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REVIEW

The significance of histological diagnosis in renal allograft biopsies in 2014

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Summary

In 2014, the renal allograft biopsy still represents the best available diagnostic 'gold' standard to assess reasons for allograft dysfunction. However, it is well recognized that histological lesion observed in the biopsy is of limited diagnostic specificity and that the Banff classification as the international diagnostic standard represents mere expert consensus. Here, we review the role of the renal allograft biopsy in different clinical and diagnostic settings. To increase diagnostic accuracy and to compensate for lack of specificity, the interpretation of biopsy pathology needs to be within the clinical context, primarily defined by time post-transplantation and patient-specific risk profile. With this in mind, similar histopathological patterns will lead to different conclusions with regard to diagnosis, disease grading and staging and thus to patient-specific clinical decision-making. Consensus generation for such integrated diagnostic approach, preferably including new molecular tools, represents the next challenge to the transplant community on its way to precision medicine in transplantation.

Introduction

The first renal transplant biopsy in a human was performed in 1952 in a living donor kidney transplant recipient who became anuric by day 21. The slides were recently discovered at the Paris Necker Hospital and upon review show a combination of T cell and antibody-mediated rejection [1]. Since then, renal transplant biopsies have remained the gold standard to determine the cause of graft dysfunction [2,3]. Biopsy findings change the clinical diagnosis in an average of 36% of patients (27–46%) and therapy in 59% [4–10]. The diagnostic sensitivity of the biopsy depends on the size, number and content of cores. The specificity of the biopsy is impossible to measure because no other, 'more true' diagnostic gold standard for comparison is available. However, results showing that the biopsy findings correlate with the clinical course in 80–89% of cases are reassuring [7,11]. The expectation is that molecular diagnostics will increase the specificity and sensitivity of the biopsy, which is subject of active investigation and has been reviewed comprehensively elsewhere [12]. Today, the renal allograft biopsy is not only meant to diagnose, but also to grade and stage a disease entity once identified and by this to give prognostic information with the aim to guide tailored treatment in the individual patient. The morphological spectrum of pathological lesions in a transplant kidney biopsy is limited, meaning that the same lesions need to be interpreted in different ways, depending on the clinical context and current understanding of relevant diseases. We here review the role of the renal allograft biopsy in different

clinical and diagnostic settings, primarily defined by time post-transplantation and population-specific disease risks. The aim is to summarize current knowledge in this regard and to set the stage for a consensus process of how to diagnostically interpret the same histological lesions in a different context. It can be anticipated that with further evolution of treatment options and increasing understanding of disease mechanisms ongoing review and refinement of such consensus will be required.

Kidney transplant biopsy in the protocol biopsy setting

The diagnostic value of protocol biopsies at implantation for predicting graft performance post-transplantation is controversial [13]. This is likely due to the complexity of donor, recipient and clinical variables before, at and post-organ transplantation, which makes it challenging, if not impossible to predict the course of a graft from a single biopsy at the time of implantation. Indisputable is the value of an implantation biopsy as the morphological baseline in an individual patient. Interpreting the evolution of histological lesions from implantation (i.e. the pre-existing donor burden) through consecutive protocol and clinical indicated biopsies is very helpful in understanding the course and prognosis in the individual transplant recipient [14]. Implementation of post-transplantation protocol biopsy programmes in numerous transplant centers was driven by the notion that detection of subclinical pathology will allow early therapeutical intervention and thus improve long-term outcome [15–17]. Such protocol biopsy programmes were instrumental for understanding the impact of early clinical and subclinical changes in the allograft on long-term outcome as well as the time course of diseases in kidney allografts (Fig. 1) [18–21]. However, the individual patient's benefit from an unselected protocol biopsy programme approach remains controversial [22]. Depending on the overall disease prevalence in a renal transplant population, significant numbers (50–60%) of noninformative protocol biopsies are procured [14]. Compared with clinically indicated biopsies, fewer protocol biopsies lead to a change in patient management. In particular, under current immunosuppressive regimens, the rate of subclinical cellular rejections in immunologically low-risk populations is very low and alone does not seem to justify the necessity for unselective protocol biopsies [23,24]. Thus, significant resources are potentially allocated ineffectively if protocol biopsies are not used in a risk-adjusted manner, for example in presensitized patients (see below) or patients on immunosuppressive withdrawal protocols [23, 25].

