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REVIEW

Protocol biopsies after kidney transplantation

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Summary

Numerous studies have investigated features of allograft injury in renal biopsies obtained in stable kidney transplants. Evaluation of protocol biopsies has revealed a considerably high prevalence of subclinical acute rejection (SAR) and chronic allograft nephropathy (CAN) already in early phases after transplantation. The meanwhile well-established association of SAR and CAN in protocol biopsy with long-term allograft failure and the finding of superior allograft outcome after treatment of SAR in a randomized prospective study may point to clinical relevance of this procedure. In this review, potential benefits and risks associated with kidney allograft biopsy in stable renal transplant recipients are discussed.

Introduction

Short- and long-term survival after renal transplantation has significantly improved over the last decades. Nevertheless, chronic allograft failure is still the rule and represents one of the most important challenges in transplantation medicine. Chronic allograft injury, the dominant cause of late renal allograft loss, may be caused by a variety of antigen-dependent and antigen-independent mechanisms [1]. The development of more effective and less toxic immunosuppressive protocols may further improve long-term outcomes. The ultimate, up to now unachieved goal would be the induction of transplantation tolerance, i.e. a state of alloantigen-specific unresponsiveness in the absence of baseline immunosuppression. On the contrary, measures enabling early recognition of factors contributing to graft failure may critically improve outcomes after kidney transplantation. In this respect, percutaneous allograft biopsy represents an indispensable diagnostic tool, as a thorough histologic analysis allows a subtle differentiation of factors contributing to graft dysfunction. At most centers, allograft biopsies are performed exclusively in case of acute or chronic graft dysfunction. However, surveillance (protocol) biopsies performed in stable kidney transplants might allow timely recognition of pathologic conditions causing a deterioration of graft function at a later timepoint. Now, there is accumulating evidence that the performance of protocol biopsies could be a worthwhile strategy to improve long-term outcomes in kidney transplantation. Protocol biopsies may uncover histologic signs of acute rejection without associated graft dysfunction [subclinical acute rejection (SAR)] as well as the early occurrence of features of chronic allograft nephropathy (CAN), and may help to establish an individually targeted immunosuppressive regimen, which may include antirejection treatment of subclinical rejection episodes, the use of novel less toxic immunosuppressants or even reduction or withdrawal of immunosuppressive drugs. In this overview, aims, potential advantages and risks of protocol biopsies performed after kidney transplantation are discussed.

Subclinical acute rejection – definition and incidences

The occurrence of histologic signs of acute cellular rejection in the absence of graft dysfunction, a condition termed SAR, is well established in the literature [2–20]. Already in the 1980s, Burdick *et al.* [2,3] suggested that cellular infiltrates are not necessarily associated with clinically overt rejection. In their small series of kidney allograft recipients, the authors described the constant finding of mononuclear interstitial infiltrates in 1- and 4-week protocol biopsies [2,3]. Representative protocol biopsy studies evaluating signs of SAR in protocol biopsies are listed in Table 1.

In most studies evaluating surveillance biopsies, SAR is defined and classified according to the Banff scheme (Banff grade I or greater) analogous to clinical acute rejection [4–23]. Rush *et al.* defined SAR as an increase in serum creatinine <10% associated with the histologic diagnosis of acute rejection (at least i2t2) according to the Banff classification [4,5,9,11,24]. With the use of the Banff scheme, a recently published interobserver comparison revealed a high reproducibility of results (acute and chronic changes) obtained in stable renal allograft patients [16].

A number of analyses have revealed variable frequencies of SAR, whereby highest incidences were reported for biopsies obtained within the first months after transplantation (Table 1). Using the Banff scheme, most episodes of SAR were classified Banff grade I (tubulo-interstitial infiltrates). Vascular involvement (intimal arteritis) classified Banff II rejection is hardly ever found in stable allografts. Banff-borderline changes are frequently observed sometimes exceeding 50% of specimens [4–7,10,12,13,15,25]. For surveillance, biopsies performed between months 1 and 6 post-transplantation, Rush *et al.* noted a 20–50% prevalence of SAR [4,5,9,11,24]. In a recent study, Nankivell *et al.* [13] described SAR for 29% of 3-month-protocol biopsies. Comparably high prevalences of SAR have also been described by other working groups (see Table 1). Furthermore, the occurrence of SAR was reported to be accompanied with an increased expression of a variety of immune activator genes including distinct proinflammatory cytokines and cytotoxic T cymphocyte (CTL) effector molecules granzyme B and perforin [8].

