Role of Histopathology in Liver Dysfunction After Transplant

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Overview

In the liver transplant multidisciplinary team, the (hepato-)pathologist plays an integral role in graft monitoring and treatment strategies of both early and late dysfunctions.

Although early mortality rates after LT have dramatically fallen over the last three decades, the rates of late graft loss and chronic adverse effects of chronic immunosuppression have remained constant.

To increase expectation for long-term, morbidity-free survival, it is essential to understand the underlying pathophysiology of causes of late graft and patient injury and failure.

Histological abnormalities are common in protocol liver biopsies from long-surviving recipients, even from asymptomatic patients with normal biochemical tests.

An increasing body of evidence supports the role of combined humoral and cell-mediated alloimmunity in long-term liver allograft injury.

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18.1 Introduction

Graft dysfunction following liver transplant (LT) occurs as both early- and late-new onset conditions, and histological assessment plays (or should play) an integral role at all stages in the management of transplanted patients. The spectrum of pathological findings that are seen in liver allograft biopsies is broad, and its interpretation always needs careful correlation with clinical and laboratory data (original disease, immunosuppression [IS], liver tests, viral serology, immunology and radiology) [1–3]. Post-transplant liver biopsies quite frequently show complex morphological pictures reflecting more than one aetiological process; the clinical picture is also often complex in such cases and histology may help to identify the predominant cause of graft damage [3].

In other cases, when a cause of dysfunction has been tentatively identified using other diagnostic approaches, liver biopsy may provide further important information pointing to the presence of an additional or alternative cause for graft dysfunction.

Moreover, the patterns of liver enzyme abnormalities and other clinical parameters are not always clear-cut in differentiating between different conditions which not infrequently require diametrically opposite therapies (e.g. rejection versus sepsis or biliary stenting) and the liver biopsy is crucial in defining these processes [1].

Again, it's well known and demonstrated that the correlation between standard biochemical tests and biopsy findings is poor and that recurrent and de novo liver diseases after LT can be present in the face of normal biochemical function, sometimes with advanced fibrosis [4].

Finally, since a significant proportion of long-term survivors suffer adverse side effects of immunosuppression (IS), some patients might be potential candidates for IS minimization. Protocol pre-weaning and follow-up post-weaning biopsies are recommended: the first is used to exclude any histological evidence of rejection or other findings that might exclude the patient from this procedure and the latter for monitoring the effects of IS withdrawal [5].

18.2 Indications for Liver Allograft Biopsy

To date, needle biopsies after LT are performed in two main settings: (1) when indicated by clinical or biochemical signs of graft dysfunction (e.g. signs or symptoms of liver disease, imaging abnormalities of the liver, elevation of liver tests); and (2) on a protocol basis (scheduled at regular time points irrespective of liver function test [LFT] values). A third indication, almost completely abandoned since the introduction of directly acting antivirals (DAAs) for hepatitis C, has been to monitor recurrence of disease and response to therapy [4].

All transplant centres perform allograft biopsies on clinical indication while the use of protocol liver biopsies (PLBs) has been declining over the last two decades and completely abandoned by many centres.

The main causes of graft dysfunction are traditionally divided into "early" (occurring within the first 3 months after LT) and "late" (after 6 months), being able to overlap in

the intermediate time (3–6 months) [1]. They include (a) preservation/reperfusion injury; (b) portal hyperperfusion syndrome; (c) rejection; (d) surgical complications involving vascular and/or biliary structures; (e) sepsis; (f) complications of IS therapy [opportunistic infections, post-transplant lymphoproliferative disease (PTLD), de novo malignancies]; (g) recurrence of the original disease; and (h) de novo diseases [6].

As for the native liver biopsy, an adequate sample size is crucial for a reliable histological assessment. The American Association for the Study of Liver Diseases recommends two passes with a 16-gauge needle; liver biopsies with less than 11 portal tracts might not be representative [7].

