Protocol Transplant Biopsies: Are They Really Needed?

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Studies suggest that surveillance or protocol biopsies that are performed during the first year after kidney transplantation may be clinically useful in identifying early acute rejection or chronic allograft nephropathy at a point when they may be amenable to treatment. Although the benefit of this approach has yet to be evaluated in large, multicenter, prospective trials, numerous studies suggest that implementation of protocol biopsies may improve long-term graft function. In particular, a number of reports suggest that detection of chronic allograft nephropathy in early protocol biopsies is predictive of subsequent graft function and loss and that early treatment may have a dramatic effect on the outcome of the graft. Protocol biopsies also have the potential to be of great value in high-risk patients, such as those with delayed graft function, by allowing for early intervention for acute rejection. Furthermore, the procedure seems to be relatively straightforward and safe. Nevertheless, paucity of data has meant that clear proof of a benefit of early treatment of subclinical rejection and chronic allograft nephropathy detected by protocol biopsy is lacking. Moreover, the optimal timing of protocol biopsies and reliable methods to quantify the histologic changes observed in biopsy specimens have yet to be determined. This review discusses the pros and cons of protocol biopsies and considers the place of this procedure in the routine treatment of kidney transplant patients.

Protocol Biopsies Are Valuable in High-Risk Recipients

Protocol biopsies have the potential to be of great value in high-risk renal transplant recipients by allowing for early intervention. For example, delayed graft function (DGF) is strongly associated with poor long-term graft survival, and early treatment of acute rejection in such patients is vital to lower serum creatinine at 2 yr (183 versus 133 μmol/L; \( P = 0.05 \)) (11). Further evidence for a benefit of treating SCR has been provided by two reports in which early protocol biopsies were obtained at 3 mo posttransplantation; untreated SCR was found to be correlated with an increase in interstitial fibrosis and tubular atrophy in later biopsies in both studies (9,12).

A recent study has reported a continuous correlation for both acute rejection and borderline rejection, between the degree of functional graft impairment at the time of biopsy (protocol biopsies performed at 6 wk and 3 and 6 mo) and outcome at 1 yr (acute rejection, \( r = 0.40 \); borderline rejection, \( r = 0.34 \); \( P < 0.01 \)) (13). In addition, in a 10-yr study of 304 patients with stable graft function, those with borderline changes or SCR in protocol biopsy specimens at 14 d posttransplantation were found to have a higher incidence of acute rejection than those with normal biopsies (0.48 and 0.60 versus 0.23, respectively; \( P < 0.05 \)) (10). Moreover, the graft survival rates in patients with SCR in day 14 biopsies in this study were lower than those with normal or borderline changes at 1 yr (88.4 versus 97.9 and 99.1%; \( P < 0.05 \)), 5 yr (77.8 versus 96.2 and 95.9%; \( P < 0.05 \)), and 10 yr (62.3 versus 96.2 and 93.7%; \( P < 0.05 \)). These data thus suggest that use of protocol biopsies for the early detection and treatment of acute and borderline rejection may be a major factor in preserving long-term graft function.

### Table 1. Frequency of subclinical acute rejection and mild CAN in protocol biopsies

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of Patients</th>
<th>Time after Transplantation</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclinical rejection</td>
<td></td>
<td></td>
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<tr>
<td>Choi et al., 2005 (10)</td>
<td>304</td>
<td>2 wk</td>
<td>13</td>
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<td>115</td>
<td>1 wk</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 mo</td>
<td>8</td>
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<tr>
<td>Shapiro et al., 2001 (28)</td>
<td>100</td>
<td>1 wk</td>
<td>25</td>
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<tr>
<td>Nankivell et al., 2001 (9)</td>
<td>112</td>
<td>3 mo</td>
<td>29</td>
</tr>
<tr>
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<td>50</td>
<td>1 wk</td>
<td>4</td>
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<tr>
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<td>1</td>
</tr>
<tr>
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<td>35</td>
<td>1 mo</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 mo</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 mo</td>
<td>15</td>
</tr>
<tr>
<td>Séron et al., 1997 (7)</td>
<td>98</td>
<td>3 mo</td>
<td>4</td>
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<td>Rush et al., 1995 (8)</td>
<td>25</td>
<td>1 mo</td>
<td>20</td>
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<td></td>
<td></td>
<td>3 mo</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 mo</td>
<td>12</td>
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*aCAN, chronic allograft nephropathy.