In this regard, appropriate timing of protocol biopsies appears to be one of the crucial variables: Early (<1 year post-transplantation) protocol biopsies in the non-presensitized population seem to reflect the donor characteristics and peri-transplantation injury with little potential to predict the long-term future [26,27]. However, in a population of presensitized patients, early protocol biopsies can detect subclinical, early of antibody-mediated rejection (ABMR) and guide therapy accordingly [28–30]. The timing of later protocol biopsies, however, largely depends on the prior probability of the disease entity that is to be detected and thus treated earlier, for example, recurrent original disease [31]. In patients in whom noncompliance is suspected, protocol biopsies might be a management tool to detect subclinical T cell-mediated rejection (TCMR) earlier, which in these patients has been demonstrated to be associated with consecutive development of de novo donor-specific antibodies (DSA) and ABMR [32].

Per Banff consensus, pathologists apply the same classification systems to protocol biopsies that have been developed and evaluated on indication biopsies. The major challenge for histopathologists therefore remains the interpretation of subtle, often nonspecific histological changes with a lack of pathogenetic precursor lesions as the fully developed disease will be resistant to treatment in most cases. To this end, high expectations are with new molecular diagnostic tools aiming to detect disease-specific changes at the submicroscopic level [33–35] [36].

Kidney transplant biopsy in patients with acutely deteriorating allograft function

The differential diagnosis in an acutely deteriorating allograft is broad. Although time after transplantation increases the prior probability for certain findings, few noninvasive diagnostic tools are available to clinicians to guide disease-specific treatment. The biopsy therefore remains the gold standard in the assessment of an acutely deteriorating allograft.

In the first days and weeks after transplantation ischemia/reperfusion injury (acute tubular injury), acute calcineurin inhibitor toxicity (CNIT) and acute rejection are the main differential diagnosis. There is currently no reproducible grading system for acute tubular injury in place, and the morphological degree of acute tubular injury does not necessarily seem to correlate with allograft function [37]. In particular, in this context, molecular diagnostics has the potential to be superior to histology. The molecular phenotype of acute kidney injury is fairly well defined and has been demonstrated to be a better predictor of allograft function and outcome than histology [37]. The role of histology in the assessment of acute CNIT is controversial as the original description of the typical appearances of acute CNIT (including acute tubular damage with isometric cytoplasmic vacuolization, microcalcifications and vacuoliza-