18.2.1 Event-Driven Liver Allograft Biopsy

The majority of post-transplant liver biopsies are performed to assess the cause of graft dysfunction, usually suspected by abnormal liver tests and/or imaging findings. Particularly in the early post-transplant period, these "indication" biopsies are usually diagnostic and often result in a change of management. They may reveal a wide spectrum of findings reflecting one or more overlapped clinico-pathological conditions whose aetiology is related to the time since transplantation. Knowledge of the original disease, age and sex of the recipient, recent changes in IS, previous biopsy results, clinical and laboratory profiles and radiology findings must be incorporated into biopsy interpretation [2].

Some practical examples of how the liver biopsy can help to identify the cause of damage and guide to the proper therapy are given below.

T-cell-mediated rejection (TCMR) versus *sepsis*: This differential diagnosis is a frequent clinical dilemma in the early post-transplant period as both of these conditions have a cholestatic biochemical profile and require opposite treatments. Histologically, they have distinct morphologies that permit to easily differentiate between them. Since TCMR and sepsis can coexist, the biopsy may help to identify the main process.

TCMR (acute and late) versus *chronic viral hepatitis*: Although less commonly encountered than in previous years, this differential diagnosis has important therapeutic implications: unnecessary augmentation of IS can accelerate fibrogenesis in chronic HCV, while untreated acute rejection can progress to chronic rejection. The two conditions may appear histologically similar, but each of them has proper key features that drive toward the right choice. Also in this case, these may overlap and the prevalent cause of liver damage can be detected by histology.

Biliary strictures versus *early and late TCMR*: Bile duct damage and loss, usually occurring in the late post-transplant period, needs clinicopathological correlations to distinguish between chronic rejection and many other underlying causes of vanishing bile duct diseases such as recurrent primary sclerosing cholangitis (PSC), sclerosing cholangitis secondary to reflux cholangiopathy or ischemic cholangiopathy (due to angiopathic injury [e.g. prolonged preservation, non-heart beating donors, hepatic artery thrombosis/stenosis, small for size syndrome] or immunological causes [e.g. antibody-mediated rejection, chronic obliterative arteriopathy associated to chronic rejection, cytomegalovirus [CMV] infection), and adverse

drugs reactions (e.g. cholangitic drug-induced liver injury). Important histological differences among these vanishing bile duct conditions may address to the correct diagnosis, providing the appropriate treatment.

18.2.2 Protocol Liver Allograft Biopsy

In the early years of LT, PLB was part of standard practice in most transplant centres. This is no longer the case, and patients are routinely monitored with liver chemistry tests despite their acknowledged unreliability in reflecting the histological status of the graft. Nevertheless, protocol biopsy monitoring of asymptomatic long-term survivors with normal or nearly normal LFTs is controversial and most LT centres have discontinued this practice, regarding surveillance biopsies as not helpful in guiding clinical management [8].

Reasons discouraging the use of PLB include the following: (1) potentially serious complications (e.g. bleeding, bile peritonitis, gallbladder perforation: <1% and the estimated mortality rate: <0.03%), (2) costs, (3) potential sampling error, (4) interobserver variability in biopsy interpretation among pathologists, (5) available non-invasive diagnostic techniques to monitor allograft inflammation and fibrosis (they do not detect microscopic abnormalities such as chronic hepatitis and fibrosis at least until fibrosis is advanced, and they cannot differentiate between different causes of inflammation and fibrosis, which may have important implications for management; so they may be inadequate in the transplant setting where the goal is to prevent fibrosis); (6) the uncertain clinical significance of unexplained histological findings; and therefore, (7) an inability to integrate the biopsy findings into a rational clinical management algorithm [5, 8].

Arguments in favour of PLB are that (1) they can reveal histological abnormalities with potential to lead to fibrosis and graft loss, therefore allowing adjustment of IS; (2) normal liver histology may allow for reduction of IS and so lower the risk of related complications; (3) after IS withdrawal, PLB may provide early evidence that therapy should be reinstituted [4, 5, 8, 9].

18.3 Histological Abnormalities in Late Protocol Allograft Biopsies and Its Clinical Impact

Histological abnormalities are commonly observed in late post-transplant biopsies, usually in patients with altered liver function but also in up to 85% of PLB from recipients who are clinically well, with normal liver tests [5]. So, there is emerging evidence supporting the performance of protocol biopsies to assess allograft status and identify subclinical changes that can smolder in transplanted livers with apparent normal function.

