Transpl Int (2004) 17: 195–201
DOI 10.1007/s00147-004-0693-8

ORIGINAL ARTICLE

Titte R. Srinivas
Paul S. Kubilis
Byron P. Croker

# Macrophage index predicts short-term renal allograft function and graft survival

Received: 15 April 2003
Accepted: 15 July 2003
Published online: 3 April 2004


T. R. Srinivas
Division of Nephrology,
University of Florida,
Gainesville, Florida, USA

P. S. Kubilis
Florida Fish and Wildlife Conservation Commission, Gainesville,
Florida, USA

B. P. Croker (✉)
Department of Pathology,
Immunology and Laboratory Medicine,
Malcolm Randall VA Medical Center
and University of Florida,
1601 SW Archer Road,
Gainesville, Florida 32608, USA
E-mail: croker@pathology.ufl.edu
Tel.: +1-352-3746057
Fax: +1-352-3794023

**Abstract** The ability to predict renal allograft dysfunction in the short term and predict graft survival by quantifying the macrophage infiltrate in allograft renal biopsies is described. Renal allograft biopsies performed for cause in 41 consecutive patients were scored for macrophages (macrophage index, MI) by use of a modified Banff score of inflammation (BSI), and the impact of the MI on serum creatinine (SCr) levels 3 months post-biopsy (post-Bx) and on graft survival was quantified. Biopsies were stained for macrophages, individual lesions semiquantified and MI, BSI and chronic allograft damage index (CADI) obtained. The effects of pathologic indices on 3 month post-Bx SCr and graft survival were quantified by multivariate analysis and Cox regression. Glomerular and interstitial macrophage scores correlated inversely with graft survival. MI predicted an increase in SCr 3 months post-Bx ($P=0.02$). MI $>3$ (hazard ratio 23.13, $P=0.003$) also had a powerful negative predictive value on graft survival.

**Keywords** Kidney · Transplantation · Biopsy · Graft survival · Macrophages

## Introduction

Macrophages are mononuclear phagocytic cells that constitute an important component of the afferent and the efferent arms of both the innate immune response and the adaptive alloimmune response [1]. Their presence in renal allograft biopsies connotes a poor prognosis and progression to chronic allograft nephropathy (CAN) [2, 3]. The presence of activated macrophages in protocol biopsies is associated with clinical rejection [4]. Modification of the macrophage infiltrate by chemical inhibitors of macrophage function [5] or by prevention of their influx during reperfusion [6, 7] has been shown to prevent CAN. Previous, shorter-term studies of renal allograft biopsies obtained for cause from our cohort of patients have demonstrated that the degree of macrophage infiltration in renal allograft biopsies correlated with worsened renal function 6 months post-biopsy [8] and also with decreased allograft survival [1]. Our cohort of patients has been followed for up to 72 months. We also report on the long-term follow up of this cohort of patients. Since acute rejection is the single most impor-

tant event that leads to chronic rejection [9], we hypothesized that if the degree of macrophage infiltration correlated with long-term graft survival, effects on short-term renal function are a meaningful outcome to be studied, as treatment could potentially modify this parameter. In this study, we compared the macrophage index with other biopsy-derived indices such as the chronic allograft damage index (CADI) [10, 11] and the Banff score of inflammation (BSI) [12, 13, 14] with regard to their association with changes in serum creatinine levels 3 months post-biopsy and long-term graft survival. The results of our study are reported hereunder.

## Material and methods

### Patients

The study population ($n=41$) was drawn from patients who had received transplants at the University of Florida and had undergone a renal allograft biopsy between February 1993 and June 1994. Twenty-four patients underwent biopsy later than 3 months after transplantation. No protocol biopsies of patients with stable graft function were included. The underlying cause of renal failure leading to renal transplantation is summarized in Table 1. Further details can be obtained from references [1, 8, 15].

Approval for this study was obtained from the Institutional Review Board at the University of Florida. Patients were recruited for enrolment in the study according to prevailing guidelines put forth by the Institutional Review Board (IRB) at the University of Florida. Procedures for the obtaining of follow-up data on enrolled patients were also approved by the IRB at the University of Florida.

