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# Glomerular damage as a predictor of renal allograft loss

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## Abstract

Interstitial fibrosis and tubular atrophy (IF/TA) are the most common cause of renal graft failure. Chronic transplant glomerulopathy (CTG) is present in approximately 1.5-3.0% of all renal grafts. We retrospectively studied the contribution of CTG and recurrent post-transplant glomerulopathies (RGN) to graft loss. We analyzed 123 patients with chronic renal allograft dysfunction and divided them into three groups: CTG (N = 37), RGN (N = 21), and IF/TA (N = 65). Demographic data were analyzed and the variables related to graft function identified by statistical methods. CTG had a significantly lower allograft survival than IF/TA. In a multivariate analysis, protective factors for allograft outcomes were: use of angiotensin-converting enzyme inhibitor (ACEI; hazard ratio (HR) = 0.12, P = 0.001), mycophenolate mofetil (MMF; HR = 0.17, P = 0.026), hepatitis C virus (HR = 7.29, P = 0.003), delayed graft function (HR = 5.32, P = 0.016), serum creatinine  $\geq 1.5$  mg/dL at the 1st year post-transplant (HR = 0.20, P = 0.011), and proteinuria  $\geq 0.5$  g/24 h at the 1st year post-transplant (HR = 0.14, P = 0.004). The presence of glomerular damage is a risk factor for allograft loss (HR = 4.55, P = 0.015). The presence of some degree of chronic glomerular damage in addition to the diagnosis of IF/TA was the most important risk factor associated with allograft loss since it could indicate chronic active antibody-mediated rejection. ACEI and MMF were associated with better outcomes, indicating that they might improve graft survival.

Key words: Kidney transplantation; Chronic allograft nephropathy; Glomerulonephritis; Transplant glomerulopathy

## Introduction

The terminology "interstitial fibrosis and tubular atrophy (IF/TA) with no evidence of any specific etiology" recently replaced "chronic allograft nephropathy", and is used to describe biopsies with fibrosis and atrophy and with no obvious underlying pathogenesis (1). IF/TA is the most common cause of late renal graft failure (1,2). Several pathophysiological processes contribute to it, such as chronic alloimmune injury, drug nephrotoxicity, chronic hypertension, with chronic obstruction and post-transplant glomerulopathy being the most distinctive ones (2).

The incidence of chronic transplant glomerulopathy (CTG) is about 1.5-3% of all renal grafts (3-5). According to Banff 09 criteria (6), CTG, called in this classification

"chronic active antibody-mediated rejection" is characterized by C4d positive staining, the presence of circulating antidonor antibodies, morphologic evidence of chronic tissue injury, such as glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries.

The pathogenesis of CTG is not clear (3). It has been associated with circulating anti-donor human leukocyte antigen (HLA) antibodies and the deposition of the complement split product C4d in the peritubular capillaries suggesting antibody-mediated injury (7-9). It has also been suggested that the same immune process that causes

the intimal proliferation characteristic of chronic vascular involvement (humoral immunity) in arteries and arterioles is expressed in the glomeruli as chronic transplant glomerulopathy. Several aspects of solid organ transplants were discussed at the 10th Banff Conference (6), with special emphasis on antibody-mediated graft injury, and six Banff working groups were formed, one of which will evaluate glomerular lesion scoring.

Recurrence of glomerular disease after transplantation is the third most important cause of graft loss after one year (10). The prevalence is variable (6.0 to 19.4%) and can increase with time after transplant (10).

Even though histological and laboratory parameters can distinguish CTG from recurrent post-transplant glomerulonephritis (RGN), an early diagnostic assessment may be difficult in certain types of glomerular diseases such as membranoproliferative glomerulonephritis, and in some cases of IgA nephropathy and focal segmental glomerulosclerosis (FSGS) (11). Moreover, there are still controversies concerning the diagnosis, the best treatment option and the prognostic factors related to these conditions in the context of renal allograft survival.