They include the following: (A) *clinically relevant histological changes*: (1) recurrent diseases, (2) cholangiopathy, (3) fatty liver disease, (4) acute and

chronic rejection, and (5) chronic viral hepatitis; (B) *"minor" histopathological changes of uncertain clinical significance*: (1) portal venopathy and nodular regenerative hyperplasia, (2) thickening and hyalinization of small hepatic artery branches, (3) low-grade central perivenulitis, (4) mild and non-specific portal and lobular inflammation, so-called idiopathic post-transplant hepatitis (IPTH), and (5) perivenular subsinusoidal fibrosis. Findings in this category occur in up to two-thirds of biopsy samples and might represent adverse side effects of medications, viral hepatitis, unrecognized patterns of immunologic injury, and/or the effects of long-term engraftment and abnormal graft physiology (e.g. chronically hyperdynamic portal circulation). Some of these findings are likely to reflect the consequences of prolonged inflammation related to subclinical alloimmune injury and may, therefore, have repercussions for increasing immunosuppressive therapy [5, 9].

Recently, Feng et al. [10] studied 157 long-term paediatric liver recipients with stable normal LFTs and identified three distinct histopathologic phenotypes of graft injury based on presence and severity of interface activity and/or fibrosis. Interestingly, the cluster characterized by interface hepatitis significantly differed from the others by a rejection-associated gene expression profile; moreover, a greater proportion of these patients had class II Donor-specific antibodies (DSA) and higher class II DSA mean fluorescence intensity values compared to the other two clusters. The authors conclude that, at molecular level, the interface activity connotes subclinical rejection; this supports the clinical impact of liver biopsy to guide personalized immunosuppression strategies and maximize graft health and longevity.

Several studies evaluated the frequency and spectrum of histopathological abnormalities in late post-transplant liver biopsies obtained on protocol or clinically indicated basis, drawing attention to the need for implementation of standardized biopsy protocols. Sebagh et al. [11] detected a high percentage (80%) of graft abnormalities on 10-year protocol liver biopsies from 143 recipients. Although the correlation between normal LFTs and normal histology was significant (sensitivity 75%), the specificity of LFTs in reflecting histologic changes was low (54%). Thus, 53 (72%) of 74 patients with normal LFTs had abnormal histology. Twenty-year PLB from 91 patients [12] revealed important histological information on graft structure that could have been missed without this practice: 33/91 patients had normal LFTs, and among them, histology was abnormal in 27 (82%); changes of IS occurred in 11/33 (33%). At Queen Elizabeth Hospital in Birmingham [12–14], 176 patients with normal LFTs had undergone PLB between 2000 and 2006. Histology revealed abnormal findings in 119 patients (68%); unexplained IPTH (not related to recurrence disease) was identified in 78 patients (33%); 76 cases (32%) changed IS based on histology. Abraham et al. [4] studied 165 PLB taken from 100 recipients at the time of normal LFTs; 44/165 (27%) showed histological changes judged clinically significant in 19/44 (43%). More recently, Pereira et al. [14] evaluated 39 patients at 10 years after LT, with repeatedly normal LFTs, identifying 13 (33%) of them with histological dysfunction: 7 de novo autoimmune hepatitis (AIH), 3 cellular rejections and 3 IPTH.

18.4 Special Concerns for Paediatric Recipients

In contrast with adults, children undergo LT for mostly non-recurrent diseases; this makes the interpretation of long-term biopsy changes less complex in some respects. On the other hand, some complications occur more frequently in paediatric recipients, including the following [3]:

- Biliary strictures and microvascular injury: They are probably related to small blood vessels and bile ducts of recipient and paediatric donor and/or to use of reduced-size allografts. They are usually diagnosed radiologically, and liver biopsy has a limited role in this setting. If biliary strictures are non-anastomotic, radiology may not be able to distinguish among ischemic cholangiopathy, chronic rejection or recurrent primary sclerosing cholangitis, and histology might be helpful in such situation.
- Late-onset rejection: Possibly related to poor or non-compliance with IS, it includes different morphological and often overlapped forms: late-onset acute cellular rejection, isolated central perivenulitis, chronic rejection, acute and chronic antibody-mediated rejection.
- De novo autoimmune hepatitis (dn-AIH): This syndrome occurs in 5%–10% of paediatric and 1%–2% of adult recipients, and it's characterized by clinical, biochemical, serological and histological features indistinguishable from AIH in patients undergoing LT for conditions other than autoimmune disorders. The *Banff Working Group* recently classified dn-AIH as an atypical form of late rejection overlapping with autoimmunity, thus supporting the designation of "plasma cell-rich rejection" in patients without an AIH as original disease [15]. It is currently unknown, however, if the immune response is directed against alloantigens, allograft neoantigens, or self-antigens, possibly shared by donor and host cells [16]. The development of donor-specific antibodies to glutathione S-transferase T1 (GSTT1) occurring in the setting of a donor/recipient mismatch for GSTT1 has been shown to be highly predictive for development of dn-AIH, suggesting that this may be an alloimmune response [16–18]. It has been reported in up to 70% of LT recipients with GSTT1 mismatch [19].
- Idiopathic post-transplant chronic hepatitis (see below).
- Chronic hepatitis E infection [5].

In several paediatric centres, scheduled liver biopsies obtained more than 1 year post-LT with good graft function and normal/nearly normal liver biochemistry have demonstrated histological abnormalities [20]. The most common findings in late liver allograft biopsies are a gradual development of unexplained chronic hepatitis and graft fibrosis, discussed further below.

18.4.1 Idiopathic Post-Transplant Chronic Hepatitis and Graft Fibrosis

Histologically, IPTH is diagnosed on the basis of a predominantly portal-based mononuclear inflammatory infiltrate without conspicuous damage to bile ducts or

portal vessels but associated with variable limiting plate disruption; lobular inflammatory changes are also commonly present [21]. Although the aetiology of this finding is uncertain, there is emerging evidence suggesting that IPTH (especially in paediatric recipients, in whom recurrent disease can be largely be excluded as a cause of late graft dysfunction) may represent a hepatitic variant of rejection or de novo AIH and that these three entities might be part of an overlapping spectrum of immune-mediated injury [10, 22, 23]. Evidence supporting this viewpoint is the frequent association of IPTH with auto- and alloantibodies [10], previous repeated episodes of rejection, improvement by increasing IS [20] and molecular profiling showing upregulation of TCMR-associated genes [10].

Correlation with clinical data is of paramount importance as IPTH may represent, in some cases, early manifestation of recurrent diseases (primary biliry cholangites (PBC), AIH, hepatitis C (HCV)), viral infection (included hepatitis E (HEV)) and drug-induced liver injury [13]. In particular, HEV infection (regarded as a zoonotic infection with animals such as pigs acting as viral reservoir) has recently been recognized as a possible chronic disease in immunosuppressed adult and paediatric patients after solid organ transplantation. Kamar et al. [24] reported that more than 60% of solid organ transplant patients infected with HEV develop chronic hepatitis; dose reductions of immunosuppressive therapy resulted in viral clearance in more than 30% of patients. Halac et al. [25] found a high prevalence of chronic hepatitis E infection in liver transplanted children with histological IPTH.

Regardless of its aetiology, IPTH appears to be clinically important as it may lead to progressive fibrosis until cirrhosis in both adult and mainly paediatric recipients [13]. In fact, several PLB-based studies have clearly shown that IPTH is a common finding in children after LT and is associated with a high risk of developing progressive liver fibrosis as a result of an indolent and subclinical ongoing allograft injury [5, 22]. Most found that 1-year protocol biopsy samples from children with normal biochemical liver function were mostly normal, and they did not provide sufficient additional information on graft histology [20]. However, histological examinations of 5- and 10-year PLB from children recipients have detected increased graft hepatitis and fibrosis [20].