### Biopsies

Biopsy specimens were processed in paraffin and frozen, and electron microscopic sections were stained with KP-1 (monoclonal antibody directed against CD68 marker on monocytes) [1] and/or Kon-7 (monoclonal antibody directed against thromboxane synthetase) [8, 15, 16]. Biopsies were performed for the clinical indications of delayed graft function (DGF; defined as need for dialysis in the first week post-transplantation) ($n=7$) or for a rise in the level of serum creatinine after initial stability ($n=34$; mean rise in plasma creatinine above baseline, 1.2 mg/dl). Negative controls included native nephrectomies performed for renal cell carcinoma ($n=6$), and positive controls comprised transplant nephrectomies performed for severe rejection ($n=4$). Maintenance immunosuppression was cyclosporine, azathioprine and prednisone. Primary acute rejection therapy was intravenous methylprednisone pulse. OKT3 was employed for steroid-resistant acute rejection. Serum creatinine level was measured at baseline, time of biopsy ($Cr_0$), 3 months post-biopsy ($Cr_{3mo}$) and at 6 months post-biopsy ($Cr_{6mo}$), and yearly thereafter. Renal allograft survival was calculated as the interval from transplantation to resumption of maintenance dialysis or re-transplantation. The study population for development of chronic renal dysfunction was adjusted for death due to non-renal causes and for patients lost to follow-up. Other details of the study population and follow-up to 36 months post-biopsy are available in references [1] and [8]. For the Cox regression analysis of graft loss risk, 30 patients had complete non-missing data for the relevant outcome and predictor variables. Ten of these 30 patients lost their allografts prior to 60 months post-biopsy. For the linear regression analysis of change in serum creatinine levels at 3 months post-biopsy, 24 patients had complete non-missing data for the relevant outcome and predictor variable. Biopsies were re- graded for this study by use of Banff '97 criteria [17]. Histologic analysis was as previously described [1, 8, 15]. Briefly, histologic scores were determined from semiquantitative cut-off points based on Banff intervals. The scores were added to enable indices to be derived. The indices were then evaluated for their impact on renal allograft function and survival. Cortical structures were scored for acute inflammatory changes and chronic lesions according to Banff criteria, with appropriate modifications for derivation of the CADI. Mononuclear inflammation-producing glomerulitis (g), tubulitis (t), interstitial inflammation (i) and endovasculitis (v) were scored semiquantitatively from 0 to 3 by use of Banff intervals. Chronic changes involving the glomeruli (cg), tubules (ct), interstitium (ci) and blood vessels (cv) were scored from 0 to 3, based on Banff intervals. We scored global glomerulosclerosis (gs) and mesangial matrix increase (mm) to derive a CADI, using Banff intervals (intervals for gs and mm: 0 = 0%, 1 = < 25%, 2 = 25%–50% and 3 = > 50%). Mononuclear inflammation involving atrophic tubules was classified separately from tubulitis in intact tubules. Macrophage infiltration was

**Table 1** Underlying cause of renal failure leading to renal transplantation

| Cause | Number | Percentage |
|---|---|---|
| IgA nephropathy | 4 | 9.8 |
| Glomerulonephritis | 9 | 22 |
| Polycystic kidney disease | 6 | 14.6 |
| Diabetic nephropathy | 3 | 7.3 |
| HTN | 12 | 29.3 |
| Hereditary nephritis | 1 | 2.4 |
| Chronic pyelonephritis | 1 | 2.4 |
| Unknown | 5 | 12.2 |
| Total | 41 | 100 |

scored on KP 1-stained or Kon 7-stained sections exactly as unspecified mononuclear infiltration was scored for g, t, i and v (Mac G, Mac I, Mac T and Mac V). We used these defined scores to obtain the following indices: BSI, defined as the sum of g, t, i and v (range 0–12); macrophage index (MI), defined as the sum of Mac G, Mac I, Mac T and Mac V (range of values 0–12); CADI, as defined above (range of values 0–18). It should be noted that the CADI in this study differs from that used by Isoniemi and colleagues [10, 18] in that the range of grading for each parameter was based on Banff '97 criteria [17] and is designated as CADI*.