We hypothesize that glomerular involvement in a fibrotic graft might represent an additional negative prognostic factor and ultimately could further reduce graft survival. It is known that in kidney transplantation, chronic allograft nephropathy might be followed or not by glomerular involvement. Furthermore, patients might present other diseases with pronounced glomerular involvement, among them recurrent glomerulonephritis and transplant glomerulopathy. These are different physiopathogenic conditions and their impact on graft survival has not been fully addressed. Therefore, we compared groups with glomerular involvement to patients with IF/TA without glomerular involvement, since all patients have some degree of renal fibrosis.

## Patients and Methods

### Study population

All 1990 biopsies of deceased and living related kidney transplant patients performed at the Federal University of São Paulo between 2000 and 2003, with at least 6 months of graft function, were retrospectively reviewed. A total of 450 biopsy-proven diagnoses of IF/TA were initially identified. Biopsies were not included in the study if the patients: 1) were recipients of a previous organ transplant, 2) were recipients of a multi-organ transplant; 3) had a high immunological risk at the time of transplantation, defined as having a previously measured panel-reactive antibody grade >60%, 4) did not have a second later biopsy to be re-examined, 5) had a current or previous polyoma virus infection and/or tumors, 6) had a *de novo* post-transplant glomerulonephritis or an RGN with an unknown primary etiology of chronic renal disease, and 7) had hemolytic uremic syndrome and microangiopathic abnormalities.

Inclusion criteria were: 1) patients with biopsy-proven IF/TA and 2) with available clinical and laboratory data in their medical records.

Finally, we selected 123 patients who were divided into three groups: group 1 was the CTG group (N = 37) and group 2 was the RGN group (N = 21), including only IgA nephropathy (N = 7), membranoproliferative glomerulonephritis (N = 9), and diffuse proliferative glomerulonephritis (corresponding to the predominance of proliferation of endocapillary cells in the renal biopsy, N = 5). Since chronic transplant glomerulopathy could present histologically as glomerular sclerosis, we decided to exclude the cases of recurrent, classical, nephrotic-associated FSGS from the present analysis due to the fact that it is usually easy to establish the clinical diagnosis of recurrent FSGS. Membranous glomerulonephritis was excluded because we had two cases and the recurrence of the disease was late along the post-transplantation course (45 and 120 months). For group 3 (N = 65), we selected all biopsies with IF/TA without any grade of glomerular damage. We then randomized the sample and selected a number of cases that was almost double those with CTG to compose this group.

### Operational definitions

Delayed graft function was defined as the requirement of dialysis during the first week after transplantation without rejection and/or technical problems. Early rejection was defined when patients with graft dysfunction presented a biopsy that matched Banff 05 criteria or when the dysfunction resolved after a minimum of 3 doses of methylprednisolone over three days, in the absence of other causes of dysfunction. Any rejection before the 3rd month of transplantation was classified as early rejection and, after this period, as late rejection. Systemic arterial hypertension was defined as a repeated elevated blood pressure exceeding 140 x 90 mmHg or when patients were using at least one antihypertensive drug. New onset of diabetes after transplantation was defined when fasting plasma glucose was  $\geq 126$  mg/dL ( $\geq 7$  mM), when a random blood sugar level  $\geq 200$  mg/dL ( $\geq 11.1$  mM) was detected, accompanied by symptoms, or an oral glucose tolerance test value  $\geq 200$  mg/dL (12). Renal function was measured by serum creatinine and by creatinine clearance calculated with the Cockcroft-Gault equation.

### Outcomes

Graft loss was defined as permanent return to dialysis or estimated glomerular filtration rate  $< 15$  mL $\cdot$ min $^{-1}$  $\cdot$ 1.73 (m $^2$ ) $^{-1}$ . For the calculation of functional graft survival, patients who died with a functioning graft were eliminated from the study.

