Protocol Transplant Biopsies in Kidney Allografts: Why and When Are They Indicated?

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Protocol biopsies have long been a controversial issue in kidney transplantation. Relative risk of complications of the procedure must be weighed against the possible benefit, and the patient must be fully informed before protocol biopsies can be obtained. Risk of performing kidney allograft biopsies has diminished over recent years, with the routine use of smaller gauge biopsy needles, bioptic devices to obtain tissue cores, and ultrasound guidance for the biopsy procedure. The allograft kidney is located closer to the body surface than the native kidney, making localization of the organ for biopsy more straightforward than for native kidney. As reviewed by Wilkinson, safety of protocol biopsies has been established. In cited series totalling >3500 protocol biopsies, the rate of serious complications varied from 0.4 to 1% and very rarely led to graft loss. The authors of one large multicenter study concluded that "it is ethically justifiable to ask renal transplant recipients to undergo protocol biopsies in clinical trials and routine care" (1).

It is important to sample adequate renal cortex in graft biopsies to make pathologic observations on the tissue reliable and the procedure worthwhile for patient treatment. This is especially true in the setting of protocol biopsies, where the potential for benefit to the patient should outweigh any risk. Banff criteria define adequate cortical sampling as a specimen that contains at least 10 glomeruli and two arteries. Sampling of the medulla may also be desirable to optimize detection of early polyoma virus infection. It is recommended that at least two tissue cores be obtained, preferably at some distance from each other, to optimize sampling of pathologic processes such as rejection and infection, which may be focal in the allograft (2). Sampling with a 16-gauge needle provides a better tissue sample, with safety comparable to use of an 18-gauge needle (3).

The benefit of protocol biopsies has long been recognized in heart allografts. Routine protocol biopsies have been implemented in this setting because clinical signs and symptoms of rejection generally emerge only relatively late in the course of the rejection reaction or when rejection is very severe. Protocol biopsies are routinely obtained using an intravascular approach, which remains the gold standard (4). As in the renal allograft, multiple tissue samples are recommended for adequate sampling (5). Routine sampling, particularly within the first few months posttransplantation, ensures recognition of rejection at a time when therapeutic intervention is most likely to be efficacious. Unfortunately, these biopsies are limited to the myocardium just beneath the endocardial surface, with no sampling of arteries, so that despite the relatively invasive nature of the biopsy procedure, the information provided by these biopsies is limited compared with biopsies of the renal allograft.

Rise in serum creatinine is the most widely used marker to detect rejection or other pathologic processes in kidney allografts. However, creatinine is not a very sensitive marker of fall in GFR, and "subclinical" rejection, including vascular rejection or antibody-mediated rejection, or other processes may supervene in the graft with very minor or no change in serum creatinine (6–9). Incidence of "subclinical" rejection varies from center to center, for reasons summarized nicely by Wilkinson, including variations in HLA matching, incidence of delayed graft function (DGF), and immunosuppressive protocols, but certainly occurs to some extent in most if not all center populations of renal allograft recipients studied. Emergence of "chronic rejection" in allografts with no documented episodes of acute rejection may well be due, at least in part, to immune reactions that did not cause detectable rise in serum creatinine but resulted in structural injury to the graft.

Unlike other solid organs obtained for allografting, kidney allografts can be used successfully even if there is significant pre-existing disease in the donor organ at the time of harvesting. Kidneys from so-called "extended criteria" donors are commonly used for allografting, and these organs often have significant vascular disease, glomerulosclerosis, and/or interstitial fibrosis with tubular atrophy and loss. Indeed, it is routine to obtain a preimplantation "protocol" biopsy from most deceased donor kidneys for rapid processing to assess suitability for implantation. Baseline postimplantation biopsies with optimum fixation and processing enable detection and quantification of glomerulosclerosis, small- and large-vessel chronic vascular disease, extent of fibrosis and tubular atrophy and loss, any unsuspected pre-existing disease, and ischemic injury, which have prognostic implications for graft survival (10). Even allografts from live donors may have significant baseline pathology (11). It is important to have an accurate baseline assessment of these changes so that any glomerulosclerosis, vascular

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disease, and interstitial fibrosis and tubular atrophy in any allograft biopsies obtained at later time points posttransplantation can be interpreted correctly as either pre-existing or evolving in the allograft (10). Protocol postimplantation biopsies may also reveal very early complement deposition in patients who are at high risk for antibody-mediated rejection (12).

Another context in which kidney allograft protocol biopsies are performed routinely is in the setting of DGF postimplantation. In the context of an already elevated serum creatinine, often requiring dialysis, rejection may supervene and be undetectable by clinical assessment or laboratory testing. Many if not most centers perform routine biopsies, often at weekly intervals, throughout the period of delayed function. If rejection is detected, then appropriate therapy can be initiated in a timely manner to prevent significant allograft injury and improve outcome.

As noted by both Rush and Wilkinson, other processes that are detectable by protocol biopsies include chronic calcineurin inhibitor (CNI) toxicity, polyoma virus infection, and early fibrosis of any cause. Early identification of these processes can lead to more successful interventions to prevent progression, including reduction or discontinuation of CNI if morphologic evidence of toxicity is detected and reduction of immunosuppression to control polyoma virus nephropathy. If evolving fibrosis is detected in the allograft, then therapy with angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors (13) can be initiated. Other potential antifibrogenic strategies, including cis-retinoic acid (14), mycophenolate mofetil (13), blockade of endothelin (15), and/or anti–TGF-β (16), may be efficacious as well. In addition, potentially fibrogenic medications such as the CNI can be reduced or avoided, if appropriate. A variety of techniques are available for quantification of allograft fibrosis (17), which are increasingly easy to use and becoming more widely disseminated. In experienced hands, estimates of fibrosis and tubular atrophy and loss based on routine trichrome staining without histomorphometry may provide useful information and may even be more useful than collagen-specific staining, which may underestimate early matrix deposition, tubular atrophy with edema, and tubular loss with parenchymal retraction (*e.g.*, [18]).