### Statistical analysis

The purpose of the statistical analysis was to determine the significance and strength of association between the various pathologic indices determined at biopsy and the risk of graft loss post-biopsy or the change in Cr values measured at 3 months post-biopsy. The indices evaluated in this fashion were the MI, CADI, BSI, Banff category (normal vs suspicious or higher categories) and the glomerular and interstitial components of the macrophage index (Mac G and Mac I, respectively). Each index was analyzed both as a univariate predictor and after control for transplantation–biopsy interval (TBI) and serum creatinine level at the time of biopsy ($Cr_0$). Overall graft survival was estimated by the Kaplan–Meier method.

We used Cox proportional hazards regression analysis [19, 20, 21] to assess the impact of scores and indices on the risk of graft loss post-biopsy. For the purposes of analysis, patient follow-up was administratively censored at 60 months. Hazard ratios (HRs) were estimated before and after correction for TBI and $Cr_0$, together with 95% confidence intervals (95% CI). Since values of TBI and $Cr_0$ were significantly skewed to the right, we log-transformed these covariates prior to analysis to improve model fit. Each index was modeled as a risk factor that defined two patient groups whose index scores were above or below a cut-off point based on our previous study. In order to determine if graft loss risk increased as index scores increased, we also modeled each of the indices as a continuous predictor. In the case of the Banff category, categories were ordered by increasing level of progression, and the ranks of this ordering were modeled as a continuous predictor.

We used least-squares regression analysis [22] to assess the significance and strength of association between indices and scores and serum creatinine level at $Cr_{3mo}$. We computed regression coefficients both by univariate analysis and by using a multivariate approach that controlled for TBI and $Cr_0$. We log-transformed $Cr_{3mo}$, TBI, and $Cr_0$, prior to analysis, to improve model fit. High-score and low-score patient groups were defined by use of the cut-off points developed in the modeling of graft loss risk. Since $Cr_{3mo}$ values were log-transformed prior to analysis so that we could improve model fit, we coded index score binary predictors so that the exponentiated linear regression coefficient could be interpreted as a creatinine multiplying factor (CrMF), i.e., the multiplicative factor by which the predicted post-biopsy creatinine would be expected to change in the high-score patient group relative to the low-score patient group. In order to determine if Cr at 3 months post-biopsy tended to increase as index scores increased, we also modeled each of the indices as a continuous predictor of post-biopsy creatinine.

Model fit for both Cox and linear regression models was evaluated by standard techniques and graphic display of residuals. Although statistical power was likely to be low for detecting interactions, due to small sample size, all possible two-way interactions between predictors were evaluated for statistical significance. Only one interaction term was added at a time to a multivariate model, which was then compared with the strictly additive model. No statistically significant interactions were detected. All model fitting was performed with SAS version 8.2 (SAS Institute, Cary, N.C., USA). We used S-Plus 2000 (Insightful, Seattle, Wash., USA) to calculate Cox model $GR^2$s. All reported $P$ values are for two-tailed tests. $P$ values less than 0.05 were considered to be statistically significant. Those between 0.05 and 0.10 were considered marginally significant.

## Results

### Biopsy-derived indices as predictors of graft loss

Univariate analysis showed that CADI and BSI and log-(TBI) were significantly associated with an increase in graft loss in the post-biopsy period (up to 60 months). $Cr_0$ was not a significant predictor of graft loss risk. After correction for log-(TBI) and $Cr_0$ in the Cox model, the CADI, MI, Mac G score and Mac I score were significant predictors of graft loss. When the indices were modeled as two patient groups above and below a cut-off point after correction for log-(TBI) and log-$Cr_0$, Mac G $>1$ (HR = 4.8, $P = 0.0303$), Mac I $>1$ (HR = 5.2, $P = 0.0674$), MI $>3$ (HR = 22.62, $P = 0.0037$) and CADI $>9$ (HR = 6.63, $P = 0.0232$) were significant predictors of graft loss. The impact of an increasing TBI in association with an increase in the index values on graft survival is illustrated graphically in the context of the MI in Fig. 1. The Banff category (other than normal) attained marginal significance (HR = 3.96, $P = 0.0959$) as a predictor of graft loss in this model. Increase in the overall model $R^2$, after a significant index score had been sequentially included, was highest for MI and CADI and lowest for Mac G and Mac I scores (data not shown).

