immunosuppression used
Diagnostic criteria/terminology

**Key Points**

- Histological abnormalities are commonly present in late post-transplant biopsies from adult and paediatric liver allograft recipients. These include protocol biopsies from patients who appear to be clinically well with good graft function.
- Recurrent disease is the commonest recognized cause of abnormal graft histology in late biopsies from adults, but is very uncommon in the paediatric population. The features of recurrent disease may be modified by the effects of immunosuppression and interaction with other graft complications, resulting in changes that are complex and difficult to interpret.
- In cases where graft dysfunction has more than one possible aetiological factor (e.g. recurrent hepatitis C and rejection), liver biopsy may help to identify the main cause of graft damage.
- Problems exist with the terminology used to describe late inflammatory changes that are not obviously related to recurrent disease. Although late rejection (with “hepatic features”), *de novo* autoimmune hepatitis and “idiopathic” post-transplant chronic hepatitis are generally regarded as distinct entities, there is emerging evidence to suggest that they may all be part of an overlapping spectrum of immune-mediated injury in the long-term liver allograft.
- Recent studies suggest that “idiopathic” chronic hepatitis is an important cause of late graft fibrosis, in some cases leading to cirrhosis, particularly in the paediatric population. Further studies are required to determine which patients with late graft hepatitis are likely to progress to fibrosis or cirrhosis and whether such patients may benefit from treatment with immunosuppression
- Further studies are also required to determine the role of protocol biopsies in identifying patients in whom immunosuppression can be safely reduced or withdrawn completely in the hope of achieving “operational tolerance”.

The overall appearances of late AR may thus resemble those seen in chronic viral or autoimmune hepatitis and late AR, therefore, needs to be distinguished from other causes of post-transplant chronic hepatitis, including viral infection, autoimmune hepatitis (recurrent or *de novo*), and “idiopathic” post-transplant hepatitis. In terms of clinical management, the most important distinction concerns late AR and hepatitis C, which will be discussed later. The other conditions presenting with graft inflammation at this stage are all likely to have an allo/auto-immune basis and should, therefore, benefit from an increase in immunosuppression. Late rejection with features of CP often presents with raised transaminase levels, contrasting with the cholestatic liver biochemistry that is more typically seen in early



**Fig. 1. Late rejection presenting with features of severe central perivenulitis.** Liver biopsy 11 years post-transplant for PSC from a patient in whom there had been a recent reduction in immunosuppression because of concerns about renal impairment and suspected biliary sepsis. An autoantibody screen was negative. (A) Prominent inflammation and confluent necrosis are present in two adjacent perivenular regions and are associated with central-central bridging necrosis. (B) By contrast, a portal tract shows only mild inflammation not involving the bile duct or portal vessels.

portal-based AR [33,34,41]. It tends to be less responsive to immunosuppression and is associated with an increased frequency of adverse outcomes – these include further episodes of acute rejection [36,42], progression to chronic rejection [32,33,39,42–45], and the development of *de novo* autoimmune hepatitis [39,43]. In some cases, CP may lead to the development of centrilobular fibrosis [38,39]. Recognition of these changes at an early stage and instigation of appropriate immunosuppressive therapy may prevent the development of more serious graft complications such as progression to chronic rejection. Many cases of late acute rejection appear to be related to suboptimal immunosuppression [46,47], although this is not always the case [48]. Treatment in such cases requires re-instigation of adequate baseline immunosuppression in addition to high-dose corticosteroids, similar to the regimen used for treating early acute rejection.

# Frontiers in Liver Transplantation

**Table 5. Summary of the main diseases (excluding neoplasms) that are thought to recur following liver transplantation.**

Recurrent Disease	Frequency (%)	Histological Features (in biopsies >12 months post-transplant)	Comments
Hepatitis B	<10	Chronic hepatitis (typically mild). Fibrosis now rarely more than mild in severity.	High frequency in 1980s/early 1990s (15-85%). Incidence and clinical impact now greatly reduced by use of anti-viral therapy pre- and post-transplant.
Hepatitis C	>90	Chronic hepatitis, typically resembling changes seen in the native liver. More severe cases may be associated with prominent interface hepatitis ± confluent or bridging necrosis. Some cases have “autoimmune features” Progressive disease common - 20-50% cirrhotic by 5-10 years.	Commonest indication for liver transplantation in many centres. Most cases result in graft damage, severity variable. Most frequent cause of late graft failure.
Primary biliary cirrhosis (PBC)	20-50	Lymphocytic or granulomatous cholangitis. Portal mononuclear inflammation-typically focal. May precede development of typical bile duct lesions by several years. Progressive disease associated with ductopenia and features of chronic cholestasis. Progression to cirrhosis rare.	Most cases have mild/asymptomatic disease, frequently diagnosed on protocol biopsies. Rare cases (<1%) progress to graft failure
Primary sclerosing cholangitis (PSC)	20-30	Fibrous cholangitis (rarely seen in liver biopsies). Diagnosis more often based on findings of chronic cholestasis, ductopenia, ductular reaction and a “biliary pattern” of fibrosis. Approximately 25% have bridging fibrosis or cirrhosis by 5 years post-transplant.	More frequently clinically symptomatic than recurrent PBC. Approximately 10% progress to graft failure. Histological and radiological features difficult to distinguish from ischaemic cholangiopathy. Diagnosis therefore requires exclusion of other causes of biliary tract disease.
Autoimmune hepatitis (AIH)	20-30	Portal tract plasma cell-rich inflammatory infiltrate associated with interface hepatitis. Lobular inflammation frequently present - severe cases include foci of confluent/bridging necrosis. Lobular inflammatory changes may resemble “central perivenulitis” and can occur as the first manifestation of recurrent disease.	Most cases occur as a result of suboptimal immunosuppression and respond to immunosuppressive therapy. Diagnosis based on a combination of biochemical, serological, and histological findings.
Alcoholic liver disease (ALD)	10-30	Fatty change common (>60% of cases) Steatohepatitis and fibrosis less common. Progression to cirrhosis rare.	Recurrent alcohol consumption common, but serious graft complications are rare.
Non-alcoholic Fatty Liver Disease (NAFLD)	20-40	Fatty change common (60-100% of cases). 10-40% progress to steatohepatitis and up to 12% become cirrhotic.	Risk factors for NAFLD often persist and may be exacerbated by immunosuppressive drugs and other transplant-related factors. Many people with recurrent NAFLD have normal LFTs.