In one study [26], 158 asymptomatic children underwent PLB. The most common histological abnormality was chronic hepatitis: 22%, 43%, and 64% at 1, 5, and 10 years, respectively. Autoantibody positivity was the only factor predictive of chronic hepatitis, being present in 13% and 10% of children with normal biopsy at 5 and 10 years, respectively, and 72% and 80% of those with chronic hepatitis at 5 and 10 years, respectively, but only four children fulfilled the criteria for de novo AIH. Prevalence and severity of fibrosis also increased: 52%, 81%, and 91% at 1, 5, and 10 years, respectively. By 10 years, 50% had progressed to bridging fibrosis or cirrhosis.

Other subsequent studies have shown a high frequency of unexplained graft inflammation (22%–74%) and fibrosis (27%–97%) in scheduled biopsies obtained >1 year after LT; the majority occurred in the context of normal liver function blood tests [27–30]. In particular, Sanada et al. [30] showed that surveillance biopsy at 2 years after LT is an unnecessary examination because the serum ALT level reflects portal inflammation; instead, PLB at 5 years is an excellent tool to detect early

reversible graft fibrosis because no serum markers reflect this finding. Others [29, 30] identified late graft fibrosis in the absence of hepatitis, relating it to risk factors for ischemic biliary complications (e.g. early technical or transplant-related factors such as prolonged cold ischaemia time, young age at the time of transplantation, and the use of partial grafts). At Beatrix Children's hospital in Groningen, a study group [31] observed that, from 1 to 5 years after LT, the prevalence of fibrosis increased from 31% to 65% but remained stable at 10 years (69%); however, the proportion of patients with severe fibrosis rose from 10% at 5 years to 29% at 10 years.

18.5 Unexplained Graft Dysfunction and Progressive Fibrosis: The Role of Humoral Alloreactivity

Compared to other solid organ allografts, the liver is an immunologically privileged organ with an inherent tolerogenic capacities that confer (relative) resistance to alloimmunity (both T-cell- and antibody-mediated). This is due to its unique anatomic and functional features that favour the absorption and elimination of alloantibodies (e.g. donor-specific antibodies [DSAs]) and dampening of T-cell responses. Nevertheless, the protection against various forms of rejection is not complete, and the complex interaction between humoral immune system and liver allograft can cause antibody-mediated tissue injury under specific circumstances [32]. In fact, not all recipients with DSA will develop clinicopathologic evidence of graft injury, and susceptibility is dependent on: (1) antibody class, titre and specificity and (2) density and distribution of target antigens, possibly related to co-existing pathologies [15]. However, it has to be disproven yet that all DSAs are pathogenic and some consequences may be subclinical or may develop over long periods of time.

Post-transplant DSA can be "preformed" (memory alloimmunity) or develop "de novo" (primary/de novo alloimmunity). Recipients with sensitizing events (pregnancies, transfusions, previous transplant, implants [ventricular assist devices, homografts]) or inflammatory events (major surgeries, major infections, recent vaccinations) prior to transplant are at higher risk to develop alloimmune memory responses [33]. De novo DSA risk factors include low IS, young age, low model of end-stage liver disease (MELD), cyclosporine versus tacrolimus use, previous transplants, infections and inflammatory events (e.g. preservation-reperfusion injury) [34]. Preformed DSAs are present as an estimated 13%–17% of LT recipients, and an additional 8% develop de novo DSA within the first year after transplant [32]. In most cases (an estimated 85%), preformed DSAs disappear a few months after LT [35].

Notably, DSAs (mostly de novo DQ class II) are found in 50%–60% of children recipients, and its presence has been associated with graft inflammation, fibrosis, and de novo AIH [28, 36, 37]. Recipients keeping preformed DSA or developing de novo DSA (usually in the context of reduced IS) directed at human leukocyte antigens (HLA) class II (especially DQ and IgG3 subclass) are at higher risk for antibody-mediated rejection (AMR). AMR in LT is an area of study in its infancy compared with kidney and heart. Its exact incidence is unknown because the

diagnosis is difficult to establish, which has contributed to scepticism about whether AMR occurs in the post-OLT setting. Only one prospective study reported the incidence of AMR de novo-DSA mediated in LT [37]. It occurred in 6% of patients (9/152) after a median follow-up of 22 months after LT, and in 43% (9/21) of those who developed de novo-DSA.

