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CLINICAL RESEARCH

Glomerular C3 Deposition Is an Independent Risk Factor for Allograft Failure in Kidney Transplant Recipients With Transplant Glomerulopathy

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Introduction: Transplant glomerulopathy (TG) becomes increasingly prevalent in kidney transplant recipients over time, and it is strongly associated with allograft failure. To date, our prognostic biomarkers and understanding of the processes of immunologic injury in TG are limited.

Methods: This is a retrospective cohort analysis of kidney transplant recipients with TG (double contours of the glomerular basement membrane as defined by the chronic glomerulopathy score). Glomerular deposition of the complement protein C3 was determined, and its association with allograft survival was analyzed by Cox regression analysis.

Results: Of the 111 patients with TG, 72 (65%) had allograft failure, with a median follow-up time of 3 years from biopsy diagnosis of TG. C3-positive compared to C3-negative patients did not differ with respect to cause of end-stage renal disease, induction or maintenance immunosuppression, or sensitization. A greater proportion of patients with glomerular C3 deposition developed allograft failure compared to those with no C3 deposition (78% vs. 55%, $P = 0.01$). C3 deposition was independently associated with allograft failure in multivariate analyses (adjusted hazard ratio [HR] = 1.38, 95% confidence interval [CI] = 1.13−1.69, $P = 0.002$). There was no association between C4d or C1q deposition and allograft failure. Chronicity score was also associated with allograft failure in multivariate analysis (adjusted HR 1.26, 95% CI 1.12-1.41, $P = 0.0001$).

Conclusion: In this cohort of patients with TG, glomerular C3 deposition was independently associated with a higher risk of allograft failure. These findings identify glomerular C3 as a novel prognostic indicator in patients with TG.

Kidney Int Rep (2019) **4,** 582–593; https://doi.org/10.1016/j.ekir.2019.01.018
KEYWORDS: chronic rejection; complement; glomerulopathy; pathology; transplant

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Transplant glomerulopathy (TG) is characterized by the pathologic finding of duplication of the glomerular basement membrane on light or electron microscopy. A diagnosis of TG strongly predicts kidney allograft failure.[1−4] Transplant glomerulopathy results from chronic repetitive injury to the glomerular endothelium, and, in most cases, this process is attributed to alloimmune injury due to recurrent episodes of antibody-mediated rejection (ABMR). However, a significant proportion of cases of TG may be the result of other non-alloimmune processes that are injurious to the glomerular endothelial cells. Some such proposed mechanisms include injury from acute cellular rejection, thrombotic microangiopathy, autoantibodies, and hepatitis C virus. The cumulative incidence of TG increases over time, with up to 20% of allograft biopsy specimens demonstrating TG within 5 years after transplantation.[5] The incidence of TG is reportedly greater (up to 55% of patients) in certain high-risk populations such as positive crossmatch transplant recipients.[1] Recent studies have found that 38% of allografts fail within 5 years after the diagnosis of TG compared to only 5% in patients

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Received 16 January 2019; revised 22 January 2019; accepted 28 January 2019; published online 4 February 2019

without TG.[6] To date, our understanding of the mechanisms of immune injury resulting in TG are limited, and no effective therapeutics have been shown to reverse the poor outcomes in TG.[7] The numerous gaps in our understanding of the pathogenesis of TG impede our ability to accurately diagnose, prognosticate, and treat patients with TG.

The importance of the complement system in TG was highlighted in a recent study. Intragraft gene transcripts demonstrated upregulation of complement cascade genes in TG with allograft loss compared to those with a functioning allograft.[8] Traditionally, the deposition of C4d, the inactive complement breakdown product of the classical pathway, on peritubular capillaries is used as an indicator of complement injury due to active ABMR. However, the usefulness of C4d in the chronic setting and in TG is not clear, as reports of C4d positivity in TG vary widely.[5,9–12] Similar to the inconsistencies in the presence of C4d in TG, many studies of patients with TG have found that up to 30% of these patients lack donor-specific antibodies (DSAs).[5,13] These observations have led to theories about the involvement of other, as-yet undescribed immune mechanisms, such as other components of the complement cascade, in the glomerular injury of TG.

In prior studies, we demonstrated glomerular deposition of the complement protein C3 worsens native glomerular disease (focal segmental glomerulosclerosis and C3 glomerulonephritis).[14,15] Others also have observed that complement activation can exacerbate progression of native glomerular disease.[16–18] Glomerular C3 deposition is well described in TG with a scarcity of IgG, IgA, and C1q deposition.[4] The significance of glomerular complement C3 deposition and whether or not it is associated with outcomes in TG are unknown. These observations have led us to question whether we are missing an important immunologic mechanism of glomerular injury and allograft destruction in TG. We hypothesized that patients with evidence of complement-mediated glomerular injury in TG are at higher risk for allograft failure. To test this hypothesis, we analyzed the association of glomerular complement C3 deposition with allograft failure in a cohort of patients with TG to determine its prognostic significance.

MATERIALS AND METHODS

Patient Population and Study Design

This study was approved by the University of Wisconsin Madison Institutional Review Board and the Human Subjects Committee. All clinical and research activities performed were in accordance with the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008 ethical standards for human subjects. Patients eligible for this study included all kidney transplant recipients with a diagnosis of transplant glomerulopathy on biopsy between 1 January 2011 through 31 December 2014 performed at the University of Wisconsin Hospital and Clinics. Patients included in the study were required to have biopsy-proven transplant glomerulopathy, as defined by the Banff consensus guidelines[19] of a chronic glomerulopathy (cg) score of $\geq$1a. To meet the Banff criteria for a cg score of $\geq$1a, glomerular basement membrane double contours needed to be evident in $\geq$3 glomerular capillaries with associated endothelial cell swelling and/or subendothelial widening by electron microscopy or $\geq$1 nonsclerotic glomerular capillaries with double contour formation by light microscopy. Immunofluorescence staining on biopsy specimens was also required for study inclusion. Patients were excluded if the Banff cg score was 0 or if the biopsy specimen lacked immunofluorescence.

Clinical Data and Definition of Primary Outcome

Data were obtained from the Wisconsin Allograft Recipient Database (WisARD). Date of biopsy that first demonstrated a cg score $\geq$1a was used as the date of diagnosis of TG. The primary outcome was allograft failure following a biopsy diagnosis of TG. Allograft failure was defined as the combined endpoint of re-transplantation, return to dialysis, or patient death. Patients were followed until graft loss (re-transplantation or return to dialysis), death, or last available follow-up. Laboratory data (serum creatinine, urine protein-to-creatinine ratio, and DSAs) were obtained at the date of biopsy. Estimated glomerular filtration rate was determined from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[20] Data were extracted on maintenance immunosuppression and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use at the time of biopsy. Data were obtained on treatment for ABMR within 1 month of the biopsy diagnosis date.

