Beneficial Effects of Treatment of Early Subclinical Rejection: A Randomized Study

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Abstract. The prevalence of subclinical rejection, by the Banff criteria, is approximately 30% in the first 3 mo in renal transplant recipients. A randomized study was performed to determine whether the treatment of subclinical rejection with corticosteroids was associated with improved outcomes in these patients. Seventy-two patients, stratified by donor source, were randomized to biopsies at 1, 2, 3, 6, and 12 mo (Biopsy group), or to 6- and 12-mo biopsies only (Control group). Patients were analyzed by "intent to treat" and were followed for a minimum of 2 yr. Patients in the Biopsy arm of the study had a significant decrease in early (months 2 and 3) and late (months 7 to 12) acute rejection episodes, a reduced chronic tubulointerstitial score at 6 mo, and a lower serum creatinine at 24 mo than did patients in the Control arm. There was a trend toward an increase in infectious morbidity, but no increase in mortality, in the patients randomized to the Biopsy group. The results of this study suggest that early protocol biopsies and the treatment of subclinical rejection with corticosteroids may lead to better histologic and functional outcomes in renal transplant recipients.

A major cause of late renal transplant loss is chronic rejection (1). The development of chronic rejection is most consistently correlated with acute rejection episodes (2–5). However, the reduction in acute rejection episodes reported in recent years has not improved long-term graft outcome (6), suggesting that the incidence of chronic rejection has not been affected.

An explanation for this apparent paradox may lie in the existence of more subtle forms of pathogenic inflammation that are undetected. In support of this concept, Isoniemi et al. demonstrated that patients who had not had clinical rejection episodes developed chronic rejection in inverse proportion to the amount of immunosuppression that they had received (7). Moreover, Roberti et al. reported poorer long-term outcomes in patients with stable renal function and a urine flow cytometric pattern consistent with acute rejection, than in similar patients with normal flow cytometry (8). Finally, Tsamandas et al. showed that treatment of clinically silent tubulitis led to an improvement in renal function in a significant proportion of patients with chronic rejection (9). These observations suggest that silent inflammation to the graft may be associated with the development of chronic rejection. It is therefore important to determine both the prevalence and pathogenic significance of subclinical allograft inflammation.

Our group has reported that in the first 3 mo, renal transplant patients with stable graft function have a 30% prevalence of histologic findings that meet the Banff criteria for grade 1 acute rejection (10). Moreover, our subsequent study showed that the cumulative inflammation sustained by the graft over the first year, even if subclinical, correlated with graft dysfunction (11). Therefore, we conducted a randomized study, the aim of which was to determine whether corticosteroid treatment of subclinical rejection in the first 3 mo posttransplant would have a beneficial effect on renal allograft histology and/or function.

Materials and Methods

The study was approved by the University Medical Ethics Committee, and participants gave informed consent. Patients were randomized to protocol biopsies at 1, 2, 3, 6, and 12 mo (Biopsy group) or to biopsies at 6 and 12 mo only (Control group). Patients in the Biopsy group were treated with high-dose corticosteroids if subclinical rejection (defined below) was found in the protocol biopsies done at 1, 2, or 3 mo. We decided not to biopsy patients in the Control group at these time points, because it was felt that it was not ethical to subject patients to the risk of a biopsy and withhold treatment if histologic evidence of rejection was present.

The primary end point of the study was a 50% reduction in acute or chronic pathology in protocol biopsies at 6 mo in the Biopsy group. In a previous study, we reported on the prevalence of acute and chronic pathology in protocol biopsies done at 6 mo in a cohort of 60 patients (12). On the basis of those findings, it was estimated that 32 patients were required in each arm of the study, for an alpha of 0.05 with 80% power. Secondary end points for the study were renal function (i.e., serum creatinine) and the incidence of clinical rejection episodes.

