Protocol Biopsies: Utility and Limitations

Yihung Huang and Evan Farkash

As both T cell and antibody–mediated rejection can have a subclinical phase, protocol biopsies provide an early opportunity to intervene before the onset of clinical allograft dysfunction. Protocol biopsies are usually done after reperfusion to establish baseline, between 3 and 6 months to identify subclinical rejection, and at 6-12 months to assess chronicity and persistent inflammation that have prognostic implication. Treatment of both subclinical T cell and antibody–mediated rejection prevents progression of rejection and development of interstitial fibrosis/tubular atrophy or transplant glomerulopathy. Although subclinical rejection has become less frequent in low-risk patients on triple immunosuppression containing tacrolimus, protocol biopsies may still be useful in selected population. Protocol biopsies are more likely to benefit patients at higher risk for rejection, including those who are highly sensitized, transplanted across donor-specific antibody barriers, or on calcineurin inhibitor/corticosteroids sparing regimens. Interstitial fibrosis on protocol biopsies, especially in conjunction with persistent inflammation, predicts lower allograft survival.

Key Words: Protocol biopsies, Subclinical rejection, Allograft survival

PRACTICE OF PROTOCOL BIOPSIES

Unless clinical evaluation or laboratory/imaging workup points to a clear etiology, a transplant kidney biopsy is generally indicated to diagnose the cause of allograft dysfunction, including persistent delayed graft function (DGF), new creatinine elevation from established baseline, and new-onset proteinuria. Even when clinical evaluation strongly suggests a particular diagnosis, such as acute rejection in the setting of nonadherence to immunosuppression, biopsy is still needed to clarify the variety and severity of rejection to guide treatment. Although detection of noninvasive biomarkers such as BK viremia, donor-specific antibody (DSA), and urinary chemokines may be helpful, they generally have moderate positive predictive values and do not preclude the need of kidney biopsies to confirm the diagnoses.[1-3]

Indication vs Protocol Biopsies

The degree of laboratory abnormality that should trigger a biopsy is a matter of clinical judgment. A 25% increase in serum creatinine certainly indicates a significant change in allograft function, whereas smaller fluctuation may be explained by laboratory variability and hydration status. However, in patients with high immunologic risk and predisposed to acute rejection, even a mild decline in glomerular filtration rate (GFR) may raise enough concerns to mandate a biopsy. In transplant centers with screening programs, detection of high level BK replication or DSAs may lead to biopsies even in the setting of stable kidney function. Following treatment of rejection, biopsies may be indicated to document histologic resolution.

In addition to indication biopsies, some programs also perform protocol biopsies around predefined post-transplant milestones regardless of allograft function. As discussed previously, there is no agreed upon threshold of kidney dysfunction for indication biopsies, and protocol biopsies essentially set the threshold at zero. It should be noted that although the terms protocol and surveillance biopsies are often used interchangeably, they emphasize different aspects of the screening program. These biopsies are scheduled per protocol, and they are often surveillance in nature given the absence of usual clinical indications. However, a recipient coincidentally found to have increased creatinine on the day of a prescheduled protocol biopsy would actually have a clear indication. In one observational single-center study, only <50% of protocol biopsies performed at 3 and 6 months were considered true surveillance biopsies without any graft dysfunction.[4]

Rationale and Practice of Protocol Biopsies

Creatinine rise and proteinuria are not sensitive indicators of kidney dysfunction and may not manifest until the underlying pathology is diffuse or advanced. Although entities like hyper/early acute rejection and recurrent focal segmental glomerulosclersis develop over hours or days and rapidly become clinically apparent, other causes of graft dysfunction develop over a longer period with a subclinical phase that eludes routine laboratory monitoring. Protocol biopsies are therefore needed to detect these subclinical pathologies. In addition, protocol biopsies can also track chronic histologic changes in different compartments of the allograft, providing a more detailed picture of the allograft health than laboratory values alone.

Protocol biopsies are not universally practiced. A recent survey of US kidney transplant programs found that only 17% perform protocol biopsies routinely on all recipients,[5] whereas others reserve protocol biopsies for higher risk patients. In addition to their clinical application, surveillance biopsies are also often part of transplant research protocols. The low incidence of clinical rejection during the first post-transplant year with modern immunosuppression increases the importance of subclinical rejection as a potential end point.[5] Also, as incidence of allograft failure has become low in short-term studies, the chronicity score

From the Division of Nephrology, University of Michigan, Ann Arbor, MI; and Renal Pathology, Department of Pathology, University of Michigan, Ann Arbor, MI.

Financial Disclosure: The authors declare that they have no conflicts of interest to disclose.