Analysis of Allograft Pathology

Allograft biopsy was performed for clinical indication (increase in serum creatinine and/or proteinuria). Fixed sections of allograft tissue were stained with hematoxylin and eosin, periodic acid–Schiff, and Masson's trichrome stain for pathologic analysis. C4d staining was performed by immunohistochemical stain on frozen sections. Pathologic assessment of all allograft biopsy specimens was analyzed by clinical renal pathologists. Pathologic diagnosis and scoring of Banff criteria were performed using the Banff consensus guidelines for transplant pathology using the defined criteria for scoring on a scale of 0 to 3.[19] Microvascular inflammation was calculated from the sum of peritubular capillaritis and glomerulitis scores (microvascular

inflammation score range 0−6). Chronicity score was calculated from the sum of chronic glomerulopathy, interstitial fibrosis, tubular atrophy, and vascular fibrous intimal thickening scores (chronicity score range 0−12). Immunofluorescent staining of C1q, C3, IgA, IgM, and IgG was performed on frozen sections. Clinical renal pathologists performed qualitative assessment of the degree of immunofluorescent staining of C1q, C3, IgA, IgM, and IgG on allograft biopsy specimens. The degree of immunofluorescent stain intensity was reported on a scale of 0 to 3, with 0 representing negative staining; 1, low-intensity staining; 2, medium-intensity staining; or 3, high-intensity staining.

Protocol for Treatment of Antibody-Mediated Rejection

Antibody-mediated rejection treatment protocols at our institution were performed as previously described.[21,22] Briefly, treatment is based on both the severity and time after transplantation of the rejection episode. Rejection diagnosed >3 months after transplantation is treated with dexamethasone, 100 mg i.v. with taper, and i.v. Ig, 200 mg/kg i.v. every 2 weeks × 3. Rituximab, 375 mg/m^2 i.v. single dose, is added based on clinical characteristics. Clinical characteristics of patients more likely to receive rituximab include younger age, better kidney function, higher DSA, diffuse C4d, greater microvascular inflammation, and lower chronicity score. Baseline immunosuppression is also increased by 25%. Maintenance immunosuppression regimen is triple therapy with tacrolimus (12-hour trough goal 5−7 ng/dl at 6 months following transplantation), mycophenolic acid 720 mg twice daily, and prednisone 5 mg daily. Immunosuppression doses are adjusted if adverse events occur.

Statistical Analysis

Continuous variables were expressed as the mean with SD or as median with interquartile range, as appropriate. Comparisons between groups were performed using *t* tests and Kruskal−Wallis tests. Categorical variables were expressed as frequencies with proportions and compared between groups using χ^2 or Fisher exact tests. Time-to-event data estimates were obtained using Kaplan−Meier curves and log-rank test. Cox proportional hazard models were used to assess hazard ratios and 95% confidence intervals between patient or biopsy specimen characteristics with the composite primary outcome of allograft failure. Then sequential adjustment of parameters with $P < 0.05$ was used in a stepwise fashion in a multivariable Cox regression model to assess the independent association of patient or biopsy specimen characteristics with the composite endpoint of allograft failure. Validity of proportional hazards assumptions were tested using Schoenfeld residuals. Two-sided *P* values <0.05 were considered statistically significant. All analyses were performed using MedCalc, Version 18.5 (MedCalc Software, Ostend, Belgium) or Stata Statistical Software, Release 13 (StataCorp, College Station, TX).

RESULTS

Demographic and Clinical Characteristics at Time of Transplant

Of the 1154 kidney transplant recipients who underwent allograft biopsy between the years 2011 to 2014, 111 patients (10%) were diagnosed with TG (Figure 1). The median time from transplantation to the biopsy diagnosis of TG was 8.3 years (range 4 months to 32.3 years). Transplant glomerulopathy was defined as chronic glomerulopathy (cg) score ≥1a based on Banff consensus guidelines.[19] Of the 111 patients diagnosed with TG, 8% (n = 9) had a cg score of 1a; 31% (n = 34) had a cg score of 1b; 16% (n=18) had a cg score of 2; and 45% (n = 50) had a cg score of 3. The mean age at time of biopsy diagnosis of TG was 51.3 ± 12.9 years (Table 1). The major causes of end-stage renal disease (ESRD) included diabetic nephropathy or glomerulonephritis.

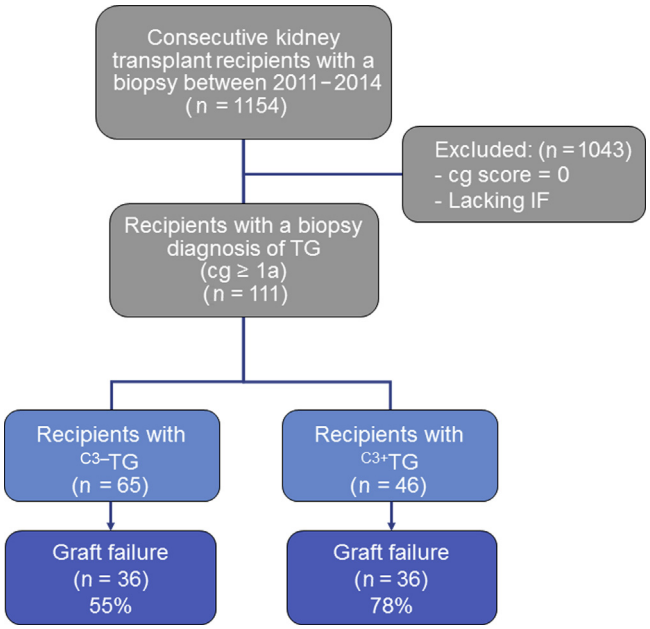

Figure 1. Flow diagram of study cohort and outcomes in transplant recipients with transplant glomerulopathy. From 2011 through 2014, there were 1154 kidney transplant recipients who underwent allograft biopsy. Patients were excluded if the cg score on biopsy was 0 or if the biopsy specimen lacked immunofluorescence studies. There were 111 transplant recipients included in the study with transplant glomerulopathy on biopsy (as defined by a chronic glomerulopathy [cg] score of ≥1a). All patients with transplant glomerulopathy (TG) were grouped into $^{C3-}$TG (C3 score of 0) or $^{C3+}$TG (C3 score ≥1) TG. Allograft failure occurred in 55% of the patients with $^{C3-}$TG compared to 78% in $^{C3+}$TG ($P = 0.01$). IF, immunofluorescence.

The majority of patients were white and male, and only 2% were hepatitis C positive. Patients had a mean of 3.7 ± 1.5 human leukocyte antigen (HLA) mismatches. The majority of patients received basiliximab (45%) as an induction agent. The cohort of 111 patients with TG was stratified according to C3 deposition on biopsy specimens as either C3-negative TG ($^{C3-}$TG, C3 score of 0 on immunofluorescent staining, n = 65) or C3-positive TG ($^{C3+}$TG, C3 score of ≥ 1 on immunofluorescent staining, n = 46). There were no significant differences between $^{C3-}$TG compared to $^{C3+}$TG in terms of age, sex, race, cause of ESRD, hepatitis C status, donor status, sensitization, number of HLA mismatches, or induction regimen (Table 1).

