ORIGINAL ARTICLE

Re-evaluation of glomerulitis using occlusion criteria based on the Banff 2013 revision: a retrospective study

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SUMMARY

The presence of occlusion/near-occlusion of glomerular capillaries was recently added to the existing definition of glomerulitis (g). We retrospectively re-evaluated 135 renal allograft biopsies regarding g to ensure no antibody-damaged grafts were missed. Previous and revised g scores (pg and rg, respectively) were compared for clinicopathologic correlations. The g score did not change in 100 (74.1%) biopsies. Thirty-five (25.9%) biopsies were changed to a lower score. Sensitivity and specificity of pg and rg for the presence of donor-specific antibodies (DSA) were 76% vs. 58% and 70% vs. 79%, respectively. Pg score indicated graft loss with 65% sensitivity and 63% specificity, whereas rg showed 46% sensitivity and 71% specificity. Area under the curve (AUC) values in ROC analysis for DSA and graft loss were as follows: pg, 0.773; rg, 0.693; and pg, 0.635; rg, 0.577, respectively. A comparison of the two AUC values revealed a significant difference between pg and rg only for DSA ($P = 0.0076$). Pg and post-transplant time of biopsy independently predicted graft loss, whereas rg did not. In conclusion, revised g scores showed lesser sensitivity but higher specificity for DSA and graft loss. Recent definition of g missed antibody-mediated rejection in few cases, and it was not an independent predictor for graft loss.

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Key words
Banff classification, biopsy, glomerulitis, renal transplant

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Introduction

Glomerulitis (g) was initially described as the presence of mononuclear cell infiltrate and endothelial swelling in glomeruli capillaries and graded according to the percentage of affected glomeruli [1]. In the Banff 2013 meeting, the definition of g was revised and the presence of occlusion/near-occlusion of ≥1 glomerular capillaries along with endothelial swelling was added to the existing definition. This change was based on the results of the study of the Banff Glomerulitis Working Group who showed that a definition including occlusion/near-occlusion had better interobserver agreement and correlation with C4d scores and gene profiles of endothelial injury. They declared that no further support could be obtained by the use of immunohistochemistry [2].

Glomerulitis has previously been defined in several ways depending on (i) the type of inflammatory cells within the capillary lumens such as T lymphocytes, macrophages, and/or neutrophils [3–5], (ii) the presence

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of inflammatory cells regardless of endothelial enlargement [5,6], (iii) endothelial cell enlargement along with the presence of inflammatory cells [3,7], and (iv) the presence of occlusion [4,5,8,9]. Among the types of cellular infiltrate, the predominance of macrophages was associated with indices of ABMR [3,4]. Occlusion of the capillary lumen was not described by any objective criteria. It is easy to interpret total occlusion because one sees the diminished patency of the lumen with full obliteration; however, the minimum threshold for near-occlusion is not clear. Nickeleit defined glomerulitis as the presence of ≥3 endocapillary cells within a glomerular capillary loop, typically with >75% luminal occlusion [10], whereas Banff describes g as the occlusion of ≥1 capillary loops with endothelial enlargement and mononuclear cells [1,11]. Early ultrastructural changes in glomerular capillaries were shown as endothelial swelling and vacuolization with loss of fenestration, subendothelial electrolucent widening, and early duplication of glomerular basement membrane [7,12,13]. Occlusion was related to abundant cytoplasms packed with prominent organelles [12].

Microvascular injury (glomerulitis, peritubular capillaritis (ptc), thrombosis), C4d positivity, and the presence of donor-specific antibodies (DSAs) are the components of main triad of acute/active ABMR. Recent studies demonstrated a new phenotype, so-called C4d-negative ABMR, in which C4d is negative but microvascular inflammation and DSAs are present [2,14]. Therefore, the microvascular inflammation sum score (g + ptc ≥ 2) was introduced as a hallmark of antibody-associated endothelial injury for the diagnosis of ABMR. Thus, identifying glomerulitis and correct grading became more important [2]. Furthermore, glomerulitis is not only a diagnostic tool but has also been shown as a strong predictor for graft loss [3,15,16].

