

Transplant glomerulopathy: clinico-pathologic features

Glomerulopatia do transplante: análise clínico-patológica

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ABSTRACT

Transplant glomerulopathy is a sign of chronic kidney allograft damage. It has a distinct morphology and is associated with poor allograft survival. We aimed to assess the prevalence and clinic-pathologic features of transplant glomerulopathy, as well as determine the functional and histological implications of its severity. We performed a single-centre retrospective observational study during an eight-year period. Kidney allograft biopsies were diagnosed and scored according to the Banff classification, coupled with immunofluorescence studies. The epidemiology, clinical presentation, outcomes (patient and graft survival) and anti-HLA alloantibodies were evaluated. Transplant glomerulopathy was diagnosed in 60 kidney transplant biopsies performed for clinical reasons in 49 patients with ABO compatible renal transplant and a negative T-cell complement dependent cytotoxicity crossmatch at transplantation. The estimated prevalence of transplant glomerulopathy was 7.4% and its cumulative prevalence increased over time. C4d staining in peritubular capillaries (27.6%) was lower than the frequency of anti-HLA antibodies (72.5%), the majority against both classes I and II. Transplant glomerulopathy was associated with both acute (mainly glomerulitis and peritubular capillaritis) and chronic histologic abnormalities. At diagnosis, 30% had mild, 23.3% moderate and 46.7% severe transplant glomerulopathy. The severity of transplant glomerulopathy was associated with the severity of interstitial fibrosis. Other histological features, as well as clinical manifestations and graft survival, were unrelated to transplant glomerulopathy severity.

Key-Words: Antibody; glomerulopathy; histology; kidney; transplant.

RESUMO

A glomerulopatia do transplante é um sinal de lesão crónica do enxerto renal. Apresenta uma morfologia distinta e está associada a uma pior sobrevida do enxerto. O objectivo do estudo foi avaliar a prevalência e características clínico-patológicas da glomerulopatia do transplante, bem como determinar as implicações funcionais e histológicas da sua gravidade. Estudo retrospectivo e observacional realizado num único centro durante um período de oito anos. As biópsias de enxerto renal foram diagnosticadas e avaliadas segundo a classificação *Banff*, complementadas com estudos de imunofluorescência. Análise da epidemiologia, apresentação clínica, *outcomes* (sobrevida do

paciente e enxerto) e aloanticorpos anti-HLA. A glomerulopatia de transplante foi diagnosticada em 60 biópsias realizadas por motivos clínicos, em 49 receptores de transplante renal ABO compatível e com *crossmatch* antilinfocitário negativo por citotoxicidade dependente do complemento aquando transplante. A prevalência estimada da glomerulopatia do transplante foi 7.4%, a prevalência cumulativa aumentou ao longo do tempo. A deteção de C4d nos capilares peritubulares (27.6%) foi inferior à dos anticorpos anti-HLA (72.5%), a maioria contra as classes I e II. A glomerulopatia do transplante associou-se tanto as lesões histológicas agudas (principalmente glomerulite e capilarite peritubular), como crónicas. Aquando diagnóstico, 30%, 23.3% e 46.7% apresentavam glomerulopatia do transplante leve, moderada e severa, respectivamente. Verificou-se uma associação entre a gravidade da glomerulopatia do transplante e a severidade da fibrose intersticial. Os outros indicadores histológicos, as manifestações clínicas e a sobrevida do enxerto, foram independentes da gravidade da glomerulopatia do transplante.

Palavras-Chave: Anticorpo; glomerulopatia; histologia; rim; transplante.

■ INTRODUCTION

Transplant glomerulopathy (TG) is a unique pathologic entity distinct from other forms of chronic allograft injury and is associated with poor kidney transplant outcomes¹. It develops as a maladaptive/remodelling response to sustained or episodic endothelial injury, and is now widely accepted as the cardinal histologic phenotype of chronic antibody-mediated rejection². It primarily is an endothelial pathology affecting kidney microcirculation and includes a constellation of histologic features on light and electron microscopies³, with immunofluorescence typically negative or with non-specific IgM and C3⁴. Morphologic features of TG evolve over time⁵.