Particularly high rates of acute rejection were obtained in studies evaluating biopsies performed in patients with delayed graft function (DGF). In a recent study, Qureshi *et al.* [17] reported about 50% acute rejection rates for recipients with DGF. Lower rejection rates were reported by Jain *et al.* [10]. But incidences of SAR were significantly lower in patients with immediate graft function (4%) when compared to patients with DGF (18%) [10].

Table 1. Features of subclinical acute rejection (SAR) in protocol biopsies.

Author	Timing of Bx	*N*	Immunosuppression	Evaluation	Results
Burdick *et al.* [2,3]	1 and 2 weeks	14	Aza, steroids	Morphometry	Interstitial infiltrate in all biopsies
Rush *et al.* [5]	1, 2, 3, 6 and 12 months	25	CyA, Aza, steroids (OKT3 induction)	Banff	SAR in 24%, 52%, 28%, 28%, 12%
Seron *et al.* [6]	3 months	98	ATG or OKT3, CyA, steroids	Banff, morphometry	SAR: 4%
Lipman *et al.* [8]	2.5–4 months	32	CyA, Aza, steroids	Molecular markers, Banff	SAR: increased TCR, TNF-α, IL-10, IL-2, IL-4, IFN-γ, IL-15, FasL granzyme B, perforin
Legendre *et al.* [25]	3 months, 2 years	41	Induction, Aza (CyA), (steroids)	Banff	SAR: 23%, 16% (Cad); 0%, 0% (Liv*)
Jain *et al.* [10]	7 days	83	CyA or Tac, Aza, steroids	Banff	SAR: 18% (DGF), 4% (non-DGF)
Shapiro *et al.* [12]	8.2 ± 2.6 days	28	Tac (MMF or Aza), steroids (induction)	Banff	SAR: 25%
Nankivell *et al.* [13]	3 months	112	CyA, Aza, steroids (OKT3 induction)	Banff	SAR: 29%
Veronese *et al.* [14]	2 and 12 months	32, 26	Not specified	Banff, immunohistochemistry	SAR: 32% (2 months), increased granzyme B expression
Gloor *et al.* [15]	3 months	114	Tac, MMF, steroids (induction)	Banff	SAR: 2.6%
Qureshi *et al.* [17]	7–10 days (DGF)	65	CyA, Aza or MMF, steroids (ATG induction)	Banff	SAR (DGF): 50.8%
Shishido *et al.* [18]	1, 2, 3 and 5 years	95	CyA, Aza or Miz, steroids (ALG induction)	Banff, CADI	SAR in Bx with CAN: 50%, 32%, 19%, 16%

HLA, human leukocyte antigen; ALG, antilymphocyte globulin; ATG, antithymocyte globulin; Aza, azathioprine; Bx, biopsy; CADI, chronic allograft damage index; CAN, chronic allograft nephropathy; CyA, cyclosporin A; DGF, delayed graft function; Miz, mizoribine; MMF, mycophenolate mofetil; *N*, number of patients; SAR, subacute acute rejection; Tac, tacrolimus; TCR, T-cell receptor; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; Cad, cadaveric.
*HLA-identical living-donor kidneys.

Chronic allograft damage in stable kidney allografts – a predictor of late graft failure

There is increasing evidence that protocol biopsies may be a valuable tool to uncover early signs of clinically inapparent chronic allograft damage. Numerous studies have evaluated the prevalence of chronic renal allograft damage in surveillance biopsies (Table 2).

