ORIGINAL ARTICLE

The relationship between proteinuria and allograft survival in patients with transplant glomerulopathy: a retrospective single-center cohort study

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SUMMARY

Proteinuria and transplant glomerulopathy (TG) are common in kidney transplantation. To date, there is limited knowledge regarding proteinuria in different types of TG and its relationship to allograft survival. A retrospective cohort analysis of TG patients from indication biopsies was performed to investigate the relationship of proteinuria, histology, and graft survival. One hundred and seven (57.5%) out of 186 TG patients lost their grafts with a median survival of 14 [95% confidence interval (CI) 10–22] months after diagnosis. Proteinuria ≥ 1 g/24 h at the time of biopsy was detected in 87 patients (46.8%) and the median of proteinuria was 0.89 (range 0.05–6.90) g/24 h. TG patients with proteinuria ≥ 1 g/24 h had worse 5-year graft survival (29.9% vs. 53.5%, $P = 0.001$) compared with proteinuria <1 g/24 h. Proteinuria was associated with graft loss in univariable Cox regression [hazard ratio (HR) 1.25, 95% CI, 1.11–1.41, $P < 0.001$], and in multivariable analysis (adjusted HR 1.26, 95% CI 1.11–1.42, $P < 0.001$) independent of other risk factors including creatinine at biopsy, positive C4d, history of rejection, and Banff lesion score mesangial matrix expansion. In this cohort of TG patients, proteinuria at indication biopsy is common and associated with a higher proportion of graft loss.

Introduction

Transplant glomerulopathy (TG) is a morphological diagnosis that featured glomerular basement membrane double contours or glomerulus multi-lamination regardless of etiology [1]. It can be classified by either the pathological change severity based on Banff lesion score chronic allograft glomerulopathy (cg) or the etiology regarding the effect of antibody-mediated rejection (ABMR). The presence of TG in pathology is associated with poor allograft survival [2]. However, not all TG patients lose their graft function immediately following the diagnosis. A reliable tool that allows simple and easy stratification of risk in TG patients for graft loss is needed to provide information on prognosis and further treatment options.

Proteinuria (PU) is frequently encountered for recipients after kidney transplantation because it may reflect severe damage to the integrity of the glomerular tuft [3,4]. A previous study showed that proteinuria is an independent risk factor for graft loss regardless of graft function and histologic change for all kidney transplant recipients [5]. Several studies found high level of proteinuria significantly impacted graft survival of TG patients [6,7]. However, the detailed relationship between different levels of proteinuria and graft survival in different types of TG patients needs further analysis in a relatively large patient cohort.

This study aims to explore the relationship between proteinuria and renal allograft survival in TG patients. It is hypothesized that proteinuria is a reliable marker of structural glomerular damage and an indicator for poor allograft outcome, even more objective than the semiquantitative assessment of TG histological severity.

Patients and methods

Patient population

This retrospective study included all consecutive patients who underwent allograft biopsy in Campus Mitte and Virchow, Charité Universitätsmedizin Berlin (Berlin, Germany) between January 2000 and December 2018. This study included all adult kidney transplant recipients who have biopsy-proven TG, as defined by the Banff consensus guideline [1]. In patients with more than one biopsy and with different diagnoses of TG, the biopsy with the severest clinical and pathological changes was chosen for this study. We excluded patients with potentially confounding histological diagnoses such as recurrent/*de novo* glomerulopathy, thrombotic microangiopathy (TMA), and hepatitis C virus-associated membranoproliferative glomerulonephritis (MPGN).

Clinical and laboratory data

Clinical and laboratory data were extracted from the transplant database system (TBase) in our center [8,9]. The data were prospectively collected from the hospital system since 2000 at each follow-up visit. Graft survival time was calculated from the diagnosis of TG until the patient's initiating maintenance dialysis or death. Patients were followed until graft loss, recipient death, or last available follow-up. The last follow-up was 31st December 2018 for patients not experiencing graft loss. For patients with more than 60 months of graft survival until the end of 2018, the survival time was set as

60 months. Patient death with a functioning graft was censored.

Total proteinuria was measured routinely at each visit from 24-h urine collections at the time of allograft biopsy or four weeks around biopsy without special treatment in 165 patients. For patients ($n = 18$) without a 24-h urine test, spot urinary protein concentrations (mg/l) were converted to 24-h urine test unit (g/24 h) assuming a urinary output of 2 l/24 h [10]. In three patients, only dipstick's results were available at the time of biopsy. Negative dipsticks were estimated to be 0.075 g/24 h (= the average urinary protein concentration between 0.0 and 0.15 g/24 h proteinuria); trace dipsticks results were imputed as 0.325 g/24 h (= the average urinary protein concentration between 0.150 and 0.499 g/24 h); positive (+) dipsticks results were calculated as 1.0 g/24 h (= the average urinary protein concentration between 0.5 and 1.5 g/24 h); and double-positive (++) dipsticks were imputed as 3.0 g/24 h (= the average urinary protein concentration between 1.5 and 4.5 g/24 h) [11]. Data on proteinuria were also collected at the following time points: 3 ± 1, 6 ± 2, and 12 ± 3 months before the biopsy. Furthermore, follow-up proteinuria information was obtained at 3 ± 1, 6 ± 2, 12 ± 3 months after biopsy.

Renal allograft pathology

Ultrasound-guided graft biopsy was performed when clinically indicated due to rising creatinine and/or proteinuria. A qualified biopsy involved at least one interlobular artery and no less than seven glomeruli [12]. The histological analysis was performed by two independent pathologists in a consensus way. The pathological changes were graded on a scale of 0–3 according to the 2017 Banff classification.

Complement split product C4d was detected by indirect immunofluorescence on paraffin sections of formalin-fixed tissue using polyclonal anti-C4d antibody (Dianova, Hamburg, Germany). Banff diagnostic categories were determined based on the 2018 Banff classification reference guide [1].

Statistical analysis

The time of baseline was set as the time of biopsy when TG was diagnosed in this cohort. Continuous variables with normal distribution were summarized as mean ± standard deviation, and other continuous variables were expressed as medians with ranges. For categorical variables, the *N* and percentages in each category

were shown. For analysis of different parameters from the two groups, the Student's t-test and Mann–Whitney U-test were used for continuous variables and Chi-square test (or Fisher's exact test, if appropriate) for categorical data. Wilcoxon signed-rank test was used to compare the changes of proteinuria over time in different groups.