Clinical Characteristics at Time of Biopsy Diagnosis of Transplant Glomerulopathy

At time of biopsy diagnosis of TG for the overall cohort (n = 111), the mean serum creatinine was 2.2 ± 0.9 mg/dl, the median degree of proteinuria was 2.0 [0.9−3.4] g/g, and 71% of TG patients were DSA positive (Table 2). When stratified by glomerular C3 deposition, patients with $^{C3+}$TG compared to $^{C3-}$TG had a higher serum creatinine ($^{C3+}$TG: 2.4 ± 1.1 vs. $^{C3-}$TG: 2.0 ± 0.7 mg/dl, $P = 0.01$), a higher degree of proteinuria ($^{C3+}$TG: 2.7 [1.7−4.6] vs. $^{C3-}$TG: 1.6 [0.9−3.4] g/g, $P = 0.0009$), and were less likely to receive treatment with i.v. corticosteroids and i.v. Ig. ($^{C3+}$TG: 4% vs. $^{C3-}$TG: 29%, $P = 0.001$) (Table 2). Of patients with positive DSA values, the median sum mean fluorescence intensity values between $^{C3-}$TG and $^{C3+}$TG were similar. The majority of patients in both the $^{C3-}$TG and $^{C3+}$TG groups were on triple maintenance immunosuppressive therapy with prednisone, calcineurin inhibitor, and mycophenolate.

Histologic Characteristics at Time of Biopsy Diagnosis of Transplant Glomerulopathy

At time of biopsy diagnosis of TG, 51% of the overall cohort had pertitubular capillary deposition of C4d, and 61% had chronic active ABMR by the most recent Banff criteria[19] (Table 3). When stratified by glomerular C3 deposition, the Banff scores for chronic glomerulopathy (cg), C4d deposition, glomerulitis, and the chronicity score were not significantly different between $^{C3-}$TG and $^{C3+}$TG (Table 3). Pathologic diagnoses of thrombotic microangiopathy or chronic active ABMR, as defined by the Banff criteria,[19] were also not

Table 1. Baseline demographic and clinical characteristics of kidney transplant recipients

Variable	Overall n = 111	C3− n = 65	C3+ n = 46	*P* value[a]
Demographics				
Age at transplantation, yr, mean ± SD	42.3 ± 12.8	43.2 ± 12.2	41.5 ± 13.6	0.50
Age at biopsy, yr, mean ± SD	51.3 ± 12.9	52.1 ± 12.4	50.2 ± 13.5	0.50
Sex (male), n (%)	64 (58%)	33 (51%)	31 (67%)	0.08
Race (white), n (%)	102 (92%)	60 (92%)	42 (91%)	0.90
Cause of ESRD, n (%)				
DM	27 (24%)	12 (19%)	15 (33%)	0.09
HTN	5 (5%)	4 (6%)	1 (2%)	0.90
GN[b]	39 (35%)	22 (34%)	17 (37%)	0.70
PKD	10 (9%)	8 (12%)	2 (4%)	0.20
Other[c]	30 (27%)	19 (29%)	11 (24%)	0.50
Hepatitis C positive, n (%)	2 (2%)	0 (0%)	2 (4%)	0.07
Transplant characteristics				
Prior transplant, n (%)	12 (11%)	7 (11%)	5 (11%)	1.00
Deceased donor, n (%)	61 (55%)	34 (52%)	27 (59%)	0.50
PRA (%), median [IQR]	0 [0−19][d]	5 [0−14][e]	0 [0−56][f]	0.72
HLA total mismatches, mean ± SD	3.7 ± 1.5	3.5 ± 1.5	3.9 ± 1.5	0.10
Induction agent				
Alemtuzumab, n (%)	25 (23%)	12 (18%)	13 (28%)	0.30
Basiliximab, n (%)	50 (45%)	29 (45%)	21 (46%)	1.00
Thymoglobulin, n (%)	22 (20%)	13 (20%)	9 (20%)	1.00
Other, n (%)	11 (10%)	8 (12%)	3 (6%)	0.40

DM, diabetes; ESRD, end-stage renal disease; GN, glomerulonephritis; HLA, human leukocyte antigen; HTN, hypertension; IQR, interquartile range; PKD, polycystic kidney disease; PRA, panel reactive antibody.
[a]*P* value indicates group differences for C3− transplant glomerulopathy (TG) compared to C3+ TG.
[b]Glomerulonephritis diagnoses (n = 39): IgA nephropathy (n = 12), membranous nephropathy (n = 6), lupus nephritis (n = 6), antineutrophil cytoplasmic antibody vasculitis (n = 3), focal segmental glomerulosclerosis (n = 3), hemolytic uremic syndrome (n = 2), Alport syndrome (n = 1), chronic glomerulonephritis (n = 5), and thin basement membrane (n = 1).
[c]Other diagnoses (n = 30): reflux nephropathy (n = 6), hypoplasia (n = 3), obstructive (n = 2), renal artery thrombosis (n = 2), prune belly (n = 1), hepatorenal (n = 1), ischemia (n = 1), cystinosis (n = 1), unknown (n = 13).
[d]Seventy missing values.
[e]Forty missing values.
[f]Thirty missing values.

Table 2. Clinical characteristics at time of biopsy diagnosis of transplant glomerulopathy (TG)

Variable	Overall n = 111	C3− n = 65	C3+ n = 46	*P* value[a]
Laboratory characteristics				
Serum creatinine (mg/dl), mean ± SD	2.2 ± 0.9	2.0 ± 0.7	2.4 ± 1.1	0.01
eGFR (ml/min per 1.73 m^2), mean ± SD	41 ± 21	43 ± 20	38 ± 23	0.30
UP/Cr (g/g), median [IQR]	2.0 [0.9–3.4][b]	1.6 [0.5–2.7][c]	2.7 [1.7–4.6][d]	0.0009
Class I or II DSA positive, n (%)	73 (71%)[e]	50 (77%)[f]	23 (50%)[g]	0.02
Class I DSA positive, n (%)	37 (36%)[e]	25 (40%)[f]	12 (30%)[g]	0.32
Class II DSA positive, n (%)	65 (64%)[e]	45 (71%)[f]	20 (51%)[g]	0.04
DSA (sum MFI) (for patients with positive DSA)				
Class I, median [IQR]	1510 [841–5251]	1510 [910–5251]	1620 [640–6330]	0.85
Class II, median [IQR]	6663 [1721–21,775]	9416 [2212–22,822]	2759 [1442–10,306]	0.15
Class I + II, median [IQR]	6663 [2138–21,782]	9870 [3025–22,738]	3886 [1405–10,692]	0.08
Medications, n (%)				
Prednisone	108 (97%)	64 (98%)	44 (95%)	0.60
Calcineurin inhibitor	94 (85%)	55 (85%)	39 (85%)	1.00
Mycophenolate	98 (88%)	54 (83%)	44 (96%)	0.07
ACE inhibitor or ARB	47 (42%)	24 (37%)	23 (50%)	0.20
ABMR treatment, n (%)				
i.v. Steroids + i.v. Ig	21 (19%)	19 (29%)	2 (4%)	0.001
i.v. Steroids + i.v. Ig + rituximab	7 (6%)	6 (9%)	1 (2%)	0.20

ABMR, antibody-mediated rejection; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; MFI, mean fluorescence intensity; UP/Cr, urine protein-to-creatinine ratio.
[a]*P* value indicates group differences for C3− TG compared to C3+ TG.
[b]Five missing values.
[c]Three missing values.
[d]Two missing values.
[e]Eight missing values.
[f]Three missing values.
[g]Five missing values.