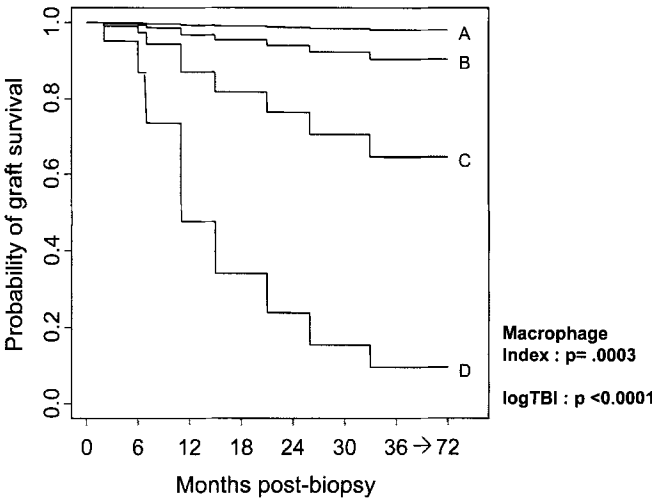


**Fig. 1** Predicted probability of graft survival for selected patient strata defined by MI and transplantation–biopsy interval. Stratum A: Macrophage Index (MI) < 3 and Transplant-Biopsy Interval (TBI) < 3 months (3 mo); Stratum B: MI < 3 and TBI > 3 mo; Stratum C: MI > 3 and TBI < 3 mo; Stratum D: MI > 3 and TBI > 3 mo

These results are displayed in Table 2. For the most part, when indices were modeled as continuous predictors of graft loss risk, there was an incremental increase in risk of graft loss for every unit increase in the value of modeled indices (data not shown).

Biopsy-derived indices as predictors of serum creatinine levels 3 months post-biopsy

Univariate regression analysis indicated that CADI, BSI, Mac I and MI were significantly associated with an increased $Cr_{3mo}$. Banff category, Mac G, and $Cr_0$ were not significant predictors of $Cr_{3mo}$. The transplantation-to-biopsy interval was marginally significant as a predictor of $Cr_{3mo}$ ($P = 0.0746$). The strength of association between predictor variables and $Cr_{3mo}$ was highest for CADI ($R^2 = 0.50$) followed by Mac I, BSI and MI ($R^2 = 0.43$, 0.40 and 0.30, respectively).

**Table 2** Biopsy-derived indices as predictors of graft loss. Indices were modeled by use of cut-off points

| Variable | Hazard ratio | Hazard ratio 95% CIs | *P* |
|---|---|---|---|
| Log-(TBI) | 2.20 | 1.18–3.19 | 0.008 |
| Mac G > 1 | 5.03 | 1.23–20.59 | 0.025 |
| Mac I > 1 | 3.94 | 1.05–14.8 | 0.042 |
| MI > 3 | 23.13 | 2.9–184.25 | 0.003 |
| CADI > 9 | 5.38 | 1.29–22.37 | 0.021 |
| BSI > 5 | 2.6 | 0.61–11.08 | 0.196 |
| Banff category other than normal | 4.16 | 0.85–20.38 | 0.078 |