The receiver operating characteristics (ROC) analysis was performed to analyze diagnostic accuracy for five-year graft loss. The optimal cutoff point for proteinuria was defined at the maximal Youden index. Kidney allograft survival, censored for patient death with functioning graft, was estimated using the Kaplan–Meier curves and log-rank test.

Cox proportional hazard models were performed to assess hazard ratios (HR) and 95% confidence intervals (CI) for factors associated with kidney allograft loss. Potential predictors for graft loss were included in the model based on their presence at the time of biopsy and associations in the literature. The associations of variables were assessed with a Spearman correlation in case of co-linearity. A backward elimination manner was adopted with a P value criterion of 0.157 [13]. The proportional hazards assumption for the Cox model was tested using Schoenfeld residuals. The model discrimination and goodness-of-fit were determined by Harrell's C concordance statistics (C-index) as well as Grønnesby and Borgan test, respectively. All statistics were performed using STATA version 15.1 (Stata Corp, College Station, TX, USA) and GRAPHPAD PRISM 8.0 (GraphPad Software, La Jolla, CA, USA) software. Two-sided P value <0.05 is considered statistically significant.

Ethics

The patient information was collected routinely according to local data protection regulations in TBase for regular follow-up in our center. According to local law, no formal institutional review approval by the ethics committee of Charité Universitätsmedizin Berlin is needed for retrospective scientific analysis of own patient data. The procedure of the study conformed to the Declaration of Helsinki 2000 as well as the Declaration of Istanbul 2008.

Results

Incidence of proteinuria in TG patients

Among 2375 indication allograft biopsies performed from 2000 to 2018, 346 biopsies (346/2375, 14.6%)

from 251 patients were diagnosed with TG. After excluding potentially confounding cases with recurrent/de novo glomerulonephritis (n = 32), TMA (n = 31), and hepatitis C virus-associated MPGN (n = 2), a total of 186 patients were enrolled (Fig. 1). These patients were diagnosed with TG at the median of 73.5 (range 2–232) months after transplantation. The mean proteinuria level was 1.43 ± 1.46 (median 0.89, range 0.05–6.90) g/24 h and abnormal urine protein excretion (≥0.3 g/24 h) was found in 148 of 186 biopsy-proven TG patients (79.6%). More than half (99/186, 53.2%) of patients' urine protein excretion was lower than 1 g/24 h, and only 24/186 patients (12.9%) proteinuria was ≥3 g/24 h.

Demographics and clinical variables

The average age for transplantation was 43.3 ± 15.0 and 76.9% (143/186) of patients received their first transplant graft. Patients had a mean of 3.1 ± 1.4 human leukocyte antigen (HLA) mismatches. The baseline creatinine was 1.6 ± 0.6 (median 1.5, range 0.6–3.7) mg/dl and majority of patients received tacrolimus (117/186, 62.9%) as a maintenance immunosuppressant. The patient's serum creatinine was 2.7 ± 1.1 (median 2.5, range 0.9–8.2) mg/dl at the time of biopsy (Table 1).

Proteinuria and graft survival

Patients were followed for a median of 109 (from 5 to 272) months after transplantation and a median of 27 (from 0.3 to 143) months after diagnosis of TG by biopsy. The primary outcome was allograft loss (return to chronic dialysis or dead with functioning graft) following the diagnosis of TG. After 5 years, 107/186 patients (57.5%) had started maintenance dialysis and five patients (2.7%) died with a functioning graft. 35/186 (18.8%) patients without graft loss were censored at end of observation, and only 39/186 (21.0%) grafts were still functioning more than 5 years after the diagnosis of TG. Estimated death-censored 5-year graft survival was 33.0% (95% CI 25.4–40.7%).

In patients with subsequent graft loss, the proteinuria further increased at 3, 6, and 12 months after the diagnosis of TG (Wilcoxon signed-rank test, $Z = -3.40$, -3.52, -2.65; $P = 0.001$, <0.001, 0.008, respectively), while it remained stable in patients without graft loss (Wilcoxon signed-rank test, $Z = -0.98$, -0.62, -0.85; $P = 0.327$, 0.533, 0.398, respectively). Significant differences were also detected between the two groups in
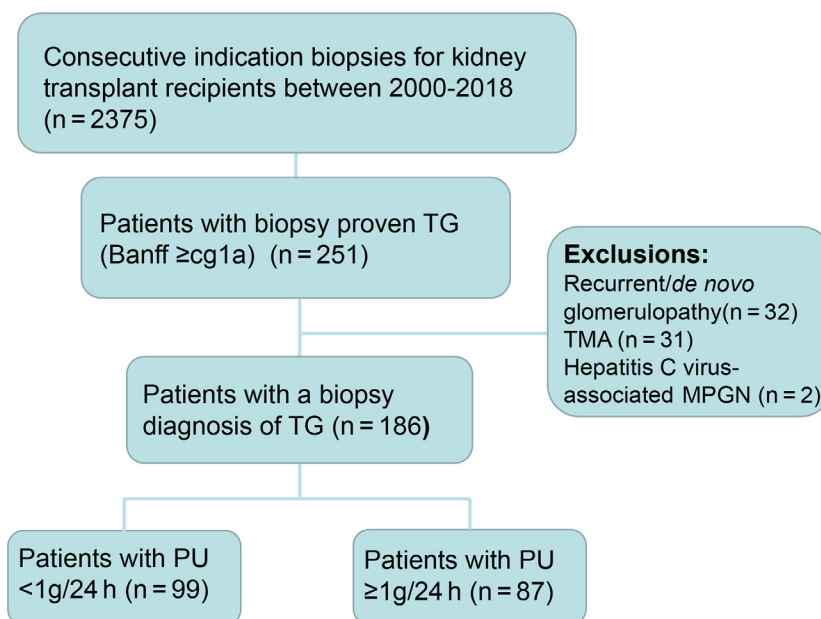
Figure 1 Flow chart of patient enrollment. Between January of 2000 and December of 2018, a total of 2375 indication biopsies due to rising creatinine and/or increase of proteinuria were performed, of which the TGlesions were found in 346 biopsies of 251 recipients. After exclusion of cases with recurrent/de novo glomerulonephritis ($n = 32$), TMA ($n = 31$), and hepatitis C virus-associated MPGN ($n = 2$), a total of 186 patients with biopsy-proven TG were enrolled in this retrospective study. cg, chronic allograft glomerulopathy; MPGN, membranoproliferative glomerulonephritis; PU, proteinuria; TG, transplant glomerulopathy; TMA, thrombotic microangiopathy.