Two overlapping liver allograft AMR phenotypic expressions have been recently recognized: acute and chronic AMR. Acute AMR (aAMR) usually occurs within the first several weeks after LT in highly sensitized patients, but it can also appear later in some recipients with de novo DSA usually in the setting of low IS levels. Clinically, aAMR is characterized by unexplained allograft dysfunction with thrombocytopenia, hypocomplementaemia and circulating immune complexes. Current stringent criteria for its diagnosis include: (1) positive serum DSA; (2) histopathological evidence of diffuse microvascular injury/microvasculitis (often mixed with T-cell-mediated rejection); (3) strong and diffuse C4d staining in the portal microvasculature, sinusoids or central veins; (4) exclusion of other causes of a similar type of injury.

Chronic AMR (cAMR) is less well defined, but strongly linked to serum class II DSA (either preformed or de novo) and associated with late acute T-cell-mediated rejection, chronic rejection and fibrosis, particularly in the paediatric population. Unlike aAMR, cAMR is a slowly evolving process with a number of potential histopathological lesions, but most commonly it appears as indolent low-grade lymphoplasmacytic portal and perivenular inflammation (described above as IPTH) accompanied by slowly progressive non-inflammatory fibrosis with peculiar patterns and the potential to evolve toward fibrous septa and cirrhosis. Adjunctively, cAMR doesn't have typical clinical or biochemical features, and many cases are observed in PLB from clinically well recipients, with normal/near normal LFTs [38–41].

Thus, although not so frequent as in other allografts, the increasing amount of evidences suggests that AMR is much less uncommon than previously thought and it is probably misunderstood and underestimated in most cases labelled as "unexplained" graft injury and failure, both in early and in late post-LT time [42].

Moreover, the recent trends in LT (including the rising use of marginal donor organs, advances in the treatment of recurrent diseases and attempts to minimize IS) are likely related to the increase of the humoral reactivity-related immunologic consequences. More work is needed to decipher the complex interactions between the humoral immune system and the liver allograft and to understand the ways by which alloantibodies incur tissue injury after LT.

18.6 Role of Protocol Biopsy in Immunosuppression Minimization

With improvement of patient outcomes following LT, efforts are directed toward a long-term graft and patient health, by maintaining an optimal balance between the effectiveness and side-effects of individual IS [43, 44]. Patient and graft survival are affected by many factors, including the consequences of both over-IS

(e.g. nephrotoxicity, cardiovascular events, metabolic disorders, opportunistic infections and cancers) and under-IS (e.g. rejection), recurrence of the original disease, unexplained chronic hepatitis and graft fibrosis. Ideally, the choice between protecting the graft versus protecting the recipient might be mitigated by the study of liver allograft tolerance (e.g. absence of graft rejection without IS) [45].

The *Banff Working Group on Liver Allograft Pathology* [5] proposed working definitions for biopsy changes that: (1) are conducive to lowering IS and compatible with operational tolerance; and (2) raise concern for closer follow-up and perhaps increased IS. The practical goal of a stratified IS approach is to improve the quality of life and outcomes through (1) minimized exposure to complications of chronic IS in an individually tailored manner and (2) maintained graft function and structure by preventing uncontrollable acute or indolent chronic rejection. In particular, optimization of IS is significantly important for paediatric recipients with greater potential post-transplant longevity.

LT is a unique clinical setting in that up to 20% of highly selected, long-surviving, allograft recipients with normal/near normal LFTs can be weaned off IS without rejecting their grafts [35]. This clinical situation, defined as operational tolerance (OT), is probably the most extreme manifestation of the well-documented intrinsic tolerogenic properties of the liver. Clinico-pathological features associated with successful weaning include longer time since LT (>3 years), lack of humoral sensitization (DSA-), paucity of previous cellular rejection episodes, already minimized IS, non-autoimmune primary liver diseases and lower recipient age at time of transplantation [9].