After the introduction of the revision from the Banff 2013 meeting, we recognized that we began to overlook glomerulitis when we used the new criteria, which necessitate the presence of occlusion/near-occlusion of the glomerular capillary lumen as a criterion. We therefore retrospectively rescored 135 renal allograft biopsies for cause in terms of glomerulitis and compared the results of this reassessment with our previous evaluation. We searched for any change in the diagnosis of ABMR with possible significant clinical results after this revised definition.

Materials and methods

Patients

Randomly selected renal allograft indication biopsies of ABO-compatible, cross-match–negative renal transplant obtained from 135 patients with complete clinical, laboratory, and pathologic data were included. The study covered a 9-year period between 2007 and 2015, and all patients from the same center with a standardized therapy were selected. All biopsies were performed for clinical indication at the time of graft dysfunction. For patients with multiple biopsies, only the first biopsy was included. Data on age, sex, primary kidney disease, time of renal transplantation, type and age of donor, and cause of graft failure were reviewed from medical records.

Immunosuppressive protocol

Induction therapy with ATG-Fresenius (2 mg/kg/day) was used in transplantations from deceased donors. Immunologically risky living donor transplantations received induction therapy with ATG or basiliximab, and other patients received no induction. All patients were treated using a triple-maintenance immunosuppressive regimen including a calcineurin inhibitor (cyclosporine or tacrolimus), azathioprine or mycophenolate mofetil/mycophenolate sodium, and prednisone. All of the patients received prednisone, beginning with a dose of 120 mg daily, with a rapid taper reaching a maintenance dose of 10 mg daily within the first month, and 5 mg daily within the first year. Target blood levels were 50–150 and 5–10 ng/ml for cyclosporine and tacrolimus in the post-transplant maintenance period, respectively.

Histopathologic evaluation

All cases were evaluated under light microscopy according to the Banff criteria by a nephropathologist (YO) who was blinded to the previous pathology report and clinical data [1,2,17]. Scoring was performed using hematoxylin–eosin-, periodic acid–Schiff (PAS)-, periodic acid methenamine silver (PAMS)-, and trichrome-stained slides depending on the lesion scored. Both previous and revised (Banff 2013) g scores were evaluated in the same attempt. Previous g criteria corresponded to the presence of mononuclear cell infiltrate and endothelial swelling in glomeruli capillaries. Revised g criteria differed from the previous g by the presence of occlusion or near-occlusion in addition to the present criteria. Both g scores based on the percentage of glomeruli affected as <25%, 25–75%, and >75% for g1, g2, and g3, respectively. C4d staining (anti-C4d antibody, polyclonal; Cell Marque, The Hague, the Netherlands) on paraffin-embedded tissue blocks was performed using immunohistochemistry with an automatic staining

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system (Ventana BenchMark XT, IHC/ISH automated staining platforms, Roche diagnostics Ltd, Rotkreuz, Switzerland). Linear and circumferential staining in peritubular capillaries was regarded as positive according to the recent Banff scoring system (C4d > 0) [2]. Banff 2013 diagnostic categories and related criteria were used for the final pathologic diagnosis, and all cases were reviewed for this purpose.

DNA extraction and HLA class I and II typing

Genomic DNA was extracted from 10 ml peripheral blood in EDTA vacutainers using the modified salting out method [18]. HLA class I (HLA-A and HLA-B) and class II (HLA-DRB1 and HLA-DQB1) genotypes of patients and donors were analyzed using polymerase chain reaction–sequence-specific oligonucleotide (PCRSSO) with Luminex technology (One Lambda Inc., Canoga Park, CA, USA).

Anti-HLA antibody screening

Serum samples were tested for the presence of anti-HLA class I and II antibodies using Luminex kits (One Lambda Inc.). The cutoff for a positive reaction was set at the normalized MFI value of 1000 or greater.