proteinuria change at six and twelve months before biopsy (Mann–Whitney *U*-test $Z = -2.14$, -2.84; $P = 0.032$, 0.005, respectively) other than three months before biopsy ($Z = -1.31$, $P = 0.191$ by Mann–Whitney *U*-test; Fig. 2a). The utility of proteinuria at the time of biopsy for differentiating those TG patients with graft loss was evaluated by ROC analysis. Proteinuria at the time of biopsy yielded an area under the ROC curve (AUC) of 0.64 (95% CI 0.56–0.72, $P < 0.001$; Fig. 2b). The optimal cutoff point for proteinuria according to the maximum Youden index (0.26) was 0.92 g/24 h with a sensitivity of 59.8% (95% CI 50.3–68.6%) and specificity of 65.8% (95% CI 54.9–75.3%). Kaplan–Meier survival analysis results showed a significantly inferior allograft survival in TG patients with higher level of proteinuria ($P = 0.001$; Fig. 2c).

Proteinuria and patient characteristics

For subsequent analyses, the cohort of 186 patients with TG was stratified according to the optimal cutoff value of 0.92 g/24 h. Patients with proteinuria ≥0.92 g/24 h had lower age at the time of transplantation (40.8 ± 14.1 vs. 45.8 ± 15.4, $P = 0.021$), and less pre-existing anti-HLA donor-specific antibodies (DSA; 4.4% vs. 13.7%, $P = 0.040$).

Patients with proteinuria ≥0.92 g/24 h had higher peak panel reactive antibody (pPRA) level ($Z = -2.11$, $P = 0.035$ by Mann–Whitney *U*-test). Incidence of hypertension (69.2% vs. 44.2%, $P < 0.001$) and delayed graft function proportion (39.6% vs. 23.2%, $P = 0.040$) were also higher in patients with proteinuria ≥0.92 g/24 h.

No significant differences were found between the two groups in terms of biopsy age, gender, previous transplantation count, donor type, cold ischemia time, number of HLA mismatches, cause of end-stage renal disease, baseline creatinine, creatinine at biopsy, immunosuppressive regimen, *de novo* DSA status, and comorbidities (Table 1).

Proteinuria and renal allograft histology

The distribution of the histopathological features was shown as Banff lesion score percentage in Fig. 3. Forty patients (21.5%) had cg 1, 58 patients (31.2%) had cg 2, and 88 patients (47.3%) had cg 3. Ninety-nine TG patients (53.2%) encountered transplant glomerulitis (Banff scored g ≥ 1), and 67 patients (36.0%) had peritubular capillaritis (Banff scored ptc ≥ 1). Forty-two of the 186 patients (22.6%) had positive staining of C4d (Banff scored C4d ≥ 1) and interstitial fibrosis/tubular

Table 1. Patients' demographics and clinical characteristics.

Variables	Overall (n = 186)	PU < 0.92 g/24 h (n = 95)	PU ≥ 0.92 g/24 h (n = 91)	P value*
At the time of transplantation				
Age (years, mean ± SD)	43.3 ± 15.0	45.8 ± 15.4	40.8 ± 14.1	**0.021**
Gender (m/f)	106/80	48/47	58/33	0.069
Previous kidney Tx (0/1/2/3)	143/38/4/1	76/18/0/1	67/20/4/0	0.133
Donor living/deceased	74/112	38/57	36/55	0.951
Cold ischemia time (h, median, range)	11 (3.5, 31.2)	12.8 (4.2, 24)	10 (3.5, 31.1)	0.166
HLA mismatches (mean ± SD)	3.1 ± 1.4	3.2 ± 1.4	2.9 ± 1.4	0.115
Peak PRA (%, median, range)	0 (0, 100)	0 (0, 99)	0 (0, 100)	**0.035**
Peak PRA ≥5% n (%)	38 (20.4%)	13 (13.7%)	25 (27.5%)	**0.020**
Peak PRA ≥85% n (%)	11 (5.9%)	4 (4.2%)	7 (7.7%)	0.364
Pre-transplant PRA (%, median, range)	0 (0, 98)	0 (0, 98)	0 (0, 96)	0.300
Pre-Tx PRA ≥5% n (%)	19 (10.2%)	8 (8.4%)	11 (12.1%)	0.409
Pre-Tx PRA ≥85% n (%)	4 (2.2%)	2 (2.1%)	2 (2.2%)	0.965
Pre-existing DSA n (%)	17 (9.1%)	13 (13.7%)	4 (4.4%)	**0.040**
Etiology of ESRD				
Glomerulonephritis	32	14	18	0.172
Interstitial nephritis	24	11	13	
Polycystic disease	10	8	2	
Htn/nephrosclerosis	13	8	5	
Diabetic nephropathy	12	6	6	
Other causes	59	25	34	
Unknowing etiology	36	23	13	
After transplant and before biopsy				
Baseline Cr (mg/dl, mean ± SD)[†]	1.6 ± 0.6	1.6 ± 0.6	1.7 ± 0.6	0.271
Delayed graft function (y/n/ns)	58/121/7	22/70/3	36/51/4	**0.040**
Calcineurin inhibitors (Tac/CyA)	117/37	61/21	56/16	0.623
mTOR inhibitors	27 (14.5%)	13 (13.7%)	14 (15.4%)	0.742
Steroid-free regimen n (%)	33 (17.7%)	19 (20%)	14 (15.4%)	0.410
History of rejection n (%)	56 (30.1%)	29 (30.5%)	27 (29.7%)	0.899
At the time of biopsy				
Age (years, mean ± SD)	50.1 ± 14.9	52.1 ± 15.3	48.1 ± 14.2	0.066
Time from Tx to biopsy (months, median, range)	73.5 (2, 232)	65 (5, 232)	78 (2, 224)	0.123
Cr (mg/dl, mean ± SD)	2.7 ± 1.1	2.7 ± 1.1	2.8 ± 1.1	0.633
Proteinuria (g/24 h, median, range)	0.89 (0.05, 6.90)	0.37 (0.05, 0,90)	2.09 (0.94, 6.90)	**<0.001**
De novo DSA (y/n)	146/40	75/20	71/20	0.878
BKV viremia n (%)	13 (7.0%)	9 (9.5%)	4 (4.4%)	0.251
CMV viremia n (%)	18 (9.7%)	11 (11.6%)	7 (7.7%)	0.370
Hypertension[‡] n (%)	105 (56.5%)	42 (44.2%)	63 (69.2%)	**<0.001**
PTDM n (%)	19 (10.2%)	11 (11.6%)	8 (8.8%)	0.530
BMI (kg/m², mean ± SD)	25.3 ± 4.5	25. 9 ± 4.7	24.7 ± 4.3	0.075
ACEI/ARB therapy (y/n)	134/52	61/34	73/18	**0.015**