From April 1992 to August 1995, 120 adult renal transplants were performed in our center. Patients were excluded from the study if they received a kidney from an HLA-identical living-related donor.
11), or because of graft failure or near failure before randomization (n = 13). Of the 96 eligible patients, 20 declined to participate. The remaining 76 patients were stratified by donor source upon entry into the study. Thirty-nine patients were randomized to the Biopsy group and 37 to the Control group, at which time the study was closed. Extra patients were recruited into the study to allow for dropouts. There were four patient exclusions after randomization, one from the Control group and three from the Biopsy group. The exclusion from the Control group was a patient transferred out of Province within 1 mo of initiation of the study. The three patients excluded from the Biopsy group were two who withdrew on days 1 and 30 after randomization, and one who was excluded because of early (i.e., before 3 mo) recurrence of thrombotic thrombocytopenic purpura refractory to treatment. This left 36 patients in both the Biopsy and Control groups. All patients have been followed for a minimum of 2 yr, and were analyzed by "intent to treat."

Immunosuppression was a standardized triple therapy protocol. This consisted of cyclosporin A (CsA; Sandimmune; Sandoz, Basel, Switzerland) 3 mg/kg per d by intravenous infusion for 3 to 5 d, followed by oral CsA sufficient to achieve target trough levels of 250 to 350 μg/L in the first 3 mo and 150 to 200 μg/L thereafter. CsA levels were measured in whole blood, 12 h after dosing, using the monoclonal TDx system (Abbott Laboratories, Abbott Park, IL). The CsA level reported at the time of a protocol biopsy was the average of the last three recorded levels. Azathioprine was given at 1 to 2 mg/kg per d. Prednisone was started at 1 mg/kg per d and was tapered to 15 mg/d over 2 wk, when possible. Diltiazem SR, 60 to 90 mg twice a day, was given to all patients from the time of transplant.

Core renal biopsies were done in the clinical investigation unit, using a spring-loaded 18-gauge gun after ultrasound localization. Acute inflammation and chronic changes were scored according to the Banff schema, 1993 to 1995 version (13). As reported previously, chronic glomerular and vascular changes (CG + CV) and chronic interstitial and tubular changes (CI + CT) were grouped for analysis (14). Two cores of tissue were obtained in most instances. Adequacy of tissue for examination was as reported previously (11). Two groups of renal pathologists reported independently on the biopsies. Both groups were blinded to the clinical status of the patient. The local pathologist (Dr. Gough) reported the biopsies on the day they were done, and communicated the findings to the local transplant physicians. All slides were then coded and sent out of Province to the second group of pathologists (Drs. Slezek and Trpkov). Differences in reporting of the biopsies were resolved by consensus between the pathologists, such that only one diagnosis (and biopsy score) was entered in the final database.

Of the 144 possible protocol biopsies during the first 6 mo in the Biopsy group, 136 (approximately 94%) were obtained. Thirty-four protocol biopsies were done in each group at 6 mo. Although the intent was to perform biopsies in all patients at 12 mo, these were obtained in only 32 of 35 patients in the Biopsy group and in 27 of 34 patients in the Control group. The reasons for not obtaining 12-mo biopsies in the Control group included patient refusal (two patients), severe chronic renal failure with biopsy-proven chronic rejection at 6 mo (two patients), and severe medical illness, anticoagulation, or anteposition of the bowel (one patient each). Because of these missing data, the 12-mo protocol biopsies were excluded from the analysis.

Acute rejection episodes were classified as "clinical" or "subclinical." A clinical rejection episode could be diagnosed at the time of, or between, protocol biopsies. At the time of protocol biopsy, a diagnosis of clinical rejection required an acute inflammatory score ≥4 (acute rejection ≥ grade 1) and an increase in the serum creatinine >10% from the baseline of the preceding 2 wk (months 1 to 3), or the preceding month (months 6 and 12). Between protocol biopsies, clinical rejection was defined as an increase in the serum creatinine >10% from the baseline for which an alternative diagnosis was excluded (e.g., obstruction). The diagnosis of clinical rejection was supported either by a biopsy or by a subsequent decrease in the serum creatinine in response to an increase in steroids, or OKT3 (Ortho Biotech, North York, Ontario, Canada) administration. A diagnosis of subclinical rejection required an acute inflammatory score ≥4 in a protocol biopsy and an increase in serum creatinine <10% from the defined baseline.