## Chronic rejection (CR)

Classical cases of chronic rejection (CR), described in the late 1980s and early 1990s, were associated with ductopenia and an obliterative arteriopathy, usually progressing to graft failure within the first 12 months of transplantation [1,27]. Improvements in immunosuppression have resulted, not only in a reduced prevalence of graft failure from CR (currently less than

1–2%), but also in a different pattern of presentation. More cases now occur later (>12 months post transplant) with a more insidious presentation and an indolent course, in some cases running for a period of several years without progressing to graft failure [6,49]. Histological features may also be different to those seen in classical cases of chronic rejection. One important example is the development of features of chronic cholestasis including a ductular reaction and periportal fibrosis [49], producing a “biliary

pattern” resembling that seen in biliary obstruction. Late CR may thus resemble changes seen in other chronic biliary diseases associated with ductopenia in the liver allograft - these include recurrent primary biliary cirrhosis (PBC), recurrent primary sclerosing cholangitis (PSC), and ischaemic cholangiopathy. Before attributing changes to late CR, imaging of the biliary tree should be carried out to exclude others cause of large duct disease, including recurrent PSC. Features favouring a diagnosis of CR include bile duct inflammation, senescence-related changes in bile ducts (atrophy, nuclear pleomorphism, disordered polarity, and cytoplasmic eosinophilia) [50], and the presence of CP [30]. In cases where there is a problem in the distinction between late CR and recurrent biliary disease (PBC or PSC), review of previous biopsies may help to identify CR as the more likely diagnosis. Most cases of late CR will have one or more previous biopsies showing features of acute rejection, often including prominent bile duct injury and other features suggesting transition to early chronic rejection. In some cases CP lesions may progress to centrilobular fibrosis and less commonly to the development of bridging fibrosis or cirrhosis. The latter typically has a veno-centric pattern, which is probably related to rejection-induced occlusive lesions in hepatic and portal veins [30,49]. In cases where diagnostic histological features are lacking, knowledge of the clinical context (e.g. suboptimal immunosuppression levels) and review of preceding biopsies (which usually include one or more specimens with AR) should help to identify CR as the most likely cause for graft dysfunction.

### Disease recurrence

Recurrent disease is the commonest recognized cause of graft dysfunction in patients surviving >12 months post-transplant. A summary of the main diseases that recur in the liver allograft is presented in Table 5. The prevalence and clinical significance of disease recurrence vary considerably. For some conditions (e.g. hepatitis C), recurrence is common and has an important impact on graft function and graft survival. Other diseases recur in a

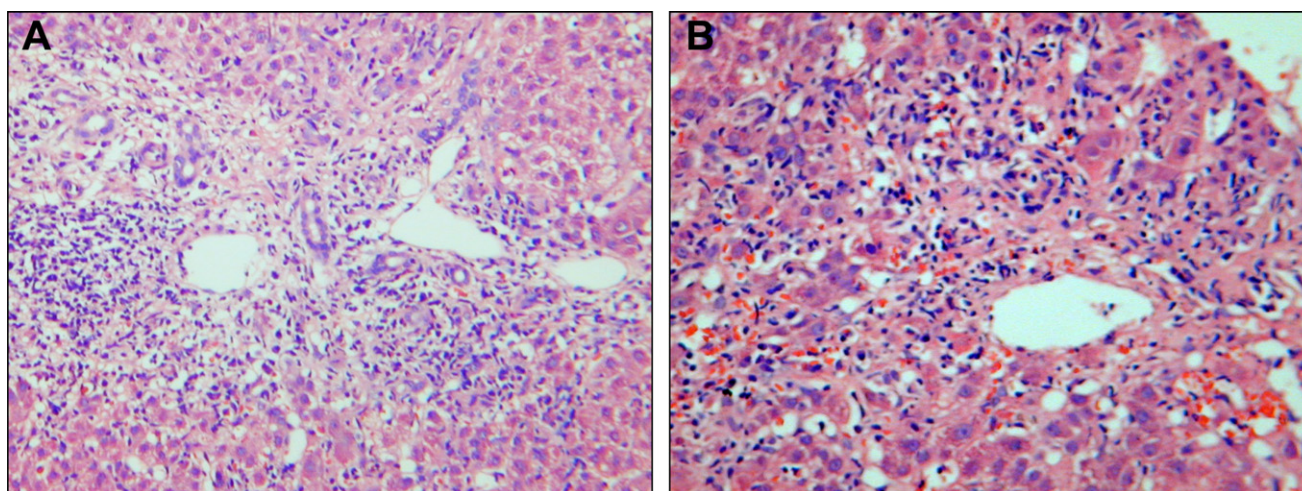
mild/subclinical form (e.g. PBC) or respond readily to treatment (e.g. autoimmune hepatitis) and thus have little or no impact on graft or patient survival.

There are a number of factors that influence histological assessment of disease recurrence in the liver allograft [1,27,51,52]. Histological features of some recurrent diseases overlap with other complications of liver transplantation. The most important example is hepatitis C and acute rejection. Other examples are recurrent biliary disease (PBC, PSC) having overlapping features with CR and recurrent PSC with ischaemic cholangiopathy. Interactions between recurrent disease and other graft complications (e.g. hepatitis C and rejection) can produce complex changes that are difficult to interpret. The effects of immunosuppression should also be considered. Viral infections behave more aggressively in this setting - histological manifestations of this include more severe inflammatory activity and more rapid progression to fibrosis and cirrhosis or, in a small number of cases, atypical cholestatic features (“fibrosing cholestatic hepatitis”). By contrast immune-mediated diseases such as autoimmune hepatitis (AIH) or PBC should be prevented from recurring or progress more slowly when adequate levels of immunosuppression are maintained, but may be “unmasked” if immunosuppression is reduced for any reason.

The histological features of most recurrent diseases are similar to those occurring in the native liver and will not be described in detail here. Some of the main changes that are seen in biopsies obtained >12 months post-LT are summarized in Table 5. For a more detailed description of the pathology of recurrent disease, the reader is referred elsewhere [1,27].

### Recurrent hepatitis C

End-stage liver disease due to chronic HCV infection is the leading indication for transplantation in many centres. Re-infection is universal and begins within a few hours of implanting the new liver [53]. Most cases (>80%) develop graft inflammation related to HCV, but the severity and clinical consequences of graft re-infection are very variable [54].