significantly different between $^{C3-}$TG and $^{C3+}$TG (Table 3). The scores for inflammation were slightly higher in $^{C3-}$TG (peritubular capillaritis, microvascular inflammation, tubulitis, and interstitial inflammation), but chronicity (tubular atrophy) was greater in $^{C3+}$TG compared to $^{C3-}$TG. The deposition of the complement protein C1q was significantly higher in $^{C3+}$TG compared to $^{C3-}$TG ($^{C3+}$TG 1.0 ± 0.9 vs. $^{C3-}$TG 0.4 ± 0.6, $P < 0.0001$). Deposition of IgM was also greater in $^{C3+}$TG compared to $^{C3-}$TG ($^{C3+}$TG: 1.8 ± 0.8 vs. $^{C3-}$TG: 1.1 ± 0.8, $P < 0.0001$). Representative images of the typical pathologic features observed in transplant recipients with TG are shown in Figure 2. Double contour formation of the glomerular basement membrane is evident by light microscopy (blue arrows), and glomerular deposition of C3 was observed in regions of the glomerular capillary loops and mesangium (Figure 2a and b). Glomerular C3 deposition was located primarily in glomerular capillary walls (70% of $^{C3+}$TG) (Table 4). C3 deposition was in a focal segmental pattern with a granular quality (Table 4). Glomerular C3 deposition correlated with IgM ($R^2 = 0.47$, $P < 0.0001$) and also correlated with glomerular C1q deposition ($R^2 = 0.42$, $P < 0.0001$). The proportion of glomeruli with global glomerulosclerosis for the entire cohort with TG was 18% (Supplementary Table S1). Global glomerulosclerosis was similar between $^{C3-}$TG compared to $^{C3+}$TG (16% vs. 22%, respectively, $P = 0.12$, Supplementary Table S1).

C3 Deposition Was Associated With Allograft Failure in Transplant Glomerulopathy

Overall, 72 (65%) of the 111 transplant recipients with TG reached the primary outcome of allograft failure after a biopsy diagnosis of TG over a median follow-up time of 3.0 years (range 1 day to 6.7 years). The proportion of transplant recipients with uncensored graft failure was significantly greater in the $^{C3+}$TG group compared to $^{C3-}$TG (78% vs. 55%, $P = 0.01$) (Figure 1). Kaplan–Meier survival analysis found a significantly greater proportion of patients with uncensored allograft failure in $^{C3+}$TG compared to $^{C3-}$TG ($P = 0.008$) (Figure 2c). Kaplan–Meier analysis performed for death-censored allograft failure also demonstrated a significantly higher proportion of allograft failure in $^{C3+}$TG compared to $^{C3-}$TG ($P = 0.03$). When C3 deposition was stratified by intensity of deposition on biopsy specimens, patients with a higher C3 score had a greater proportion of patients with allograft failure compared to those with less C3 deposition ($P = 0.05$) (Figure 2d).

A Cox proportional hazards model was used to test the association between the baseline characteristics, clinical characteristics at time of diagnosis of TG, and histologic characteristics with allograft failure in TG.

Table 3. Histologic features at time of biopsy diagnosis of transplant glomerulopathy (TG)

	Overall N = 111	C3− n = 65	C3+ n = 46	*P* value[a]
Banff score (score range 0–3), mean ± SD				
Tubulitis (t)	0.1 ± 0.4	0.2 ± 0.4	0.0 ± 0.2	0.03
Interstitial inflammation (i)	0.2 ± 0.6	0.3 ± 0.6	0.1 ± 0.3	0.04
Vasculitis (v)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.00
Peritubular capillaritis (ptc)	0.5 ±0.7	0.6 ± 0.7	0.3 ± 0.5	0.02
Glomerulitis (g)	0.8 ± 0.9	0.9 ± 0.9	0.6 ± 0.8	0.10
Microvascular inflammation (mvi)[b]	1.0 ± 1.2	1.2 ± 1.3	0.6 ± 1.0	0.008
C4d	0.9 ± 1.1	1.1 ± 1.2	0.7 ± 0.9	0.09
Arteriolar hyaline thickening (ah)	1.5 ± 1.2	1.3 ± 1.2	1.7 ± 1.2	0.10
Mesangial matrix increase (mm)	1.2 ± 0.8	1.1 ± 1.0	1.2 ± 0.7	0.60
Tubular atrophy (ct)	1.4 ± 0.8	1.3 ± 0.7	1.6 ± 0.7	0.05
Chronic interstitial fibrosis (ci)	1.4 ± 0.7	1.3 ± 0.7	1.5 ± 0.7	0.08
Vascular fibrous intimal thickening (cv)	1.1 ± 0.9	1.2 ± 0.9	1.0 ± 0.7	0.30
Vhronic glomerulopathy (cg)	2.0 ± 0.9	2.1 ± 1.0	1.9 ± 1.0	0.50
Chronicity score[c]	5.9 ± 2.2	5.8 ± 2.4	6.0 ± 2.0	0.70
Immunohistochemistry, n (%)				
ptc C4d	56 (51%)	34 (52%)	22 (48%)	0.64
Immunofluorescence score (score range 0–3), mean ± SD				
C1q	0.6 ± 0.8	0.4 ± 0.6	1.0 ± 0.9	<0.0001
C3	0.8 ± 1.1	0.0 ± 0.0	2.0 ± 0.9	<0.0001
IgA	0.3 ± 0.8	0.2 ± 0.6	0.5 ± 1.0	0.05
IgG	0.2 ± 0.6	0.1 ± 0.4	0.3 ± 0.7	0.07
IgM	1.4 ± 0.8	1.1 ± 0.8	1.8 ± 0.8	<0.0001
Electron microscopy findings, n (%)				
Immune complexes	36 (37%)[d]	13 (24%)[e]	23 (53%)[f]	0.003
Hyaline aggregates	11 (11%)	6 (9%)	5 (11%)	0.94
Pathologic diagnoses, n (%)				
Chronic active ABMR[g]	68 (61%)	42 (65%)	26 (57%)	0.47
cg with DSA+ (C4d−, mvi−)[h]	20 (18%)	13 (20%)	7 (15%)	0.52
cg with mvi+ (DSA−, C4d−)[h]	5 (5%)	3 (5%)	2 (4%)	0.95
cg with DSA−, C4d−, mvi−[h]	12 (11%)	6 (9%)	6 (13%)	0.53
cg with DSA n/a, C4d−, mvi−[h]	5 (5%)	1 (1%)	5 (11%)	0.03
Thrombotic microangiopathy	1 (1%)	1 (1%)	0 (0%)	1.00

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; n/a, not available; ptc, peritubular capillary.
[a]*P* value indicates group differences for C3− TG compared to C3+ TG.
[b]Score range 0–6.
[c]Score range 0–12.
[d]Fourteen missing values.
[e]Eleven missing values.
[f]Three missing values.
[g]Chronic active ABMR defined as cg≥1a and (DSA+ or C4d+) and (C4d+ or mvi≥2).
[h]mvi− defined as mvi<2. mvi+ defined as mvi≥2.