**Table 3** Biopsy-derived indices as predictors of Cr at 3 months. Indices were modeled as continuous predictors. *MF* multiplying factor

| Variable | Creatinine MF | MF 95% CIs | MF = 1 *P* |
|---|---|---|---|
| Log-(TBI) | 1.10 | 0.99–1.2 | 0.0746 |
| Log-($Cr_0$) | 1.20 | 0.99–1.45 | 0.4410 |
| Mac G score | 1.15 | 1.0–1.32 | 0.0758 |
| Mac I score | 1.45 | 1.00–2.11 | 0.0004 |
| MI | 1.16 | 1.00–1.35 | 0.0018 |
| CADI | 1.13 | 0.99–1.28 | 0.0007 |
| BSI | 1.13 | 0.99–1.28 | 0.0085 |
| Banff category (normal vs other than normal) | 0.917 | 0.84–1.00 | 0.1774 |

In the multivariate analysis, after correction for TBI and $Cr_0$, CADI, Mac I score, MI and BSI were significant predictors of $Cr_{3mo}$. In addition, the Mac G score attained marginal significance as a predictor of $Cr_{3mo}$. $Cr_{3mo}$ was predicted to increase by a factor of 1.13 per unit increase in the CADI ($P = 0.0007$), 1.45 per unit increase in the Mac I score ($P = 0.0004$), 1.13 per unit increase in the BSI ($P = 0.009$), 1.16 per unit increase in the MI ($P = 0.002$) and by a factor of 1.15 per unit increase in the Mac G score ($P = 0.076$). These results are tabulated in Table 3.

When indices were modeled as two patient groups above and below a cut-off point Mac G > 1 (P = 0.0555), Mac I > 1 ($P = 0.0174$), MI > 3 ($P = 0.0197$), CADI > 9 ($P = 0.0412$) and BSI > 5 ($P = 0.0394$) emerged as significant predictors of graft dysfunction 3 months after the biopsy (Table 4).

## Discussion

The presence of macrophages in renal allografts has been shown to confer a poor prognosis and correlates with markers of fibrosis and vascular sclerosis [2, 3].

**Table 4** Biopsy-derived indices as predictors of renal function at 3 months. Indices and scores were modeled by use of cut-off points. *MF* multiplying factor

| Variable | Creatinine MF | MF 95% CIs | *P* |
|---|---|---|---|
| Log-TBI | 1.10 | 0.99–1.20 | 0.0746 |
| Log-$Cr_0$ | 1.20 | 0.99–1.45 | 0.4410 |
| Mac G > 1 | 1.38 | 1.00–1.91 | 0.0555 |
| Mac I > 1 | 1.48 | 1.00–2.20 | 0.0174 |
| MI > 3 | 1.47 | 1.00–2.17 | 0.0197 |
| CADI > 9 | 1.56 | 1.00–2.44 | 0.0412 |
| BSI > 5 | 1.60 | 1.00–2.56 | 0.0394 |
| Banff category other than normal | 1.17 | 1.00–1.36 | 0.3986 |

Macrophages function as antigen-presenting cells, elaborate and respond to cytokines and mediate tissue injury. Vaso-active products secreted by activated macrophages may contribute to the renal dysfunction observed in acute rejection [2]. The degree of macrophage infiltration correlates with worsened renal function 6 months post-biopsy [8].

The incidence of acute rejection has declined in recent years, whereas the negative prognostic impact of an episode of acute rejection has increased [23]. The long-term prognosis for graft survival after an episode of acute rejection depends, to a large extent, on the histologic severity of the episode [19, 24, 25, 26], the time from transplantation to biopsy [26] and whether or not renal function returns to baseline after treatment of the rejection [27]. Given the fact that early inflammatory changes in protocol biopsies translate into chronic lesions at later sampling points [28], we hypothesized that a risk factor that impacts graft function at 6 months post-biopsy and long-term graft survival might affect renal function at an earlier time point. We chose the 3 month post-biopsy time point as it would be a clinically meaningful time point where renal function would be expected to return to baseline after successful treatment of an episode of acute rejection.

Univariate analysis of our data pointed to a significant negative effect of a longer transplantation-to-biopsy interval on long-term allograft survival and, to some extent, on renal function at 3 months post-biopsy. This is in concordance with the fact that late episodes of acute rejection have a worse prognosis [26]. Taking into account that the CADI was developed as an index that looked at a defined time point (2 years post-transplantation) [10] and the varying times from transplant to biopsy in this study, we corrected the multivariate model for the transplantation-to-biopsy interval. The MI, CADI, Mac I score, Mac G score and the BSI were significant predictors of graft function 3 months post-biopsy and had a negative impact on long-term allograft survival. These findings complement our previous observations [1, 8].