Interobserver reproducibility of glomerulitis scoring

We conducted an interobserver reproducibility analysis for both previous and revised g scoring because the Banff 2013 revision was based on better interobserver agreement than the initial definition [2]; 20 representative cases showing a switch to a lower score from each subcategory of g ($n = 12$) and no change in g ($n = 8$) were selected by the first nephropathologist (YO). A senior nephropathologist (IK), who was blinded to both clinical data and scores by YO, scored these 20 cases according to both definitions of g in the same attempt.

Data analyses

Data are given as mean and standard deviation (mean ± SD) or median (IQR) throughout the document where appropriate. Proportions are written as percentages. The paired-sample t-test was used to test the difference of means of g scores. Spearman's correlation analysis was performed to assess the degree of correlations between clinicopathologic parameters. Only significant correlations with ρ values of >0.30 were recorded. Sensitivity and specificity, as well as negative and positive predictive values, were calculated for both previous and revised g scores. The association of DSA and the prediction of graft loss with both previous and revised g scores were tested using ROC analysis by calculating area under the curve (AUC) values. The difference between AUC values was tested using the previously described DeLong method [19] with MEDCALC software (version 16.0). Graft loss was defined as return to dialysis and censored for patient death with a functioning graft. The Kaplan–Meier test was performed for graft survival comparing survival with the log-rank test. Cox regression analysis was used to calculate cumulative hazard functions and to develop multivariate models. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software for Windows version 21.0 (IBM Corp, Armonk, NY, USA). P value less than 0.05 was considered significant in all comparisons.

Weighted kappa was used to evaluate the interobserver reproducibility of glomerulitis by both definitions. The thresholds for kappa values were as follows: <0.01 = poor agreement; 0.01–0.20 = slight agreement; 0.21–0.40 = fair agreement, 0.41–0.60 = moderate agreement, 0.61–0.80 = substantial agreement, and >0.80 = almost perfect agreement [20]. Weighted kappa analysis was performed manually in Excel (Microsoft Excel 2010) and verified using VASSARSTATS (http://vassarstats.net).

This retrospective study was approved by the institutional ethics committee of Istanbul University, Istanbul Faculty of Medicine (issue number: 2016/172).

Results

Patient characteristics

One hundred and thirty-five renal allograft biopsies were included in this study. Indications for biopsies were described as an increase in serum creatinine ($n = 83$, 61.5%), new-onset proteinuria ($n = 16$, 11.9%) or both ($n = 34$, 25.2%), or delayed graft function ($n = 2$, 1.5%) over a median follow-up period of 39 (IQR 15–92) months after transplantation.

The study population included 88 males and 47 females. DSA data were available for 117 patients. The patients' demographic data and clinical characteristics are displayed in Table 1. For the 135 patients, the Banff 2013 transplant biopsy diagnoses using the previous g definition were T cell-mediated rejection (TCMR) in 17 (12.6%), ABMR in 22 (16.3%) (including four C4d-negative ABMR), mixed rejection in 13 (9.6%),

Table 1. Demographic data and clinical characteristics of the study cohort (n = 135).

	All patients
Age (years) (mean ± SD)	38.96 ± 12.7 (14–70)
Male:Female, *n* (%)	88 (65.2):47 (34.8)
Primary disease, *n* (%)	
Glomerulonephritis	29 (21.5)
Diabetic nephropathy	9 (6.7)
Hypertensive nephrosclerosis	4 (3)
Interstitial nephritis/pyelonephritis	6 (4.4)
Vesicoureteral reflux nephropathy	19 (14.1)
Polycystic kidney disease	3 (2.2)
Amyloidosis	3 (2.2)
Others	7 (5.2)
Unknown	55 (40.7)
Donor type (deceased/living), *n* (%)	30/105 (22.2/77.8)
DSA positivity (n = 117), *n* (%)	50 (42.7)
C4d positivity (C4d > 0), *n* (%)	54 (40)
Post-transplantation biopsy time (months), median [IQR]	39 [15–92]
Postbiopsy follow-up time (months), median [IQR]	21 [8–41]
Maintenance immunosuppression, *n* (%)	
Cyclosporin, MMF, steroids	24 (17.8)
Tacrolimus, MMF, steroids	54 (40)
Cyclosporin, azathioprine, steroids	6 (4.4)
Tacrolimus, azathioprine, steroids	8 (5.9)
Others	43 (31.9)