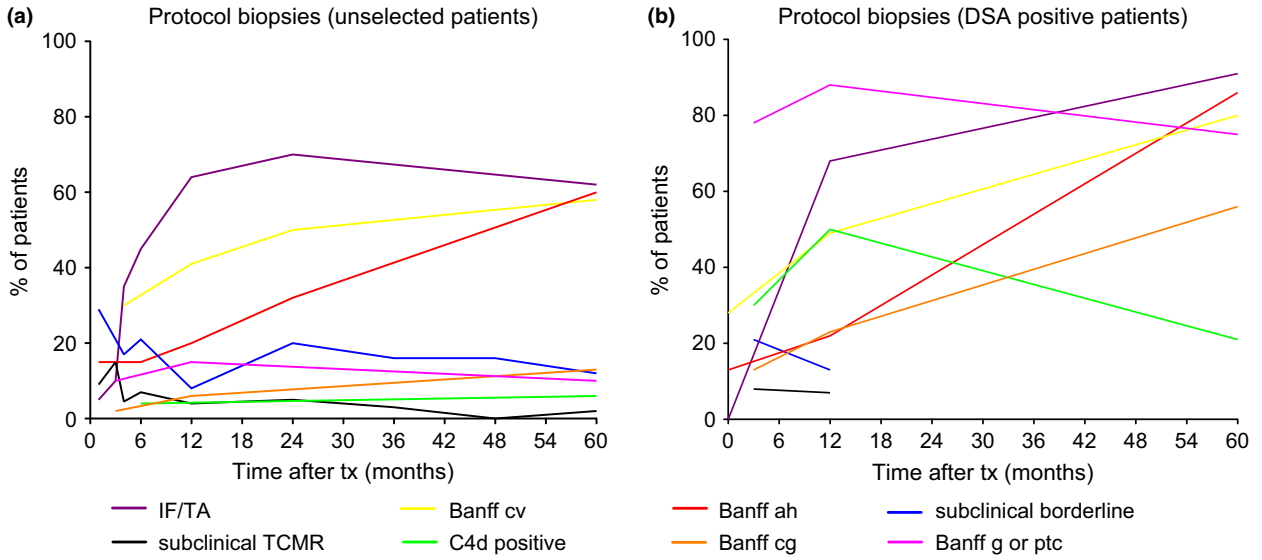

Figure 1 Time course of selected acute and chronic lesions in transplant kidney biopsies in different clinical settings. The plotted curves represent approximations based on meta-analysis of representative studies as indicated. (a) In unselected protocol biopsies, the main burden of interstitial fibrosis/tubular atrophy is established within the first year after transplantation. Chronic arterial and arteriolar lesions develop later but will affect the majority of patients in the long term. The prevalence of subclinical rejections decreases with time after transplantation. Transplant glomerulopathy is an infrequent and late lesion in this patient cohort [15,16,19–21,24,28,58,86–92]. (b) In contrast, in protocol biopsies selectively taken in presensitized patients, transplant glomerulopathy occurs relatively early after transplantation and increases steadily. Other chronic lesions such as interstitial fibrosis/ tubular atrophy and vascular lesions show a similar pattern compared with panel (a) but appear to be more advanced at 5 years after transplantation. Microcirculation inflammation is a frequent finding even late after transplantation [28–30].

tion of arteriolar smooth muscle cells) originates from the early eras of calcineurin inhibitors with incomparably high drug doses applied to comparable young donors with usually pristine organs [38]. Today older and marginal donors with comorbidities (hypertension, diabetes, donation after cardiac death) associated with similar histologic features essentially eliminate any diagnostic specificity for CNIT in the biopsy. Thus, CNIT is frequently a diagnosis of exclusion.

Acute rejection is divided into the two broad categories of TCMR and ABMR. The Banff classification to grade rejection represents international diagnostic consensus in that regard, which is regularly reviewed and adapted based on our increasing understanding of allograft rejection [11,39,40]. Acute TCMR is graded according to severity, reflected in the degree of interstitial inflammation and tubulitis, and according to the compartment that is affected. Thus, endothelialitis or endarteritis used to be regarded as the most severe form of TCMR. This concept, however, is currently under review as it has been shown that a significant proportion of patients with this type of rejection in fact do have DSA [41]. This is reflected in the most recent version of the Banff classification in which endothelialitis is regarded as a morphological feature of TCMR and/or ABMR if additional criteria are met, that is, presence of DSA [40].

Currently, the main challenges emerge at the low end of the spectrum of both cellular and antibody-mediated rejections [40]: What is the true nature of borderline rejections? What is the impact of vascular rejections/ endothelialitis with neither concomitant interstitial inflammation nor tubulitis, so-called isolated v-lesions? Can we accurately diagnose ABMR in the absence of C4d?