### Histological analyses

Biopsy specimens of all cases were evaluated and graded according to the Banff 05 criteria (12). IF/TA was divided into three grades according to the Banff 05 clas-

sification, which evaluates the severity of IF/TA: grade I corresponding to mild IF and TA (<25% of cortical area), grade II corresponding to moderate IF and TA (26-50% of cortical area), and grade III corresponding to severe IF and TA (>50% of cortical area; may include non-specific vascular and glomerular sclerosis, but severity is graded by tubulointerstitial features). At least four stained slides were used to quantify histological changes in each biopsy, one stained with hematoxylin-eosin, one with Masson's trichrome, one with periodic acid-Schiff, and one with silver. All biopsy specimens were also reviewed by two independent observers (pathologists) before inclusion in the study. The severity of acute and chronic lesions in each renal compartment was calculated by applying concordance criteria among these observers. The kappa index was used to assess inter-rater reliability when observing or otherwise coding qualitative/categorical variable (kappa >0.70 is considered to be satisfactory).

The kappa index was 0.85 (95%CI = 0.75-0.95), and observed agreement 0.90. When there was a discrepancy between the two pathologists, the mean values for each variable was used as the final grade of lesion severity.

#### **Criteria for the definition of chronic transplant glomerulopathy**

The criteria were: light microscopy showing double contours of the glomerular basement membrane in at least 10% of the most severely affected tuft, 2) negative immunofluorescence or few deposits of IgM and/or C3, 3) electron microscopy revealing reduplication of the glomerular basement membrane and subendothelial accumulation of electron-lucent material, 4) C4d positive staining, and 5) presence of circulating antidonor antibodies (not available in 90% of the patients).

Immunofluorescence analysis was available for 71% of the RGN cases, 60% of the CTG cases, and 30% of the IF/TA cases. C4d positive staining and electron microscopy were not routinely performed (only when indicated by the assistant physician), being available for 50% (31% from IF/TA patients, 71% from RGN patients, and 73% from CTG patients), and 52% (31% from IF/TA patients, 76% from RGN patients, and 78% from CTG patients) of all renal biopsies, respectively.

The Banff 05 and Banff 07 classifications were used for the diagnosis of IF/TA and CTG, respectively. Light microscopy and immunofluorescence were used for RGN cases. Electron microscopy was used only in cases in which light and immunofluorescence microscopy techniques were not able to clarify the diagnoses.

#### **Statistical analysis**

Ages of donors and recipients were considered to be continuous and categorical variables (donor's age above or below 39 years, recipient's age above or below 34 years) based on the median donors' and recipients' ages. The pres-

ence of proteinuria was recorded as a numerical variable in grams per day (24-h urine collections). In further analysis, proteinuria was categorized into  $\geq 1$  and  $\geq 0.5$  g/24 h. Chi-square and Fisher exact tests were performed to compare demographic covariates between groups when appropriate. Data are reported as means  $\pm$  SD. The Student *t*-test and ANOVA were used to compare the mean values among groups, with the level of significance set at  $P < 0.05$  (two-tailed). The Kaplan-Meier method was used to estimate graft survival, not including the death of patients with a functioning graft. Statistical significance was determined by log-rank comparisons of survival curves using two-sided *P* values. Data are reported as means  $\pm$  SD and, when appropriate, as median and ranges. A multivariate model, the Cox proportional hazards model, was used to analyze the relationship between graft loss and the other covariates, including those that were significant in univariate analysis or the variables that we considered clinically relevant. The Cox proportional hazard model (backward step) was used to obtain the final model of significant predictors and confounding variables were analyzed when required. There was no confusion when the exponential coefficient (hazard ratio, HR) did not change more than 10%. The Stata statistical software (SPSS) 12.0 was used for all statistical analyses (Stata Corporation, USA).