**Diagnosis of CAN by Protocol Biopsy**

The frequency of CAN in early protocol biopsies up to 6 mo posttransplantation has been reported in a number of studies, with incidences of up to 79% (Table 1) (7,8,12,14,15). Evidence suggests that the first few months after transplantation are critical in the development of CAN and that protocol biopsies may be a valuable means of detecting early signs of chronic allograft damage that have yet to become clinically apparent. In particular, early protocol biopsies have shown that the presence of tubulointerstitial damage and vascular chronic damage are powerful predictors of allograft survival (16). Furthermore, serial protocol biopsies have shown that both interstitial and vascular chronic damage rapidly increase during the first 6 mo after transplantation, then slowly thereafter (17). It thus follows that early detection and treatment of CAN could have a dramatic effect on the outcome of the graft. This premise is supported by the findings of a number of studies that have investigated the effect of CAN diagnosed in protocol biopsies on outcome.

In one study of 98 kidney transplant recipients, protocol biopsies were performed at 3 mo posttransplantation (7). Compared with patients without CAN, those with CAN detected by protocol biopsy had an increased incidence of acute rejection before the biopsy (3.9 versus 24.3%, respectively; \( P = 0.003 \)), a higher mean cyclosporine level prebiopsy (214 versus 242 ng/ml; \( P = 0.049 \)), and lower actuarial graft survival (94.4 versus 80.5%; \( P = 0.024 \)). Conversely, late allograft outcome in patients with borderline changes in this study was comparable to that of patients with normal histology (7). An additional study—in which a chronic graft damage score was used to categorize the degree of chronic changes—has reported that early chronic pathology, detected by protocol biopsy at 6 mo, predicted graft function and loss at 2 and 3 yr posttransplantation (15). In particular, patients with chronic graft damage scores >6 had a higher rate of graft loss than those with scores <6 (Figure 1). Similarly, CAN diagnosed in protocol biopsies at 3 and 6 mo has been shown to predict renal allograft function at 2 yr posttransplantation (18). In addition, graft survival was found to be significantly better in patients without CAN versus those with CAN detected in protocol biopsies performed at 4 (91 versus 74%; \( P < 0.05 \)) or 14 (94 versus 75%; \( P < 0.05 \)) mo (19).

Protocol biopsies have also been reported to be useful in the detection of CAN in pediatric transplant recipients. CAN was observed in 30.4% of protocol biopsies that were taken from stable allografts of pediatric patients approximately 100 d after renal transplantation; creatinine clearance decreased in the following year in patients in whom CAN was detected, even when renal function had been normal at the time of biopsy (20). Conversely, creatinine clearance did not change in patients with normal biopsies (20).
prevent adverse long-term graft outcome. However, it is extremely difficult to diagnose graft rejection in patients with DGF on purely clinical grounds. Accordingly, there is a strong need for a means of promptly diagnosing acute rejection in patients with DGF, a need that may be met by protocol biopsies.

The usefulness of protocol biopsies in detecting SCR in patients with DGF was examined in a study of 83 renal transplant patients, 33 of whom had DGF (4). SCR was detected in 7-d protocol biopsy specimens in 18% of patients with DGF versus 4% of patients with early graft function. Half of the acute rejection episodes in the patients with DGF were clinically undiagnosed and were detected only by protocol biopsy, leading the study authors to recommend that protocol biopsies should be used in all patients with DGF. In another study of 410 kidney transplants, protocol biopsies were performed at 7 and 10 d posttransplantation; the risk for graft failure from acute rejection in patients with DGF was found to be 2.91 and was similar to the risk from early acute rejection in patients without DGF (2.95) (21). After taking the effects of acute rejection into account, the risk of graft failure as a result of prolonged DGF was reduced to 1.76. This suggests that much of the reduced graft survival associated with DGF may be explained by silent acute rejection, indicating that protocol biopsies and treatment of SCR during prolonged DGF may be warranted.