The clinical presentation of TG lags behind the initial histologic stages of the disease⁵. Its reported prevalence, based on biopsies performed for clinical reasons, varies from 1.6 to 7%⁴⁻⁹, but these numbers clearly underestimate its true prevalence.

The aim of this work was to assess the prevalence and clinico-pathologic features of TG, as well as determine the functional and histological implications of TG severity.

■ SUBJECTS AND METHODS

■ Renal transplant patients

From January 2004 to December 2011, a total of 1261 kidney allograft biopsies were evaluated at the Renal Morphology Laboratory of the Hospital de Curry

Cabral. After the exclusion of biopsies with insufficient/inadequate material (n = 96), biopsies of patients with missing follow-up (n = 191) and donor kidney biopsies (n = 308), 666 cases were available for analysis. Of these, 60 were diagnosed with TG based on glomerular double contours, after excluding other conditions with similar histological changes (n = 2, one with membranoproliferative glomerulonephritis and another one with cortical necrosis). The biopsies were performed to investigate worsening renal function and/or proteinuria.

Clinical and laboratory data of patients were extracted from their medical records, including demographic and clinical variables, factors related to renal transplantation, post-transplantation day at biopsy, arterial hypertension, serum levels of creatinine and proteinuria at biopsy, history of acute rejection episodes and graft outcome. A semiquantitative analysis of the Panel-reactive antibodies (PRA) was performed: 0, 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (> 75%).

■ Anti-HLA antibody analysis

Recipient sera taken in the peribiopsy period was screened for Human Leukocyte Antigens (HLA) antibodies and sera found to have anti-HLA class I and/or class II antibodies was further evaluated to determine HLA specificities using the Luminex technology.

■ Histopathology

The biopsies were diagnosed and scored according to the Banff classification^{10,11}.

Transplant glomerulopathy was diagnosed by light microscopy based on double contours of the glomerular basement membrane¹⁰; the diagnosis was supported by immunofluorescence studies, which showed mesangial IgM and/or C3 or negative immunofluorescence findings. Electron microscopy studies were not performed.

■ Indirect Immunofluorescence Staining for C4d

C4d staining was done in all biopsies with available frozen tissue. Scoring of C4d staining was based on the percentage of stained tissue on immunofluorescence with an intensity $\geq 1+$ and a linear, circumferential staining pattern of peritubular capillaries. It was graded as negative, focal (1-50% of sampled capillaries) or diffuse ($\geq 50\%$ of sampled capillaries) positive¹².

■ Statistical analysis

Parametric variables were expressed as means \pm standard deviation (SD), non-parametric variables were expressed as median and interquartile range (IQR) and, qualitative data were described as percentages. Categorical variables were compared using the Chi-square test. Fisher's exact test was used on a 2x2 table with ≥ 1 cells with an expected frequency ≤ 5 . Differences between groups (i.e., TG severity/increasing Banff "cg" score from cg1–cg3) were analysed with the Kruskal-Wallis test. Graft survival was analysed by Kaplan-Meier and Cox proportional hazards modelling. All p-values were two-tailed and values < 0.05 were considered statistically significant. Confidence intervals included 95% of predicted values. Analyses were carried out using SPSS software (version 20; SPSS Inc. Chicago IL, USA).

■ RESULTS

■ Prevalence of TG and clinical findings

Of the 666 renal allograft biopsies included in the present study, TG was diagnosed in 60 biopsies, in 49 patients. The prevalence of TG in our renal transplant population, based on biopsies performed for clinical reasons, was 7.4%.