## **Results**

#### **Demographic analysis of the enrolled population**

The groups were matched according to gender (58% males), recipient age ( $34 \pm 13$  years) and transplantation time. The immunosuppressive treatment (intention to treat) corresponded to cyclosporine A, prednisone and azathioprine for 80% of the patients. All patients were treated with prednisone (data not shown). The mean period of follow-up after transplantation was  $50 \pm 29$  months (range 6-140 months). During this period, 24 grafts were lost at  $51 \pm 32$  months (range 6-115 months) due to noncompliance with treatment ( $N = 1$ ), acute rejection ( $N = 3$ ), IF/TA ( $N = 10$ ), and chronic antibody-mediated rejection ( $N = 10$ ). The groups differed in mean period of time from transplantation to graft biopsy. Even though serum creatinine levels did not differ among groups at the time of the biopsy, the CTG group had higher serum creatinine levels ( $2.3 \pm 1.7$  mg/dL) compared to the RGN ( $1.6 \pm 0.5$  mg/dL) and IF/TA groups ( $1.9 \pm 0.6$  mg/dL). Twenty-four-hour proteinuria differed significantly between the CTG ( $1.8 \pm 1.8$  g/24 h) and IF/TA ( $0.2 \pm 0.5$  g/24 h) groups, as well as between the IF/TA and RGN ( $2.1 \pm 2.2$  g/24 h) groups.

The presence of glomerulitis in the different groups was as follows: CTG ( $N = 1$ ) = 30%; RGN ( $N = 6$ ) = 29%; IF/TA 0% ( $P = 0.000$ ), and the presence of hyalinosis was: CTG = 73%, RGN = 48%, IF/TA = 31% ( $P = 0.000$ ).

Univariate analysis showed that dialysis time, age and gender of donor, type of donor, age of recipient, positive



hepatitis B virus and hepatitis C virus (HCV) serology, post-transplant diabetes mellitus, time of ischemia, delayed graft function, and type and number of acute rejection episodes were not significantly different among groups. In contrast, the number of antihypertensive drugs, proteinuria  $\geq 0.5$  g/24 h at 1st year post-transplantation, cytomegalovirus (CMV) antigenemia, the use of angiotensin converting enzyme inhibitor (ACEI), the use of mycophenolate mofetil (MMF), and the degree of HLA matching with a living donor differed significantly among groups (see Table 1).

There was a difference in mean period of time from transplantation to graft biopsy among groups. The grade of IF/TA by Banff 05 ( $\geq$  grade II) was significantly different ( $P = 0.000$ ) among CTG ( $N = 29$ , 78%), RGN ( $N = 14$ , 23%) and IF/TA ( $N = 17$ , 28%) patients.

#### Allograft survival and multivariate analysis

Graft survival is shown in Figures 1 and 2 according to

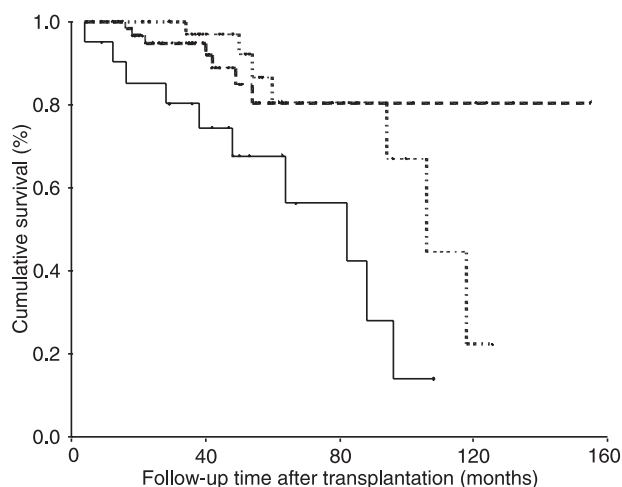
time of transplantation and time between biopsy and last follow-up. Patients in the CTG and RGN groups had a faster progression of allograft dysfunction and a lower allograft survival than those in the IF/TA group ( $P = 0.013$ ) after the diagnosis of the morphologic lesion. Allograft survival at the 2-year post-diagnostic biopsy was 75% for CTG, 58% for RGN and 85% for IF/TA. On the other hand, allograft survival at the 1st, 5th and 7th year post-transplant was statistically different among groups ( $P = 0.004$ ), with the following respective values: CTG = 97, 80 and 67%; RGN = 90, 67 and 14%; IF/TA = 100, 84 and 80%.