The optimal scoring system for evaluating chronic allograft damage in protocol biopsies is not yet defined. Frequently, chronic lesions are classified according to the Banff classification. By the Banff scheme CAN is recognized and semiquantitatively scored according to the presence of interstitial fibrosis, tubular atrophy, and transplant vasculopathy [21–23]. However, the Banff classification may lack the sensitivity to detect early chronic changes and thus might underestimate chronic allograft injury [26–28]. Furthermore, because of sampling errors, a substantial number of biopsies might not be properly classified using the Banff definition of CAN [26]. As an alternative, chronic damage was evaluated by morphometric quantitation of specific lesions, such as interstitial fibrosis, intimal widening or deposition of collagen III [27–29]. Finally, some authors used indices of chronic allograft damage, such as the chronic graft damage (CGD) score or the chronic allograft damage index (CADI), which are based on numerical scoring of various histologic alterations compatible with chronic rejection [18,30–33].

In a substantial proportion of transplants histologic features of CAN may occur already early after transplantation, suggesting that the first few months after transplantation are crucial in the development of CAN (Table 2). Analysis of protocol biopsies performed within the first 6 months revealed prevalences of CAN (Banff) up to 40%. Nankivell *et al.* [20] described the natural course of CAN in a study evaluating 959 protocol biopsies performed serially up to 10 years after transplantation. In this analysis, 120 kidney-pancreas recipients and one patient receiving a kidney allograft alone were evaluated. The authors reported frequent mild chronic injury (grade I CAN) at 1 year (94%) predicted by early tubulointerstitial damage from ischemic injury and acute (clinical or subclinical) rejection. At this time-point chronic glomerulopathy scores, glomerulosclerosis and fibrointimal vascular thickening were minimal. Beyond 1 year, a later phase of CAN characterized by chronic glomerular and microvascular injury was common. At 10 years, severe CAN was found in 58.5% of patients, signs of CNI toxicity in almost all recipients.

Most importantly, many studies have pointed out that early CAN detected in protocol biopsies may ultimately result in deterioration of graft function. Therefore,

Table 2. Features of chronic allograft damage in protocol biopsies.

Author	Timing of Bx	*N*	Immunosuppression	Evaluation	CAN
Isoniemi *et al.* [31]	2 years	89	Not reported	CADI	Score <2: $N = 44$; score >2: $N = 45$
Dimeny *et al.* [33]	6 months	99		CGD score	Score: 4.7 ± 2.9
Seron *et al.* [6]	3 months	98	ATG or OKT3, CyA, steroids	Banff, morphometry	CAN: 42%
Legendre *et al.* [25]	3 months, 2 years	41	Induction, Aza (CyA), (steroids)	Banff	CAN: 26, 48% (Cad); 0%, 0% (Liv*)
Bicknell *et al.* [51]	1 week, 3 and 6 months	51	Tac versus CyA (Aza), steroids	Molecular markers	At 1 week higher collagen III and TIMP-1 mRNA levels in CyA-treated patients
Seron *et al.* [34]	3 months	280	Six different schemes	Banff	CAN (+RTV): 30.9%; CAN (–RTV): 7.4%
Lehtonen *et al.* [32]	1 year	102	CyA, Aza or MMF, steroids	CADI	Score: 2.92 ± 1.67
Nankivell *et al.* [13]	3 months	112	CyA, Aza, steroids (OKT3 induction)	Banff	CAN: 24% (3 months)
Hueso *et al.* [37]	1 year	51	CyA-based in 88% of patients	Banff, TGF-β1	CAN: 53%; TGF mRNA: no correlation with histology
Moreso *et al.* [27]	4 and 12 months	40	Six regimens, in most cases CyA-based	Banff, morphometry	Increase in Vinterstitium/cortex and Vintima/artery at 4 months
Veronese *et al.* [14]	2 and 12 months	32, 26	Not specified	Banff	CAN: 58% (12 months)
Baboolal *et al.* [28]	3, 6 and 12 months	51	Tac versus CyA, Aza, steroids	Banff, morphometry, TGF-β	CAN: 4%, 12%, 49%; TGF-β increased (CyA > Tac)
Seron *et al.* [26]	4 and 14 months	155	Five regimens, in most cases CyA-based	Banff	CAN: 40%, 52.9%
Shishido *et al.* [18]	1, 2, 3 and 5 years	95	CyA, Aza or Miz, steroids (ALG induction)	Banff, CADI	CAN (1 year): 48% (CADI score: 3.5 ± 1.3)