On unadjusted survival analysis, C3 deposition (hazard ratio [HR] = 1.27, 95% confidence interval [CI] = 1.06–1.52, $P = 0.009$), arteriolar hyaline thickening (HR = 1.37, 95% CI = 1.12–1.67, $P = 0.003$), and chronicity indices (chronic interstitial fibrosis, tubular atrophy, vascular fibrous intimal thickening, and chronicity score (HR = 1.23, 95% CI = 1.11–1.36, $P = 0.0001$)) were associated with allograft failure (Table 5). C3 deposition (adjusted HR [aHR] = 1.38, 95% CI = 1.13–1.69, $P = 0.002$), arteriolar hyaline thickening (aHR = 1.28, 95% CI = 1.04–1.58, $P = 0.02$), and chronicity score (aHR = 1.26, 95% CI = 1.12–1.41, $P = 0.0001$) remained independent risk factors for allograft failure in TG after adjustment for all of the predictors significantly associated with allograft outcomes (C3, arteriolar hyaline thickening, tubulitis, chronic interstitial fibrosis, tubular atrophy, vascular fibrous intimal thickening, and chronicity score). The same multivariate analysis was performed for death-censored graft failure, which also demonstrated C3 deposition (aHR = 1.56, 95% CI = 1.21–1.99, $P = 0.0005$) and chronicity score (aHR = 1.35, 95% CI = 1.17–1.56, $P < 0.0001$) were independently associated with allograft failure in TG. Proportional hazards test did not violate the proportional hazards assumption by Schoenfeld residuals ($P > 0.14$). Tubulitis was associated with a reduced risk of allograft failure on univariate analysis; however, this was not retained in multivariate analysis. Baseline characteristics and clinical characteristics at time of biopsy were not associated with

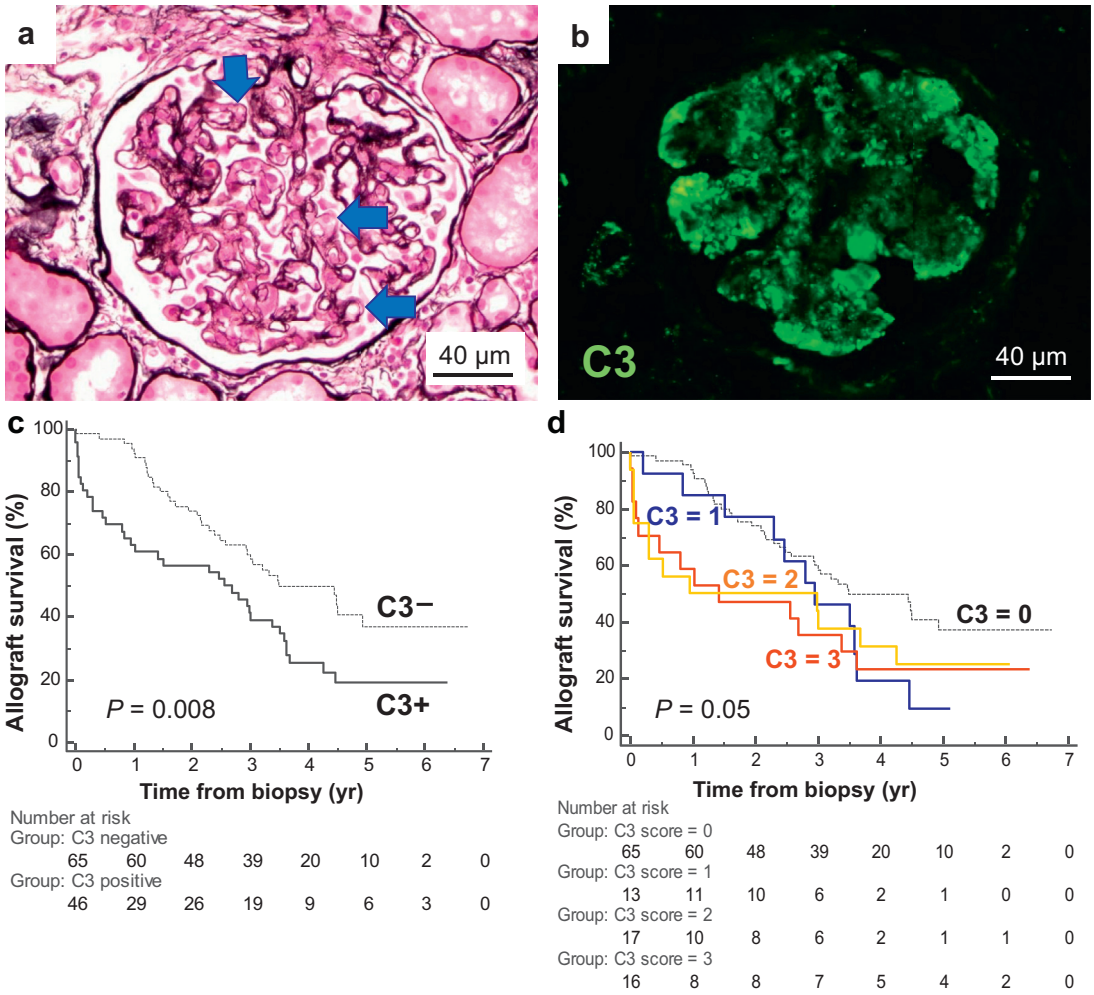

Figure 2. C3 complement deposition in transplant glomerulopathy corresponded with allograft failure. (a) Representative silver-stained allograft biopsy with transplant glomerulopathy is shown. Blue arrows highlight areas of double contour formation of the glomerular basement membrane (original magnification ×400). (b) C3 deposition in transplant glomerulopathy (TG) was seen in endothelial and mesangial areas of the glomerulus (original magnification ×400). (c) $^{C3+}$TG (C3 score ≥1) had a higher proportion of patients with allograft failure compared to $^{C3-}$TG transplant recipients (C3 score of 0) ($P = 0.008$). (d) C3 deposition was stratified into groups with no C3 (C3 = 0), low (C3 = 1), medium (C3 = 2), or high (C3 = 3) level of staining. Higher levels of C3 deposition corresponded to a higher proportion of patients with allograft failure ($P = 0.05$).

allograft failure in the cohort of transplant recipients with TG. If the 2 groups, cg (with DSA−, C4d−, mvi−) and cg (with DSA not available, C4d−, mvi−), were removed from the risk assessment for allograft failure, there was an 18% higher risk of allograft failure associated with C3 deposition by univariate analysis (HR = 1.18, 95% CI = 0.95–1.47, $P = 0.1$).

Table 4. Glomerular C3 immunofluorescence characteristics at time of biopsy diagnosis of transplant glomerulopathy

	C3+ n = 46	C3=1 n = 13	C3=2 n = 17	C3=3 n = 16
Intraglomerular location				
Capillary wall	32 (70%)	8 (62%)	13 (76%)	11 (69%)
Mesangial	7 (18%)	4 (31%)	1 (6%)	2 (13%)
Mesangial and capillary wall	7 (18%)	1 (8%)	3 (18%)	3 (19%)
Intraglomerular distribution				
Segmental	32 (70%)	7 (54%)	12 (71%)	13 (81%)
Global	14 (30%)	6 (46%)	5 (29%)	3 (19%)
Intrarenal distribution				
Focal	32 (70%)	8 (62%)	11 (65%)	13 (81%)
Diffuse	14 (30%)	5 (39%)	6 (35%)	3 (19%)
Staining pattern				
Granular	46 (100%)	13 (100%)	17 (100%)	16 (100%)
Linear	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Kaplan−Meier survival curves demonstrated the proportion of transplant recipients with allograft failure was not significantly different between those positive compared to negative for C1q deposition ($P = 0.51$), peritubular capillary C4d deposition ($P = 0.99$), class I DSA ($P = 0.56$), or class II DSA ($P = 0.51$) (Figure 3).