SD, standard deviation; DSA, donor-specific antibody; IQR, interquartile range; MMF, mycophenolate mofetil or sodium.

borderline changes in 38 (28.1%), suspicious ABMR in 20 (14.8%), glomerulonephritis in 15 (11.1%), and others in 10 (7.4%).

Glomerulitis scoring

The mean glomerulitis scores were 0.86 ± 1.05 and 0.53 ± 0.79 for the previous and revised scoring, respectively ($P < 0.001$). Overall, underscoring was detected in 35 (25.9%) biopsies, whereas scores did not change in 100 (74.1%) (Table 2). Overscoring was not observed in any biopsies. Illustrative examples of underscoring of g and g with occlusion are given in Fig. 1a and b, respectively.

Table 2. Changes in glomerulitis scoring according to the previous and revised Banff 2013 definitions.

N = 135		Revised g score			
		g0	g1	g2	g3
Previous g score	g0	69	0	0	0
	g1	13	18	0	0
	g2	4	6	10	0
	g3	1	5	6	3

g, glomerulitis.

As microvascular inflammation sum score (g + ptc) was introduced as a diagnostic criterion for C4d-negative ABMR, which suggests antibody-mediated endothelial injury, we grouped our cases into three subgroups such as (g + ptc) < 2, [(g0) + ptc] ≥ 2 and [(g ≥ 1) + ptc] ≥ 2 according to both g scores. All 72 cases in (g + ptc) < 2 group and all three cases in [(g0) + ptc] ≥ 2 group remained in the same group for both g scoring. However, among 60 cases with [(g ≥ 1) + ptc] ≥ 2, 11 changed to the (g + ptc) < 2 and four to [(g0) + ptc] ≥ 2 group. Regarding the Banff 2013 criterion for microvascular inflammation score, only these 11 cases lost their light microscopic signature of antibody–endothelium interaction with the revised g scoring. DSA was present in 4/8 (50%) (not available in three cases), and C4d and cg were each noted in 2/11 (18%) cases. Banff diagnoses according to the Banff 2013 criteria using the previous g score were as follows: three glomerulonephritis, one C4d-positive ABMR, three suspicious ABMR, two mixed rejection (with C4d-negative ABMR component), and two TCMR. A change in glomerulitis scoring resulted in a diagnosis conversion in only five, in which the revised g score

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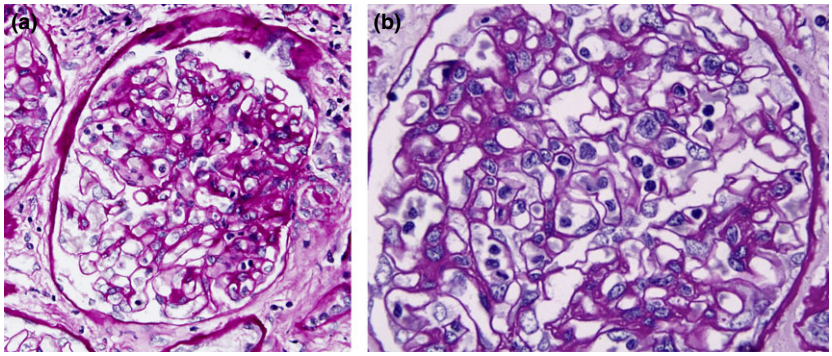