Multivariate analysis using the Cox model revealed the following predictive factors for allograft outcome: use of ACEI (HR = 0.12, 95%CI = 0.03-0.41,  $P < 0.001$ ), use of MMF (HR = 0.17, 95%CI = 0.03-0.80,  $P = 0.026$ ), serum creatinine  $\leq 1.5$  mg/dL by the 1st year post-transplant (HR = 0.20, 95%CI = 0.05-0.69,  $P < 0.001$ ), and proteinuria  $\leq 0.5$  g/24 h by the 1st year post-transplant (HR = 0.14, 95%CI =

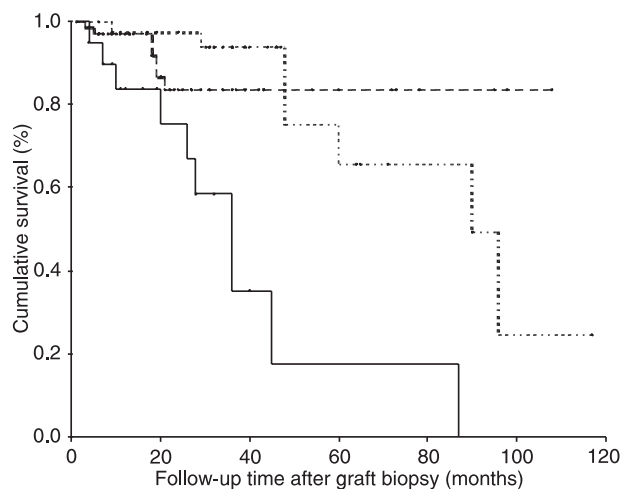
**Table 1.** Demographic, laboratory, clinical, and histological characteristics of the patients.

	CTG (N = 37)	RGN (N = 21)	IF/TA (N = 65)	P <sup>+</sup>	P*
Recipient age (years)	38 $\pm$ 12	35 $\pm$ 13	32 $\pm$ 13	ns	ns <sup>a</sup> , 0.042 <sup>b</sup>
Donor age (years)	39 $\pm$ 9	33 $\pm$ 12	38 $\pm$ 15	ns	ns <sup>a</sup> , ns <sup>b</sup>
Number of antihypertensive drugs in use	2 $\pm$ 0.85	1.43 $\pm$ 0.9	1.58 $\pm$ 0.8	0.020	0.043 <sup>a</sup> , ns <sup>b</sup>
Proteinuria (g/24 h) at 1st year post-Tx	1.0 $\pm$ 2.1	1.7 $\pm$ 3.8	0.2 $\pm$ 0.9	0.020	ns <sup>a</sup> , ns <sup>b</sup>
Total acute rejection episodes	0.5 $\pm$ 0.61	0.25 $\pm$ 0.5	0.54 $\pm$ 0.7	ns	0.182 <sup>a</sup> , 0.996 <sup>b</sup>
Early rejection episodes <3 months (N, %)	14 (38%)	5 (24%)	29 (45%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Late rejection episodes $\geq 3$ months (N, %)	4 (11%)	2 (10%)	8 (12%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Deceased donor (N, %)	8 (22%)	6 (29%)	23 (35%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
HLA matching living donor (N, %)	29 (78%)	15 (71%)	42 (65%)	0.014	ns <sup>a</sup> , ns <sup>b</sup>
Delayed graft function (N, %)	8 (22%)	3 (15%)	17 (26%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
New onset diabetes after Tx (N, %)	7 (19%)	2 (10%)	2 (3%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Anti-HBV antibodies (N, %)	6 (16%)	1 (5%)	5 (8%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Anti-HCV antibodies (N, %)	8 (22%)	3 (15%)	9 (14%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
CMV antigenemia (N, %)	10 (27%)	16 (76%)	6 (9%)	0.001	ns <sup>a</sup> , ns <sup>b</sup>
Mean transplant time at graft biopsy (months)	41 $\pm$ 26	23 $\pm$ 20	26 $\pm$ 24	0.003	0.011 <sup>a</sup> , 0.014 <sup>b</sup>
Serum creatinine (mg/dL)	2.3 $\pm$ 1.7	1.6 $\pm$ 0.5	1.9 $\pm$ 0.6	ns	ns <sup>a</sup> , ns <sup>b</sup>
Proteinuria (g/24 h) at 1st year post-Tx	1.0 $\pm$ 2.1	1.7 $\pm$ 3.8	0.2 $\pm$ 0.9	0.020	ns <sup>a</sup> , ns <sup>b</sup>
IF/TA ( $\geq$ grade II Banff 05 classification)	29 (78%)	14 (67%)	17 (26%)	0.000	ns <sup>a</sup> , ns <sup>b</sup>
ACEI therapy (N, %)	29 (78%)	12 (57%)	65 (100%)	0.000	ns <sup>a</sup> , ns <sup>b</sup>
Initial immunosuppressive therapy					
CsA (N, %)	34 (92%)	18 (86%)	53 (82%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Tacrolimus (N, %)	1 (3%)	2 (10%)	11 (17%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Rapamycin (N, %)	4 (11%)	2 (10%)	3 (5%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
Azathioprine (N, %)	24 (65%)	17 (81%)	49 (75%)	ns	ns <sup>a</sup> , ns <sup>b</sup>
MMF (N, %)	7 (19%)	0 (0%)	12 (18%)	0.023	ns <sup>a</sup> , ns <sup>b</sup>