Surveillance for urine and serum markers of rejection, when implemented routinely, may potentially enable detection of early and/or subclinical cell-mediated rejection, potentially obviating the need to do protocol biopsies to detect subclinical rejection in patients with functioning allografts. However, studies of these markers thus far largely have been done in the context of clinically overt rejection, although early rises in these markers may precede overt cell-mediated rejection (19). Whether molecular signals for all "subclinical rejection" are the same as those in early stages of what will become clinically overt acute rejection has not been addressed, and ability to detect reliably "subclinical" rejection, whether cellular or antibody mediated, has not been assessed systematically. In addition, CNI toxicity and early fibrosis are not currently detectable using these molecular surrogates. Polyoma virus infection, however, can be detected by quantification of viropathic "decoy" cells or viral antigens in the urine (20), techniques that are sensitive but may detect polyoma virus infection of urothelium not involving the allograft. Detection of viral DNA in serum may provide the closest correlation with biopsy-proven infection in the allograft, although occasionally is positive in the absence of nephropathy on biopsy (21). Biopsy of the graft is desirable to confirm the diagnosis and before reducing immunosuppression to control the viral infection.

What to do? Because protocol biopsies are safe and well tolerated in the vast majority of cases, the following recommendations seem reasonable (Table 1). Protocol peri-implantation biopsies, optimally done after vascular anastomosis, should be obtained as a baseline from all renal allografts, for assessment of any pre-existing fibrosis and/or vascular disease, to diagnose rare instances of other unsuspected pre-existing donor disease, and potentially to detect early rejection in highly sensitized patients. Performing a biopsy after vascular anastomosis provides the most immediate monitoring of acute changes and

Table 1. Recommendations for protocol biopsies[a]

Recommendation	Allograft Patient Population
Standard practice	
postimplantation	All recipients
weekly	All recipients with persistent DGF
within 3 mo	All highly sensitized/at-risk patients (unless clinical rejection has occurred)
at time of detection	Any recipient with significantly increased urine or plasma markers of PPV
Strongly recommended	
6 to 12 mo	Recipients with high CNI exposure, H/O toxicity
6 to 12 mo	Recipients with H/O prolonged DGF, rejection
Recommended for study (with interventional trials)	
1 to 3 mo	All recipients
6 to 12 mo	All recipients

[a]DGF, delayed graft function; PPV, polyoma virus, CNI, calcineurin inhibitor; H/O, history of.

could be especially critical for patients who are at risk for antibody-mediated rejection, because capillary margination of leukocytes and/or C4d deposition can be seen at early time points, as noted above. Biopsies should also be obtained at 1-wk intervals in the context of DGF, until stable recovery of function is achieved, to detect acute rejection or other processes that are evolving in the poorly functioning allograft.

Beyond these recommendations, a more selective/tailored use of protocol biopsies seems appropriate currently. As pointed out by Rush, protocol biopsies to rule out subclinical rejection potentially would be most useful in sensitized patients with significant risk factors for acute rejection. In at-risk patients (historical or current high panel reactive antibodies, child-to-mother donation, donor-specific transfusion, and previous allografts), a protocol biopsy should be obtained within the first 3 months, unless clinical rejection has supervened in the meantime, to rule out subclinical rejection. Biopsies should also be obtained from patients with urine or serum evidence of possible polyoma virus nephropathy to establish the diagnosis (and rule out subclinical rejection) before reducing immunosuppression. Protocol graft biopsies should be seriously considered in stable allografts at 6 months to 1 year to rule out subclinical toxicity in patients with significant cumulative CNI exposure or with a history of episode(s) of acute CNI toxicity. Six- to 12-month biopsies are also recommended to rule out emerging fibrosis in patients with a history of DGF that was slow to resolve or with a history of acute rejection, both consistently associated with later allograft fibrosis and dysfunction.

As proposed by both authors, multicenter, randomized trials in transplantation should be designed and implemented to evaluate yield and efficacy of protocol biopsies within 3 months of transplant in unselected populations of kidney allograft recipients. Trials should include standardized therapy of any detected subclinical acute rejection and follow-up comparison with outcome in a control cohort. Large multicenter trials of 6-month to 1-year protocol biopsies should also be carried out to evaluate kidney allografts for emergence of fibrosis/sclerosis, with fibrosis carefully quantified and compared with any baseline fibrosis to determine progression. Such trials would optimally include interventional strategies should fibrosis be detected, with later protocol biopsies as study end points.

Finally, although not normally defined as protocol biopsies, the threshold for biopsy of slowly deteriorating allografts and of allografts that fail to return to baseline function after therapy for acute rejection should be low. These biopsies could enable identification of active pathologic processes or emerging fibrosis in the allograft and initiation of early interventions to arrest or delay progression to overt dysfunction and later graft loss. Hopefully, proactive monitoring of intragraft injury and early intervention can alter the pattern of relentless decrements in structure and function that occur in the majority of renal allografts. Protocol biopsy of the kidney allograft is the current gold standard to achieve that goal.

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