Rejection episodes, defined either on clinical grounds or by pathologic criteria, occurring at any time posttransplant, were treated with a 2-wk tapering course of high-dose steroids (starting either at 1000 mg of intravenous Solumedrol or 200 mg of oral prednisone, with taper). OKT3 was given to only 12 patients (5 mg intravenously for 7 d), five as induction therapy (two in the Biopsy group and three in the Control group) and seven for steroid-resistant rejection (four in the Biopsy group and three in the Control group).

Results

Pretransplant patient demographics are given in Table 1. In the Control group, there were trends toward poorer HLA-B locus matching (P = 0.10) and the use of older donors (P = 0.09). The mean CsA levels and the total prednisone doses were not significantly different between the groups in the first month posttransplant (Table 2). Before the initiation of protocol biopsies at month 1, there were 12 nonprotocol biopsies in each of the Biopsy and Control groups for episodes of graft dysfunction.

At 2 yr, graft survival was 97% (35 of 36) in the Biopsy group compared with 83% (30 of 36) in the Control group. Three patients died with a functioning graft, one in the Biopsy group and two in the Control group. The patient who died in the Biopsy group was presumed to have died of a cardiac arrhythmia, as no abnormalities were found at autopsy. Of the two patient deaths in the Control group, one patient died of septic shock from a urinary tract infection from a cecal "bladder" (an "Indiana pouch"); the other patient died of multiple complications after perforation of a gastric ulcer. Four other
Table 1. Pretransplant demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Biopsy Group (n = 36)</th>
<th>Control Group (n = 36)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA match&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-A</td>
<td>1 (0.2) 81%</td>
<td>1 (0.2) 60%</td>
<td>NS</td>
</tr>
<tr>
<td>HLA-B</td>
<td>1 (0.2) 64%</td>
<td>0 (0.2) 40%</td>
<td>0.10</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1 (0.2) 61%</td>
<td>1 (0.2) 72%</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female</td>
<td>15/21</td>
<td>11/25</td>
<td>NS</td>
</tr>
<tr>
<td>Living/cadaveric</td>
<td>5/31</td>
<td>6/30</td>
<td>NS</td>
</tr>
<tr>
<td>Recipient age (yr)</td>
<td>41 ± 2</td>
<td>41 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>30 ± 3</td>
<td>36 ± 3</td>
<td>0.09</td>
</tr>
<tr>
<td>PRA at transplant (%)</td>
<td>1.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Cold ischemic time (min)</td>
<td>926 ± 76</td>
<td>1014 ± 77</td>
<td>NS</td>
</tr>
<tr>
<td>Delayed graft function</td>
<td>7 of 36 (19%)</td>
<td>9 of 36 (25%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup> PRA, panel reactive antibody.
<sup>b</sup> Values represent median (minimum, maximum) and the percentage of patients matched for ≥1 antigen at that locus.

grafts were lost in the Control group, two to chronic rejection and one each to renal infarction and to acute rejection resulting from noncompliance.

The incidence of clinical rejection episodes was similar in both the Biopsy and Control groups in the first month posttransplant (Table 2). This was true for rejection episodes occurring in the first 2 wk, as well as for those occurring between 3 and 4 wk posttransplant. The incidence of clinical rejections was greater in the Control group during months 2 and 3 posttransplant (P = 0.02). Clinical rejection episodes were similar between the patient groups from 4 to 6 mo. However, from 7 to 12 mo there were more patients in the Control group that had at least one clinical rejection episode compared with the Biopsy group (33% versus 11%, P = 0.03).