Table 1

Demographic and clinical characteristics of patients with transplant glomerulopathy

	n = 49
Age (years; mean \pm SD)	52 \pm 12.5
Male (%)	57.1
Hepatitis C seropositivity (%)	15
Cadaveric donor (%)	76.7
Expanded criteria donor (%)	44.4
Retransplantation (%)	16.7
HLA-A-B-DR mismatches (median and IQR)	4 (3 - 4)
PRA (semiquantitative analysis; median and IQR)	0 (0 - 2)
Prior acute rejection (%)	40.8
T-cell/ antibody mediated rejection (%)	85/ 25
Characteristics at time of biopsy	
Post-transplantation time (years; mean \pm SD)	8.2 \pm 5.5
Serum creatinine (mg/dL; mean \pm SD)	2.8 \pm 1.2
Proteinuria (g/day; mean \pm SD)	3.7 \pm 3
Arterial hypertension (%)	70

Semiquantitative analysis of PRA: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 ($\geq 75\%$)

The cumulative prevalence of TG increased with time, from 16.3% at 3 years, to 26.5% at 5 years, 51% at 7 years, and 61.2% at 9 years post-transplant.

The demographic and clinical details of kidney transplantation recipients with TG are outlined in Table 1. All patients were submitted to an ABO compatible renal transplant and had a negative T-cell crossmatch at transplantation using an anti-human globulin enhanced complement dependent cytotoxicity assay. The median HLA-A-B-DR mismatch was 4 and no kidney transplant was HLA identical. In the current study, 40.8% of patients with TG had experienced prior T-cell (85%) or antibody-mediated acute rejection (25%) episodes; 10.2% of patients had multiple previous acute rejection episodes.

Transplant glomerulopathy was diagnosed at a mean of 8.2 years post-transplant, with a mean serum creatinine of 2.8 mg/dL and with 78% of patients with urine protein levels ≥ 1 g/day.

■ Anti-HLA antibodies

Of the 60 biopsies, 40 had available sera for anti-HLA analysis. Alloantibody was detected in 72.5% of cases, either against class I HLA (17%), or class II HLA (7%), or both (76%). Donor-specific antibodies

Table II

Status of circulating anti-HLA antibodies in transplant glomerulopathy biopsies in the peribiopsy period

	n = 40
Anti-HLA panel-reactive antibodies	
Negative (%)	27.5
Positive (%)	72.5
– Anti-HLA class I antibodies (%)	17
– Anti-HLA class II antibodies (%)	7
– Both class I and class II antibodies (%)	76
Donor specific anti-HLA antibodies	
Negative (%)	17.2
Positive (%)	58.6
– Anti-HLA class I antibodies (%)	59.3
– Anti-HLA class II antibodies (%)	37.5
Indeterminate (%)	24.1
– Anti-HLA class I antibodies (%)	14.8
– Anti-HLA class II antibodies (%)	29.2

Human Leukocyte Antigens (HLA)

(DSA) were identified in 58.6% anti-HLA antibody containing-sera, with 59.3% and 37.5% DSA for class

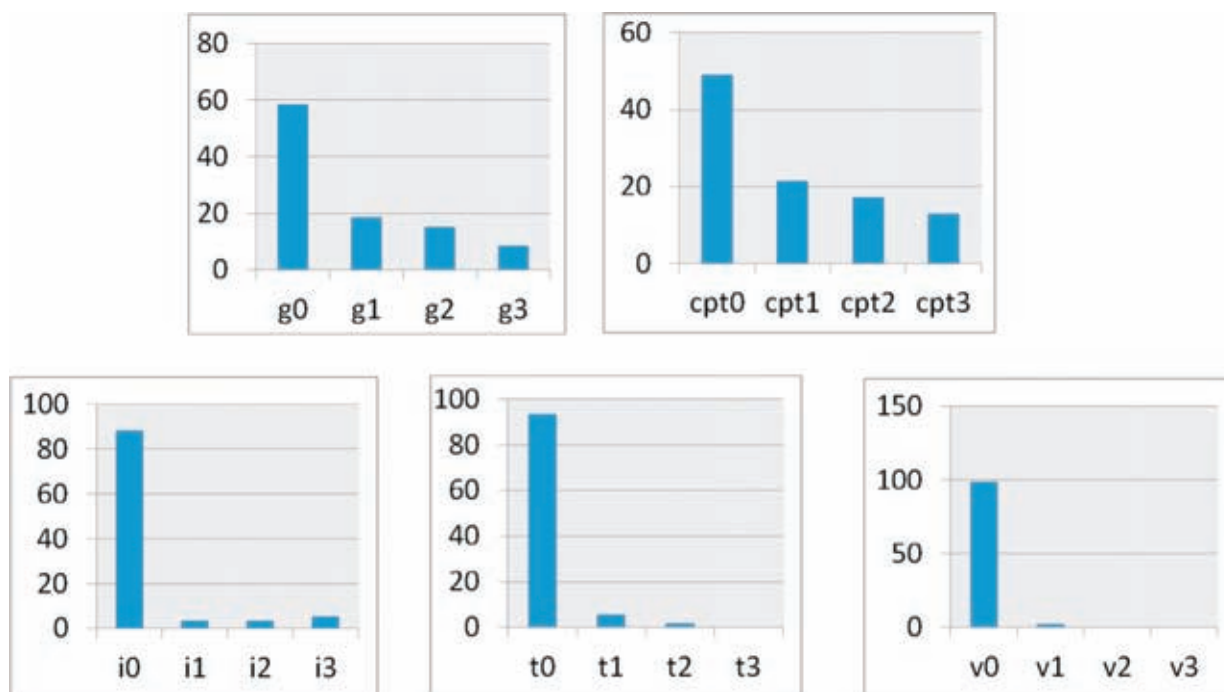
I and class II, respectively (Table II). It is important to note that in a non-negligible percentage of cases, donor specificity could not be identified (reported as “Indeterminate” in Table II).

