Protocol biopsy: what is the rationale and what is the evidence?

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**Introduction**

The incidence of acute rejection (and the proportion of grafts lost during the first year after renal transplantation) have markedly decreased after the introduction of cyclosporin A. The reduction of the rate of graft loss after the first year, however, has been much less impressive. Chronic transplant nephropathy has become the most common cause of late graft failure [1]. Chronic allograft nephropathy is strongly correlated with the number of acute rejection episodes during the first year after renal transplantation [2,3]. In the past, the possibility of graft failure was suspected only when a sustained and irreversible decline of renal function was evident, usually in the context of proteinuria and hypertension. Unfortunately, by the time the clinical diagnosis was confirmed by histology renal scarring was usually too advanced to make delayed treatment a promising proposition.

Traditionally, renal allograft biopsies were performed mainly in the setting of acute graft dysfunction. Recently, there has been a change of paradigms. Several reports suggested that acute rejection episodes and chronic allograft nephropathy are often subclinical without causing a measurable decrease in renal function. This raises the issue of biopsies of stable allografts (protocol biopsies) and the clinically useful information they provide.

**Subclinical acute rejection in patients with a stable renal graft**

The clinical presentation of acute rejection episodes after renal transplantation varies. Today, the most common presentation is a rise in serum creatinine for which no obvious cause is found. In addition, however, several investigators have recently documented the presence of subclinical acute rejection, i.e. histopathological evidence of acute tubulitis in the presence...
of stable or even improving renal function. Shapiro and co-workers [4] performed biopsies 1 week after transplantation in patients with good graft function. About 21% of patients with normal or improving renal function had borderline histopathological findings and 25% had frank acute tubulitis (Banff 1A to Banff 2A). Similar observations were made by Rush and his colleagues [5,6] in protocol biopsies 3 months after transplantation. They found borderline changes or even rejection in, respectively, 21 and 33% of patients with stable renal function. This observation led the authors to postulate that >50% of the rejection episodes are subclinical at some time after renal transplantation without acute impairment of renal function. The histological severity of such subclinical rejections did not differ from that of patients with a decline in glomerular filtration rate during the acute rejection episode. Using similar protocols, other investigators found that the frequency of acute or borderline rejection was much less (1–17%) (Table 1). Individual bias and sampling problems make the interpretation of the different observations difficult. Controversy continues about the significance and biological relevance of an interstitial infiltrate in renal transplants of stable patients [7].

**What are the consequences of a biopsy-proven rejection without impairment of renal function?**

Recent reports provide evidence in favour of treating biopsy-proven subclinical rejection episodes. Rush et al. [5] showed that corticosteroid treatment of subclinical rejection in the early post-operative period (i.e. months 1–3) is associated with better outcome. A decrease in the frequency of early (months 2 and 3) and late (months 7–12) acute rejection episodes was noted. This was accompanied by a decrease in the chronic tubulointerstitial score at 6 months post-transplant. In patients treated as a result of the findings of protocol biopsies, the serum creatinine concentration was lower at 24 months post-transplant compared to untreated controls. An additional observation further documents the value of protocol biopsies: when subclinical rejection episodes occurring later than 6 months after transplantation were treated with corticosteroids, treatment at this point in time failed to prevent subsequent deterioration of renal function. This finding indicates that a reversible rejection process may be present early after renal transplantation (<6 months). Beyond 6 months after transplantation, however, such rejections can no longer be fully reversed by standard immunosuppressive therapy [5]. Nevertheless, the controversy about the management of Banff borderline rejection continues. While the benefit of treating borderline rejection in the setting of graft dysfunction has been documented beyond doubt, it is still not certain whether the patient with stable graft function diagnosed by protocol biopsy derives benefit from treatment [8].

**Acute rejection in patients with delayed graft function**

Both delayed graft function and episodes of acute rejection are strongly associated with poor long-term graft outcome. In the patient with delayed graft function, it is difficult or impossible to diagnose graft rejection on clinical grounds. Early treatment of acute rejection is of vital importance to prevent an adverse long-term graft outcome. Consequently, there is a need for a method to promptly diagnose acute rejection in patients with delayed graft function. Such a method is provided by the protocol biopsy.

Jain et al. performed protocol biopsies in patients with delayed graft function 7 days after transplantation. About 18% of patients with delayed graft function had acute rejection compared to only 4% (P<0.05) of patients with early graft function. Borderline rejection was present in 12 vs 8%, respectively. Half of the acute rejection episodes that had occurred in patients with delayed graft function had remained clinically undiagnosed and were picked up only by the protocol biopsies. In patients with delayed graft function who required dialysis, none of the episodes of acute rejection had been diagnosed on clinical grounds. If protocol biopsies had not been performed, these episodes would have been missed completely [8,9]. Similar results were reported by Shapiro et al. [4]. They found acute rejection in biopsies performed 1 week after transplantation in 21% of the patients with delayed graft function and borderline rejection in about 36% (Table 2).
Chronic allograft nephropathy in patients with stable graft function

Chronic allograft nephropathy is the most common cause of late renal allograft failure. It is characterized by slow deterioration of renal function. Unfortunately, when biopsies are performed only after renal function has deteriorated, by the time the diagnosis is established by histology, the degree of scarring is usually advanced and often beyond the point of no return.