Address correspondence to Yihung Huang, MD, MS, 3914 Taubman Center, 1500 E Medical Center Drive, Ann Arbor, MI 48109. E-mail: yihung@med.umich.edu

1548-5595/$36.00

http://dx.doi.org/10.1053/j.ackd.2016.09.002

on 1-year protocol biopsy, which correlates with subsequent graft failure, is increasingly used as surrogate end points for long-term outcomes.[6]

Protocol biopsies are done with the same techniques as indication biopsies, and the usual contraindications, such as coagulopathy and uncontrolled hypertension, apply to both. Given their less urgent nature, transplant centers usually apply a stricter safety standard on protocol biopsies. For example, centers may withhold protocol biopsies if ongoing anticoagulation or antiplatelet therapies are deemed unsafe to interrupt. In a single-center study, protocol and indication biopsies were found to have similar incidences of complications.[7] Nonetheless, recipients undergoing protocol biopsies likely have a higher cumulative risk of biopsy-related complications because of the overall higher number of biopsies.

Efficacy of Screening

Similar to any other screening programs, protocol biopsies should be pursued with clear goals in mind. Not all screening programs produce improvement in health outcomes, with one example being the detection of early-stage prostate cancer that may be inconsequential. In order for protocol biopsies to be beneficial, there should be evidence that treatment of subclinical pathologies leads to better outcomes. In addition, subclinical pathologies need to be reasonably prevalent to ensure a favorable risk-benefit ratio of protocol biopsies. These parameters are influenced by the immunologic risk of the patient population and the prevailing immunosuppression regimen at a transplant center. Also, protocol biopsies ideally should reliably distinguish between pathologic and benign findings to avoid overdiagnosis and overtreatment. How protocol biopsies fulfill or fall short of these criteria will be discussed subsequently.

CLINICAL SUMMARY

- Protocol biopsies are needed to detect rejection in the subclinical phase.
- Treatment of subclinical rejection may help prevent progression and subsequent development of interstitial fibrosis/tubular atrophy and transplant glomerulopathy.
- Interstitial fibrosis on protocol biopsies, especially in conjunction with persistent inflammation, predicts lower allograft survival.

FINDINGS ON PROTOCOL BIOPSIES AND TREATMENT OF SUBCLINICAL REJECTION

Implantation Biopsies

Centers that perform protocol biopsies usually have one done by the surgeons at the time of implantation. One main purpose is to evaluate the degree of donor disease, which may be substantial even in some donors with low kidney donor profile index. The kidney donor profile index includes history of hypertension and diabetes, which may be under-reported in deceased donors with scant prior medical contacts. Even in living donor kidneys with rigorous predonation workup, implantation biopsies may still uncover unexpected donor disease, especially in those older than 60 years or with hypertension.[8]

In addition, implantation biopsies provide the baseline of comparison and help ascertain the chronicity of changes seen on all subsequent biopsies. It is therefore crucial that representative sampling of different compartments is obtained. Although some surgeons might prefer the wedge technique to avoid damage to larger blood vessels, the core needle technique, the same approach as subsequent post-transplant percutaneous biopsies, should ideally be used. Compared with core needle biopsies, wedge biopsies underestimate vascular lesions and overestimate glomerulosclerosis because of its shallower sampling.[9] These discrepancies can lead to inaccurate assessment of chronic changes in allografts.

Beyond serving as the baseline of comparison, it is less clear whether implantation biopsy has an additional prognostic value. Clinicians often use the findings on implantation biopsies to gauge the appropriate length of DGF and nadir post-transplant serum creatinine, although this practice is not clearly supported by evidence. A systematic review of implantation biopsy did not show consistent associations between implantation biopsy findings and post-transplant outcomes, either in DGF or estimated GFR (eGFR).[9] However, most studies reviewed included only wedge biopsies, which may explain the poor correlation.

Timing of Protocol Biopsies During the First Post-Transplant Year

The main objective of protocol biopsies during the first post-transplant year is the detection and treatment of subclinical T cell and antibody–mediated rejection. There is no consensus on the optimal timing of protocol biopsies during this period. Although older studies in the era of less potent immunosuppression showed the highest prevalence of subclinical rejection at 1 month,[10,11] increasing use of induction therapy has reduced the incidence of early rejection and limits the utility of early protocol biopsies.[12] In addition, some tubulointerstitial inflammation as late as 6 weeks post-transplant may represent peritransplant injury-repair response rather than rejection based on molecular phenotyping.[13] Therefore, many programs now wait until 3-6 months post-transplant to perform the first protocol biopsy. By then, waning protection from induction and de-escalation of maintenance immunosuppression likely again increase the prevalence of subclinical rejection. Nonetheless, as discussed subsequently, recipients with increased risk of early rejection, such as those transplanted across DSA barriers, may still benefit from early protocol biopsies.

Detection and Treatment of Subclinical T Cell–Mediated Rejection

Subclinical T cell–mediated rejection (TCMR; Banff grade $\geq$ 1A) is generally treated the same as clinical rejection manifesting as allograft dysfunction, and this approach is well supported by evidence from the