Biopsies in which either or both the amount of cellular infiltrate and the degree of tubulitis do not meet the threshold for a diagnosis of acute TCMR are designated as 'borderline/ suspicious for acute rejection' [42]. Policies how to treat those patients vary but a concomitant significant increase in serum creatinine (approx. 20% from baseline) is a widely accepted condition to treat. There is still uncertainty among clinicians and pathologists as to whether these minor infiltrates represent clinically relevant alloreactivity or not. Molecular studies in fact have shown that only 1/3 of biopsies diagnosed as borderline had a molecular phenotype similar to TCMR [43]. The remaining 2/3 molecularly resembled nonrejection biopsies. Interestingly, borderline biopsies morphologically but not molecularly falling short for a diagnosis of rejection showed a higher degree of inflammation within areas of interstitial fibrosis and tubular atrophy; a finding that is disregarded for a diagnosis of rejection. While clinically ambiguous, the borderline category remains part of the current Banff classifica-

tion as there is still insufficient scientific data to be certain about its nature. [40]. In particular, within the first days after transplantation, these minor infiltrates may reflect response to injury rather than true immunological alloreactivity [43]. To address this unmet need at the 2013 Banff conference, a working group was established to conduct multicenter studies with the aim to determine the diagnostic and clinical significance of borderline lesions under current immunosuppression and sensitive DSA and polyomavirus screening standards [40].

A single lymphocyte underneath the arterial endothelium is regarded as acute rejection Banff type II, even in the absence of any interstitial inflammation or tubulitis. Banu Sis and colleagues presented their results during the 2013 Banff conference on allograft pathology, showing that biopsies with isolated v-lesions are similar with regard to their response to treatment and graft survival to v-lesions with concomitant interstitial inflammation and tubulitis, thus represent rejection [40]. This is entirely in concordance with a recently published study comparing 23 biopsies with isolated v-lesions with matched biopsies showing endothelialitis plus inflammation and biopsies without any evidence of rejection [44].

Within the last decade, the understanding of acute ABMR has vastly improved; a fact that is not necessarily attended by effective treatment for this disease. Antibody-mediated rejection has been recognized as one of the main reasons for allograft failure [45,46]. Chronic ABMR will be discussed below. Acute ABMR is characterized by microcirculatory inflammation within the capillary bed, namely glomerulitis and capillaritis. Reproducible grading of these variables with respect to clinical significance remains challenging but has recently been further refined [40,47,48]. Following others, two publications have lately shown again the detrimental effect of microcirculation inflammation (glomerulitis and capillaritis) on graft survival, its association with DSA and the development of transplant glomerulopathy [49,50].

Only recently endothelialitis affecting the arteries has been convincingly shown to be part of the spectrum of ABMR in some cases, which is reflected in the updated 2013 Banff classification of allograft pathology [40,41]. Thrombotic microangiopathy can also be seen and may be the only morphological evidence of ABMR apart from C4d positivity [51]. The latter used to be an inevitable prerequisite for a pathological diagnosis of ABMR in pre-C4d versions of the Banff classification for ABMR [39]. After the adoption of C4d, ABMR diagnosis was dependent on the detection of the complement split product as the evidence of DSA acting on the allograft. Since 2013, this is no longer required given there is sufficient degree of microcirculatory inflammation (glomerulitis and peritubular capillaritis), further histological evidence of acute tissue injury (thrombotic microangiopathy or endothelialitis or acute tubular damage) and positive testing for DSA [40]. These fundamental changes to the diagnostic Banff criteria of ABMR take into consideration that C4d staining is highly specific for the interaction of DSA with the allograft but suffers from limited sensitivity and interlaboratory reproducibility [52] (reviewed in [53]). Several studies have now convincingly shown that microcirculation injury in the presence of DSA is a strong predictor of chronic graft injury, independent of C4d [28,49,54,55].

Kidney transplant biopsy in patients with chronically deteriorating allograft function, the 'Late Allograft Biopsy'