The mean corticosteroid dose received during months 2 and 3 was significantly higher in the Biopsy compared with the Control group (P = 0.05), whereas from months 4 to 6 the dose of corticosteroids was equivalent between the groups (P = NS, Table 2). The mean corticosteroid dose received during months 7 to 12 was significantly higher in the Control compared with
Table 3. Clinicopathologic diagnosis at protocol biopsy

<table>
<thead>
<tr>
<th>Category</th>
<th>Biopsy Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Month 1</td>
<td>Month 2</td>
</tr>
<tr>
<td>Acute score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal (0 to 1)</td>
<td>7 of 35 (20%)</td>
<td>8 of 34 (24%)</td>
</tr>
<tr>
<td>borderline (2 to 3)</td>
<td>4 of 35 (11%)</td>
<td>7 of 34 (20%)</td>
</tr>
<tr>
<td>subclinical (≥4)</td>
<td>15 of 35 (43%)</td>
<td>11 of 34 (32%)</td>
</tr>
<tr>
<td>clinical (≥4)</td>
<td>9 of 35 (26%)</td>
<td>8 of 34 (24%)</td>
</tr>
<tr>
<td>Chronic score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total chronic (≥2)</td>
<td>1 of 35 (3%)</td>
<td>0 of 34 (0%)</td>
</tr>
<tr>
<td>CI + CT (≥2)</td>
<td>0 of 35 (0%)</td>
<td>0 of 34 (0%)</td>
</tr>
<tr>
<td>CG + CV (≥2)</td>
<td>1 of 35 (3%)</td>
<td>0 of 34 (0%)</td>
</tr>
</tbody>
</table>

* CI + CT, chronic interstitial and tubular changes; CG + CV, chronic glomerular and vascular changes.

Chronic pathology was defined as a Banff chronic score ≥2.

The results of the 1-, 2-, 3-, and 6-mo protocol biopsies are shown in Table 3. The prevalence of subclinical rejection in the Biopsy group at these time points was 43, 32, 27, and 15%, respectively. In contrast, at 6 mo the prevalence of subclinical rejection was 32% in the Control group (P = 0.09, versus the Biopsy group). The mean total acute inflammatory score in the 6-mo protocol biopsy was not different between the groups (Biopsy group: 1.91 ± 0.29 versus Control group: 2.44 ± 0.35, P = NS). There was, however, a trend toward less tubulitis in the Biopsy compared with the Control group (tubulitis score: 0.79 ± 0.14 versus 1.12 ± 0.18 respectively, P = 0.16). Mild endothelialitis was present in only one patient in the Control group, whereas there was no glomerulitis in either group.

At 6 mo, the mean total chronic score was 0.50 ± 0.13 in the Biopsy group and 1.02 ± 0.31 in the Control group (P = 0.12). The decrease in the chronic score observed in the Biopsy group was largely accounted for by a reduction in the scores for the chronic interstitial and tubular changes (CI + CT score), which was 0.21 ± 0.09 in the Biopsy group versus 0.62 ± 0.18 in the Control group (P = 0.05). Moreover, there were significantly more patients in the Control group with a CI + CT score ≥2 compared with the Biopsy group (24% versus 6%, P = 0.04).

At 24 mo, the serum creatinine was significantly lower in the Biopsy group compared with the Control group (133 ± 14 μmol/L versus 183 ± 22 μmol/L, P = 0.05) (Figure 1). Moreover, the Biopsy group had twice as many patients with excellent renal function at 24 mo (i.e., serum creatinine <130 μmol/L) than the Control group (66% versus 35%, P = 0.01). In addition, renal function was more stable from 6 to 24 mo in the Biopsy group compared with the Control group (ΔCr6–24 = −2 ± 5 μmol/L versus 46 ± 19 μmol/L, P = 0.02). Indeed, more patients in the Control group had a rise in serum creatinine (ΔCr6–24) of ≥20 μmol/L compared with the Biopsy group (38% versus 14%, P = 0.02).