**Fig. 2. Recurrent hepatitis C with severe inflammatory activity.** Liver biopsy 21 months post-transplant from a man who presented with acutely deranged LFTs (AST  $15\times$  normal) shortly after antiviral therapy had been stopped because of nephric abscess. He became HCV-RNA positive, having previously been HCV-RNA negative. (A) Portal tract contains a moderately dense infiltrate of mononuclear inflammatory cells. This is not associated with significant inflammation of bile ducts or portal vessels. There is moderate interface hepatitis. (B) There is prominent perivenular inflammation with confluent hepatocyte necrosis (“central perivenulitis”).

## Frontiers in Liver Transplantation

Histological features in biopsies obtained >12 months post-LT are mostly similar to those that are seen with chronic HCV infection in the native liver. There is typically a predominantly portal-based mononuclear inflammatory infiltrate including lymphoid aggregates. Interface hepatitis and lobular inflammation are usually mild in severity. Mild steatosis may also be present. In addition to confirming a diagnosis of recurrent HCV (and excluding other causes of graft dysfunction), liver biopsies are used to assess disease severity and progression. Histological abnormalities are often present in protocol biopsies from HCV-positive patients who are clinically well, with apparently normal graft function [5,6] and the changes seen in these specimens may have implications for prognosis and treatment [54]. For example, the presence and severity of fibrosis at one year have been shown to be predictive for subsequent progression to cirrhosis and graft failure [55–58] and this information may help to identify patients who are most likely to benefit most from anti-viral therapy [59]. Liver biopsies are also used to monitor treatment responses [60]. The scoring systems used for grading and staging HCV in the native liver have also been applied to post-transplant biopsies. However, they should be used cautiously in this setting, particularly in cases with atypical features or the possibility of a dual pathology (e.g. HCV and rejection).

Non-invasive markers are increasingly used to assess the severity of HCV infection and monitor disease progression in the native liver and have also more recently been used in a similar manner in liver allograft recipients. These include serum biomarkers and transient elastography, both of which have been shown to reliably diagnose advanced fibrosis in HCV-positive patients [61,62] and to have predictive value in identifying patients likely to progress rapidly to graft fibrosis [63,64]. Caution should be applied to the interpretation of these non-invasive tests in the transplant population, as there are other possible causes of graft fibrosis, but it is likely that they will lead to a changing role for liver biopsy in the assessment of allograft damage in HCV-positive patients.

There are three important differences that apply to the histological assessment of HCV in the liver allograft. Firstly, the disease tends to behave in a more aggressive manner. This may be manifested by more severe inflammatory activity, sometimes including areas of confluent and bridging necrosis (Fig. 2), which are very rarely seen with HCV infection in the non-immunosuppressed person. In such cases, the possibility of an additional cause for lobular necro-inflammation should be considered, the main ones being late rejection and *de novo* autoimmune hepatitis [30]. In cases where it is difficult to make a distinction between aggressive recurrent HCV and other causes of lobular necro-inflammation (usually manifested as central perivenulitis), knowledge of relevant clinical events may help to identify the most likely cause (Fig. 2). For example, a recent reduction in immunosuppression would favour a diagnosis of rejection, whereas high viral RNA levels favour HCV as a more likely cause for graft dysfunction. There is also more rapid progression to fibrosis and cirrhosis – approximately 20–50% of patients are cirrhotic 5–10 years post transplant [54,65]. Secondly, there may be atypical features, some of which reflect the effects of immunosuppression – these include cholestatic features resembling so-called fibrosing cholestatic hepatitis (FCH), first described as a complication of HBV infection, and features resembling autoimmune hepatitis. FCH mainly presents during the first few months after transplantation, when levels of immu-

nosuppression are highest [66,67]. It is rarely seen >12 months post-LT and will not, therefore, be discussed further here. Thirdly, there are important interactions with other graft complications, particularly rejection – these may produce complex histological changes that are difficult to interpret.

### *HCV with “autoimmune features” (“plasma cell hepatitis”)*

Recent studies have described biochemical, immunological, and histological features resembling AIH in patients transplanted for HCV. These have occurred in two main settings. Some have presented as a complication of anti-viral therapy with interferon (IFN) and may represent an alloimmune response triggered by IFN-induced stimulation of the host immune system [68–71]. The overall prevalence in the largest reported series was 20% [70]. A similar mechanism has been postulated for the development of rejection in HCV-positive patients following treatment with IFN (discussed below). Other studies have documented histological features suggestive of AIH unrelated to anti-viral therapy in approximately 15% of patients with recurrent HCV [72,73]. In addition to having plasma cell rich portal and lobular inflammatory infiltrates – giving rise to the term “plasma cell hepatitis” (PCH) [73] – most cases are associated with features of central perivenulitis. Cases with autoimmune features have a worse outcome than those with “typical” recurrent HCV. Whilst some cases are associated with auto-antibodies and/or raised serum IgG levels, supporting a diagnosis of AIH [72], others appear to be related to suboptimal immunosuppression, suggesting that these changes may represent a form of superimposed rejection [73]. The presence of a plasma cell rich infiltrate in the native liver of patients transplanted for HCV has recently been identified as a risk factor for the subsequent development of PCH, suggesting that some patients have an immunological predisposition to develop PCH [74].

### *Hepatitis C and rejection*

The relationship between HCV and rejection is complex. The immunosuppressive therapy used to treat episodes of AR is an important risk factor predisposing to more severe HCV infection [75,76]. Conversely, a higher incidence of AR and CR has been observed in HCV-positive patients compared with those transplanted for other disease [77–81]. This association probably reflects a number of factors – these include different approaches to immunosuppression in HCV-positive patients, shared pathways of immune-mediated damage between HCV and rejection and the effects of anti-viral therapy, which may result both in non-specific augmentation of the host’s immune system and increased hepatocellular metabolism of immunosuppressive drugs following viral clearance from hepatocytes [81–83].