■ TG and other histologic lesions

At diagnosis, 18 of 60 biopsies (30%) had mild, 14 (23.3%) moderate and 28 (46.7%) severe TG (cg1, cg2, cg3, correspondingly).

The histopathology of allograft biopsies with TG is shown in Figs. 1 and 2.

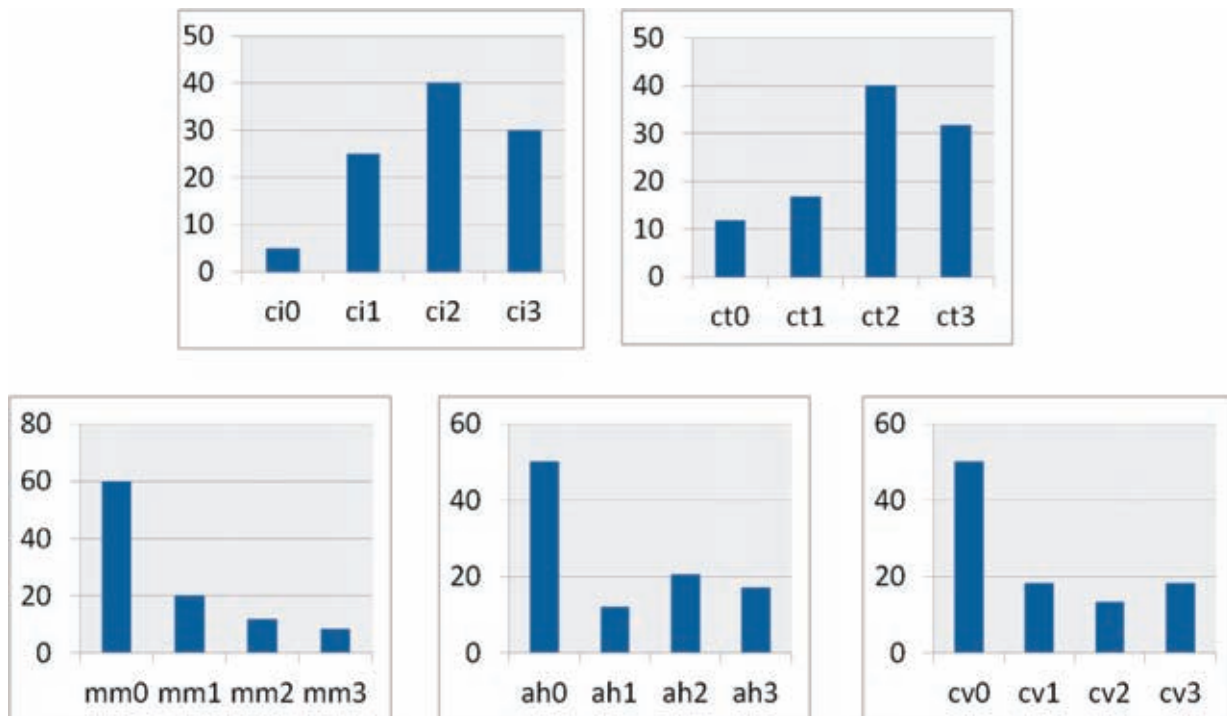
We observed glomerulitis (Banff “g” score) and peritubular capillaritis (Banff “cpt” score) in 41.7% and 51.1% of TG biopsy specimens, respectively. Other morphologic indicators of activity were uncommon: interstitial inflammation (Banff “i” score), tubulitis (Banff “t” score) and intimal arteritis (Banff “v” score).