By multiple regression analysis, the early variates (i.e., months 1 to 3) correlating independently (r² = 0.38) with an elevated serum creatinine at 24 mo were randomization to the Control group, HLA-B mismatching, clinical rejection episodes in weeks 3 and 4, and low CsA levels in months 1 and 3. Not unexpectedly, a greater proportion of the variance for the 24-mo serum creatinine was obtained by including later variates (i.e., months 4 to 12). In this latter analysis, independent predictors (r² = 0.62) of a high 24-mo serum creatinine were a low 1-mo CsA level, clinical or subclinical rejection episodes during months 4 to 6, the chronic biopsy score at 6 mo, and clinical rejection episodes during months 7 to 12. Early variates correlating independently (r² = 0.36) with clinical rejection episodes in months 7 to 12 were randomization to the Control group, HLA-B mismatching, delayed graft function, clinical rejection episodes in weeks 3 and 4, and low 1- to 3-mo CsA levels.

We have reported previously that three outcomes can be identified based on a ΔCr6–24 of ±20 μmol/L (14). In the

![Figure 1](image-url)
current study, variates correlating independently ($r^2 = 0.42$) with the $\Delta Cr_{6-24}$ were the 6-mo chronic biopsy score and clinical rejection episodes during months 7 to 12. The adjusted odds ratio (OR) and 95% confidence intervals (CI) for a $\Delta Cr_{6-24} \geq 20 \mu mol/L$ increased for each increase of 1 in the 6-mo CI + CT score (OR = 2.5; 95% CI, 1.01 to 6.15), and for every clinical rejection episode during months 7 to 12 (OR = 17.4; 95% CI, 3.6 to 85.4). The adjusted OR for a 6-mo CI + CT score $\geq 2$ increased (I) if delayed graft function had been present (OR = 5.5; 95% CI, 1.1 to 27.8); (2) for each rejection episode during weeks 3 and 4 posttransplant (OR = 2.5; 95% CI, 1.1 to 18.1); and (3) if randomization had been to the Control group (OR = 6.7; 95% CI, 1.1 to 41.3).

Complications of the biopsy were infrequent. However, one patient in the Biopsy group had bleeding after the 1- and 3-mo protocol biopsies, with the latter resulting in temporary urinary tract obstruction from clots. One patient in the Control group had two episodes of bleeding, one after a nonprotocol biopsy at 4 mo for graft dysfunction, which required embolization of the bleeding site, and one after the protocol biopsy at 6 mo that resolved spontaneously.

There was a trend toward more bacterial infections in the Biopsy group. In particular, pneumonia was seen in eight instances in the Biopsy group and in three instances in the Control group ($P = 0.11$). However, herpes zoster was seen in four patients in the Biopsy group and in two patients in the Control group, whereas invasive cytomegalovirus was seen in two patients in the Biopsy group and in four patients in the Control group ($P = NS$).

Discussion

This randomized study demonstrates that corticosteroid treatment of early (i.e., months 1 to 3) subclinical rejection is associated with better outcomes in renal transplant patients. Specifically, there was a decrease in early (months 2 and 3) as well as late (months 7 to 12) clinical rejection episodes, a decrease in the chronic tubulointerstitial score at 6 mo, and a lower serum creatinine at 24 mo in those patients randomized to treatment. In the treated patients, there was a trend toward more infectious morbidity from pneumonia, but no increased mortality, compared with those randomized to the control group.

Although not statistically significant, randomization resulted in the Control group of patients being slightly less matched at the HLA-B locus, and receiving kidneys from slightly older donors. Although these imbalances may have contributed to the adverse outcomes observed in the Control group, multivariate analysis demonstrated that randomization to the Control group was an independent predictor of these outcomes.

Analysis of the U.S. Renal Data System database showed that both short- and long-term renal allograft outcomes can be predicted by two early events: delayed graft function and acute rejection episodes (15). In the present study, multivariate analysis demonstrated the convergence of these early nonimmune and alloimmune inflammatory events as risk factors for the 6-mo chronic allograft histology and the incidence of late acute rejection episodes (months 7 to 12). Moreover, the treatment of early subclinical rejection with corticosteroids appears to have decreased the late impact of these two early risk factors.