With the awareness that there was little progress in long-term allograft outcome, it became clear that the term chronic allograft nephropathy needed to be discontinued to identify the specific diseases that lead to allograft failure [56]. El-Zoghbi *et al.* [57] were able to show that the reasons for allograft failure can be identified in >80% of cases based on careful clinical–pathological assessment. Recurrent or de novo glomerular diseases, including transplant glomerulopathy, chronic cellular and antibody-mediated rejections (beyond the first year) as well as polyomavirus nephropathy, were shown to account for the largest proportion of graft losses. Other studies confirmed the detrimental effect of microcirculation injury in the context of DSA on long-term allograft outcome [45,46]. In contrast to previous concepts, in these studies, chronic CNIT was rarely identified as the main cause of allograft failure, potentially due to the lack of specific diagnostic histologic features in the biopsy. In the past, progressive arteriolar hyalinosis was considered the hallmark of chronic CNIT. However, this has been shown to be less specific than previously thought: While several studies have concordantly demonstrated that progressive arteriolar hyalinosis is a constant feature in renal allografts, the specific underlying cause is difficult to determine and frequently multifactorial [58,59]. While there is little doubt that long-term use of CNI contributes to chronic vascular, glomerular and tubulo-interstitial changes, the benefits (e.g., preventing de novo DSA formation and ABMR) from potent immunosuppression including CNI seem to outweigh its side effects in most patients, at least until we have more effective treatments, in particular to control the antibody–response in the individual patient.

In the transplant, recurring glomerular pathologies comprise a heterogeneous group of diseases. All glomerulonephritides, either immune complex-mediated or pauci-immune, have the contingency to recur in the transplant. One of the largest studies has identified recurrent glomerular disease as the third most common reason for graft loss

[60]. Recurrence rates, however, and the impact on graft survival, vary substantially depending on the entity [61,62]. While dense deposit disease almost invariably recurs, IgA nephritis and membranous glomerulonephritis come back in about one-third of cases [61,62]. Lupus nephritis and ANCA-associated vasculitis have little propensity to recur. One ought to consider that recurrent and de novo disease in the transplant might be seen at an early subclinical stage under ongoing immunosuppression, which may alter the presentation of the disease. Therefore, the true time course of recurrent glomerulonephritis is very difficult to assess as there are limited data from protocol biopsies addressing this question [63,64]. Other glomerular lesions with a high risk of recurrence are atypical HUS (due to factor I and factor H mutation) and FSGS. It is beyond the scope of this article to review the entire topic of recurrent glomerular diseases and we refer to comprehensive reviews in this matter [61,62].

'Chronic rejection' is often considered in a patient with progressively deteriorating graft function over a longer period of time. From a histopathological perspective, however, this category must not be used as a waste basket for any biopsy showing an inflammatory infiltrate associated with scarring. All disease processes at some point are associated with inflammation in areas of interstitial fibrosis and tubular atrophy. To date, only chronic tissue injury to the microcirculation (transplant glomerulopathy or peritubular capillary basement membranes multilayering (PTCBMML) seen by EM) or to arteries (de novo and/or accelerated arterial fibrous intimal thickening) are accepted as rejection lesions [40]. This is based on numerous association studies and consecutive consensus for generating diagnostic standards. However, these lesions are not entirely specific as similar chronic remodeling features can be observed after nonrejection-mediated severe endothelial injury, for example, after hemolytic uremic syndrome or malignant hypertension [65]. Therefore, features of chronic microcirculation injury require an additional activity component (microcirculation inflammation and evidence of DSA) to suggest a chronic-active antibody-mediated process or intimal inflammatory infiltration indicating a cellular rejection component. Thus, intimal arterial fibrosis is ambiguous and may either be seen in the context of a chronic-active ABMR or chronic-active TCMR [66]. Transplant glomerulopathy or PTCBMML seen in isolation in a patient with chronically deteriorating graft function with no detectable DSA at the time is considered insufficient for a diagnosis of chronic ABMR. The finding may, however, raise the possibility of previous episodes of acute ABMR with irreversible microvascular remodeling and now decreased DSA titers, for example, after treatment. Clinico-pathological correlation and consideration of previous biopsy results is crucial in these difficult cases, as rejection is not a one-time confined event but rather an ongoing dynamically evolving disease process.