DISCUSSION

We found that glomerular C3 deposition was associated with allograft failure in kidney transplant recipients with TG. This association was independent of other demographic, clinical, therapeutic, and histologic characteristics (with the exception of chronicity and arteriolar hyalinosis). In addition, with greater intensity of glomerular C3 deposition, there was a

Table 5. Risk factors for allograft failure in patients with TG

	Unadjusted			Multivariate[a]		
Predictor	**HR**	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Baseline characteristics						
Age at biopsy	1.01	0.98–1.02	0.61			
Sex	0.84	0.53–1.35	0.47			
Race	1.48	0.68–3.23	0.32			
ESRD due to GN	0.92	0.56–1.49	0.72			
ESRD due to DM	1.08	0.64–1.82	0.78			
ESRD due to HTN	1.84	0.66–5.10	0.24			
ESRD due to PKD	1.01	0.44–2.33	0.98			
ESRD, other	0.90	0.53–1.54	0.71			
Deceased donor	0.75	0.46–1.20	0.22			
Prior transplant	0.50	0.22–1.14	0.10			
HLA mismatch	1.06	0.90–1.25	0.46			
Induction agent						
Alemtuzumab	1.29	0.75–2.22	0.37			
Basiliximab	0.80	0.50–1.27	0.33			
Thymoglobulin	1.24	0.71–2.17	0.44			
Other	0.89	0.38–2.05	0.78			
Clinical characteristics at time of biopsy						
Serum creatinine	1.13	0.87–1.47	0.38			
eGFR	1.00	0.99–1.01	0.73			
UP/Cr	1.05	0.95–1.16	0.36			
DSA positive	0.82	0.48–1.40	0.47			
Medications						
Prednisone	2.28	0.32–16.40	0.41			
CNI	0.56	0.31–1.03	0.06			
Mycophenolate	0.75	0.37–1.52	0.43			
ACE inhibitor or ARB	1.44	0.91–2.30	0.12			
ABMR Treatment						
i.v. Steroids+i.v. Ig	0.70	0.38–1.27	0.24			
i.v. Steroids+i.v. Ig+rituximab	0.37	0.09–1.53	0.17			
Biopsy characteristics						
Tubulitis (t)	0.24	0.08–0.74	0.01	-	-	-
Interstitial inflammation (i)	0.56	0.30–1.40	0.07			
Peritubular capillaritis (ptc)	1.02	0.72–1.45	0.91			
Glomerulitis (g)	1.10	0.87–1.40	0.42			
Microvascular inflammation (mvi)	0.98	0.80–1.19	0.82			
C4d	0.99	0.81–1.22	0.95			
Arteriolar hyaline thickening (ah)	1.37	1.12–1.67	0.003	1.28	1.04-1.58	0.02
Mesangial matrix increase (mm)	1.18	0.90–1.55	0.22			
Tubular atrophy (ct)	1.56	1.16–2.10	0.003	-	-	-
Chronic interstitial fibrosis (ci)	1.67	1.22–2.28	0.001	-	-	-
Vascular fibrous intimal thickening (cv)	1.43	1.10–1.87	0.008	-	-	-
Chronic glomerulopathy (cg)	1.26	0.99–1.61	0.06			
Chronicity score	1.23	1.11–1.36	0.0001	1.26	1.12-1.41	0.0001
C1q	1.22	0.92–1.63	0.17			
C3	1.27	1.06–1.52	0.009	1.38	1.13-1.69	0.002
IgA	0.92	0.70–1.22	0.57			
IgG	1.23	0.85–1.78	0.28			
IgM	1.09	0.83–1.41	0.54			
Chronic active ABMR	1.01	0.62–1.64	0.95			

ABMR, antibody-mediated rejection; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CI, confidence interval; DM, diabetes; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GN, glomerulonephritis; HLA, human leukocyte antigen; HR, hazard ratio; HTN, hypertension; MFI, mean fluorescence intensity; PKD, polycystic kidney disease; PRA, panel reactive antibody; TG, transplant glomerulopathy; UP/Cr, urine protein-to-creatinine ratio
[a]Multivariate model includes the variables t, ah, ci, ct, cv, chronicity score, and C3.

stepwise increase in the proportion of patients with allograft failure in TG.

The involvement of the complement system in antibody-mediated glomerular injury has been appreciated since the 1960s.[23,24] More recently, researchers have demonstrated the importance of complement activation and the presence of glomerular C3 deposition in a multitude of native glomerular

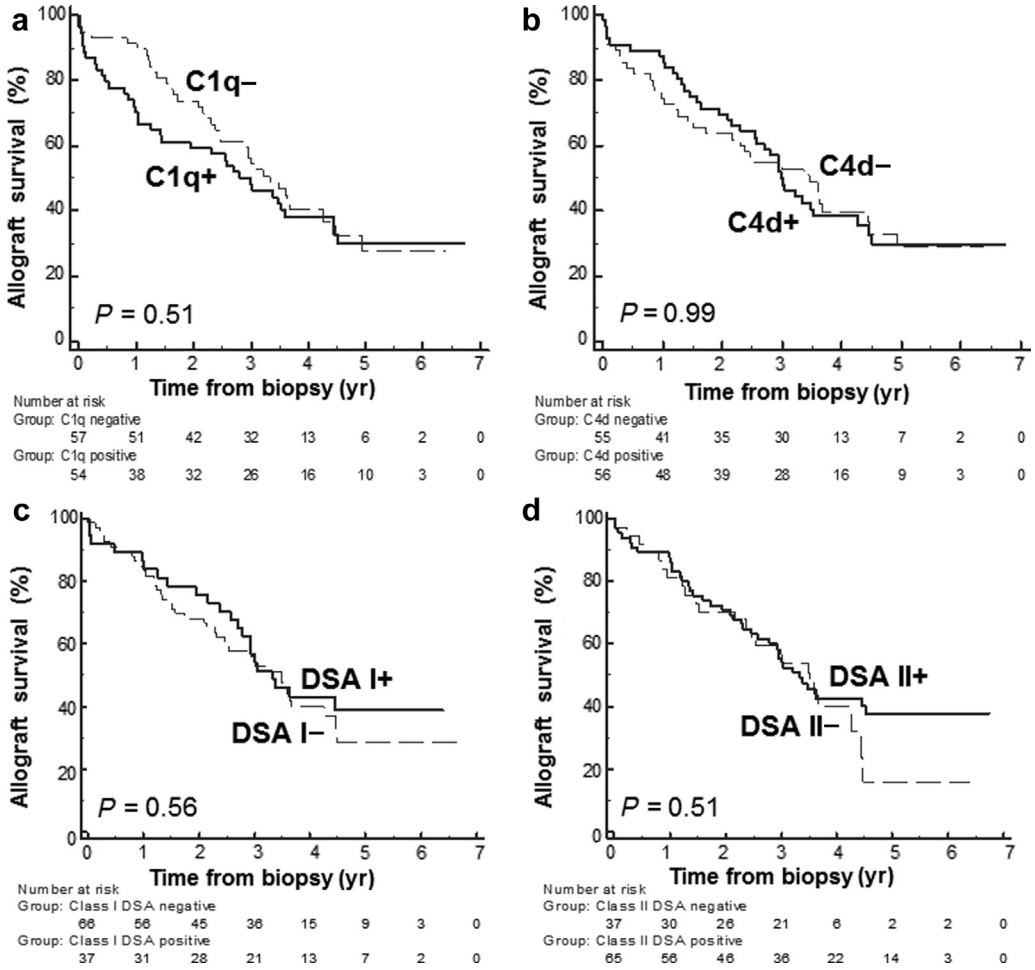

Figure 3. Kaplan–Meier curves of allograft survival demonstrated no differences according to presence or absence of C1q deposition, C4d deposition, or donor-specific antibody (DSA) in patients with transplant glomerulopathy (TG). (a,b) Transplant recipients with TG had no significant difference in allograft survival based on the deposition of the complement proteins C1q or C4d on biopsy ($P = 0.51$ and $P = 0.99$, respectively). (c,d) Allograft survival was similar among TG patients with and without DSA (class I DSA: $P = 0.56$; class II DSA: $P = 0$ .51).