ALG, antilymphocyte globulin; Aza, azathioprine; Bx, biopsy; Cad, cadaveric; CADI, chronic allograft damage index; CAN, chronic allograft nephropathy; CGD score, chronic graft damage score; CyA, cyclosporin A; LR, living-related; Miz, mizoribine; MMF, mycophenolate mofetil; *N*, number (of patients); RTV, renal transplant vasculopathy; SAR, subacute acute rejection; Tac, tacrolimus; ATG, antithymocyte globulin; TGF, transforming growth factor. *HLA-identical living-donor kidneys.

chronic renal graft damage detectable in well-functioning kidney allografts, may be a valuable predictor of late graft loss. Accordingly, protocol biopsies could be a worthful tool for future studies evaluating strategies aimed to treat CAN.

Nankivell *et al.* [13] reported Banff chronic nephropathy for 24% of 3-month protocol biopsies. The occurrence of chronic changes, i.e. chronic intimal vascular thickening of small arteries and interstitial fibrosis, in 3-month biopsies was associated with graft loss and decline of renal function [13]. Seron *et al.* [6] described a prevalence of CAN in about 42% of protocol biopsies performed 3 months after transplantation. Graft survival for patients with CAN in a 3-month biopsy was reported to be significantly lower than that reported for patients without CAN [6]. In a subsequent study, the authors described a significantly lower 10-year allograft survival rate for patients showing CAN with renal transplant vasculopathy (RTV; 3-month protocol biopsy) (41%) when compared to patients with CAN without RTV (82%) or patients without CAN (95%). These data point to a particular role of chronic vasculopathy (CV) as a predictor of low long-term allograft survival [34]. Legendre *et al.* [25] demonstrated the frequent occurrence of CAN in recipients of cadaveric transplants. Interestingly, CAN was not detected in 3-month and 2-year protocol biopsies performed in patients receiving a human leukocyte antigen (HLA)-identical living-donor allograft. Using the CADI score, Isoniemi *et al.* [31] reported a significant correlation of the 2-year score with transplant function at 6 years. Of the patients with a low CADI score (<2) 7% were in clinical chronic rejection at 6 years when compared to 42% of patients with a CADI score >2 and stable graft function at 2 years. Dimeny *et al.* [33] evaluated 6-month protocol biopsies using an in-house scoring system, the CGD score, which includes scoring of vascular intimal hyperplasia, glomerular mesangial changes, focal and diffuse lymphocytic infiltration, interstitial fibrosis and tubular atrophy. In this study, a strong association between the CGD score and the risk of late graft loss was observed. Patients with a score ≥6 had a significantly higher graft loss rate than patients with a score <6 (2 years: six of 35 vs. two of 54, $P = 0.037$; 3 years: 10 of 35 vs. two of 54, $P = 0.002$). Furthermore, patients with a CGD score > 6 had worse graft function and a higher degree of albuminuria at 2 years [33]. A limitation of sum scores however might be, that adding up scores for individual parameters that are independent might lead to an overestimation of particular pathogenic mechanisms. Furthermore, if scores are not linear (i.e. scores 1 does not exactly reflect 50% of score 2) and scored parameters are not biologically equivalent, interpretation of sum scores requires caution.

In a recent report, Moreso *et al.* [27] evaluated the time course of intimal thickening and interstitial widening in serial protocol biopsies. Interestingly, a significant increase in these parameters was observed at 4 months when compared with donor biopsies, but no further increase was observed at 1 year. Quantitation of intimal thickness in this study was proposed to allow a more accurate estimation of chronic vascular injury. Remarkably, no correlation between morphometric analysis of intimal thickness and Banff scores of acute and chronic vascular lesions was found, obviously because the degree of intimal thickness was in most instances below the threshold of the CV-score. The authors proposed the use of quantitation of these parameters for prospective treatment studies as this approach reduces the necessary study sample size when compared with semiquantitative schemes such as the Banff classification. The morphometric analysis of chronic changes in protocol core biopsies may be a useful interim end-point for prospective studies planned to modify the natural history of chronic allograft failure [27].