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BMI, body mass index; Cr, creatinine; CyA, Cyclosporine A; DSA, anti-HLA donor-specific antibodies; ESRD, end-stage renal disease; HLA, human leukocyte antigen; Htn, hypertension; mTOR, mammalian target of rapamycin; ns, not specified; PRA, panel reactive antibody; PTDM, post-transplant diabetes mellitus; PU, proteinuria; Tac, tacrolimus; Tx, transplantation.

*P values indicated group differences for proteinuria <0.92 g/24 h compared with proteinuria ≥0.92 g/24 h. Bold values indicated statistical significance.

[†]Three months after transplantation.

[‡]Defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg regardless of antihypertensive medications.

atrophy (Banff scored ci and ct ≥ 1) was found in more than half of TG patients (Fig. 3).

At the time of biopsy when TG was diagnosed, 150 patients (80.6%) were identified as ABMR including 98 patients (52.7%) with chronic-active ABMR (c-aABMR) and 52 patients (28.0%) with chronic ABMR (cABMR) [1]. The remaining 36 patients (36/186, 19.4%) were diagnosed with isolated transplant glomerulopathy (iTG). There were no significant differences between two groups in i, t, v, g, ptc, C4d, cict, cv, mm, ah scores when stratified by proteinuria (cutoff = 0.92 g/24 h). Patients with ≥0.92 g/24 h proteinuria had higher proportion in cg3 (56.0% vs. 38.9%, *P* = 0.041). No significant difference was found between two groups in other diagnostic categories (*P* = 0.413; Table 2).

Clinical and histological variables associated with graft loss

Donor-specific antibodies (regardless of the types) and hypertension at the time of biopsy had no impact on graft survival (Fig. 4a–c). Interestingly, there was no inferior allograft survival in TG patients without blockade of the renin–angiotensin–aldosterone system (RAAS; *P* = 0.734; Fig. 4d). Patients with blockade of RAAS showed significantly higher urine protein level compared with patients without blockade of RAAS (*Z* = −2.43, *P* = 0.015 by Mann–Whitney *U*-test).

The severity of TG as defined by Banff lesion score cg had no significant influence on graft survival (*P* = 0.350; Fig. 5a). Patients with Banff lesion score mm >1 had significantly inferior allograft survival (*P* = 0.005; Fig. 5b). C4d positive ABMR patients also had worse outcomes compared to C4d negative ABMR patients (*P* = 0.008; Fig. 5c), while no significant allograft survival difference was found between ABMR and iTG (*P* = 0.187; Fig. 5d).

Predictors of graft loss

A univariable and multivariable Cox regression analysis was performed to analyze the relationship between the proteinuria and allograft survival in TG patients in greater detail. When analyzed as a continuous variable, proteinuria increased 5-year graft loss after diagnosis of TG (unadjusted HR 1.25 for every 1 g/24 h increase in proteinuria, 95% CI 1.11–1.41, *P* < 0.001; Table 3). TG patients with normal proteinuria <0.3 g/24 h at time of biopsy had the lowest risk for graft loss at 5 years after diagnosis (42.1%, 16/38 patients; Fig. 2c), while TG patients with proteinuria ≥1 g/24 h had an unadjusted

2.35-fold higher risk for graft loss (95% CI 1.33–4.17, *P* = 0.003) with 70.1% 5-year graft loss (61/87 patients). The effect of proteinuria on graft loss was highest in TG patients with nephrotic range proteinuria (≥3 g/24 h; unadjusted HR 2.96, 95% CI 1.49–5.89, *P* = 0.002; Table 3).

The adjusted model included potential confounding factors, including DSA, history of rejection, creatinine at the time of biopsy, hypertension, Banff lesion score cg, Banff lesion score mm, and C4d. Three variables including DSA (*P* = 0.772), hypertension (*P* = 0.805), and Banff lesion score cg (*P* = 0.362) were removed from the final model and no violation of nonproportionality of hazards (*P* = 0.116) was found in this model. History of rejection (per rejection episode; HR 1.27, 95% CI 1.03–1.42, *P* = 0.028), proteinuria at the time of biopsy (per g/24 h; HR 1.26, 95% CI 1.11–1.42, *P* < 0.001), creatinine at the time of biopsy (per mg/dl; HR 1.51, 95% CI 1.32–1.72, *P* < 0.001), positive C4d (vs. neg; HR 2.05, 95% CI 1.33–3.16, *P* < 0.001), and Banff mesangial matrix lesion score (mm >1; HR 2.00, 95% CI 1.25–3.19, *P* = 0.004) were independent risk factors for allograft loss (Table 4). Harrell's C-statistics of the overall final multivariable analysis was 0.74. Further analysis revealed that creatinine at the time of biopsy had the highest predictive power with *C*-statistics of 0.71, followed by proteinuria at the time of biopsy (*C*-statistics = 0.61). The result of the Grønnesby and Borgan test was nonsignificant (*P* = 0.270), indicating adequate goodness-of-fit.