The distinction between HCV infection and rejection as a cause for graft dysfunction continues to be major problem clinically. Non-invasive methods are not reliable in making the distinction and this, therefore, remains a common indication for liver biopsy. Unfortunately, the two conditions also have overlapping histological features, making the assessment of liver allograft biopsies difficult. Both conditions are characterised by predominantly portal-based inflammation. Other features typically seen in acute rejection, which are also recognized to occur with HCV infection in the native liver, are bile duct inflammation, portal venous endothelitis, and portal tract eosinophilia [84,85], although these changes are rarely more than mild in severity. Features that are helpful in distinguishing recurrent HCV

**Table 6. Comparison of histological changes occurring in hepatitis C infection and acute cellular rejection of the liver allograft.** (A) Portal and periportal changes; (B) Lobular changes.

**A**

	Hepatitis C	Rejection
Portal inflammation	Mainly mononuclear cells (lymphoid aggregates)	Mixed infiltrate (lymphocytes, macrophages, blast cells neutrophils, eosinophils)
Interface hepatitis	Variable (generally mild)	Usually mild
Bile duct inflammation	None/mild (lymphocytes)	Variable, may be prominent (mixed infiltrate)
Bile duct loss	None	Variable (in cases progressing to chronic rejection)
Venous endothelial inflammation	None/mild	Variable, may be prominent
Fibrosis	Yes	No (except in cases with chronic hepatic features)

**B**

	Hepatitis C	Rejection
Lobular inflammation		
Severity	Generally mild	Variable
Pattern	Spotty	Confluent
Distribution	Random	Perivenular
Associated features	Lobular disarray	Hepatic vein endothelitis
Cholestasis	Rare (except FCH-like cases)	Common
Fatty change	Yes (macrovesicular)	No
Acidophil bodies	Common	Less numerous

hepatitis from rejection are summarized in Table 6. In most cases, the time of occurrence and pattern of inflammation enable the main cause of graft damage to be identified with a reasonable degree of confidence. Most episodes of AR occur during the first 3 months of transplantation, a time at which portal inflammatory changes related to recurrent HCV infection are unlikely to occur. Conversely, AR is rare >12 months post-LT, whereas this is a time at which portal hepatitis related to recurrent HCV is likely to be present. A problem in the assessment of late biopsies from HCV-positive patients concerns the possibility of late rejection presenting with features of chronic hepatitis or central perivenu- litis, both of which are discussed earlier.

Biopsies in which the distinction between HCV and AR is difficult are likely to have changes reflecting a combination of both conditions [86,87] (Fig. 3). In the majority of such cases, rejection-related changes are mild in severity – recurrent HCV is best regarded as the primary diagnosis and anti-rejection therapy is not indicated [87,88]. Increased immunosuppression should only be considered as a treatment option if AR is at least moderate in severity, or if there are features suggesting progression to CR.

Immunohistochemical studies have also been used in the differential diagnosis between hepatitis C and rejection. These include staining for HCV antigens as a marker of HCV infection [89–91], C4d as a marker of rejection [92–94], hepatocellular expression of the interferon-inducible MxA protein as a marker of chronic HCV infection [95], and the cell-cycle protein mini-chromosome maintenance (mcm) protein mcm-2 to identify the rate of proliferation in portal lymphocytes (higher in rejection than HCV) [96]. Some of these approaches may help to identify

the main cause of graft damage in cases where routine histological findings are inconclusive [89,96].