Besides histomorphologic evaluation, a variety of molecular markers for chronic injury have been tested in the protocol biopsy setting including matrix proteins or profibrotic factors. Nicholson *et al.* [35] investigated the impact of collagen III deposition on long-term allograft function. A percentage area of collagen III of more than 40% was found to be associated with a lower glomerular filtration rate at 24 months [35]. Laine *et al.* [36] found biochemical evidence for an increased rate of apoptotic cell death of tubular cells in protocol biopsies with chronic allograft damage. In a few recent studies, associations between the expression of profibrotic genes and the development of chronic damage were investigated [28,37]. A 1-year protocol biopsy study revealed no association between the expression of transforming growth factor (TGF)-β and distinct histologic changes including interstitial fibrosis or with clinical parameters [37]. In biopsies with established signs of CAN performed because of deterioration of graft function, however, higher levels of TGF-β expression were observed [37]. Recently, Baboolal *et al.* [28] investigated the expression of profibrotic growth factors and renal injury in protocol biopsies performed at 3, 6 and 12 months after transplantation. The authors reported an early and progressive disease in mRNA of TGF-β, thrombospondin, and fibronectin. In this study, expression of these genes was associated with a significant increase in interstitial fibrosis [28].

The relationship between SAR and CAN

Based on the observation that early and especially late acute rejection represents an important predictor for the

subsequent development of CAN [1,38,39], it can be speculated that also SAR may effect deterioration of graft function in the long term and that timely therapeutic intervention improves clinical outcomes. Nevertheless, mononuclear infiltrates may not always indicate rejection as in some recipients maintain excellent long-term function despite signs of subclinical rejection. Indeed, tubulitis is well known to occur in other pathologic conditions not related to rejection, including acute tubular necrosis. Accordingly, in the Banff scheme, some forms of tubular infiltrates, such as tubulitis in atrophic tubules are not regarded as diagnostic lesion for rejection [23]. Furthermore, the possibility of an enhancement of graft acceptance by infiltrates of distinct immune cells was speculated [40]. Nevertheless, several studies suggest that SAR may contribute to chronic allograft damage [13,18,20], and in a randomized prospective study, treatment of SAR with high-dose steroids was found to improve long-term allograft outcome [11,24]. Nankivelli *et al.* [13] reported a significant association between subclinical rejection at 3 months and severity of CAN at 12 months. Interestingly, mononuclear infiltration within a specific compartment (tubulitis, interstitial inflammation or vasculitis) strongly correlated with chronic damage within the same compartment at a later time-point (tubular atrophy, interstitial fibrosis and intimal thickening, respectively) [13]. In a recent report evaluating 1-, 3- and 5-year biopsies obtained in 95 pediatric patients, Shishido *et al.* [18] reported an association of CAN with features of SAR (see Table 1). Remarkably, an increased CADI score in a subsequent biopsy was observed for as many as 70% of cases with subclinical acute inflammation in a prior biopsy when compared to 33% paired biopsies without SAR [18].

Does treatment of SAR prevent development of CAN?

In a randomized study, Rush *et al.* [7,11] reported improved allograft outcome after treatment of early acute subclinical rejection with high-dose steroids. Thirty-nine recipients of a kidney allograft were randomized to protocol biopsies at 1, 2, 3, 6 and 12 months or only at 6 and 12 months. Patients from the first group received high-dose corticosteroids in case of SAR in 1-, 2- or 3-month biopsies. At 24 months, a lower rate of early or late acute rejection, a lower rate of SAR at 6 months, a decrease in chronic histologic changes and an improvement of 24 months allograft function was found for patients subjected to high-dose steroid therapy in case of SAR when compared with nontreated control patients. At 2 years, graft survival was 97% (biopsy group) and 83% (control group). At 5 years, follow-up graft survival was 88% and 72%, respectively [11]. These data support a high clinical relevance of early detection and treatment of SAR. Future studies testing larger sample sizes will have to confirm the randomized trial data from Winnipeg.