The chronic tubulointerstitial score (CI + CT) at 6 mo was predicted independently by delayed graft function and by acute clinical rejection episodes that occurred between 3 and 4 wk posttransplant, both of which were distributed equally between the two groups. The third independent predictor of the 6-mo CI + CT score was group randomization, which occurred at 1 mo posttransplant. Because subclinical rejection was diagnosed and treated from months 1 to 3 in the Biopsy group, the decreased 6-mo CI + CT score in these patients could have resulted from the interruption by corticosteroids of a nonimmune and/or alloimmune inflammatory program set in motion during the first month posttransplant (16). Indeed, the increased incidence of clinical rejections in the Control group during months 2 and 3 may represent the earliest evidence of failure to control such a program.

Clinical rejection episodes diagnosed late posttransplant are widely accepted as being predictive of poor graft outcome (2,5,14,17,18). In this study, clinical rejection episodes that occurred between 7 and 12 mo were strong independent predictors of both the 24-mo serum creatinine and the stability of renal function from 6 to 24 mo ($\Delta Cr_{6-24}$). Independent risk factors for 7- to 12-mo clinical rejection episodes were randomization to the Control group, HLA-B mismatching, delayed graft function, clinical rejection episodes in weeks 3 and 4, and low 1- to 3-mo CsA levels. To our knowledge, this is the first report that has identified early predictors for clinical rejection episodes occurring after 6 mo. Moreover, the critical importance of early interventions (i.e., the attainment of adequate CsA levels and the detection and treatment of subclinical rejection in months 1 to 3) in the prevention of late rejections and long-term graft dysfunction is emphasized.

We have reported that subclinical rejection at 6 mo is an independent predictor of an elevated serum creatinine at 24 mo (14). Although not reaching statistical significance, the prevalence of subclinical rejection in the Control group was double that of the Biopsy group. However, treatment of subclinical rejection at 6 mo with corticosteroids was not successful in preventing deterioration of renal function between 6 and 24 mo. We speculate that inflammatory programs may be present as early as 6 mo posttransplant, which are no longer sensitive to standard immunosuppressive therapy. Therefore, patients with subclinical rejection in the 6-mo protocol biopsy may be considered for secondary prevention studies that evaluate novel treatment strategies.

The precise mechanism(s) by which corticosteroids were able to exert their beneficial effect in the Biopsy group is not entirely clear. However, inflammatory cytokines and reactive oxygen intermediates, which are generated during alloimmune and/or ischemia-reperfusion injury, can induce activation of the transcription factor nuclear factor-$\kappa B$ (NF-$\kappa B$) (19,20). Corticosteroids are known to inhibit the activation of NF-$\kappa B$ (21), which is essential for the transcription of a number of genes associated with inflammation (e.g., tumor necrosis factor-$\alpha$, interleukin-1 [IL-1], IL-12, RANTES, monocyte chemotactic protein-1, vascular cell adhesion molecule-1, and...
intercellular adhesion molecule-1) (22). Therefore, corticosteroid treatment of subclinical rejection may have inhibited NF-kB-dependent inflammatory programs initiated by early non-immune (i.e., ischemia-reperfusion) and/or alloimmune injury to the graft.

In summary, this study suggests that treatment of early subclinical rejection with corticosteroids decreases early chronic pathology, late clinical rejection episodes and improves long-term graft function in renal transplant recipients. We hypothesize that the beneficial effect of corticosteroids is due to the interruption of early immune and nonimmune programs of tissue injury, which may otherwise become self-sustaining and refractory to standard immunosuppression. Frequent surveillance of the allograft with protocol biopsies may be required in the early posttransplant period until noninvasive tests to detect subclinical inflammation are developed. The conclusions of this study, however, must be viewed as preliminary, and will require confirmation by studies that involve a larger sample of patients.

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References