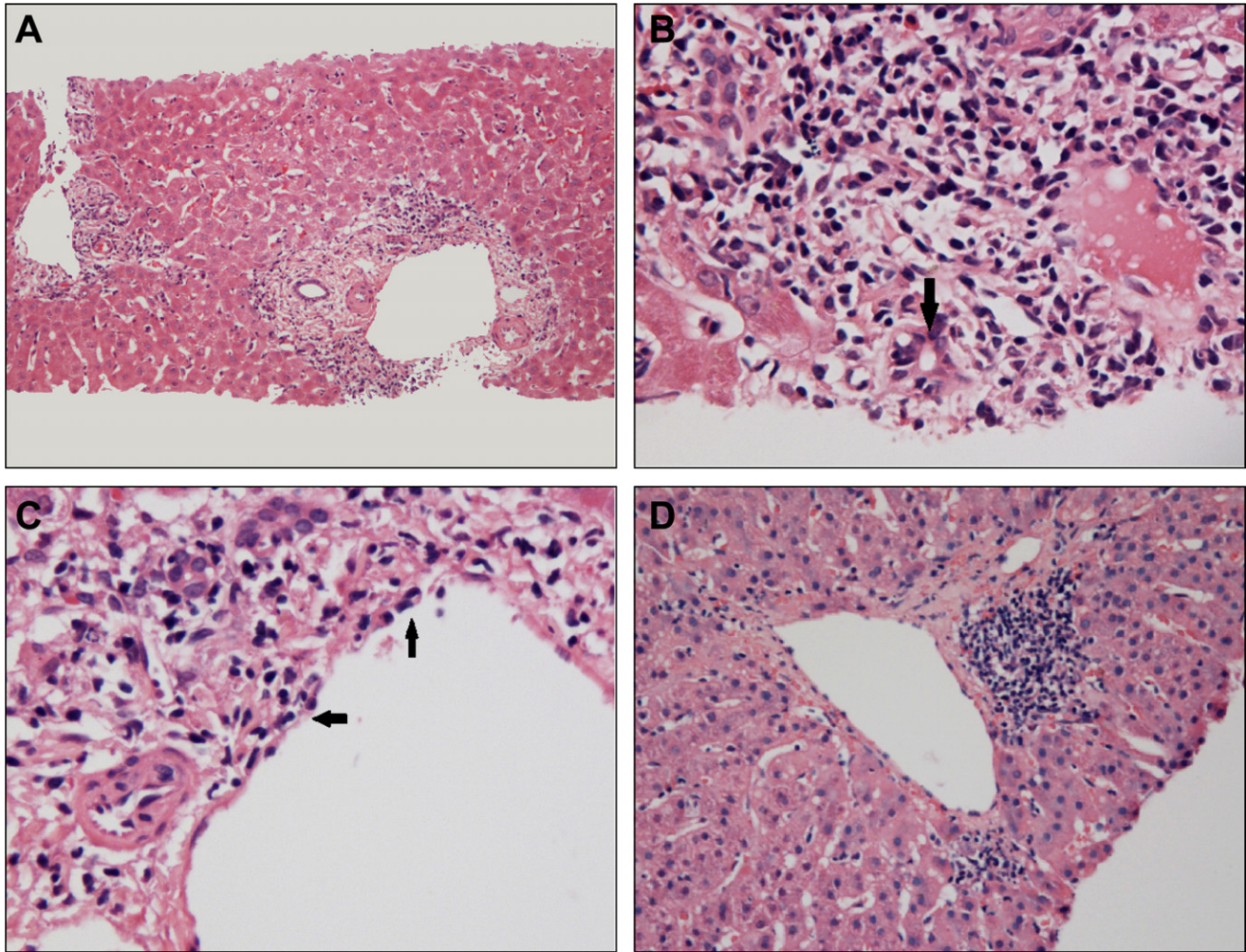
**De novo disease**

This term has been used to describe patients who are transplanted for one type of primary liver disease and subsequently develop features suggesting a different primary liver disease. A number of diseases listed in Table 5 can develop *de novo* following liver transplantation. This applies to viral infection (hepatitis B and C), which may be acquired from the donor liver or other sources, AIH and non-alcoholic fatty liver disease (NAFLD). Histological features are generally similar to those seen in recurrent disease.

*De novo autoimmune hepatitis*

The most important example of *de novo* disease is *de novo* AIH. Numerous studies have described classical biochemical, immunological, and histological features of AIH developing in patients transplanted for other diseases [35,43,97–106]. A higher frequency has been reported in children (5–10%) compared with adults (1–2%), possibly related to immunosuppressive drugs interfering with normal T cell maturation in the immature immune system. Most cases present >1 year post-LT and respond well to increased immunosuppression, but some have progressed to cirrhosis or graft failure. Histological features closely resemble those occurring with AIH in the native liver and recurrent AIH in the liver allograft. They include a plasma-cell rich mononuclear



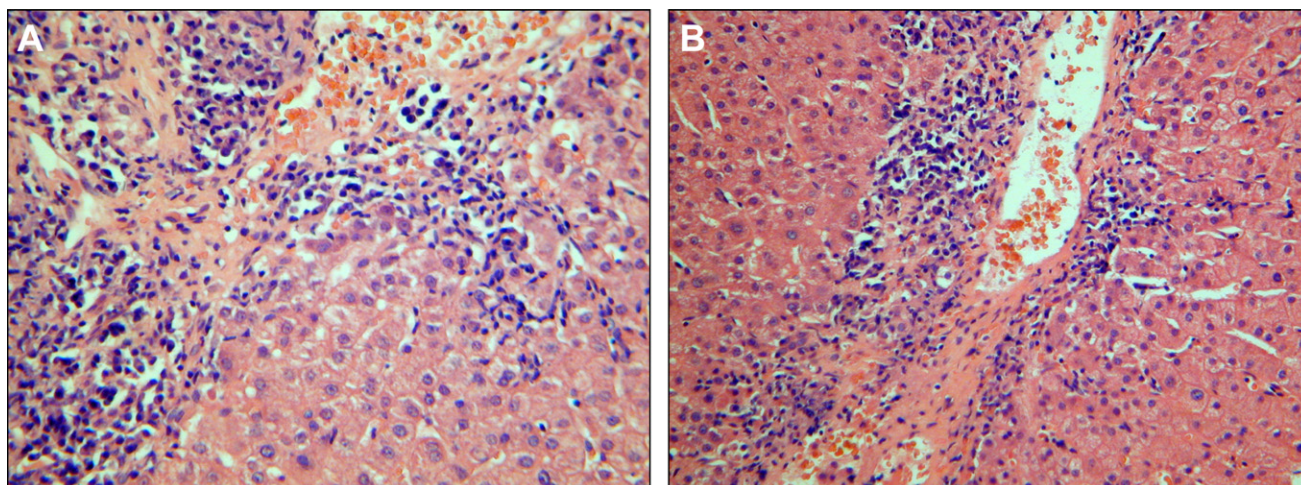


**Fig. 3. Features in keeping with recurrent hepatitis C and rejection.** Liver biopsy 3 years post-transplant from an HCV-positive patient who presented with a raised AST ( $7\times$  normal) (A) Features compatible with mild chronic hepatitis C include portal tracts with a light infiltrate of mononuclear inflammatory cells, lymphoid aggregate formation, mild interface hepatitis, and mild steatosis. Additional features suggesting the presence of co-existent rejection are (B) prominent bile duct injury (arrow), (C) portal vein endothelitis (arrows), and (D) small foci of central perivenulitis.

portal inflammatory infiltrate associated with varying degrees of interface hepatitis (Fig. 4). Lobular inflammation and necrosis tend to be more prominent than in the native liver [98,100] and can include features of central perivenulitis [30]. It is worth noting that autoantibodies are frequently present without signs of graft dysfunction, particularly in the paediatric population [35,102,107], and liver biopsy is, therefore, required to determine the nature of any damage present.

Many studies have identified overlapping areas between *de novo* AIH and rejection – these include autoantibodies being present, sometimes transiently, in otherwise typical episodes of rejection (acute and chronic) [35,102,108,109], previous acute rejection episodes being a risk factor for the development of *de novo* AIH [43,99,104] and, as discussed earlier, features of *de novo* AIH arising in the setting of under-immunosuppression [73] or when the recipient's immune system is stimulated by anti-viral treatment with interferon [68–71]. Perhaps the most convincing evidence for an alloimmune response has come from two Spanish groups, who both found that the *de novo* AIH occurred exclusively in glutathione-S-transferase T1 (GSTT1) negative recipients of a

GSTT1-positive donor liver and was associated with the development of donor-specific anti-GSTT1 antibodies [100,101,103,105]. As the GSTT1 enzyme is expressed by hepatocytes in GSTT1-positive individuals, the development of *de novo* AIH in the setting of a donor/recipient mismatch for GSTT1 may thus represent a form of late rejection, in which immune-mediated injury is mainly directed towards hepatocytes [17]. However, some studies have failed to demonstrate anti-GSTT1 antibodies as an important factor in the development of *de novo* AIH [110–112] – such cases may be associated with the development of autoantibodies directed towards other potential target antigens expressed by hepatocytes. Examples include, antibodies to cytokeratin 8/18 [110] and atypical LKM antibodies to carbonic anhydrase III or subunit beta 1 of proteasome [113]. The *de novo* development of antibodies to the bile salt export pump (BSEP) protein, in children who became cholestatic following transplantation for BSEP deficiency, can likewise be regarded as an alloimmune response to BSEP proteins expressed in the canaliculi of the liver allograft [114–116]. The distinction between alloimmune and autoimmune responses becomes blurred with