Humoral kidney allograft rejection in renal allograft protocol biopsies

Recent reports reinforce an important role of humoral immunity as mediator of allograft rejection [41,42]. Deposition of the C4 complement split product C4d along the endothelium of peritubular capillaries (PTC) may represent a specific marker of antibody-mediated allograft injury. For biopsies performed because of acute allograft dysfunction, incidences of C4d deposition were reported to be 25–50% [41,42].

The C4d may represent a valuable tool to uncover subclinical humoral rejection in protocol biopsies. Four studies evaluating C4d staining in protocol biopsies were recently published, three of them in abstract form. Sund *et al.* [43] reported endothelial C4d deposition in 11 of 37, 1-week kidney allograft biopsies performed in living-donor recipients. Nine of the C4d-positive patients showed signs of clinical rejection, two patients had stable graft function. Roberts *et al.* [44] evaluated C4d deposition in 1-week allograft protocol biopsies obtained in 53 kidney transplant recipients. The authors described C4d deposits for six of 53 patients. Furthermore, Nickerson *et al.* [45] evaluated prevalences of C4d staining in 1–6-month protocol biopsies. In this retrospective analysis, a substantial proportion of biopsies with SAR (25%) was found to be associated with peritubular C4d deposits. Fiebeler *et al.* [46] performed C4d staining in 80 renal transplant recipients subjected to protocol biopsies 6 and 12 weeks and 6 months after transplantation. Ten of 130 evaluated biopsies were found to be focally (<50% of the PTCs) C4d-positive. Five of these biopsies showed histologic signs of acute tubulo-interstitial rejection. In one patient, three serial biopsies showed strong C4d deposits without clinical signs of rejection and stable transplant function [46]. These reports suggest that humoral rejection, suspected because of peritubular C4d deposits, may occur also in stable transplant recipients. In a recent retrospective study, a substantial fraction of patients with chronic graft dysfunction were shown to stain-positive for C4d in PTC. Regele *et al.* [47] demonstrated a strong association of C4d staining with particular signs of chronic rejection, i.e. transplant glomerulopathy and multilayering of peritubular basement membranes. Positive C4d staining in a first biopsy (without evidence of transplant glomerulopathy) was found to be associated with the finding of transplant glomerulopathy in a subsequent biopsy, even if C4d staining was negative at this

time-point. These data suggest that early C4d staining might predict the occurrence of morphologic lesions reflecting chronic humoral rejection. Future studies will have to clarify if also patients with subclinical C4d-positive rejection are at-risk for chronic allograft damage.

Maintenance immunosuppression and subclinical rejection

There is evidence that the type of maintenance immunosuppression could influence the prevalence of SAR or features of chronic allograft damage [15,20,28]. In a recent report, Gloor *et al.* [15] reported an extremely low incidence of SAR in renal transplant recipients receiving tacrolimus-based immunosuppression. In this study, only three of 114, 3-month protocol biopsies showed signs of cellular rejection according to the Banff scheme. With respect to the earlier reported higher incidences (31%) under cyclosporin A (CyA), mycophenolate mofetil (MMF) and steroids [9], the authors discussed effective prevention of SAR by tacrolimus. These data are in line with earlier studies reporting a lower incidence of clinical acute rejection for patients receiving tacrolimus-based immunosuppression [48–50]. Furthermore, in a recent randomized analysis comparing tacrolimus- and CyA microemulsion-based immunosuppression, Babolaal *et al.* [28] investigated the expression of profibrotic growth factor, TGF-β, thrombospondin and fibronectin and histologic features of chronic renal injury. Importantly, the use of CyA was found to be associated with a markedly increased expression of TGF-β and a significantly higher degree of interstitial fibrosis when compared with the use of tacrolimus. Furthermore, in this study, impaired renal function at 12 months was reported for patients receiving CyA. In contrast, a previously published randomized trial revealed no difference in glomerular mRNA expression of TGF-β1 between CyA- and tacrolimus-treated renal allograft recipients [51]. Significant differences, however were reported for collagen III and tissue inhibitor of metalloproteinase 1 (TIMP-1), a profibrotic tissue inhibitor of metalloproteinases at 1 week post-transplantation (higher levels in CyA-treated patients).