Scoring categories of Banff classification (acute/active lesion): glomerulitis (“g” score), peritubular capillaritis (“cpt” score), interstitial inflammation (“i” score), tubulitis (“t” score) and intimal arteritis (“v” score)

Figure 1

Histologic findings at the time of diagnosis of transplant glomerulopathy (acute/active lesion scoring)



Scoring categories of Banff classification (chronic lesion): interstitial fibrosis (“ci” score), tubular atrophy (“ct” score), mesangial matrix expansion (“mm” score), arteriolar hyaline thickening (“ah” score) and vascular fibrous intimal thickening (“cv” score)

Figure 2

Histologic findings at the time of diagnosis of transplant glomerulopathy (chronic lesion scoring)

Interstitial fibrosis (Banff “ci” score) and tubular atrophy (Banff “ct” score) were commonly seen in biopsies with TG, followed by other chronicity indexes: mesangial matrix expansion (Banff “mm” score), arteriolar hyaline thickening (Banff “ah” score) and vascular fibrous intimal thickening (Banff “cv” score).

■ C4d deposition in peritubular capillaries

C4d deposits in peritubular capillaries was observed in 16 (27.6%) biopsy specimens with TG at the time of diagnosis, with either diffuse staining in 11 (68.8%) or focal staining in five (31.2%).

Positive C4d staining was associated with anti-HLA antibodies (OR 9.3, 95% CI 1.1-82.6, $p = 0.03$). Nevertheless, about half (51.7%) of the C4d negative TG cases had circulating alloantibodies in the peri-biopsy period.

■ Functional and histological implications of TG severity

Although arterial hypertension, allograft dysfunction and proteinuria were commonly seen in biopsies with TG, their severity did not relate significantly with higher Banff “cg” scores ($p = \text{NS}$).

After a follow-up of 9.9 ± 5.2 years, one (2%) patient expired and 34 (69.4%) lost their allograft from causes other than patient’s death. No difference was observed in death-censored graft survival between TG groups (Figs. 3 and 4).

The severity of interstitial fibrosis (Banff “ci” score) increased with higher Banff “cg” scores ($p = 0.042$). Overall, the frequency and severity of other histologic lesions (Banff “g, cpt, i, t, v, ct, mm, ah and cv” scores) did not increase significantly with growing severity of TG ($p = \text{NS}$).

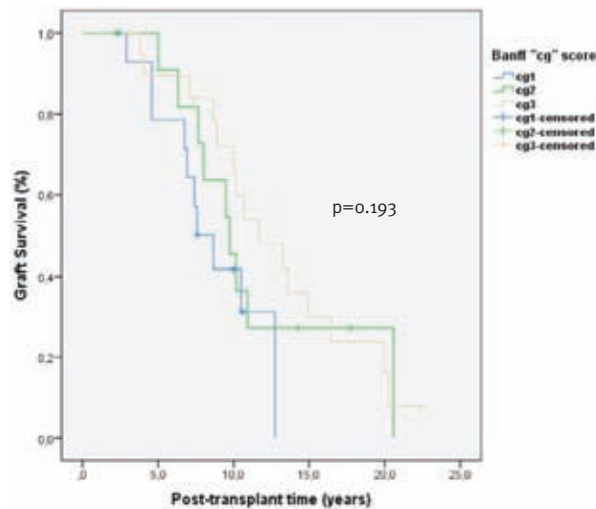


Figure 3
Cumulative death-censored graft survival according to the severity of transplant glomerulopathy (Kaplan-Meier analysis)