Discussion

Our findings help to define and clarify the clinicopathologic features as well as the prognostic significance of proteinuria for TG patients. In this study, we demonstrate a high incidence of proteinuria at the time of biopsy in TG patients and provide further evidence that proteinuria is a significant independent factor for allograft loss. In addition, with rising proteinuria, there is an increasing proportion of graft loss in TG patients, while cg score is not associated with outcome. The fact that histologic scores do not predict outcome while the simple clinical feature of proteinuria has an independent and increasing relationship with graft loss clearly suggests the clinical prognostic utility of this marker across different diagnostic categories.

Our results are in line with previous studies demonstrating a strong and independent relationship of proteinuria with graft survival of kidney transplant patients [3,14,15]. Our study provided additional insight into
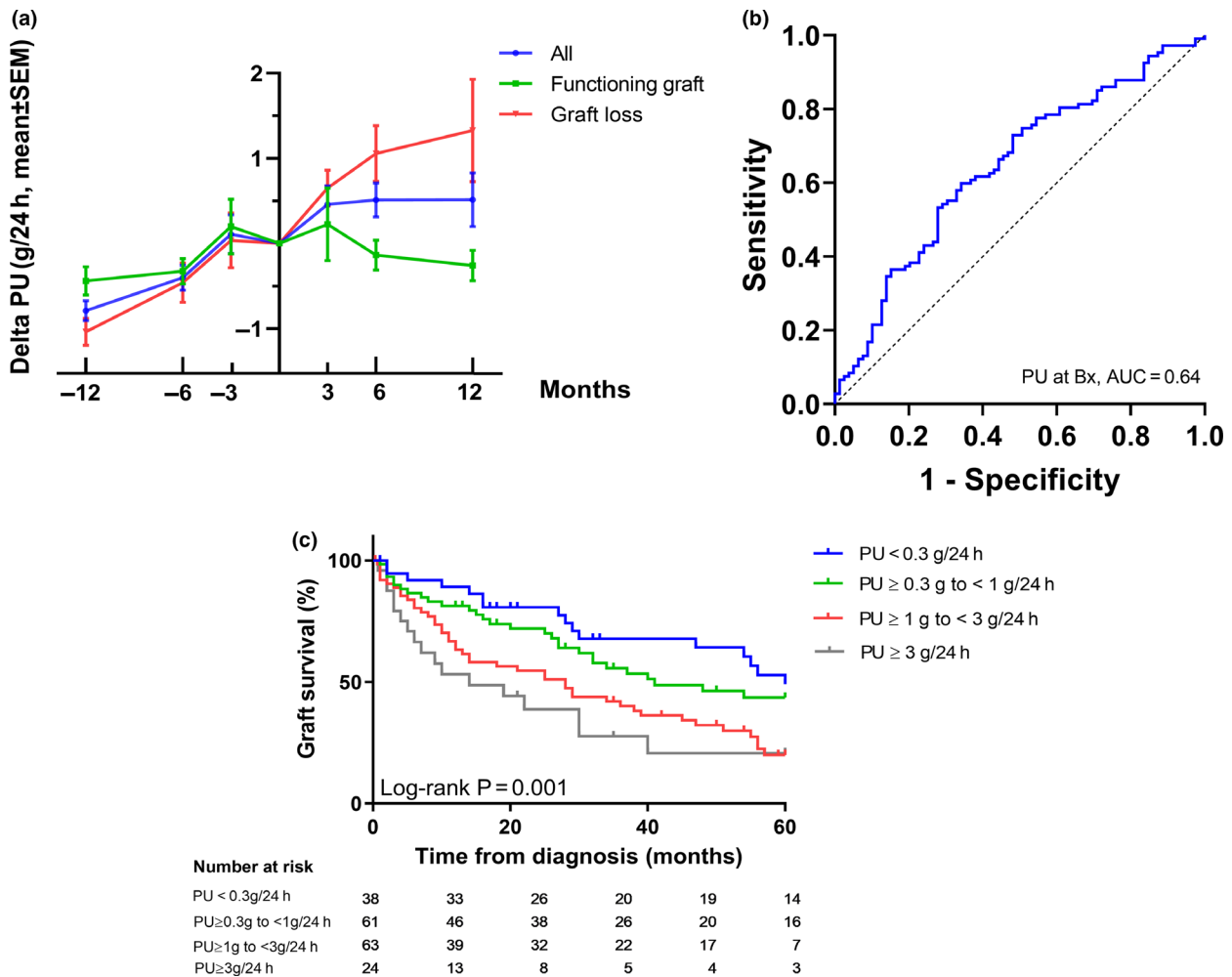
Figure 2 Proteinuria changes over time and association with allograft survival. (a) Changes of urine protein level based on the level at the time of biopsy (n = 156, 149, 147, 156, 138, 121, respectively). (b) ROC curves of association between proteinuria and graft loss at 5 years after TG. AUC was shown and the corresponding P value < 0.001. (c) TG patients were stratified into groups with different levels of proteinuria, the higher level of proteinuria corresponded to a higher risk of graft loss (P = 0.001). AUC, area under the ROC curve; PU, proteinuria; SEM, standard error of the mean.

the prognostic value of proteinuria at the time of diagnostic biopsy of TG as well as dynamic proteinuria changes of during follow-up. The existing studies regarding the influence of proteinuria on the survival of TG patients showed conflicting results, which might be due to the small sample size, the different criteria on patient inclusion and different patient cohorts [7,16–20]. Compared with previous studies, our study excluded patients with secondary TG, such as recurrent/ *de novo* glomerulonephritis, TMA, and hepatitis C virus-associated MPGN, which might control the effect of these confounders to improve the reliability of the results [21]. The overall graft survival rate was 42.5% in our study, which was lower than the previous study (5-year allograft survival 57.1%) [21]. This might be

related to the fact that only indication biopsy was performed in our center. Our results demonstrate that proteinuria at the time of TG diagnosis by indication biopsy is a major risk factor for further allograft loss, irrespective of the reasons for the development of TG and pathologic grade.