CTG = chronic transplant glomerulopathy; RGN = recurrent post-transplant glomerulonephritis; IF/TA = interstitial fibrosis and tubular atrophy; Tx = transplant; HLA = human leukocyte antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; CMV = cytomegalovirus; ACEI = angiotensin-converting enzyme inhibitor; CsA = cyclosporin A; MMF = mycophenolate mofetil. ANOVA: P<sup>+</sup> = mean of all groups; P\* = <sup>a</sup>between CTG and RGN; <sup>b</sup>between CTG and IF/TA; ns = non-significant.



**Figure 1.** Graft survival of patients with chronic transplant glomerulopathy (CTG, dotted line), recurrent post-transplant glomerulonephritis (RGN, solid line) and interstitial fibrosis and tubular atrophy (IF/TA, dashed line). Graft survival after transplantation was statistically different among the groups ( $P = 0.004$ , Kaplan Meier-graft survival curves).



**Figure 2.** Graft survival after biopsy of patients with chronic transplant glomerulopathy (CTG, dotted line), recurrent post-transplant glomerulonephritis (RGN, solid line) and interstitial fibrosis and tubular atrophy (IF/TA, dashed line). Graft survival after allograft biopsy was markedly reduced for patients with RGN and CTG compared with IF/TA ( $P = 0.013$ , Kaplan Meier-graft survival curves).

0.03-0.54,  $P = 0.004$ ). Factors associated with allograft loss were: a positive HCV serology (HR = 7.29, 95%CI = 1.98-26.85,  $P = 0.003$ ) and delayed allograft dysfunction (HR = 5.32, 95%CI = 1.36-20.82,  $P = 0.016$ ). In addition, having some degree of glomerular damage increased the risk of allograft loss more than the diagnosis of IF/TA by itself (HR = 4.04, 95%CI = 1.33-15.51,  $P = 0.015$ ). In contrast, CMV antigenemia, gender, type of donor, the presence of early rejection, steroid-resistant rejection, and the time between transplantation and diagnosis were not statistically relevant (see Table 2).

## Discussion

There is extensive evidence that incipient histological changes characteristic of IF/TA can be visualized in the allograft before the transplant function deteriorates and the serum creatinine level is not the best predictor for renal allograft dysfunction in the early stages (13-16).