An increasing value of the scores and indices above added to our ability to predict graft function and survival, over and above that which we would expect on the basis of knowledge of the time from transplantation to biopsy and the serum creatinine levels at the time of biopsy. We cannot make a meaningful statement about the lack of effect of the Banff category in our model, given the fact that the sample was skewed towards lower grades of rejection. It should also be noted that the BSI in the context of this study is derived from a single biopsy sample and does not represent the summed scores from multiple protocol biopsies. However, the results of our study support the overall concept of lesion scoring and the "sum score" approach [29, 30] in addition to routine histopathologic grading of biopsy specimens.

That the BSI, CADI and MI take into account the presence of glomerular inflammation or scarring could account for their additional prognostic value, in view of the negative prognostic impact of glomerulitis in the transplant biopsy [31].

In the context of this study, the MI provides an index of ongoing inflammatory activity at a single sampling time point in the post-transplantation period that probably confers an increased risk for long-term allograft loss. Whereas the CADI mainly quantifies chronic lesions at a defined time point, it does take into account a component of acute interstitial inflammation in the i score, which was a significant predictor of both graft loss and dysfunction in our study. The MI could be affording greater precision in our ability to quantify interstitial inflammation. That the presence of inflammatory infiltrates in allografts exhibiting late decline in function, prompting performance of a biopsy, was associated with the worst outcome, is not inconsistent with the results of previous studies. This could represent the cumulative effects of acute inflammation and tissue damage in the context of a chronically damaged graft. In fact, when we added the CADI and the MI, the term thus derived emerged as a highly significant predictor of short-term graft dysfunction and long-term graft loss (unpublished observations). The findings of Nankivell et al. [28], indicating that inflammatory changes observed in early protocol biopsies translate into fibrosis in later biopsies, raise the possibility that treatment of acute inflammation could modulate progression to fibrosis. Thus, one could use biopsy-derived indices to classify patients at high risk for graft loss, in an effort to recruit subjects for secondary prevention trials or as an intermediate efficacy endpoint in clinical trials [24, 32, 33].

A potential limitation of our study is the fact that biopsies were all performed for cause and were, therefore, not performed at a single time point. The effects of donor age were not studied, except for the effect that parameter has on the CADI [34]. We also do not know if the MI changes with time or treatment. Regardless, the outcome of these variables would be expected to reduce or blur potentially significant features, not enhance the significance of the positive findings. Therefore, the robust nature of the positive findings are expected to persist.

The implications of the MI in the context of other immunosuppression protocols remain to be tested. This is, of course, the difficulty when a disease with a long natural history is studied, as indicated by the longer half-lives that are expected in renal transplantation under modern immunosuppression regimes. In addition, therapies with shorter-term goals, such as the prevention or treatment of acute rejection, will become the standard of care without the testing of the ultimate endpoint of failure [35, 36]. If we wait for the endpoint of graft failure, the studies become increasingly difficult and

expensive. For these several reasons it will be valuable for one to have surrogate endpoints for graft failure [25, 32]. This study has shown that the MI is a robust predictor of short-term renal allograft function and long-term graft loss and is, therefore, an important surrogate endpoint. There are a number of theoretical reasons why this will continue to be valid.

Macrophage activation is involved in the afferent and efferent limbs of adaptive immunity, innate immunity, inflammation and scarring [1, 37]. These pathways could account for the variety of risk factors for chronic allograft failure, including prior acute rejection (adaptive alloimmune response) and risk factors that are not traditionally considered adaptive immune responses but may represent innate immune responses such as donor age and gender, and ischemia time [38]. Therefore, pathways of macrophage infiltration and activation potentially hold pivotal positions in responding to allograft injury and producing fibrogenic responses seen in chronic allograft nephropathy [39, 40]. While there is considerable experimental and circumstantial evidence for this hypothesis, the specific mechanisms and pathways need [2, 3, 39, 40] to be further substantiated in the context of clinical transplantation. Potential targets for intervention derived from these pathways are expected to have important basic, practical and clinical value. This report supports the effort required for additional larger and more recent studies.