**Fig. 4. Chronic hepatitis with features suggesting a diagnosis of *de novo* autoimmune hepatitis.** Liver biopsy 2 years post-transplant from a patient transplanted for metastatic gastrointestinal stromal tumour, who developed abnormal LFTs (AST 2× normal). (A) A portal tract contains a dense plasma cell rich inflammatory infiltrate associated with interface hepatitis. (B) A similar infiltrate is present in a perivenular region where it is associated with features of central perivenulitis. The patient was subsequently found to have autoantibodies and a raised serum IgG.

time following transplantation – whilst early acute rejection episodes are MHC-restricted and epitope specific, the resultant graft damage may lead to T cell responses to other graft antigens occurring in a non-MHC restricted fashion and may also break tolerance to self antigens, thus allowing the development of autoimmune responses [117,118].

*De novo non-alcoholic fatty liver disease (NAFLD)*

The distinction between recurrent and *de novo* NAFLD is often difficult. Liver transplant patients are at risk for developing a number of features of the metabolic syndrome, such as diabetes mellitus, weight gain, hypertension, and hyperlipidaemia and are thus predisposed to the development of NAFLD [119,120]. Steatosis in the donor liver has also been identified as a risk factor for the development of steatosis in late post-transplant biopsies, although the mechanism for this is uncertain [121]. Several studies have identified cases of NAFLD, which appear to have arisen *de novo* following liver transplantation [119,121–126]. However, some of these have occurred in patients who were transplanted for cryptogenic cirrhosis and/or had risk factors for the metabolic syndrome prior to transplantation and could thus be regarded as having recurrent rather than *de novo* disease [119,122,123,125,127]. Interactions between hepatitis C infection, insulin resistance, and NAFLD also appear to be important in the pathogenesis of recurrent HCV and *de novo* NAFLD [126,128–131].

Fatty change, apparently unrelated to recurrent disease, is present in 18–40% of post-transplant biopsies [7,120,121,125,126] and is also a common finding in protocol biopsies obtained from patients with normal liver function tests (LFTs) [7,9,121]. Steatosis is mainly macrovesicular and usually mild in severity. Features of steatohepatitis, also typically mild in severity, are present in 1–13% of cases. Perisinusoidal fibrosis was present in 29% of patients with *de novo* NAFLD in one study [121] – however, this lesion is difficult to assess in the liver allograft as a similar pattern of fibrosis may also occur as part of the spectrum of “hepatic structural abnormalities”, which are discussed later. More severe fibrosis is unusual, but occasional cases have progressed to cirrhosis.

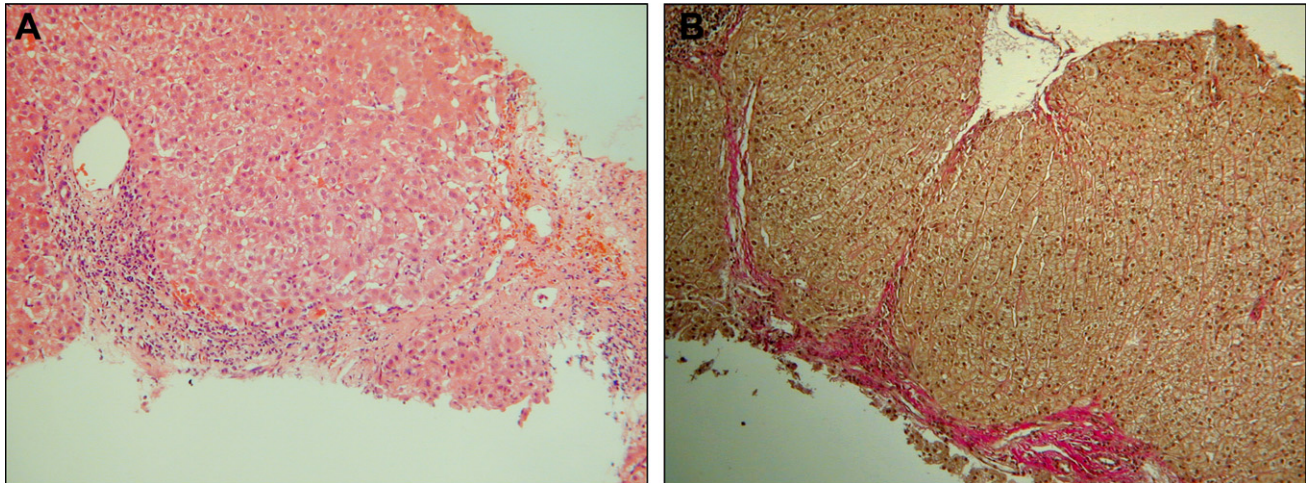
**Other histological findings in late post-transplant biopsies**

*Idiopathic post-transplant hepatitis (IPTH)*

The terms “idiopathic chronic hepatitis” or IPTH, have been used to describe cases with histological features of chronic hepatitis that are not obviously related to recognized cause of chronic graft hepatitis, such as chronic viral infection or autoimmune hepatitis (recurrent or *de novo*) (Table 7). The reported frequency varies considerably, possibly reflecting a number of factors that have been discussed earlier (Table 4). The variable terminology used to describe unexplained inflammatory changes in late post-transplant biopsies may account for at least some of the apparent discrepancies between individual centres. Other terms used in this context include “portal/parenchymal mononuclear inflammation” [132], “portal lymphocytic inflammation” [133], “non-specific inflammation” [134], “graft inflammation” [14], “interface hepatitis” [135], and “non-specific hepatitis” [10]. Overall, it has been estimated that features compatible with a diagnosis of IPTH can be observed in 10–50% of patients undergoing protocol biopsy >1 year post-transplant [16] and up to 60% of

**Table 7. Causes of chronic hepatitis in the liver allograft.**

Viral disease (recurrent or acquired)	
	Hepatitis B
	Hepatitis C
	Hepatitis E
	Other
Recurrent autoimmune disease	
	Autoimmune hepatitis
	Primary biliary cirrhosis
	Primary sclerosing cholangitis
De novo autoimmune hepatitis	
Unknown (? Late rejection)	