Figure 1 Mononuclear cells within the capillary lumens with endothelial enlargement but without capillary occlusion. This case changed from g1 to g0 with the inclusion of the Banff 2013 criteria (a) periodic acid–Schiff (PAS, 400×). An example of glomerulitis with occlusion/near-occlusion of the capillary lumens (b) (PAS, 400×).

missed ABMR or suspicious ABMR components in two and three cases, respectively. In one case, the ABMR diagnosis with the previous g scoring was retained with the revised g scoring because of C4d positivity. Graft loss was seen in seven patients [three glomerulonephritis, one mixed rejection, two suspicious ABMR, one TCMR (Grade IIB) diagnosis using previous g] in the follow-up. For these seven patients having graft loss, the Banff diagnoses using the revised g score were three glomerulonephritis, one borderline changes, one with C4d positivity only, and two TCMR.

Correlates of glomerulitis scores

Previous and revised g scores were highly correlated with each other ($\rho = 0.793$, $P < 0.001$). The correlation analysis of clinicopathologic characteristics revealed that DSA ($\rho = 0.505$, $P < 0.001$), C4d ($\rho = 0.457$, $P < 0.001$), cg ($\rho = 0.423$, $P < 0.001$), and ptc ($\rho = 0.610$, $P < 0.001$) were significantly associated with the previous g scores. Although the revised g showed similar correlations with C4d ($\rho = 0.463$, $P < 0.001$) and ptc ($\rho = 0.603$, $P < 0.001$), the degree of correlation was slightly lower for DSA ($\rho = 0.385$, $P < 0.001$) and higher for cg ($\rho = 0.535$, $P < 0.001$) than the previous g.

The distribution of cases for ptc, cg, C4d, and DSA according to the previous and revised g scores is given in Fig. 2. This illustrates no significant difference between g score groups regarding associated lesions.

Sensitivity analysis for glomerulitis scoring and ROC analysis

One of the study end points was the presence of DSA. Calculations for sensitivity and specificity for both previous and revised g scores resulted in 76% vs. 58% and 70% vs. 79% for this first end point, respectively. The second end point was graft loss. Sensitivity and specificity for both previous and revised g scores resulted in 65% vs. 46% and 63% vs. 71% for graft loss, respectively. Negative and positive predictive values are given in Table 3.

We performed ROC analysis for both previous and revised g scores for the associations with DSA and graft loss (Fig. 3a and b). For DSA analysis, previous and revised g scores had AUC values of 0.773 and 0.693, respectively. We demonstrated similar results for graft loss with both g scores (0.635 vs. 0.577). A comparison of two AUCs for the association of previous and revised g scores with DSA demonstrated a significant difference [95% CI: (0.023–0.139), $z = 2.671$, $P = 0.0076$]. According to this significance, previous g scoring was better associated with DSA than the revised g score. Although the ROC curve comparison for the prediction of graft loss between the two g scoring methods did not show a large difference, the P value was at the borderline level of significance [95% CI: (−0.0022 to 0.119), $z = 1.889$, $P = 0.0589$).

Prediction of death-censored graft survival by glomerulitis

Over a median follow-up of 21 months (IQR 8–41 months) after biopsy, 57 patients (42.2%) developed allograft failure. Regarding g scoring, the allograft failure rate of patients with g0, g1, and $g \geq 2$ were 29% (20/69), 54.8% (17/31), and 57.1% (20/35) ($P = 0.006$), and 36% (31/86), 53% (16/30), and 53% (10/19) ($P = 0.157$) considering previous and revised g scoring, respectively. When g scores were compared for the prediction of graft loss, Kaplan–Meier analysis revealed that patients with previous g0 had significantly better