Further evidence for the benefit of protocol biopsies in high-risk patients has been provided by a study conducted in 230 renal transplant patients grouped according to pretransplant antibody status (22). Humoral/vascular acute rejection was detected in 17% of biopsies performed 10 to 30 d posttransplantation, 53% of which were in recipients with antibodies to class I HLA antigens, 25% in recipients with class II antigens, and 75% with both class I and II. Protocol biopsies thus may be an effective way of detecting subclinical antibody-mediated acute rejection in sensitized high-risk renal transplant recipients.

Detection of Graft Dysfunction as a Result of Nonimmune Factors

The appropriate therapy for graft dysfunction as a result of immunologic or nonimmunologic factors differs considerably; consequently, it is crucial to identify early the cause of the dysfunction. One of the most common causes of graft failure as a result of nonimmunologic factors is cyclosporine nephrotoxicity, yet the condition is reversible if immunosuppression is modified early enough, that is, reducing the dosage of cyclosporine while increasing the dose of other immunosuppressants. Use of protocol biopsies in this setting could be valuable by identifying cyclosporine nephrotoxicity in patients with stable renal allografts. Support for this application has been provided by one study, in which protocol biopsies were performed on renal transplant recipients 12 mo after transplantation; evidence of cyclosporine nephrotoxicity was found in up to 42% of specimens (23).

Protocol biopsies may also be a useful tool to detect renal diseases such as subclinical BK virus (BKV) nephritis. Activation of BKV is increasingly common in renal transplant recipients and generally leads to a rapid decline of renal function. BKV nephropathy can be controlled only by reducing the intensity of immunosuppression; thus, early detection and treatment are vital. The importance of protocol biopsies in the diagnosis of BKV nephritis was shown by a recent study of 547 renal transplants in which biopsies were performed at 4, 12, and 24 mo posttransplantation; SCR was observed in 3.5, 2.2, and 2.0% of biopsies, respectively, and subclinical BKV nephri-
tis was detected in 2.6, 5.0, and 2.0% of samples that were taken the same time points (24). Moreover, one transplant center has detected no new cases of polyomavirus nephropathy since implementation of a monitoring program for BKV infection involving protocol biopsy coupled with urine, serum, and plasma sampling (25).

Use of Protocol Biopsies in Clinical Trials Designed to Prevent CAN

Evidence suggests that the natural history of CAN may be modified by pharmacologic intervention, yet results from large, randomized, prospective trials that evaluate therapies to prevent or treat CAN are lacking. This is due largely to the methodologic difficulties encountered in the design of such trials. In particular, the minimum sample size for a trial aimed at preventing CAN is estimated to be approximately 10 times higher than for one aimed at preventing acute rejection. The follow-up time of such trials is also lengthy, because the end point must be long-term allograft survival. Furthermore, no prospective methods to monitor the appearance/progression of CAN are available currently for the kidney; thus, clinical trials that are conducted in renal transplant patients, by necessity, need to use surrogates markers of long-term graft survival.

Histologic changes in transplant biopsies provide the earliest available evidence of graft damage. Indeed, studies have shown that CAN diagnosed in early protocol biopsy samples is an independent predictor of long-term outcome (7,15). Furthermore, the predictive value on graft survival of incipient chronic tubulointerstitial lesions in protocol biopsies has been found to be superior to other predictors of outcome, such as acute rejection or serum creatinine (7). These findings indicate that protocol biopsies from patients with stable grafts may be useful to monitor the progression of CAN and may be a valuable tool in the design of future trials aimed at modifying its natural history.