Immunosuppression minimization strategy requires close monitoring and collection of liver biopsies to monitor allograft structure. Pre-weaning biopsies are strongly recommended (1) to document baseline inflammatory and structural changes that might be confused with post-weaning injury and (2) to exclude subclinical acute cellular or early chronic rejection or significant fibrosis with architectural distortion that might signal latent immunological injury not detectable by standard liver injury test profiles or other monitoring methods [9]. Protocol follow-up biopsies during and after weaning are strongly encouraged to monitor for indolent rejection-related injury not detectable by standard LFTs [5]. Patients who do not develop symptoms or biochemical/histopathological evidence of liver injury at 1, 3, 5 and 10 years after major decreases or total withdrawal of IS should be considered operationally tolerant [5, 9]. However, OT is metastable and this makes impossible to guarantee that the graft will be spared from indolent, immune-related injury, even after the 10-year timepoint or longer [9].

Most post-off IS biopsies are triggered by an elevation of liver chemistry tests; therefore, the scarcity of PLB from stable recipients supposed to be operationally tolerant limits the understanding of this phenomenon and the ability to determine whether the tolerance is true. A few paediatric studies reported possible antibody-mediated consequences of IS weaning and late sensitization such

as de novo DSA development, tissue C4d deposits and fibrosis with peculiar topographic distribution (e.g. perivenular and subsinusoidal) [28, 30, 46–50]. Some of them [30, 49] were able to show a direct correlation between fibrosis and attempts to IS withdrawal. Conversely, the reinstatement or an increase of IS resulted in improved fibrosis [30, 48, 49]. This finding supports the possibility that fibrosis in apparently tolerant grafts is antigen dependent and, as such, a subclinical form of rejection. Progressively increasing centrilobular-based fibrosis has also been observed in children maintained on chronic baseline IS [26–28, 31, 46]. Three studies demonstrated that the observed fibrosis was associated with anti-class II DSA [28, 46, 50] and tissue C4d deposits [28], thus suggesting that humoral alloreactivity may contribute to the process of unexplained graft fibrosis late after LT. A prospective pilot study by Feng et al. [51] successfully weaned a subset of highly selected paediatric recipients who were closely monitoring for 5 years with serial liver tests, auto- and alloantibody assessment and liver biopsies. No allograft exhibited significant inflammation or fibrosis.

Certainly, non-immunological causes of injury to long-surviving allografts cannot be excluded, such as suboptimal biliary or hepatic venous drainage, especially in reduced-size paediatric graft recipients.

18.7 How to Improve Long-Term Graft Monitoring and Treatment: A Proposal

The majority of patients surviving long-term following LT have allografts that are histologically abnormal [5]. Many of these abnormalities are observed in PLB from well recipients with good graft function [4]. Changes seen in late post-transplant biopsies are often complex and reflect more than one pathological process [3]. Some of these changes are likely to reflect the consequences of prolonged inflammation related to subclinical alloimmune injury [10]. These data prompt the need for prospective, longitudinal studies of DSAs and its comparison with histology as a part of a long-term graft monitoring protocol, especially in paediatric population in whom the high incidence of progressive fibrosis is concerning. Timing of PLB after LT is not definitive, but it is recommended to be scheduled at 1, 5, 10, 15, and 20 years [20].

By performing routine serum DSA testing and scheduled liver biopsies with C4d staining, such a standardized procedure would permit: to evaluate the real magnitude (incidence and prevalence) of AMR in post-LT setting and its actual impact on long-term clinical outcome; to identify the clinico-pathological features associated to AMR in order to draw a “AMR risk profile”; to precociously identify patients with (especially chronic) AMR; to improve their therapeutic management by increasing IS; and to prevent allografts from AMR-related complications such as vascular and biliary problems and “unexplained” graft loss.

Key Points

- Standard liver chemistry tests and other non-invasive methods are insensitive compared to biopsy, which remains the most sensitive modality for evaluating subclinical parenchymal injury in long-surviving liver allografts.
- PLB can improve the management of LT recipients and should become an integral part of post-LT care as they: (1) may reveal subclinical graft dysfunction possibly related to an indolent alloimmune injury in long-term surviving patients, guiding adjustments in IS, thus preventing graft loss; and (2) may help to identify patients in whom IS can be safely reduced or even completely withdrawal in an attempt to induce operational tolerance, thus minimizing the side-effects due to chronic exposure to IS.

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