## References


1. Croker BP, Clapp WL, Abu Shamat AR, Kone BC, Peterson JC. Macrophages and chronic renal allograft nephropathy. Kidney Int Suppl 1996; 57:S42.
2. Pilmore HL, Painter DM, Bishop GA, McCaughan GW, Eris JM. Early upregulation of macrophages and myofibroblasts: a new marker for development of chronic renal allograft rejection. Transplantation 2000; 69:2658.
3. Pilmore HL, Eris JM, Painter DM, Bishop GA, McCaughan GW. Vascular endothelial growth factor expression in human chronic renal allograft rejection. Transplantation 1999; 67:929.
4. Grimm PC, McKenna R, Nickerson P, Russell ME, Gough J, Gospodarek E, et al. Clinical rejection is distinguished from subclinical rejection by increased infiltration by a population of activated macrophages. J Am Soc Nephrol 1999; 10:1582.
5. Azuma H, Nadeau KC, Ishibashi M, Tilney NL. Prevention of functional, structural, and molecular changes of chronic rejection of rat renal allografts by a specific macrophage inhibitor. Transplantation 1995; 60:1577.
6. Vos IH, Rabelink TJ, Dorland B, Loos R, Van Middelaar B, Grone HJ, et al. L-arginine supplementation improves function and reduces inflammation in renal allografts. J Am Soc Nephrol 2001; 12:361.
7. Vos IH, Govers R, Grone HJ, Kleij L, Schurink M, De Weger RA, et al. NFkappaB decoy oligodeoxynucleotides reduce monocyte infiltration in renal allografts. FASEB J 2001; 14:815.
8. Ramos EL, Barri YM, Croker BP, Clapp WL, Peterson JC, Wilcox CS. Thromboxane synthase expression in renal transplant patients with rejection. Transplantation 1995; 59:490.
9. Humar A, Hassoun A, Kandaswamy R, Payne WD, Sutherland DE, Matas AJ. Immunologic factors: the major risk for decreased long-term renal allograft survival. Transplantation 1999; 68:1842.
10. Isoniemi H, Taskinen E, Hayry P. Histological chronic allograft damage index accurately predicts chronic renal allograft rejection. Transplantation 1994; 58:1195.
11. Isoniemi H, Nurminen M, Tikkanen MJ, von Willebrand E, Krogerus L, Ahonen J, et al. Risk factors predicting chronic rejection of renal allografts. Transplantation 1994; 57:68.
12. Rush DN, Nickerson P, Jeffery JR, McKenna RM, Grimm PC, Gough J. Protocol biopsies in renal transplantation: research tool or clinically useful? Curr Opin Nephrol Hypertens 1998; 7:691.
13. Rush DN, Jeffery JR, Gough J. Sequential protocol biopsies in renal transplant patients. Clinico-pathological correlations using the Banff schema. Transplantation 1995; 59:511.
14. Rush DN, Jeffery JR, Gough J. Sequential protocol biopsies in renal transplant patients: repeated inflammation is associated with impaired graft function at 1 year. Transplant Proc 1995; 27:1017.
15. Berkes EA, Croker BP, Barri YM, Peterson JC, Wilcox CS, Ramos EL. Thromboxane synthase expression colocalizes with infiltrating macrophages in renal allograft biopsies. Kidney Int 1995; 48:1344.
16. Nusing R, Wernet MP, Ullrich V. Production and characterization of polyclonal and monoclonal antibodies against human thromboxane synthase. Blood 1990; 76:80.
17. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55:713.
18. Isoniemi H, Ahonen J, Eklund B, Hayry P, Hockerstedt K, Krogerus L, et al. Relationship between renal histology and later graft outcome. Transpl Int 1994; 7 (Suppl 1): S318.
19. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. John Wiley and Sons, New York, 1980.
20. Cox DR, Oakes D. Analysis of survival data. Chapman and Hall, London, 2002.
21. Nagelkerke NJ. A note on a general definition of the coefficient of determination. Biometrika 1991: 78:691.
22. Draper N, Smith H. Applied regression analysis, 2nd edn. John Wiley and Sons, New York, 1981.
23. Meier-Kriesche HU, Ojo AO, Hanson JA, Cibrik DM, Punch JD, Leichtman AB, et al. Increased impact of acute rejection on chronic allograft failure in recent era. Transplantation 2000; 70:1098.