The current definition of proteinuria for kidney transplant recipients is 24 h total protein excretion ≥300 mg/24 h [22]. Moreover, the clinical guideline suggests renal allograft biopsy should be performed when there is a new onset of proteinuria or unexplained proteinuria ≥3 g per gram creatinine or ≥3 g/24 h [23]. A prospective, observational cohort study including 1518 renal allograft recipients showed—similar to our study—that proteinuria >1.0 g/24 h is a specific marker
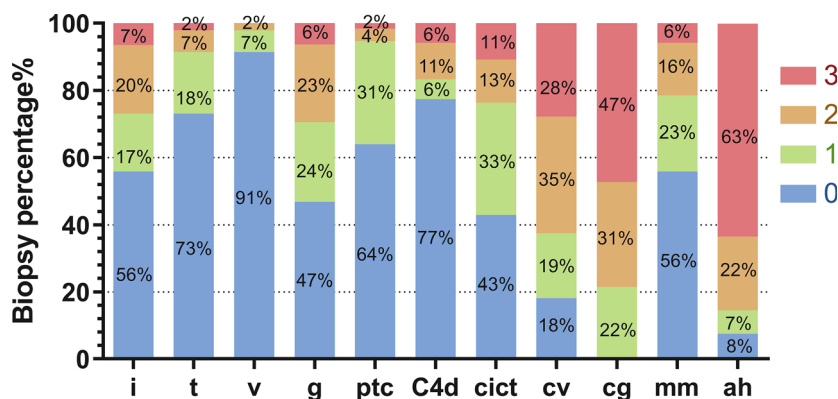
Figure 3 Banff lesion scores of TG patients (*n* = 186). The distribution of histological lesion scores from 186 allograft biopsies of TG patients was illustrated. Each score indicated a different aspect of allograft pathological change, i score (interstitial inflammation), t score (tubulitis), v score (intimal arteritis), g score (glomerulitis), ptc score (peritubular capillaritis), cict score (interstitial fibrosis + tubular atrophy), cv score (vascular fibrous intimal thickening), cg score (glomerular basement membrane double contours), mm score (mesangial matrix expansion), and ah score (arteriolar hyalinosis).

Table 2. Histologic features of enrolled biopsies

	Variables	PU <0.92 g/24 h (n = 95)	PU ≥0.92 g/24 h (n = 91)	P value*
Banff lesion score distribution (0/1/2/3)	Interstitial inflammation (i)	53/16/18/8	51/16/20/4	0.707
	Tubulitis (t)	71/15/5/4	65/19/7/0	0.196
	Intimal arteritis (v)	83/8/4/0	87/4/0/0	0.074
	Glomerulitis (g)	37/29/22/7	50/15/21/5	0.083
	Peritubular capillaritis (ptc)	62/12/10/11	57/13/11/10	0.967
	Microvascular inflammation (MVI)[†]	48/47	56/35	0.131
	C4d	79/3/9/4	65/8/11/7	0.213
	Interstitial fibrosis/tubular atrophy (cict)	40/32/15/8	40/30/9/12	0.516
	Vascular fibrous intimal thickening (cv)	15/19/27/29[‡]	17/15/34/20[‡]	0.397
	GBM double contours (cg)	0/26/32/37	0/14/26/51	**0.041**
	Mesangial matrix expansion (mm)	60/21/11/3	44/21/18/8	0.096
	Arteriolar hyalinosis (ah)	8/9/19/59	6/4/22/59	0.529
Diagnostic categories, *n* (%)	Chronic-active ABMR	51 (53.68%)	47 (51.65%)	0.413
	Chronic ABMR	29 (30.53%)	23 (25.28%)	–
	iTG	15 (15.79%)	21 (23.08%)	–

ABMR, antibody-mediated rejection; GBM, glomerular basement membrane; iTG, isolated transplant glomerulopathy; PU, proteinuria.

*P values indicated group differences for proteinuria <0.92 g/24 h compared with proteinuria ≥0.92 g/24 h. Bold value indicated statistical significance.

[†]Sum of Banff scores g + ptc (range 0–6, 0–1/2–6).

[‡]Five missing values.