Protocol biopsies may be a useful tool for evaluating the efficacy and safety of immunosuppressive regimens. In a recent study, protocol biopsies have been employed to monitor the effectiveness of steroid-free immunosuppression in pediatric recipients [52]. In this open-labeled prospective trial, immunosuppression with anti-interleukin (IL)2R antibody, tacrolimus and MMF was found to be associated with a very low incidence of SAR as assessed at 1, 3, 6 and 12 months [52].

Furthermore, surveillance biopsies may help guide changes in drug regimens and establish the optimal dose of immunosuppressants. In a recent randomized study, Gotti *et al.* [19] reported successful discontinuation or reduction of CyA or steroids based on findings in protocol biopsies performed between 1 and 2 years after kidney transplantation. Fifty-nine recipients on steroids, CyA and azathioprine were randomized to protocol biopsy or no biopsy. In the protocol biopsy group, in all five patients without significant histologic changes steroids could be safely withdrawn without subsequent occurrence acute rejection or graft loss. Furthermore, for 13 patients with signs of CyA nephropathy, CyA was discontinued or reduced. Whereas complete discontinuation of CyA led to acute rejection in all four tested patients, lowering the dose to 30–70 ng/ml trough levels in the following patients did not lead to rejection or deterioration of graft function [19]. These data may indicate that exclusion of active rejection by protocol biopsies may allow safe withdrawal of steroids.

The risk associated with renal transplant biopsy

For a long time, biopsies in patients with a well functioning graft were considered to be unethical. However, during the last decade, an increasing number of transplant centers has started to perform protocol biopsies in kidney transplant recipients. Several groups using ultrasound-guided percutaneous renal allograft biopsy reported very low complication [6,12,53]. In a large series of 1090 percutaneous renal biopsies (kidney transplants and orthotopic kidneys, ultrasound-guided), Hergesell *et al.* [54] demonstrated a very low complication rate, i.e. macrohematuria in nine patients (0.8%), necessity of blood transfusions in four patients (0.36%), minor hematomas in 2.2% and hemodynamically irrelevant arterio-venous fistulas in 9% of cases. Importantly, no loss of kidney or patient death occurred in this series [54]. Furthermore, in a recently published large multicenter study, an also very low rate of major complications (0.4%) with only one graft loss in a series of 2.127 protocol biopsies was reported [55]. Although in this analysis the clinical benefit of protocol biopsies was not formally assessed, the authors speculated that potential advantages of protocol biopsies outweigh risks associated with this procedure [55].

Conclusion

Studies dealing with the clinical value of protocol biopsies performed in stable kidney allografts have uncovered a high prevalence of SAR and features of CAN in stable allografts. Importantly, the occurrence of SAR or early chronic damage was found to be associated with the development of chronic damage and deterioration of graft function. Protocol biopsies performed during the first months post-transplantation may therefore allow

prediction of long-term allograft outcome. The important observation of Rush *et al.* [4,5,7,9,11,24], that treatment of SAR improves allograft outcomes, may represent a strong argument for the performance of protocol biopsies. However, additional randomized studies testing larger patient cohorts will have to confirm the Winnipeg data. Preliminary data suggest that protocol biopsies could be a useful tool to guide immunosuppressive therapy. Without doubt, surveillance biopsies may help to monitor the effectiveness and safety of novel immunosuppressive regimens and should be considered for clinical trials evaluating the long-term effects of novel immunosuppressive regimens. In this respect, it is important to mention that chronic allograft damage detected in protocol biopsies could be used as a surrogate marker for chronic rejection and subsequent graft failure in studies evaluating the effects of immunosuppressants on long-term outcomes. Indeed, recent studies suggest that the use of protocol biopsies (e.g. morphometric evaluation of chronic damage) could allow a significant reduction of study sample sizes and follow-up time.

Future studies testing large patient cohorts will have to clarify the true benefit (and risk) of protocol biopsy-based therapeutic consequences (e.g. antirejection treatment of SAR or changes in basal immunosuppression according to chronic features or drug toxicity) and will help to decide if potential advantages of surveillance biopsies justify the (generally low) complication risk, costs and expense of routine protocol biopsies.

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