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An Analysis of Early Renal Transplant Protocol Biopsies – the High Incidence of Subclinical Tubulitis

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To investigate the possibility that we have been underestimating the true incidence of acute rejection, we began to perform protocol biopsies after kidney transplantation. This analysis looks at the one-week biopsies. Between March 1 and October 1, 1999, 100 adult patients undergoing cadaveric kidney or kidney/pancreas transplantation, or living donor kidney transplantation, underwent 277 biopsies. We focused on the subset of biopsies in patients without delayed graft function (DGF) and with stable or improving renal function, who underwent a biopsy 8.2 ± 2.6 d (range 3–18 d) after transplantation (n = 28). Six (21%) patients with no DGF and with stable or improving renal function had borderline histopathology, and 7 (25%) had acute tubulitis on the one-week biopsy. Of the 277 kidney biopsies, there was one (0.4%) serious hemorrhagic complication, in a patient receiving low molecular weight heparin; she ultimately recovered and has normal renal function. Her biopsy showed Banff 1B tubulitis. In patients with stable or improving renal allograft function early after transplantation, subclinical tubulitis may be present in a substantial number of patients. This suggests that the true incidence of rejection may be higher than is clinically appreciated.

Key words: High incidence, protocol biopsies, subclinical acute tubulitis

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Introduction

Acute rejection after renal transplantation may be associated with a variable clinical presentation. The symptoms of fever, swelling and pain over the allograft, routinely seen in the azathioprine era (1–3), are extremely unusual today. A rise in the serum creatinine, without other obvious cause, accompanied by a renal allograft biopsy demonstrating the appropriate histopathologic picture, is the most common presentation (4). However, Rush and his colleagues (5–7), and others (8–11), have demonstrated the presence of subclinical acute tubulitis, i.e. histopathologic evidence of acute tubulitis in the presence of stable renal function. We have noted similar findings in a small number of simultaneous kidney/pancreas (SPK) recipients who underwent renal allograft biopsy, despite having stable and normal renal function, because of a rising serum lipase level (i.e. suspected pancreatic rejection) (12). This demonstration of unsuspected tubulitis raised a concern that we have been systematically underestimating the incidence of acute rejection in our patients. Accordingly, we began to perform protocol biopsies at one week, one month, and one year after transplantation in all of our adult patients undergoing renal transplantation. This report describes our initial experience with one-week biopsies.

Patients and Methods

Between March 1, 1999 and October 1, 1999, 100 adult patients undergoing cadaveric kidney or SPK transplantation, or living donor kidney transplantation, underwent 277 percutaneous renal allograft biopsies. For the purposes of this analysis, we focused on a subset of 28 patients who did not experience delayed graft function (DGF) (i.e. no dialysis required after transplantation), and who had stable or improving renal function, who underwent biopsies at approximately one week after transplantation. The other patients either had DGF (n = 14) or clinical signs (i.e. a rising serum creatinine) that warranted a biopsy (n = 46); they would have undergone a biopsy as a matter of course (nine patients did not have a one-week biopsy, and three biopsy specimens were nondiagnostic). The group of 28 patients were the subset of patients who ordinarily would have had no indication to undergo a biopsy, because their allografts were functioning well and their serum creatinine levels were either falling or were normal and stable. The mean recipient age was 48.3 ± 12.1 yr (range 22.6–71.4 yr; Table 1). Twenty-four (86%) patients had undergone cadaveric kidney transplantation, two (7%) SPK transplantation, and two (7%) living-related donor kidney transplantation. Nineteen (68%) were receiving their first kidney transplant, while six (21%) and three (11%) were undergoing their second and third kidney transplantations, respectively. The peak/most recent panel reactive antibody levels (PRA) were 22.1 ± 28.9% and 10.5 ± 19.1%. Biopsies were performed percutaneously under real-time ultrasonographic guidance, a mean of 8.2 ± 2.6 d (3–18) after transplantation.