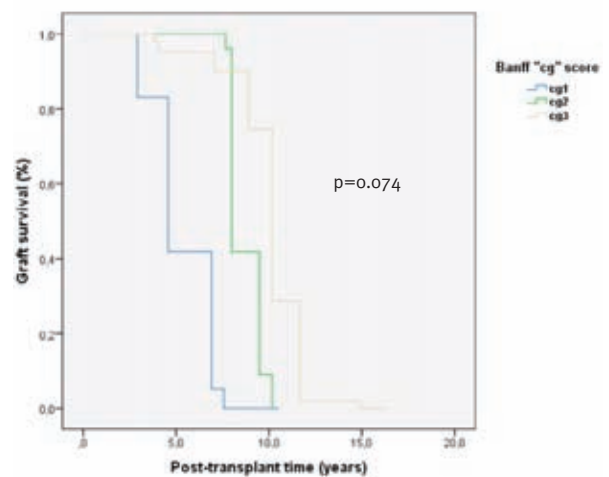


Figure 4
Cumulative graft survival according to the severity of transplant glomerulopathy (Cox regression in a multivariate model adjusted for serum creatinine, proteinuria, prior acute rejection, anti-HLA antibodies, C4d staining, interstitial fibrosis and arteriolar hyaline thickening)

DISCUSSION

Even though TG has been known since the early days of kidney transplantation, understanding of this injury pattern is still incomplete². Transplant glomerulopathy is generally thought to occur as a late complication of kidney transplantation in association with decreased function, proteinuria and hypertension. Nevertheless, TG can occur within months of transplantation and in the absence of proteinuria^{6,8,13,14}. Previous studies have consistently described a prevalence of 1.6–7% in conventional kidney transplant recipients based on biopsies performed for clinical causes^{4,9}.

In our series, TG was diagnosed by light microscopy at a mean of 8.2 years after transplantation, with a minimum of 6 months. The estimated prevalence of TG was 7.4% and we found that TG was a time-dependent pathology. We are aware that these numbers under recognize TG, since many allografts develop TG sub-clinically, and many chronically failing kidney transplants are not biopsied.

Since our study was based on biopsies performed for clinical reasons, it was not surprising to have a great fraction of cases with graft dysfunction, proteinuria and

hypertension at the time of the diagnosis of TG. The reported incidence of prior acute rejection was high (40.8%), with multiple episodes in 10.2% of recipients. The percentage of T-cell and antibody-mediated rejection episodes was similar to that of previous reports⁵.

Donor-specific antibodies, particularly HLA antigen class II antibodies, can spur insidious graft injury and, therefore, constitute a central causal factor for TG^{15–17}. The reasons why particularly HLA antigen class II antibodies (with or without anti-class I) are associated with TG are unclear. Likely, there is an influence of antibody affinity avidity, as well as an ability to engage with Fc- γ receptors. The localization of target antigens also might be a determining factor². In our review, 72.5% of TG cases had circulating anti-HLA antibodies in the peribiopsy period, the majority against both classes I and II. Anti-HLA class II DSA were detected in 37.5% cases.

The reported incidence of C4d positivity in TG is quite variable^{17,18}, probably related to criteria for biopsying patients, as well as differences in C4d detection methods and the defining criteria for C4d

positivity. In our series, 27.6% had C4d deposition in peritubular capillaries.

Sis et al.⁵ suggested that the incidence of C4d deposition in TG was lower than the incidence of circulating alloantibodies, indicating that C4d deposition along capillaries might be negative or fluctuating and suggested that C4d negativity did not necessarily exclude alloantibody-mediated glomerular damage. Our data showed that about half (51.7%) of the C4d negative TG cases had circulating alloantibodies in the peribiopsy period.