for relevant graft injury processes in kidney transplant patients [24]. Based on these cutoff values, enrolled TG patients were divided into four groups in our study and the result showed prognosis of TG patients depending on the degree of proteinuria. This result is in line with the findings of previous studies on all renal transplant recipients or rejection patients [24–26]. Our study focused on integrating 24-h proteinuria, Banff
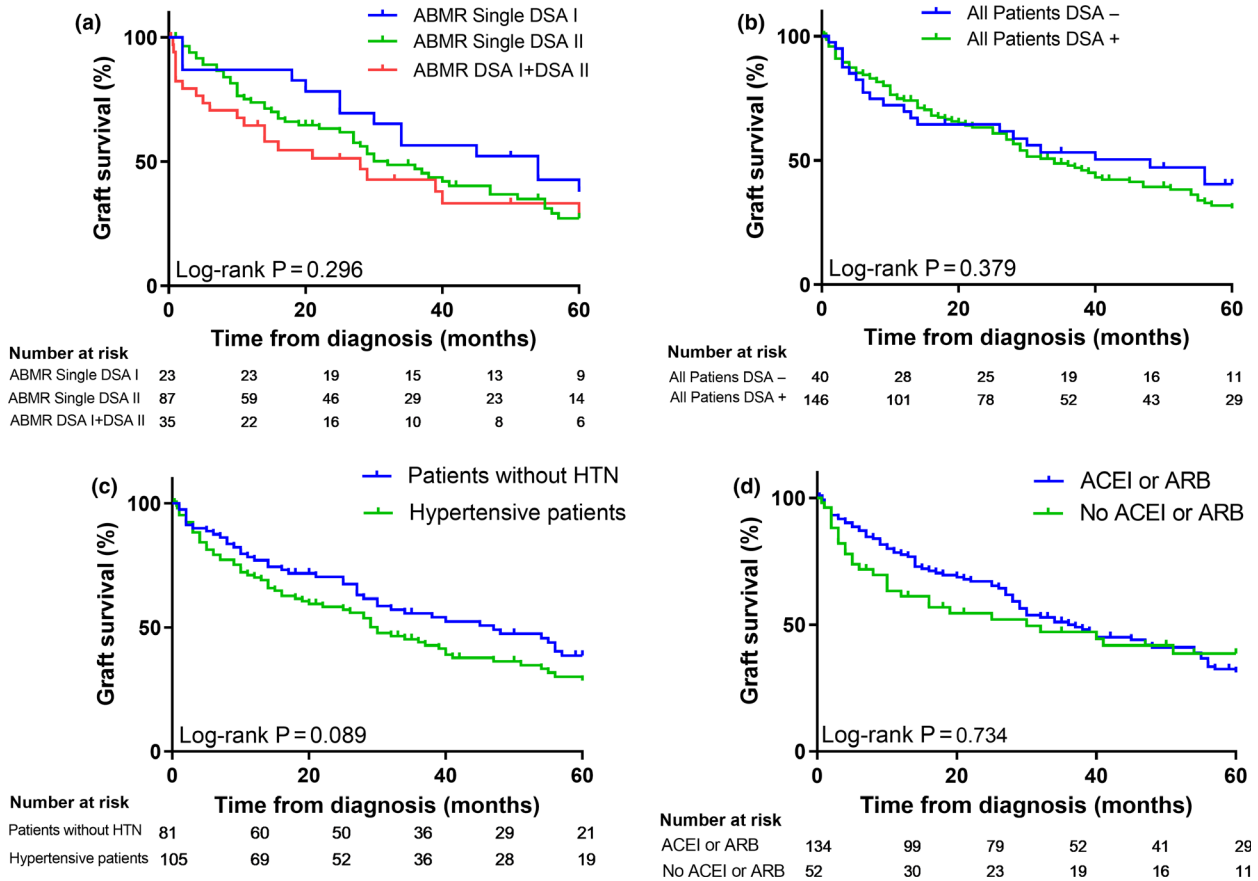
Figure 4 Kaplan–Meier survival curves for the association between DSA, hypertension, ACEI/ARB treatment, and post-biopsy graft loss. (a) ABMR patients presented no significant allograft survival differences within different status of DSA ($P = 0.296$). (b) Allograft survival in all patients with or without DSA showed no significant difference ($P = 0.379$). (c) TG patients with hypertension showed no significant difference in allograft survival ($P = 0.089$). (d) TG patients with ACEI/ARB treatment at the time of biopsy showed no superior graft outcomes ($P = 0.734$). ABMR, antibody-mediated rejection; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; DSA, anti-HLA donor-specific antibodies; HTN, hypertension; PU, proteinuria.

histological assessment, and long-term allograft survival data in TG patients and the results showed that proteinuria is an independent risk factor for graft loss in TG patients. This finding is of great importance given that proteinuria is a non-invasive marker and can be followed easily after kidney transplantation.

Blockade of the RAAS with ACEI or ARB may reduce the level of proteinuria, but study results are controversial regarding the long-term effect of these medications on allograft survival in kidney transplant patients [3,27–30]. Our data failed to show a clear improvement in proteinuria and allograft survival in TG patients treated with blockade of RAAS from the statistical perspective. This might, due to the influence of confounding variables and different drug doses, limit the interpretation of our result.

Most of the biopsies with TG had evidence of antibody-mediated injury [31]. The effect of different DSA on the survival of TG patients is still under discussion [7,16,32–34]. This may be due to different TG inclusion criteria and diagnostic methods of DSA. At the diagnosis of TG, we could not detect a significant effect of DSA on graft survival. However, as DSA is the cause for TG in around 80% of patients in our study, it is obvious that DSA is one of the most important risk factors for the development of TG.

We also reported C4d related to the graft loss independently from other prognostic factors in TG patients. This result was consistent with the results of previous studies, and our study included a larger sample size [34,35]. Of 39 patients with positive C4d deposition, all patients showed evidence of DSA before the biopsy. These results might link the antibody effect to allograft survival through complement. This can also be a possible explanation for the negative result of DSA on allograft survival to a certain extent. Taken together, our
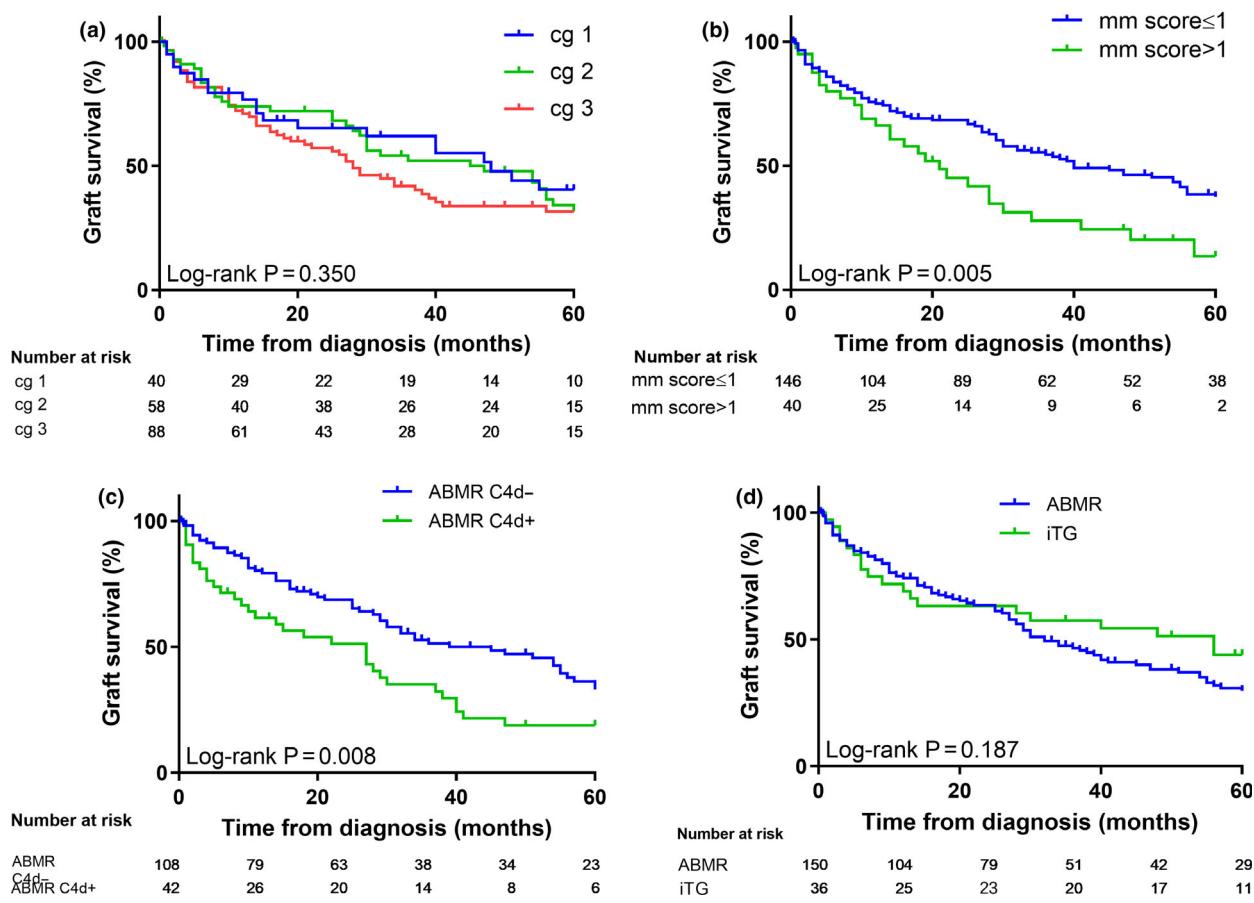
Figure 5 Allograft survival in patients with different pathological changes after the diagnosis of TG. (a)TG patients with different cg scores had no significant difference in graft prognosis (*P* = 0.350). (b) Patients with Banff lesion score mm lower than 2 had significantly better graft survival (*P* = 0.005). (c) Allograft survival probabilities in ABMR patient presence C4d were significantly lower by log-rank test (*P* = 0.008). (d) No significant difference was found in graft survival from different diagnostic categories (*P* = 0.187). ABMR, antibody-mediated rejection; cg, chronic allograft glomerulopathy; iTG, isolated transplant glomerulopathy; mm, mesangial matrix expansion.