This study reviewed 123 biopsy-proven IF/TA specimens, which were classified as IF/TA without glomerular

**Table 2.** Co-variables that significantly predicted graft loss in multivariate analyses.

Variables	P	HR	95%CI for HR	
			Lower	Upper
Initial Cox hazard model				
Use of ACEI	0.005	0.14	0.03	0.56
Use of MMF	0.046	0.14	0.02	0.97
Delayed graft function	0.020	7.94	1.39	45.31
CMV antigenemia	ns	1.71	0.49	5.97
Renal acute rejection	ns	1.58	0.37	6.60
Corticoid resistant rejection	ns	0.33	0.05	2.18
Time of transplantation between Tx and biopsy	0.038	0.97	0.95	0.99
Gender	ns	0.57	0.18	1.84
Type of donor (deceased)	ns	1.18	0.29	4.70
Hepatitis C virus antibodies	0.001	14.63	2.85	74.88
Serum creatinine (mg/dL) $\leq 1.5$ at 1st year post-Tx	0.009	0.13	0.02	0.60
Proteinuria (g/24 h) $\leq 0.5$ at 1st year post-Tx	0.004	0.11	0.02	0.51
Presence of glomerular damage	0.046	3.90	1.02	14.87
Final Cox hazard model				
Use of ACEI	0.001	0.12	0.03	0.41
Use of MMF	0.026	0.17	0.03	0.80
Delayed graft function	0.016	5.32	1.36	20.82
Hepatitis C virus antibodies	0.003	7.29	1.98	26.85
Serum creatinine (mg/dL) $\leq 1.5$ at 1st year post-Tx	0.011	0.20	0.05	0.69
Proteinuria (g/24 h) $\leq 0.5$ at 1st year post-Tx	0.004	0.14	0.03	0.54
Presence of glomerular damage	0.015	4.55	1.33	15.51

HR = hazard rate; ns = non-significant. For other abbreviations, see legend to Table 1.

damage, chronic transplant glomerulopathy and recurrent post-transplant glomerulonephritis. The biopsies showed that an early presentation of proteinuria without elevation of serum creatinine level (in most cases with glomerular lesion) was present in the CTG and RGN groups in contrast to the IF/TA group (without glomerular lesion). As cited, the three post-transplant conditions can share the same features. This makes it difficult to establish a differential diagnosis between the advanced grade of IF/TA, including chronic transplant glomerulopathy, and the recurrent post-transplant glomerulonephritis that manifests as proteinuria, considering only clinical and laboratory markers. Nevertheless, in the present study, it was possible to show that these diseases have peculiar clinical and laboratory profiles.

Although we found different stages of damage in the biopsies, the serum creatinine level at the time of the graft biopsy was not significantly different among groups. In contrast, 24-h urinary protein excretion was significantly different between CTG and IF/TA, while CTG and RGN showed high and similar proteinuria levels. As a progression marker (14,15), urinary protein excretion was a predictor of graft function loss, with the RGN group presenting the worst graft survival rate. Patients with CTG had a poorer graft survival than those without CTG. After the diagnosis of transplant glomerulopathy, the patients of this group progressed more rapidly to end-stage renal disease than those whose graft biopsy revealed IF/TA without transplant glomerulopathy.

Proteinuria, especially if elevated and persistent, is a well-defined indicator of the progressive nature of renal disease in native kidneys (5,16). Our results showed that higher levels of proteinuria after transplantation are also related to a poorer prognosis of the renal allograft. This appears to be associated with the presence of RGN. This finding emphasizes the need for biopsies to reveal the cause of proteinuria after transplantation. The impact of RGN on graft outcome is important and this diagnosis should be considered when proteinuria is present.

In addition, we found that one of the factors associated with renal allograft loss in these selected groups of patients was the presence of anti-HCV antibodies, even though it was not significant in univariate analysis. This variable was analyzed because it is a well-known risk factor for renal graft loss. In our study, patients carrying anti-HCV antibodies had 11.7 times higher risk of graft loss than those with no anti-HCV antibodies. This finding agrees with recent studies suggesting that the risk of transplant glomerulopathy increases in patients exposed to HCV and CMV infections (17,18). The presence of CMV antigenemia was not statistically significant in multivariate analysis.