The morphologic indicators of activity in TG are C4d staining and microcirculation inflammation (glomerulitis and/or peritubular capillaritis)². We observed glomerulitis and peritubular capillaritis in 41.7% and 51.1% of TG biopsy specimens, respectively. The incidence of other acute lesion scoring was residual.

Depending on the duration of the disease, chronic histologic abnormalities are seen in concurrence with TG, as a result of progression of capillary damage and subsequent ischaemic injury (segmental and/or focal glomerulosclerosis, interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, arterial fibrous intimal thickening and, sometimes, loss of peritubular capillaries)². Our data revealed a predominance of chronicity indexes, namely interstitial fibrosis (95%) and tubular atrophy (88.3%).

A major question addressed by this study was the functional and histological implications of TG severity. Our data revealed no differences in the frequency and/or severity of arterial hypertension, allograft dysfunction and proteinuria with TG severity; graft survival did not differ either. Transplant glomerulopathy was associated with acute and chronic histologic abnormalities. Nevertheless, the severity of these abnormalities was varied and, only interstitial fibrosis and TG were associated.

The results should be interpreted within the context of the study's limitations. As in all observational studies, the possibility of residual confounding or misclassification of outcomes cannot be excluded.

In conclusion, our revision assessed the prevalence and clinico-pathologic features of TG in a cohort of biopsies performed for clinical reasons. We demonstrated that the severity of TG was associated with the severity of interstitial fibrosis at the time of diagnosis of TG.

Other histologic features, as well as clinical manifestations and graft survival, were unrelated to TG severity.

Conflict of interest statement. None declared.

References

- Cosio FG, Gloor JM, Sethi S, Stegall MD. Transplant glomerulopathy. *Am J Transplant* 2008;8(3):492-496.
- Husain S, Sis B. Advances in the Understanding of transplant glomerulopathy. *Am J Kidney Dis* 2013;62(2):352-363.
- Colvin RB. Antibody-mediated renal allograft rejection: Diagnosis and pathogenesis. *J Am Soc Nephrol* 2007;18(4):1046-1056.
- Maryniak RK, First MR, Weiss MA. Transplant glomerulopathy: Evolution of morphologically distinct changes. *Kidney Int* 1985;27(5):799-806.
- Sis B, Campbell PM, Mueller T, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant* 2007;7(7):1743-1752.
- Habib R, Broyer M. Clinical significance of allograft glomerulopathy. *Kidney Int Suppl* 1993;43:595-98.
- Sijpkens YW, Joosten SA, Wong MC, et al. Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. *Kidney Int* 2004;65(6):2409-2418.
- Suri DL, Tomlanovich SJ, Olson JL, Meyer TW. Transplant glomerulopathy as a cause of late graft loss. *Am J Kidney Dis* 2000;35(4):674-680.
- Vongwiwatana A, Gourishankar S, Campbell PM, Solez K, Halloran PF. Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy. *Am J Transplant* 2004;4(1):124-129.
- Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999;55(2):713-23.
- Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: Updates and future directions. *Am J Transplant* 2008;8(4):753-760.
- Racusen LC, Colvin RB, Solez K, et al. Antibody-mediated rejection criteria – An addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003;3(6):708-714.
- Habib R, Zurowska A, Hinglais N, et al. A specific glomerular lesion of the graft: Allograft glomerulopathy. *Kidney Int Suppl* 1993;42:S104-111.
- Banfi G, Villa M, Cresseri D, Ponticelli C. The clinical impact of chronic transplant glomerulopathy in cyclosporine era. *Transplantation* 2005;80(10):1392-1397.
- Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation* 2010;90(1):68-74.
- Einecke G, Sis B, Reeve J, et al. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009;9(11):2520-2531.
- Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012;12(2):388-399.
- Al Aly Z, Yalamanchili P, Cortese C, Salinas-Madrigal L, Bastani B. C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: an uncommon finding. *Transpl Int* 2005;18(7):800-805.
- Batal I, Gimita A, Zeevi A, et al. Clinical significance of the distribution of C4d deposits in different anatomic compartments of the allograft kidney. *Mod Pathol* 2008; 21(12):1490-1498.

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