diseases including focal segmental glomerulosclerosis (FSGS),[14] IgA,[16,25,26] membranous nephropathy,[17] antineutrophil cytoplasmic antibody (ANCA)–associated crescentic glomerulonephritis,[27] postinfectious glomerulonephritis,[28] lupus nephritis,[18] and C3 glomerulopathy.[15,29,30] In this study, we found evidence that glomerular C3 deposition is an independent risk factor for allograft failure in TG. Why the glomerulus is uniquely susceptible to complement-mediated injury in native kidney disease and in kidney transplantation is an area of active investigation. All resident renal cells express complement regulatory proteins on their surface.[31,32] As evidenced by the numerous glomerular diseases in which complement plays an injurious role, it appears that the regulatory ability of these molecules can often be overcome or that the renal microenvironment allows for local activation.[33,34]

Complement activation, endothelial cell injury, and inflammatory cell infiltration lead to the structural remodeling seen in TG. Recent advances in the study of glomerular disease support moving beyond standard descriptive histopathology to incorporate disease pathogenesis into a clinically relevant classification schema.[35] We propose C3 as a novel biomarker in TG with prognostic importance for long-term allograft survival. As a biomarker, C3 staining is readily available but not always used in the assessment of transplant biopsy specimens. Tissue staining for C3 typically detects the tissue bound complement fragment C3b, and therefore represents a global marker for complement activation of all 3 pathways of the complement system. Thus, C3 deposition in TG may represent either unchecked alternative pathway activation or ongoing antibody-driven complement activation through the classical pathway.

In our study, glomerular C3 but not peritubular capillary C4d deposition corresponded to a greater risk of allograft failure in TG. Similarly, a recent study in TG found that intragraft gene transcripts associated with the complement cascade and endothelial cells were upregulated in patients with allograft failure.[8]

However, the investigators found no differences in demographics or clinical variables (PRA, DSA, C4d) when comparing individuals with allograft failure to those with a functioning allograft in TG.[8] Also, support for our observations is demonstrated by mechanistic studies in animal transplantation models. In animal models of kidney transplantation[36] and of skin transplantation,[37] C3 deficiency prolonged allograft survival and attenuated T- and B-cell function.[37,38] Taken together, these observations suggest that ongoing complement-mediated injury to the allograft may contribute to allograft loss. An association between C4d in peritubular capillaries and allograft failure in TG is noted in some,[6,39,40] but not all studies of patients with TG.[8,11,13] A wide variation in peritubular capillary C4d deposition is described in TG, with some studies reporting no C4d deposition and others up to 70% of biopsy specimens with C4d in TG.[4,5,10–12,41–44] Reasons for the variability of C4d staining includes peritubular capillary dropout and interstitial fibrosis in the chronic setting, and the transient nature of C4d staining on the peritubular capillary endothelium over days to weeks, as seen on repeat histologic assessment.[43,45] The variability in C4d deposition likely contributes to the limitations of C4d as a diagnostic and predictive marker in the chronic setting. Similar to several previous studies, we observed that chronicity scores were associated with the risk of allograft failure in TG.[13,46,47] We also reported an association between arteriolar hyalinosis and graft failure, which was demonstrated in a prior study of TG.[47] The association of arteriolar hyaline thickening and poor graft outcomes may reflect chronic vascular injury from diabetes, hypertension, or calcineurin inhibitor–related nephropathy. Some studies note an association between the presence of chronic active ABMR in TG and graft failure compared to isolated TG.[40,48] We did not observe an association between chronic active ABMR in TG and allograft failure. This is likely due to differences between our study group and prior studies in terms of the duration of follow-up and the changes in the diagnostic criteria of ABMR and TG over various eras.

How complement inhibitors factor into the therapeutic realm in TG remains to be seen. Even in renal diseases mediated purely by the alternative complement pathway, such as C3 glomerulopathy, only a subset of patients respond to currently available complement inhibitors.[49] A recent study of high-risk (persistently elevated positive cross-match) kidney transplant recipients demonstrated that preventive therapy with the terminal complement inhibitor eculizumab failed to prevent TG at 2 years' time.[50] In contrast to our study in patients with established TG, the prior study focused on the prevention of TG. In addition, the glomerular deposition of C3 in that study's patient population is unknown, making it difficult to extrapolate findings. Upstream complement activity is not affected by eculizumab, and whether more proximal blockade of the complement system, at the level above C5, prevents TG or improves allograft survival warrants study.

There are several limitations of our study. Our study was observational in nature, and selection bias of patients who underwent biopsy is possible. To address this, we performed univariate and multivariate analysis of patient demographic and clinical data, and these variables were not found to have associations with allograft failure. We observed several differences between $^{C3+}$TG and $^{C3-}$TG patient groups. Specifically, the $^{C3+}$TG group had more chronicity, worse kidney function, and higher level of proteinuria at the time of biopsy, and received less treatment for ABMR compared to the $^{C3-}$TG group; we accounted for these differences using multivariate regression analyses.

In summary, our study found that glomerular C3 complement deposition is an independent risk factor for allograft failure in patients with TG after renal transplantation. The data presented in the current study can be applied to educate and to prepare patients and clinicians for the anticipated disease course in TG with and without glomerular C3 deposition. Future studies of C3 deposition in a prospective cohort of patients with TG are needed to determine whether C3 can be used as a predictive biomarker in earlier stages of TG. If validated, these findings will improve our understanding of the pathogenesis of TG and will help in the investigation of treatment strategies and clinical trials to improve long-term outcomes among patients with TG.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The authors thank Dana Clark, MA, and Andreas Friedl, MD, for their editorial assistance and Daniel Felix, PharmD, and Christi Albert, PharmD, for their contributions to data collection.

This project was supported by the Clinical and Translational Science Award (CTSA) program through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR002373, and the KL2 Training Award (KL2TR002374). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

SUPPLEMENTARY MATERIAL

Table S1. Glomerulosclerosis at time of biopsy diagnosis of transplant glomerulopathy.