Serón et al. (16) estimated the minimum sample size of a clinical trial using the presence/absence of CAN in a protocol biopsy at 3 mo posttransplantation as the primary efficacy variable. Power calculations suggested that approximately 300 patients in each of the treatment and placebo groups would be necessary to detect a 50% reduction in the incidence of CAN at 3 mo. This sample size is similar to the numbers of patients enrolled in trials aimed at preventing acute rejection but with a shorter follow-up time. Thus, protocol biopsies could well become a useful primary efficacy variable in trials aimed at preventing CAN.

Safety of Protocol Biopsies

Use of protocol biopsies has been limited in some centers by concerns over their safety; nevertheless, a number of studies suggest that the procedure is safe (26–29). For example, a retrospective audit of sequential protocol biopsies performed in four transplant centers has revealed a low incidence of clinically significant complications after protocol biopsy. Of the 2127 biopsy events assessed for major complications and 1486 for minor ones, there were three episodes of hemorrhage and three episodes of peritonitis, and three patients required trans-fusion. No deaths were observed, and only one graft was lost; all complications presented within 4 h of biopsy (27). Similarly, of 277 renal biopsies that were performed in a single-center study, there was only one (0.4%) serious hemorrhagic complication (28). An additional study of 1171 protocol biopsies has reported rates of major complications of just 0.7% when a 16-gauge needle was used to perform the procedure and 1% when an 18-gauge needle was used (29). Performing protocol biopsies seems to be relatively straightforward, with the procedure generally taking place in the outpatient clinic and patients being discharged a few hours after the procedure. Indeed, transplant recipients’ compliance with the procedure is high, with the consent rate in one center reported to be >90% (26).

Con: The Case Against Use of Protocol Biopsies

Variation in Reported Incidence of SCR and CAN

Use of protocol biopsies to detect early SCR and CAN has yet to be put into practice extensively, and centers’ decisions to adopt protocol biopsies as part of their routine patient treatment are likely to depend on the frequency that SCR and CAN are detected in biopsy specimens and the benefit of their treatment in terms of graft survival and function. However, the incidences of SCR and CAN reported in biopsy specimens thus far have differed widely, with the incidence of SCR in the first 6 mo posttransplantation varying from 1 to 29% and CAN from 0 to 79%. Furthermore, it is difficult to draw any firm conclusions on the frequency of SCR and CAN from these studies as methodologic differences between studies make comparisons problematic.

A number of explanations exist for the differences in SCR incidence reported between studies (6). For example, one possible reason may be differences in human leukocyte antigen (HLA) matching, because it is widely known that reducing HLA mismatch reduces recipients’ risk for rejection. Alternatively, the variation may be due to differences in the incidences of DGF between studies, as DGF is a known risk factor for both SCR and clinical rejection; thus, studies that exclude patients with DGF are likely to report lower incidences of SCR. Another factor that is likely to have an impact on the occurrence of SCR is the immunosuppressive protocol used. Indeed, Nickerson et al. (30) showed that increasing baseline immunosuppression reduces the frequency of clinical but not subclinical rejection. This indicates that studies that use a higher initial level of immunosuppression may well produce a higher proportion of patients with SCR. Comparison between studies is complicated further by the fact that some studies include few patients and that precise inclusion criteria in many studies are not reported.

The reported frequency of CAN in early protocol biopsies also varies considerably, with the changes observed being relatively mild, chronic lesions, usually classified as Banff grade 1 (7,8,12,14,15). It should be noted, however, that age-related, chronic renal changes can resemble those of CAN, yet four of the five studies mentioned did not control for donor changes by undertaking a donor biopsy. The remaining study reported an incidence of donor chronic changes of 54% and an incidence of
new CAN of only 26% (14). This suggests that donor-related changes may explain a large proportion of the variability in the reported incidence of CAN. This is supported by a recent study that reported that donor variables had little effect on acute histopathology scores in protocol biopsy specimens taken at 6 to 12 mo posttransplantation, although donor variables (e.g., deceased donor, increasing age, female gender, hypertension) explained approximately one third of the variability observed in chronic histopathology scores (31). The methodologic problems associated with the reporting of the incidence of SCR may also apply to the interpretation of CAN incidence data (see above). Thus, although numerous reports suggest that the frequency of SCR and CAN in early protocol biopsies may be high, the lack of consistency between studies casts doubt on the use of these data to support adoption of protocol biopsies in the routine treatment of kidney transplant patients.