As described above, chronic tissue injury as a diagnostic component of chronic rejection is limited to vascular and glomerular changes. Inflammation in areas of tubular atrophy and interstitial fibrosis (IF/TA) is currently disregarded for a diagnosis of rejection, no matter how florid it may be. Diagnostic problems occur in cases in which there is a dense inflammatory infiltrate in IF/TA, affecting a significant proportion of the cortex with spillover into the adjacent cortex and only minimal tubulitis. This is currently regarded as borderline or even insufficient for a diagnosis of rejection if too little nonatrophic cortex is involved. Several studies however have shown that inflammation in IF/TA inversely correlates with outcome [15,19,67,68]. In addition, the 'total inflammation' score (ti), scoring the total amount of inflammation in the biopsy regardless of its distribution in scarred and nonscarred areas, was more meaningful in terms of outcome than the Banff i-score [67]. Therefore, a respective Banff working group is currently investigating whether inflammation in IF/TA should be under certain circumstances regarded as part of the rejection process.

Kidney transplant biopsy in patients with DSA

Introduction of solid phase immunoassays using single antigen beads (SAB) based on Luminex® platform has markedly increased the sensitivity and specificity of HLA-antibody testing prior to and following kidney transplantation. This does not come without difficulties as the significance of SAB-detected donor-specific and nondonor-specific anti-HLA antibodies in different clinical settings ranging from highly sensitized to nonsensitized first-transplant patients is not fully understood. Standardization of the tests and quantification of the results are also subject to ongoing debates: A comprehensive guideline on technical aspects and clinical use of antibody testing in solid organ transplantation has recently been published [69]. The guideline refers to two different clinical scenarios, which are presensitized patients and patients who develop de novo DSA after transplantation. While the first occurs after repeat transplantation, blood transfusion, or pregnancy, the latter is associated with reduction of immunosuppression, either due to nonadherence or clinical reasons. Screening for DSA at least once is recommended for all patients, regardless of their estimated risk for the presence of DSA and ABMR, within the early post-transplant period. Early protocol biopsies are recommended for high-risk, presensitized patients while a biopsy should be performed in intermediate and low-risk patients once DSA is detected. While it is unambiguous that the diagnosis of ABMR should prompt specific treatment, there is no clear guid-

ance for scenarios in which there is an incomplete ABMR phenotype, such as C4d positivity in the absence of microcirculation injury or isolated peritubular capillaritis in the absence of glomerulitis and vice versa. Only very few studies have addressed these very particular questions [70].

The mere presence of DSA has limited prognostic value on its own which highlights the validity of histopathology in this matter [28,55]. In addition, functional assessment of the graft alone will likely delay the diagnosis of ABMR in many cases: It has been shown in protocol biopsy studies that a significant proportion of patients with both preformed and de novo DSA do not present with graft dysfunction although the biopsy does show evidence of ABMR [28,32]. This is even more important as de novo DSA frequently develop beyond the first after transplantation [32]. Interestingly, the authors of the aforementioned guidelines do not conceal the uncertainty among experts about the necessity and value of an annually antibody screening in nonsensitized patients but recommend testing if there is suspicion of nonadherence [69]. This reflects the finding that formation of de novo DSA has been shown to be associated with incompliance in medication taking [31,32]. Clearly, additional tests are required to increase the prognostic power of histopathological assessment in patients with preformed or de novo DSA. Gene expression measurement has recently been shown to improve risk stratification in patients with ABMR when added to conventional light microscopy [35]. But also electron microscopy has been proven useful in the detection of early and subclinical endothelial damage in peritubular capillaries and glomeruli [71–73] .

Kidney transplant biopsy in patients with BK viremia/viruria

JC and BK are the two polyomavirus species known to cause nephropathy but only the latter is relevant in daily clinical practice as it accounts for >90% of cases. BK virus nephropathy (BKVNP) affects up to 10% of kidney transplants although routine screening of blood and urine is likely to further reduce numbers of manifest BKVNP and graft losses. The only effective treatment in case of viremia, viruria or manifest BKVNP is reduction of immunosuppression [74]. At most centers, kidney transplant recipients are routinely screened for BK viremia or viruria during the first 2 years after transplantation. A significant increase in blood or urine viral load should prompt reduction of immunosuppression. A kidney biopsy is recommended if the increase in viral load is accompanied by a significant deterioration of kidney function [74,75].