Table 3. Association of proteinuria with the allograft outcome determined by univariable Cox regression analysis

Factor	*n*	HR	95% CI	*P* value
PU at biopsy (per g/24 h)	186	1.25	1.11-1.41	**<0.001**
PU at biopsy (in 4 categories)				
Normal (<0.3 g/24 h)	38	1	–	–
Mild (≥0.3 to <1 g/24 h)	61	1.33	0.72-2.45	0.363
Moderate (PU ≥1 to <3 g/24 h)	63	2.35	1.33-4.17	**0.003**
Severe (PU ≥3 g/24 h)	24	2.96	1.49-5.89	**0.002**

CI, confidence interval; HR, hazard ratio; PU, proteinuria.

Bold *P* values indicated statistical significance.

findings suggested that persisting complement-mediated injury to the allograft might contribute more to allograft loss.

In our study, the only histological feature independently associated with graft failure was mm score. Until now, this Banff lesion score was not in the focus especially regarding TG prognosis since it is only a descriptive score [1]. Mesangial matrix expansion can often be found in chronic cases of TG by light microscopy, but it develops much later compared with endothelial and subendothelial abnormalities [36–38]. These data suggest that the appearance of mesangial

Table 4. Independent risk factors for graft loss in patients with TG

Predictors	HR	95% CI	P value	C-statistics
History of rejection (per rejection episode)	1.27	1.03–1.42	0.028	0.54
PU at biopsy (per g/24 h)	1.26	1.11–1.42	<0.001	0.61
Cr at biopsy (per mg/dl)	1.51	1.32–1.72	<0.001	0.71
C4d (positive vs. negative)	2.05	1.33–3.16	<0.001	0.56
Mesangial matrix expansion (mm score >1)	2.00	1.25–3.19	0.004	0.55

CI, confidence interval; Cr, creatinine; HR, hazard ratio; PU, proteinuria; TG, transplant glomerulopathy.

Multivariable analysis of clinical and pathological characteristics included proteinuria at the time of biopsy, DSA, history of rejection, creatinine at the time of biopsy, hypertension, Banff lesion score cg, Banff lesion score mm, C4d. C-statistics was Harrell's C concordance statistics (range 0.5–1).

matrix expansion might represent the late state of TG with severe structural damage present, which might explain its negative effect on graft survival [35]. Expansion of the glomerular mesangial matrix *per se* leads to intercapillary sclerosis thus affect the glomerular filtration rate by reducing glomerular capillary surface area [39].

Currently, there is no standard treatment for TG, thus the enrolled patients in this study were treated according to the underlying cause. While most patients presented with ABMR, we were surprised to see that iTG had an almost identical prognosis compared to ABMR, strongly dependent on the amount of proteinuria.

Our study has all the limitations of retrospective cohort studies with potential undetected confounding factors. The number of patients with TG is limited, even in large centers [19,20] thus limiting the number of confounding variables, which can be included in the multivariable analysis. Another weakness is the fact that no data on electron microscopy were available. However, here we present a large and well-characterized cohort of patients with TG. The strength of our study is the complete follow-up without loss to follow-up and dynamic information of proteinuria over time. To fully identify the prognostic importance of 24-h proteinuria in TG patients, an independent external patient cohort should be considered for the validation and development of a prognostic score. While the reproducibility of the proposed cutoff for proteinuria is limited and rather small numbers and potential confounders limit the reproducibility of the final model, we strongly believe that the importance of proteinuria over histological scores will be confirmed in future studies. This point has been proven by Premaud et al in all kidney transplant patients, in which proteinuria was the second most important risk variable after creatinine [40].

In summary, our retrospective single-center results suggested that proteinuria was highly associated with allograft loss in patients with TG after kidney transplantation. Increasing proteinuria is associated with inferior outcomes, while histological cg score was not. We were able to identify additional, independent predictors for allograft survival after diagnosis of TG such as creatinine at biopsy, C4d, history of rejection, and the Banff lesion score mm. These factors might be used in the future to better stratify high-risk patients with TG for graft loss, so that appropriate treatment can be taken.

Conflict of interest

The authors have no relevant disclosures to make.

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