**Fig. 5. “Idiopathic” post-transplant chronic hepatitis.** Liver biopsy 9 years post-transplant from a patient who underwent liver transplantation for cystic fibrosis at the age of 13 years. (A) Portal tract contains a moderately dense infiltrate of mononuclear inflammatory cells, not associated with typical features of acute or chronic rejection. An area of lobular inflammation is associated with central-portal bridging necrosis. (B) There is fibrous portal expansion with bridging fibrosis (Haematoxylin Van Gieson).

children at 10 years [12], making this the commonest overall diagnosis in annual review biopsies from adults [136] and children [12] in some centres. The prevalence increases with time – ranging from 20% to 30% during the first 3 years post-transplant to more than 60% at 10 years [12,14,135,137]. Most patients with “idiopathic” CH are clinically well with good graft function. Minor abnormalities of liver biochemistry may be present, usually a mild elevation in serum transaminases. Within the range of normal LFTs, the histological severity of hepatitis appears to correlate with serum alanine transaminase (ALT) levels [10].

Histological findings include a predominantly mononuclear portal inflammatory infiltrate, which lacks typical features of acute or chronic rejection and is associated with variable interface hepatitis (Fig. 5A). Lobular inflammatory changes are commonly present – they tend to be most marked in perivenular regions and may be associated with foci of confluent or bridging necrosis, resembling CP [30]. Recent studies have shown that chronic hepatitis unrelated to disease recurrence frequently leads to the development of fibrosis in the paediatric population, with 50–70% of children progressing to bridging fibrosis or cirrhosis by 10 years post-LT [12,14,135] (Fig. 5B). Progression to bridging fibrosis or cirrhosis has also been observed in up to 27% of adults with unexplained CH [137,138].

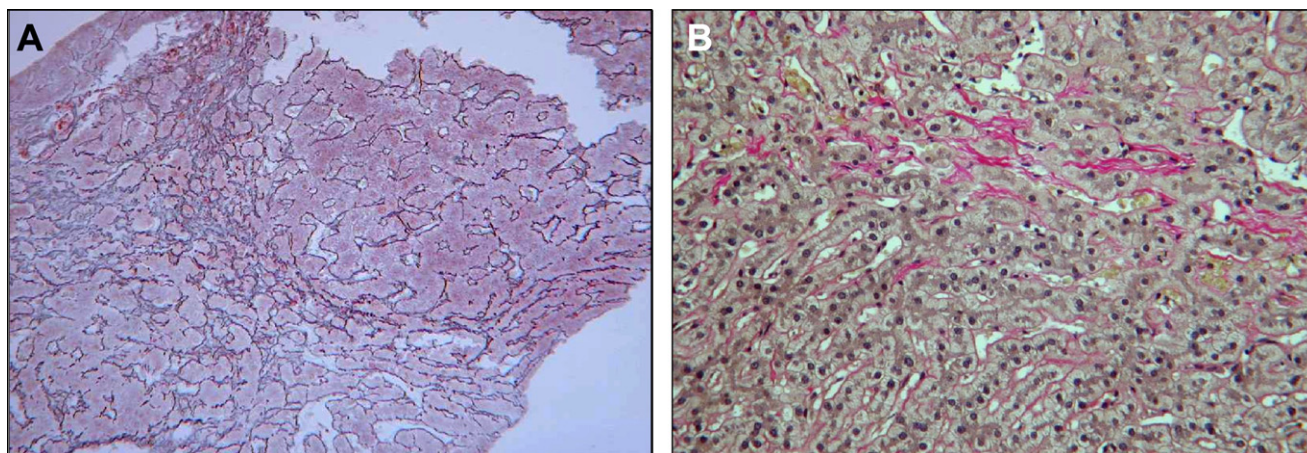
Many paediatric cases are associated with the development of autoantibodies [12] or uncharacterized serum factors reacting with normal hepatocytes and/or biliary epithelial cells [139] or with features suggestive of rejection [135], suggesting that this is likely to represent a form of immune-mediated graft damage. This hypothesis is supported by the observation that treatment of IPTH with increased immunosuppression may prevent fibrosis progression [140] and by the suggestion, discussed earlier, that late rejection may present with “hepatitic features” resembling chronic viral or autoimmune hepatitis. In cases of IPTH associated with auto-antibodies, a diagnosis of *de novo* AIH should be considered – however, many such cases have normal or only mildly elevated transaminase levels and do not thus fulfil the diagnostic criteria for *de novo* AIH [12]. Nevertheless, these observations provide further support for the concept that late rejection, *de*

*novo* AIH and IPTH may all be part of an overlapping spectrum of (allo)immune damage in the long-term liver allograft.

Before making a diagnosis of IPTH, other causes of chronic hepatitis in the liver allograft should be excluded (Table 7). Recognised viral agents, such as hepatitis B and C, cannot be identified in the great majority of patients who have graft hepatitis following liver transplantation for non-viral liver disease. A recently postulated cause of late graft inflammation is hepatitis E virus (HEV) infection, which has been suggested to lead to chronic hepatitis in the setting of immunosuppression and in a small number of cases to the development of severe fibrosis or cirrhosis [141–144]. Most cases appear to represent acquired infection and may be related to the consumption of inadequately cooked game meat or pork [145]. Treatment by reducing immunosuppression [144] or by giving anti-viral therapy [146] has proved to be effective in some cases. However, the functional significance of HEV infection has been questioned by studies showing that HEV seropositivity is present with a similar frequency in non-transplant patients with chronic liver disease [143] and in healthy adults with no evidence of liver disease [147]. A higher than expected frequency of unexplained chronic hepatitis has been seen in patients transplanted for acute liver failure due to seronegative hepatitis [138,148] – the reason for this is uncertain, but one possible explanation might be an unidentified viral agent. Features of a “non-specific” chronic hepatitis can occur as an early manifestation of recurrent autoimmune diseases, such as AIH [149], PBC [150], and PSC [151], in some cases preceding the development of more typical diagnostic features by several years.

### Other causes of graft fibrosis

There are other possible mechanisms of late graft fibrosis, particularly in the paediatric population. Centrilobular fibrosis has been identified in 10–20% of late post-transplant biopsies from children, sometimes as an isolated finding [11,152], and most likely represents organization of rejection-related central perivenulitis lesions.