Kidney biopsy may be false negative for polyomavirus nephropathy, especially in early stage, focal disease with small, inadequate biopsies with absence of medulla [76]. In most of these cases, the role of histopathology therefore is to exclude rejection due to reduced immunosuppression, exclude other differential diagnosis and to grade BKVNP, ideally to provide guidance of therapy and prognostically relevant information. Suspected BKVNP should always be confirmed by immunohistochemistry or in situ hybridization. There is currently no generally accepted classification system for BKVNP [40]. Previously published systems divide BKVNP in stages A, B and C according to the degree of inflammation and fibrosis [74,76]. It remains to be proven whether the morphological viral load (the amount of infected cells in the biopsy) is prognostically relevant. Preliminary data presented at the 2013 Banff meeting suggest that the percentage of tubular epithelial nuclei showing viral replication (e.g., demonstrated by SV40 immunohistochemistry) can be used to stage disease activity and thus guide therapy [40]. A notorious problem is the differentiation between TCMR and BKVNP, especially when the latter is resolving or if tubulitis is found distant from infected cells. This challenging differential diagnosis is frequently encountered by the pathologist as viremia detected by screening triggers reduction in immunosuppression and thus increases the risk of TCMR while inflammation due to BKVNP is resolving, creating overlap of both entities in the biopsy [77]. In such situations, detection of the replicating polyomavirus by immunohistochemistry on the one hand and presence of endothelialitis or established criteria for ABMR can be utilized to diagnose concomitant BKVNP and rejection.

Summary

Transplant kidney biopsies are still gold standard in the differential diagnosis of allograft dysfunction. Protocol biopsies have significantly contributed to the understanding of the time course of disease processes and their precursor lesions within the last two decades (Fig. 1). With this body of knowledge in mind, the benefit from protocol biopsies in an unselected approach is probably not justifying anymore the effort, risks and costs; and it has been proposed to refocus the use of protocol biopsies to patients being at high risk for recurrent disease or rejection, especially ABMR [40]. Both are considered main contributing factors to allograft failure. The histological phenotype and evolution of ABMR has been studied in depth during the last two decades, especially after the ground-breaking discovery of C4d as a highly specific marker indicating antibody-mediated injury [78,79]. This marker, however, is afflicted by its low sensitivity [54,80]. There is now convincing evidence that ABMR can be diagnosed in patients with DSA in the absence of C4d [40]. Recently published work has also shown that endothelialitis, previously considered characteristic for TCMR, can be part of the spectrum of ABMR [41].

Refinement of the morphological characteristics of ABMR leaves the pathologist with the uncertainty how to interpret subtle changes at the low end of the spectrum. Molecular studies investigating the gene expression profile on biopsy material have already been shown to successfully support light microscopy and to provide relevant prognostic information [35,81–83]. This does not only apply to ABMR but similarly to TCMR and the ongoing debate on the true nature of cellular infiltrates designated as borderline [43]. An area of uncertainty is the relevance of lymphocytic inflammation within tubular atrophy and fibrosis, a finding that is currently not taken into consideration for a diagnosis of acute rejection, but becomes nowadays more relevant with the increasing number of late allograft biopsies. There is evidence that the total inflammatory burden in the biopsy has more impact on the graft outcome than the actual infiltrate in the viable cortex alone: Patients with inflammation in areas of tubular trophy and fibrosis appear to do worse than patient with fibrosis only [15,68,84]. However to avoid overtreatment, further investigation is needed to find out whether treatment targeting these infiltrates has any effect on outcome.

Any pathological classification should be aiming to provide clinically relevant information. This has always been the mission of the Banff process, which started empirically from a classification based on expert consensus and evolved through constantly being challenged by scientific data exploring the areas of uncertainty [85]. Morphological exploration of the individual lesion, however, is an exhaustible process and integration of the 'molecular microscope' and development of evidence based integrated diagnostic systems is needed to further increase diagnostic precision in the individual transplant recipient [33].

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