Table 1: Patient demographics

n = 28	
Mean recipient age (yr)	48.3 ± 12.1
Mean date of biopsy (postoperative day)	8.2 ± 2.6 (range 3–18)
Cadaveric kidney only	24 (86%)
Simultaneous pancreas/kidney	2 (7%)
Living donor kidney	2 (7%)
1st transplant	19 (68%)
2nd transplant	6 (21%)
3rd transplant	3 (11%)
Panel reactive antibody (%)	
Peak	22.1 ± 28.9
Recent	10.5 ± 19.1
Additional baseline immunosuppression	
Mycophenolate mofetil	18 (64%)
Daclizumab	4 (14%)
OKT3	1 (4%)
Azathioprine	1 (4%)
Nothing	4 (14%)

Table 2: Incidence/severity of tubulitis

No tubulitis	15 (54%)
Borderline	6 (21%)
Banff 1A	2 (7%)
Banff 1B	4 (14%)
Banff 2A	1 (4%)

Immunosuppression was with tacrolimus and steroids, as previously described (13–15). Eighteen (64%) patients also received mycophenolate mofetil 2 g/d (15). Four (14%) patients received antibody induction with daclizumab 2 mg/kg, at the time of transplantation (followed later by 1 mg/kg at 2 and 4 weeks). One (4%) patient received OKT3 induction, one (4%) received azathioprine, and four (14%) received no additional agent.

Pathologic evaluation of the biopsies, according to the Banff criteria (16), was performed by our transplant pathologists, who were blinded regarding the clinical status of the patients. Informed consent was obtained prior to each biopsy.

Results

Six (21%) patients without DGF and with stable or improving renal allograft function had evidence of borderline histopathology, and seven (25%) had evidence of frank acute tubulitis on the one-week biopsy (Table 2). The histopathologic severity ranged from Banff 1A to Banff 2A (16). Fifteen (54%) patients had no evidence of acute tubulitis.

The serial serum creatinine and tacrolimus levels in the two groups are shown in Table 3. The mean tacrolimus level on the day of the biopsy in the patients with subclinical borderline or acute tubulitis was significantly lower (16.6 ± 4.9 ng/mL) than in the patients with no evidence of acute rejection (25.1 ± 7.6 ng/mL; $p < 0.002$).

There was a significantly higher degree of HLA-DR mismatching in the group with subclinical borderline or acute tubulitis. There were three (23%) 0 DR mismatches, six (46%) 1 DR mismatches, and four (31%) 2 DR mismatches in the group with borderline or acute tubulitis (mean DR mismatch 1.1 ± 0.8). In the group with no tubulitis, 11 (73%) were 0 DR mismatches, and four (27%) were 1 DR mismatches (mean DR mismatch 0.3 ± 0.5; $p < 0.05$ by the Likelihood Ratio test).

All patients with histologic evidence of subclinical borderline or acute tubulitis were treated. In general, patients with borderline changes received a single bolus of steroids, along with augmentation of their tacrolimus dosage. Patients with Banff 1A tubulitis or worse received a bolus and a short steroid recycle, in addition to augmentation of their tacrolimus dosage.

Of the 277 biopsies performed, there was one (0.4%) serious hemorrhagic complication, in a 40-year-old white female with normal and stable renal function, who was receiving low

Table 3: Serial renal function and tacrolimus levels prior to, on the day of, and 7 days following protocol biopsies performed 1 week after transplantation

		D − 7	D − 3	D − 1	D 0	D + 7
Borderline or acute tubulitis (n = 13)	Serum creatinine (mg/dL)	6.0 ± 3.1	2.9 ± 2.5	2.4 ± 2.0	2.1 ± 1.7	1.6 ± 0.7
	Tacrolimus levels (ng/mL)	20.0 ± 4.8	22.0 ± 6.1	19.1 ± 3.6	16.6 ± 4.9*	20.2 ± 4.2
No tubulitis (n = 15)	Serum creatinine (mg/dL)	8.8 ± 4.3	4.1 ± 3.4	2.7 ± 1.8	2.3 ± 1.4	2.1 ± 1.5
	Tacrolimus levels (ng/mL)	28.4 ± 12.0	24.7 ± 14.7	22.9 ± 6.4	25.1 ± 7.6	20.8 ± 6.0

*$p < 0.002$.

molecular weight heparin because of a history of a left ventricular mural thrombus following previous coronary artery bypass grafting. This patient had been taking coumadin pretransplantation; low molecular weight heparin had been discontinued for 24 h prior to her biopsy, which was in fact performed uneventfully. She presented 6 h later with pain and swelling over the allograft, underwent surgical re-exploration with evacuation of a large hematoma, and required multiple blood transfusions. She ultimately recovered and has normal renal function. Her biopsy showed Banff 1B tubulitis, for which she received a bolus and a recycle of steroids, and additional tacrolimus.