**Fig. 6. Nodular regenerative hyperplasia.** (A) Protocol liver biopsy 1 year post-transplant from a patient transplanted for Wilson disease. Two hyperplastic hepatocyte nodules are separated by a zone in which cell plates are compressed (Reticulin stain). (B) Perisinusoidal fibrosis is present at the periphery of a hyperplastic nodule (Haematoxylin Van Gieson).

Portal fibrosis has been observed as an isolated finding in late biopsies from children [15,153,154]. The prevalence and severity increase with time – by 10 years fibrosis was present in 69% of children biopsied, of whom >50% had bridging fibrosis or cirrhosis [15]. Graft fibrosis in these cases was not associated with inflammatory changes supporting a diagnosis of chronic hepatitis or rejection, but instead with transplant-related factors predisposing to biliary complications [15]. A biliary pattern of fibrosis had been observed in an earlier study of the same cohort of patients [153].

#### *Hepatic structural abnormalities*

Hepatic structural abnormalities are seen in 20–30% of biopsies obtained >5 years post-transplant [3,4,6155,156]. The most common manifestation is nodular regenerative hyperplasia (Fig. 6A). Other observed changes include liver cell plate disarray, perisinusoidal fibrosis (Fig. 6B), hepatocellular atrophy, and sinusoidal dilatation/congestion. Possible causes include vascular problems (particularly portal venous insufficiency), drug toxicity (e.g. azathioprine) or immune-mediated damage to sinusoidal and/or vascular endothelial cells. Many cases are observed in protocol biopsies and have no obvious clinical manifestations [3,6], but some patients develop signs of portal hypertension [155] and a few have progressed to graft failure necessitating retransplantation [4,157]. A higher prevalence of nodular changes has been observed in late biopsies from reduced size allografts – this may relate to disturbances to the hepatic micro-architecture that occur following restoration of liver volume in these cases [158].

#### *Role of liver biopsy in graft monitoring and treatment*

Much of the foregoing discussion has focused on the concept that otherwise unexplained inflammatory changes in late post-transplant biopsies are likely to have an alloimmune basis, particularly in the paediatric population, and have the potential to progress to graft fibrosis or cirrhosis. The fact that these changes are frequently seen in protocol biopsies from patients who are clinically well with normal LFTs suggests that protocol biopsies have an important role in identifying subclinical graft dysfunction in

patients surviving long-term following liver transplantation [9,28]. They should probably also prompt a low threshold for autoantibody testing, particularly in children.

On the other hand, many patients undergoing late post-transplant protocol biopsy have minimal or mild inflammatory changes with no significant fibrosis. Can it be argued that these findings might indicate a form of graft tolerance and, if so, could they be used to identify patients in whom immunosuppression might be safely reduced or withdrawn? In a recent review of 235 protocol biopsies obtained >1 year post-LT from patients with normal LFTs, biopsy-directed changes in immunosuppression were instigated in 32% of cases [9]. In 76% of the cases where a change was made, immunosuppression was reduced – usually based on lack of inflammation in a patient where there were concerns about renal impairment.

Liver biopsies are also used routinely in the assessment and monitoring of patients undergoing immunosuppression withdrawal in an attempt to induce “operational tolerance”. The majority of studies have been carried out on patients with stable graft function, usually at least 2 years post-transplant, with overall success rates in the region of 10–20% (reviewed by Demetris [23] and Sanchez-Fueyo [159]). A higher frequency of successful weaning of immunosuppression has been observed in the paediatric population [160], particularly in young children [161], possibly reflecting the immature immune system in children. Pre-weaning biopsies are mainly used to exclude the presence of rejection, but may also be able to identify other features that are predictive of tolerance – examples include the absence of significant portal inflammation [19], lack of lobular CD3+ and CD8+ T cells [19], lack of fibrosis (in HCV-positive patients [20]), and the presence of portal T cells with a regulatory phenotype (FoxP3-positive) [21,162]. Studies on peripheral blood samples have also attempted to identify markers associated with a “tolerogenic profile” [159] – examples include increased numbers of regulatory T cells (CD4+, CD25+, FoxP3+) and delta1 T cells, decreased numbers of NK cells and alterations in the ratio of dendritic cell subsets. Oligonucleotide microarrays and bioinformatics have also been used to identify more complex tolerance-related gene expression profiles (reviewed by Sanchez-Fueyo [159]). Post-weaning biopsies have been used, both to investigate

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the cause of graft dysfunction (usually rejection) and on a protocol basis. Most episodes of AR occurring in this setting are mild and respond to treatment with re-instating immunosuppression. Some studies have identified bile duct atypia/atrophy and/or focal duct loss raising the possibility of progression to early CR [20,21,163], including protocol biopsies from patients with normal liver tests. Protocol biopsies have also shown more severe periportal fibrosis in patients who are operationally tolerant compared with those maintained on immunosuppression [21,22] – it has been postulated that this may reflect a pro-fibrogenic role for portal tract T lymphocytes with a regulatory phenotype, which are also present in such cases [22,164]. However, the fact that reinstatement of immunosuppressive therapy has resulted in reducing the severity of fibrosis in apparently tolerant patients suggests that such patients may in fact have a low-grade subclinical form of rejection [22].

### Conclusions and future developments

The majority of patients surviving long-term following liver transplantation have allografts that are histologically abnormal. Many of these abnormalities have been observed in protocol biopsies from people who appear to be well with good graft function. Uncertainties relating to the pathogenesis and clinical significance of many of the pathological changes that are seen in this setting have led to many centres reducing or discontinuing protocol biopsies. The role of protocol biopsies in assessing disease severity, such as fibrosis progression in recurrent hepatitis C, is also changing with the increasing use of non-invasive methods to assess liver fibrosis. Nevertheless, there is emerging evidence to suggest that protocol biopsies may reveal clinically important changes, such as graft fibrosis and cirrhosis, particularly in the paediatric population. Furthermore, some of these changes are likely to reflect the consequences of prolonged inflammation related to subclinical alloimmune injury and may, therefore, have implications for maintaining or increasing immunosuppressive therapy. Conversely, the absence of significant inflammation or fibrosis in a late protocol biopsy may help to identify patients in whom immunosuppression can be safely reduced or even withdrawn completely in the hope of achieving “operational tolerance”. Further studies are required to devise optimal algorithms for the use of liver biopsy in the assessment of the long-term liver allograft.

### Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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