Reliability of SCR and CAN Diagnosis

Assessment of the histologic changes detected by protocol biopsy is fraught with difficulties as all transplants generate an immune response to some degree, rendering analysis of histologic criteria for rejection in protocol biopsies problematic. It is clear, therefore, that reproducible and easily interpretable methods to quantify histologic changes in protocol biopsies are needed. To this end, a number of new molecular techniques have been developed, including identification of proinflammatory transcripts in tissue or urine by PCR (32), urine spectroscopy (33), and measurement of cell activation markers. However, although these strategies are promising for the future, they remain experimental and cannot be used to predict long-term graft function or used as a basis for initiating acute rejection therapy in patients with stable kidney function.

The lack of reliable methods to quantify histologic changes in protocol biopsies has led to such specimens’ being evaluated by use of Banff ’97 criteria (34). However, Banff criteria were devised to evaluate diagnostic biopsies for which the findings are likely to be more easily interpretable, not protocol biopsies for which the degree of interstitial fibrosis is mild, particularly in early biopsies (0 to 20%) (19). This leads to a high probability of misclassification of biopsies with borderline changes or grade 1 CAN.

In one study by Roberts et al. (6), protocol biopsies were performed at days 7 and 28 after renal transplantation; the presence of SCR or borderline changes in biopsies that were taken on day 7 was found to predict the development of clinical rejection within the following 3 d. Of the 92 specimens that were analyzed at day 7, SCR was detected in 13% and borderline changes in 12%. However, a greater proportion of specimens (17.4%) were deemed inadequate by Banff criteria. Similarly, SCR was detected in 8% of specimens and borderline changes in 16% at day 28, yet 26.9% of the specimens were found to be inadequate. The large proportion of inadequate specimens in both the day 7 and day 28 biopsies observed thus calls into question the reliability of the findings. Indeed, an additional study from the same group reported a reduction in the frequency of SCR after the introduction of new agents (e.g., mycophenolate mofetil, azathioprine) into immunosuppressive regimens in recent years (13% SCR in 7-d biopsies performed during 1992 to 1995 versus 2% in those performed during 2001 to 2003), leading Roberts et al. (10) to discontinue the use of early protocol biopsies in their unit (35). This latest finding is supported by a recent study that reported a decreased incidence of SCR when mycophenolate mofetil was used as the primary immunosuppressant compared with patients without such treatment (odds ratio 0.23; P < 0.05).

In an additional study, 310 protocol biopsies were obtained from 155 patients at 4 and 14 mo posttransplantation, and lesions were graded according to Banff criteria (19). Graft survival was found to be significantly better in patients without CAN versus those with CAN at both time points (P < 0.05 for both comparisons), with the incidence of CAN progressing from 40% at month 4 to 53% at month 14. However, approximately 25% of the biopsy specimens could not be classified properly, and the increase observed in the incidence of CAN between months 4 and 14 was lower than the proportion of misclassified biopsies. This suggests that evaluation of two sequential protocol biopsies (taken at months 4 and 14) by Banff criteria is not an accurate way of monitoring the progression of CAN in renal transplant patients and that care must be taken in interpreting the results of protocol biopsy specimens.

How Should Utility of Protocol Biopsies Be Tested?

To date, good evidence supporting the general implementation of protocol biopsies into clinical practice is lacking as large, multicenter, prospective trials are yet to be carried out. Even when such trials are undertaken, any evaluation of the utility of protocol biopsies must consider the complex differences that exist between clinical trials, such as differences in patient selection, use of induction therapies, and baseline and maintenance immunosuppression. To evaluate fully the benefit of protocol biopsies in terms of long-term survival and function, outcome parameters should include a minimum of 5-yr graft survival and function end points. Thus, it could be several years before evidence supporting the benefit of protocol biopsies in renal transplant patients with stable kidney function becomes available (36).