24. Gaber LW. Role of renal allograft biopsy in multicenter clinical trials in transplantation. Am J Kidney Dis 1998; 31 (6 Suppl 1): S19.
25. Gaber LW, Moore LW, Gaber AO, First MR, Guttmann RD, Pouletty P, et al. Utility of standardized histological classification in the management of acute rejection. 1995 Efficacy Endpoints Conference. Transplantation 1998; 65:376.
26. Basadonna GP, Matas AJ, Gillingham KJ, Payne WD, Dunn DL, Sutherland DE, et al. Early versus late acute renal allograft rejection: impact on chronic rejection. Transplantation 1993; 55:993.
27. Opelz G. Critical evaluation of the association of acute with chronic graft rejection in kidney and heart transplant recipients. The Collaborative Transplant Study. Transplant Proc 1997; 29:73.
28. Nankivell BJ, Fenton-Lee CA, Kuypers DR, Cheung E, Allen RD, O'Connell PJ, et al. Effect of histological damage on long-term kidney transplant outcome. Transplantation 2001; 71:515.
29. Gaber LW, Schroeder TJ, Moore LW, Shakouh-Amiri MH, Gaber AO. The correlation of Banff scoring with reversibility of first and recurrent rejection episodes. Transplantation 1996; 61:1711.
30. Gaber LW, Moore LW, Alloway RR, Flax SD, Shakouh-Amiri MH, Schroder T, et al. Correlation between Banff classification, acute renal rejection scores and reversal of rejection. Kidney Int 1996; 49:481.
31. Messias NC, Eustace JA, Zachary AA, Tucker PC, Charney D, Racusen LC. Cohort study of the prognostic significance of acute transplant glomerulitis in acutely rejecting renal allografts. Transplantation 2001; 72:655.
32. Yilmaz S, Taskinen E, Hayry P, Isoniemi H. Protocol core biopsy as intermediate efficacy end-point in chronic kidney allograft rejection. Transplant Proc 1996; 28:491.
33. Gaber LW, Moore LW, Gaber AO, First MR, Guttmann RD, Pouletty P, et al. A multicenter report on the utilization of Banff grading in acute renal rejection. The 1995 Efficacy Endpoints Conference. Transplant Proc 1997; 29:184.
34. Lehtonen SR, Taskinen EI, Isoniemi HM. Histological alterations in implant and one-year protocol biopsy specimens of renal allografts. Transplantation 2001; 72:1138.
35. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 1998; 342:605.
36. Hunsicker LG, Bennett LE. Design of trials of methods to reduce late renal allograft loss: the price of success. Kidney Int Suppl 1995; 52:S120.
37. Luster AD. The role of chemokines in linking innate and adaptive immunity. Curr Opin Immunol 2002; 14:129.
38. Croker BP, Tisher CC. Indications for and interpretation of the renal biopsy: evaluation by light, electron and immunofluorescence microscopy. In: Schrier RW, ed. Lippincott, Williams and Wilkins, Philadelphia, 2001: 457.
39. Fukao T, Frucht DM, Yap G, Gadina M, O'Shea JJ, Koyasu S. Inducible expression of Stat4 in dendritic cells and macrophages and its critical role in innate and adaptive immune responses. J Immunol 2001; 166:4446.
40. Croker BP, Ramos EL. Pathology of the renal allograft. In: Tisher CC, Brenner BM, eds. Renal pathology with clinical and functional correlations. Lippincott, Philadelphia, 1994: 1591.