Another risk factor associated with renal allograft loss was a delayed allograft dysfunction episode. This episode increased the risk of allograft loss by 4.9. This finding has been reported by others although it is controversial (19-22). Delayed graft function increases the risk of acute rejection

by leading to an overwhelming immune response to the allograft. The presence of increased immune material leads to increased production of fibrotic material and to a decrease in functioning tubules. This increases the prevalence and severity of IF/TA (22) and consequently becomes a risk factor for allograft loss, as observed in the present study.

As cited above, the CTG and RGN groups showed lower allograft survival rates when compared to the IF/TA group. This finding is in accordance with the current literature that describes chronic rejection as the first cause of renal allograft loss, followed by death with a functioning graft and immune complex-mediated glomerulonephritis (recurrent and *de novo*). Hariharan et al. (23) have demonstrated that glomerular disease recurrence determines a 1.9 times higher relative risk of graft loss after 5 years of follow-up compared to patients without recurrence.

It is of note that the present study was not randomized or controlled and had limitations associated with its retrospective design. Immunofluorescence was not available for 29% of the patients in the RGN group because during the period of case inclusion in this study renal biopsy specimens were not routinely assessed by this technique in our service. Therefore, it is necessary to say that, without immunofluorescence analysis, eventual cases of IgA nephropathy could have been missed. Nevertheless, within the context of this study, it is not probable that the glomerulonephritis classified as recurrent (as there was a diagnosis of glomerulonephritis in native kidneys) could correspond to another post-transplant glomerulonephritis. Also, electron microscopy was not available for most cases.

The authors are aware that the absence of immunoperoxidase studies for the detection of C4d deposition is an important limitation, and this approach will be indispensable in further studies about these particular issues. It is of note that Sis et al. (24) reported that 70% of 53 CTG biopsies had anti-HLA, 36% C4d staining, 91% capillaritis, and 35% glomerulitis. However, only 53% of CTG with alloantibody showed C4d, suggesting that C4d staining has low sensitivity for chronic antibody-mediated rejection, but CTG with no endothelial stress showed good survival.

In the current study, the presence of glomerular damage was a risk factor for renal allograft loss independently of the presence of IF/TA. This finding agrees with recently published studies (25-29). Wavamunno et al. (30) showed that endothelial and subendothelial ultrastructural abnormalities in glomerular and peritubular capillaries are sensitive early markers of CTG. They are associated with C4d deposition subsequently detected by light microscopy.

Our results show that the use of MMF and renin-angiotensin system (RAS) blockade provide some protection of the renal allograft. MMF could be responsible for this effect because its immunosuppressive activity (31) also inhibits the expression of adhesion molecules on endothelial cells and the proliferation of arterial smooth cells (31-34), thereby retarding the infiltration of inflammatory cells (33,34), and

possibly delaying the onset/progression of IF/TA (32,35). Additionally, it is well-known that RAS blockade delays the progression of glomerulosclerosis and interstitial fibrosis in proteinuric diseases (36,37). The investigators have shown that RAS blockade is efficient in reducing proteinuria and delaying the rate of decline of renal function in patients with post-transplant glomerulonephritis. On the basis of these considerations, an early diagnosis of proteinuria and its cause have prognostic and relevant therapeutic consequences (38,39).

Finally, the treatment and prevention of IF/TA and renal allograft rejection remain a challenge (32,40). In fact, the numerous unanswered questions in this area motivated the present study. We searched for morphological and functional markers that could distinguish renal lesions that contribute to loss of the renal graft, particularly the contribution of the glomerular compartment. Considering the limitations of

the study design and the sample size, it was apparent that the functional parameters evaluated were not adequate to permit a differential diagnosis of the three histological groups in this context, but they could contribute to the suspicion of the type of graft involvement and to the early indication of a renal biopsy. We emphasize that more attention should be devoted to the identification of clinical and laboratory risk factors for graft loss. In addition, different histological diagnoses of graft loss were demonstrated in the present study. Certainly, the differentiation of chronic immunologic rejection from other causes of chronic allograft dysfunction is especially important in renal biopsies, because the treatment of such conditions is completely different. Chronic transplant glomerulopathy, as well as post-transplant glomerulonephritis, are well known causes of loss of renal allograft that deserve an early diagnosis and intervention.

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