An additional problem for general implementation of protocol biopsies is that the optimal timing and frequency for such biopsies has not been evaluated. Furthermore, it is unclear whether decisions for intervention should be made on serial biopsies or based on a single biopsy result. If therapy decisions are to be made on serial biopsies, then it will be necessary to analyze the results of such biopsies in a standardized manner, which is problematic. Conversely, a single biopsy is likely to result in a high degree of inaccuracy.

Treatment for SCR and CAN

The presence of mononuclear infiltrates in renal allografts does not necessarily indicate rejection, and some recipients maintain good long-term graft function despite signs of SCR (37). Furthermore, the benefit of treating SCR detected by protocol biopsy has yet to be confirmed. This is in part because the effect of not treating SCR is largely unknown, as most studies give anti-rejection treatment for SCR as soon as it is detected. In
one study conducted by Roberts et al. (6), the presence of SCR or borderline changes in biopsies that were taken on day 7 posttransplantation was found to be strongly predictive of subsequent development of clinical rejection within the following 3 d. However, in the same study, untreated SCR and borderline changes detected in day 28 biopsies did not seem to be adverse prognostic factors; no difference in outcome (measured by graft survival and mean serum creatinine) at 1 and 6 yr posttransplantation were found between patients with SCR or borderline changes and those with no rejection or borderline changes (Figure 2). Further doubt on the utility of treating SCR has been cast by the study by Nickerson et al. (30), which demonstrated that increasing baseline immunosuppression in renal transplant recipients reduced the frequency of clinical rejection but not SCR, suggesting that the benefit of treating SCR may be limited.

The appropriate management steps to be taken after detection of CAN in a protocol biopsy may also not be clearcut, because it must be proved that the CAN will respond to therapy (37). CAN detected at 5 yr is rarely therapy responsive; thus, it is likely that the appropriate management for CAN detected at 6 mo or 5 yr posttransplantation are different. It is also possible that the underlying mechanisms of CAN detected up to 1 yr posttransplantation and after this point may differ. Thus, the window of time during which CAN will respond to therapy must be established before the appropriate timing for protocol biopsies can be determined (37).

Conclusions
The recent introduction of protocol biopsies in some centers to determine the presence of CAN and SCR in stable renal allograft should be seen as a major step forward in the quest to improve long-term transplant outcomes. Paucity of data has meant that clear proof that protocol biopsies improve long-term graft function and survival is lacking and that the benefits of treating SCR detected by protocol biopsy in particular are not clearcut. However, available evidence suggests that early detection and treatment of CAN that cannot be diagnosed clinically will benefit long-term graft function. Furthermore, reports indicate that protocol biopsies are safe and that their use is likely to improve the treatment of renal transplant patients. In addition, use of protocol biopsies as a primary efficacy variable could well become a useful tool in the design of future trials aimed at preventing CAN; power calculations suggest that they may allow an important reduction in the number of patients involved in such trials, as well as reducing the follow-up time. An additional potential benefit of protocol biopsies may in differentiating chronic loss of renal function caused by immunologic causes from nonimmunologic causes. Nevertheless, future studies must focus on reliable methods to quantify histologic changes in protocol biopsies. Furthermore, large-scale, multicenter, prospective trials of protocol biopsies are required to determine the criteria and optimal timing for such biopsies and assess fully their place in the routine treatment of renal transplant patients.

References


Please see the related articles in *JASN* by Baeten *et al.* (page 294–304), which suggests that serial CD8+ phenotyping in transplant biopsies may help identify patients at risk for chronic rejection when immunosuppression is tapered, and by Rowshani *et al.* (pages 305–312), which describes the use of serial transplant biopsies to demonstrate no difference in fibrosis at 6 and 12 months between cyclosporine- and tacrolimus-treated patients.