Supplementary information is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. Bentall A, Cornell LD, Gloor JM, et al. Five-year outcomes in living donor kidney transplants with a positive crossmatch. *Am J Transplant*. 2013;13:76–85.
2. Busch GJ, Galvanek EG, Reynolds ES Jr. Human renal allografts. Analysis of lesions in long-term survivors. *Hum Pathol*. 1971;2:253–298.
3. Zollinger HU, Moppert J, Thiel G, et al. Morphology and pathogenesis of glomerulopathy in cadaver kidney allografts treated with antilymphocyte globulin. *Curr Top Pathol*. 1973;57:1–48.
4. Maryniak RK, First MR, Weiss MA. Transplant glomerulopathy: evolution of morphologically distinct changes. *Kidney Int*. 1985;27:799–806.
5. Gloor JM, Sethi S, Stegall MD, et al. Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant*. 2007;7:2124–2132.
6. Issa N, Cosio FG, Gloor JM, et al. Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. *Transplantation*. 2008;86:681–685.
7. Abreu R, Carvalho F, Viana H, et al. Morphologic patterns and treatment of transplant glomerulopathy: a retrospective analysis. *Clin Transplant*. 2017;31:e12915.
8. Kamal L, Broin PO, Bao Y, et al. Clinical, histological, and molecular markers associated with allograft loss in transplant glomerulopathy patients. *Transplantation*. 2015;99:1912–1918.
9. Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol*. 2001;12:574–582.
10. Akalin E, Dinavahi R, Dikman S, et al. Transplant glomerulopathy may occur in the absence of donor-specific antibody and C4d staining. *Clin J Am Soc Nephrol*. 2007;2:1261–1267.
11. 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;7:1743–1752.
12. Hayde N, Bao Y, Pullman J, et al. The clinical and genomic significance of donor-specific antibody-positive/C4d-negative and donor-specific antibody-negative/C4d-negative transplant glomerulopathy. *Clin J Am Soc Nephrol*. 2013;8:2141–2148.
13. Patri P, Seshan SV, Matignon M, et al. Development and validation of a prognostic index for allograft outcome in kidney recipients with transplant glomerulopathy. *Kidney Int*. 2016;89:450–458.
14. Strassheim D, Renner B, Panzer S, et al. IgM contributes to glomerular injury in FSGS. *J Am Soc Nephrol*. 2013;24:393–406.
15. Panzer SE, Laskowski J, Renner B, et al. IgM exacerbates glomerular disease progression in complement-induced glomerulopathy. *Kidney Int*. 2015;88:528–537.
16. Zhu L, Guo WY, Shi SF, et al. Circulating complement factor H-related protein 5 levels contribute to development and progression of IgA nephropathy. *Kidney Int*. 2018;94:150–158.
17. Ma H, Sandor DG, Beck LH Jr. The role of complement in membranous nephropathy. *Semin Nephrol*. 2013;33:531–542.
18. Sargsyan SA, Serkova NJ, Renner B, et al. Detection of glomerular complement C3 fragments by magnetic resonance imaging in murine lupus nephritis. *Kidney Int*. 2012;81:152–159.
19. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting report: revised diagnostic criteria for chronic active T cell–mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant*. 2018;18:293–307.
20. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
21. Parajuli S, Mandelbrot DA, Muth B, et al. Rituximab and monitoring strategies for late antibody-mediated rejection after kidney transplantation. *Transplant Direct*. 2017;3:e227.
22. Redfield RR, Ellis TM, Zhong W, et al. Current outcomes of chronic active antibody mediated rejection—a large single center retrospective review using the updated Banff 2013 criteria. *Hum Immunol*. 2016;77:346–352.
23. Cochrane CG, Unanue ER, Dixon FJ. A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. *J Exp Med*. 1965;122:99–116.
24. Wilson CB, Dixon FJ. Immunopathology and glomerulonephritis. *Annu Rev Med*. 1974;25:83–98.
25. Maillard N, Wyatt RJ, Julian BA, et al. Current understanding of the role of complement in IgA nephropathy. *J Am Soc Nephrol*. 2015;26:1503–1512.
26. Kim SJ, Koo HM, Lim BJ, et al. Decreased circulating C3 levels and mesangial C3 deposition predict renal outcome in patients with IgA nephropathy. *PLoS One*. 2012;7:e40495.
27. Haas M, Eustace JA. Immune complex deposits in ANCA-associated crescentic glomerulonephritis: a study of 126 cases. *Kidney Int*. 2004;65:2145–2152.
28. Sethi S, Fervenza FC, Zhang Y, et al. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. *Kidney Int*. 2013;83:293–299.
29. Martinez-Barricarte R, Heurich M, Valdes-Canedo F, et al. Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. *J Clin Invest*. 2010;120:3702–3712.
30. Hou J, Markowitz GS, Bomback AS, et al. Toward a working definition of C3 glomerulopathy by immunofluorescence. *Kidney Int*. 2014;85:450–456.
31. Ichida S, Yuzawa Y, Okada H, et al. Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int*. 1994;46:89–96.
32. Thurman JM, Nester CM. All things complement. *Clin J Am Soc Nephrol*. 2016;11:1856–1866.
33. Laskowski J, Renner B, Le Quintrec M, et al. Distinct roles for the complement regulators factor H and Crry in protection of the kidney from injury. *Kidney Int*. 2016;90:109–122.

34. Mathern DR, Heeger PS. Molecules great and small: the complement system. *Clin J Am Soc Nephrol*. 2015;10:1636–1650.
35. Sethi S, Nester CM, Smith RJ. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. *Kidney Int*. 2012;81:434–441.
36. Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med*. 2002;8:582–587.
37. Marsh JE, Farmer CK, Jurcevic S, et al. The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation*. 2001;72:1310–1318.
38. Peng Q, Li K, Patel H, et al. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol*. 2006;176:3330–3341.
39. Kieran N, Wang X, Perkins J, et al. Combination of peritubular C4d and transplant glomerulopathy predicts late renal allograft failure. *J Am Soc Nephrol*. 2009;20:2260–2268.
40. Lesage J, Noel R, Lapointe I, et al. Donor-specific antibodies, C4d and their relationship with the prognosis of transplant glomerulopathy. *Transplantation*. 2015;99:69–76.
41. Loupy A, Hill GS, Suberbielle C, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant*. 2011;11:56–65.
42. Haas M, Rahman MH, Racusen LC, et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant*. 2006;6:1829–1840.
43. Regele H, Bohmig GA, Habicht A, et al. Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol*. 2002;13:2371–2380.
44. Al Aly Z, Yalamanchili P, Cortese C, et al. C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: an uncommon finding. *Transpl Int*. 2005;18:800–805.
45. Nickeleit V, Zeiler M, Gudat F, et al. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. *J Am Soc Nephrol*. 2002;13:242–251.
46. Lopez Jimenez V, Fuentes L, Jimenez T, et al. Transplant glomerulopathy: clinical course and factors relating to graft survival. *Transplant Proc*. 2012;44:2599–2600.
47. John R, Konvalinka A, Tobar A, et al. Determinants of long-term graft outcome in transplant glomerulopathy. *Transplantation*. 2010;90:757–764.
48. Torres IB, Salcedo M, Moreso F, et al. Comparing transplant glomerulopathy in the absence of C4d deposition and donor-specific antibodies to chronic antibody-mediated rejection. *Clin Transplant*. 2014;28:1148–1154.
49. Bomback AS, Smith RJ, Barile GR, et al. Eculizumab for dense deposit disease and C3 glomerulonephritis. *Clin J Am Soc Nephrol*. 2012;7:748–756.
50. Cornell LD, Schinstock CA, Gandhi MJ, et al. Positive crossmatch kidney transplant recipients treated with eculizumab: outcomes beyond 1 year. *Am J Transplant*. 2015;15:1293–1302.