This consideration prompted some groups to perform protocol biopsies in an attempt to diagnose chronic allograft nephropathy at an earlier stage. Seron et al. [10], using protocol kidney biopsies, studied 98 patients with stable graft function (serum creatinine concentration <200 μmol/l, proteinuria <1 g/24 h) 3 months after transplantation. About 42% of the biopsies displayed chronic transplant nephropathy according to the Banff criteria. Patients with chronic transplant nephropathy had experienced more acute rejection episodes after transplantation. They also had higher mean cyclosporin concentrations, and graft survival was worse. In contrast, if only borderline changes were found, late allograft outcome was the same as that of patients with normal histology. There was also no difference in graft outcome whether tubulitis was present or not.

Fujisawa et al. [11] observed chronic allograft nephropathy in 30.4% of stable allografts of paediatric patients ~100 days after living-related renal transplantation. When early protocol biopsies of the allografts showed chronic allograft nephropathy, the creatinine clearance decreased within the following years even if renal function had been normal at the time of biopsy. In contrast, the creatinine-clearance did not change in the allografts in which the protocol biopsies showed normal findings.

Non-immunological causes of graft failure

Since the introduction of cyclosporin A, there have been numerous reports documenting the potential nephrotoxicity of this calcineurin inhibitor. The reported frequency of cyclosporin A nephrotoxicity following kidney transplantation varied between 10 and 54% [12,13]. According to Opelz [14], average late allograft outcome is worse when cyclosporin A doses of <3 mg/kg/day are used. The explanation for this observation is unknown, e.g. large inter-individual differences in susceptibility or insufficient precision of the pre-dosing blood cyclosporin A measurements. If more aggressive dosing regimens are adopted, however, a strong argument is provided for more frequent allograft biopsies or even protocol biopsies.

The diagnosis of cyclosporin A nephrotoxicity is extremely important, since this condition is reversible if immunosuppression is modified in time, i.e. reduction of the cyclosporin A dose and increased dosing of other immunosuppressants [15]. Only a few studies have reported the results of protocol biopsies to exclude cyclosporin A nephrotoxicity in patients with stable renal allografts. In patients treated with cyclosporin A, Takeda et al. [16] performed protocol biopsies more than 12 months after renal transplantation. Evidence of cyclosporin A nephrotoxicity was found in up to 42% of renal transplant recipients.

Other non-immunological factors influencing graft survival are infections, e.g. with cytomegalovirus. Recently, there have been several reports on BK-virus nephropathy (polyoma virus) in renal allografts. Infection with BK virus is widespread in the general population. Activation of this virus, sometimes in conjunction with graft rejection, has been reported by some authors in up to 8% of renal transplant recipients [17]. BK virus allograft nephropathy usually leads to a rapid decline of renal function. Up to 50% of patients will lose their allografts within a short time period. Specific antiviral therapy is not available. The only way to control BK-virus nephropathy is to reduce the intensity of immunosuppression.

An early diagnosis of non-immunological causes of graft dysfunction by protocol biopsy enables immunological and non-immunological factors to be distinguished. This distinction is of great clinical importance, since the respective therapies differ significantly (Table 3).

Conclusion

The recent introduction of protocol biopsies in renal allograft recipients has undoubtedly improved patient management. Timely treatment of allograft rejection that cannot be diagnosed on clinical grounds is

<table>
<thead>
<tr>
<th>Time after transplantation</th>
<th>Acute rejection (%)</th>
<th>Borderline rejection (%)</th>
<th>Normal (%)</th>
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</thead>
<tbody>
<tr>
<td>Jain et al. [8,9]</td>
<td>1 week</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Shapiro et al. [4]</td>
<td>1 week</td>
<td>21</td>
<td>36</td>
</tr>
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| Acute rejection Increase of immunosuppression Modification of immunosuppression Reduction of immunosuppression |
|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Calcineurin inhibitor toxicity                                 | Infection, i.e.                                               | BK-virus nephropathy                                           |
| Increase of immunosuppression                                  | Modification of immunosuppression                              | Reduction of immunosuppression                                |
definitely of benefit for long-term graft function. In addition, protocol biopsies are useful for identification of chronic loss of renal function from causes other than immunologically mediated rejection. Moreover, the relative safety of the biopsy procedure is well documented in several series and this investigative strategy is ethically justified [18].

Conflict of interest statement. None declared.

References