One (4%) of the 28 patients in this group died 2.8 months after kidney transplantation, of a probable myocardial infarction. This patient had also developed post-transplant diabetes mellitus. The remaining patients are alive with functioning allografts.

The focus of this analysis was placed deliberately on those patients who would not ordinarily have undergone a biopsy, i.e. those who were doing well. Patients with DGF or those with a rising serum creatinine are patients who would ordinarily undergo a biopsy to establish the etiology of their renal dysfunction. In the 14 patients with DGF, five (36%) had borderline changes, three (21%) had Banff 1A-2A tubulitis, and six (43%) had no tubulitis on the one-week biopsies. In the 46 patients with a rising serum creatinine at the time of the one-week biopsy, 14 (30%) had borderline changes, 13 (28%) had Banff 1A-2A tubulitis, and 19 (41%) had no tubulitis on the one-week biopsy.

Discussion

This report represents an initial analysis of our early experience with protocol biopsies. We confined the analysis to an ideal subgroup of patients, i.e. those without DGF, who had stable or improving renal function. These patients had no clinical indication for renal allograft biopsy, yet over 40% of them had evidence of subclinical borderline or frank acute tubulitis, ranging from Banff 1A to Banff 2A. We have previously described the successful treatment of borderline histopathology with augmented immunosuppression, and have tended to think of it as a very mild form of rejection (17). The only apparent difference between the patients with subclinical borderline or acute tubulitis and those without rejection was a significantly lower (but still conventionally therapeutic) tacrolimus level on the day of the biopsy, in the patients with borderline or acute tubulitis.

This experience confirms the work of Rush et al. who have contended that there is a substantial incidence of subclinical acute tubulitis that ordinarily goes undetected (5–7). It suggests that we may have been underestimating the true incidence of acute rejection in our patients.

The significance of the observation of a high incidence of subclinical tubulitis is subject to varying interpretations. Certainly, one possible view is that these histopathologic changes, in the absence of any evidence of renal dysfunction, are of no consequence, and that treatment with augmented immunosuppression is unnecessary, gratuitous, and perhaps harmful. Another view, and one that is consistent with the late protocol biopsy studies that have shown a high incidence of chronic allograft nephropathy (18), is that immunosuppression has become so powerful that rejection may not even be manifested by a rising serum creatinine. Unfortunately, undiagnosed, and therefore untreated subclinical acute tubulitis may behave similarly to clinically apparent acute tubulitis and increase the risk for the development of chronic allograft nephropathy. Certainly, the randomized trial data from Winnipeg suggest that renal function and graft survival are better two years after transplantation in patients undergoing more frequent biopsies, and therefore undergoing more frequent treatment for subclinical acute tubulitis (5–7). Most of the patients described in our series were already in a randomized trial of different immunosuppressive protocols, and it was not feasible to randomize them further on the basis of the results of the protocol biopsies.

The potential benefits of discovering unsuspected acute tubulitis have to be balanced against the risks associated with renal allograft biopsies. Our incidence (0.4%) of major hemorrhagic complications was reasonably low, but emphasizes that there is some risk associated with renal allograft biopsies. We have not noted an increased incidence of opportunistic infections, although this is also a potential concern.

In conclusion, we observed, in an analysis of one-week biopsies in 28 patients who had ideal clinical courses and stable renal function after transplantation, a 21% incidence of subclinical borderline changes and a 25% incidence of subclinical acute tubulitis. This observation suggests that it is possible that the true incidence of acute rejection may be higher than we have clinically appreciated.

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