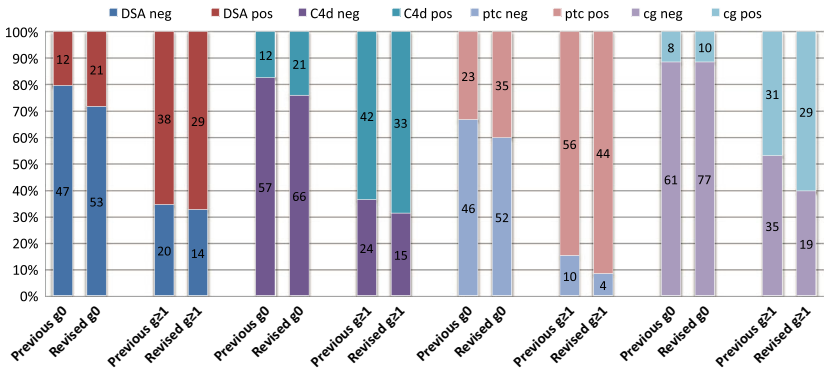

Figure 2 The distribution of antibody-mediated features according to previous and revised glomerulitis scores.

Table 3. Association of glomerulitis with DSA and prediction of graft loss with previous and revised glomerulitis (g) scores.

	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
DSA				
Previous g score	76	70	80	66
Revised g score	58	79	72	67
Graft loss				
Previous g score	65	63	71	56
Revised g score	46	71	64	53

DSA, donor-specific antibody; NPV, negative predictive value; PPV, positive predictive value; g, glomerulitis.

survival compared with those with previous g1 or g ≥ 2 ($P < 0.001$) (Fig. 4a). On the other hand, although log-rank comparison for revised g scores revealed an overall p value of 0.013, significance was present only between revised g0 and g ≥ 2 ($P = 0.006$) (Fig. 4b). There were no significant differences for revised g0 vs. g1, and g1 vs. g ≥ 2 ($P = 0.063$ and $P = 0.347$, respectively).

In univariate Cox regression analysis, both previous and revised g scores significantly predicted death-censored graft survival ($P < 0.0001$ and $P = 0.005$, respectively). Higher g scores with both definitions of g scoring revealed worse graft survival. The worst prognosis was seen in the g ≥ 2 group with both previous and revised g scores. The significant prognostic difference present between g0 and g1 categories by previous g scoring had been lost with the revised g scoring. Previous g scoring and post-transplant biopsy time were found as independent prognostic predictors in multivariate analysis after adjusted for ptc, C4d, and cg. However, the revised g score was not seen as an independent predictor for graft loss with the same multivariate model including revised g scoring. The results of univariate and multivariate Cox regression analysis regarding previous and revised g scores are given in Table 4.

Interobserver reproducibility of glomerulitis scoring

Interobserver reproducibility was tested on 20 selected cases, 11 of which were switched from g + ptc ≥ 2 with previous g scoring to g + ptc < 2 with revised g scoring by YO. Five of these included cases in which ABMR/suspicious ABMR diagnosis could not be given by the microvascular inflammation sum score using revised g. Weighted kappa values between two nephropathologists were 0.486 (moderate) and 0.531 (moderate) for previous and revised g scores, respectively. The second observer missed ABMR/suspicious ABMR component with revised g score in four cases, three of which overlapped five cases missed by the first observer.

Discussion

Glomerulitis was originally defined as the accumulation of mononuclear cells and endothelial swelling in capillary lumens of glomeruli and graded according to the percentage of glomeruli involved [1,11]. Although capillary occlusion or near-occlusion criteria were introduced previously [6,9,12], it was only recently added to the existing definition at Banff 2013 [2]. The present study demonstrated

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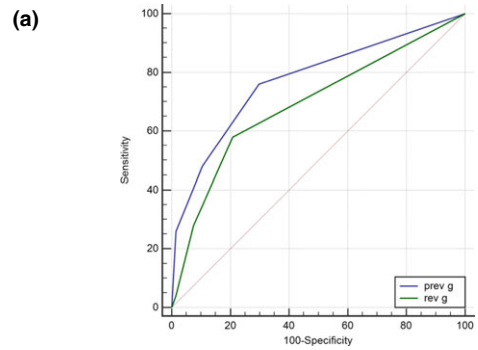
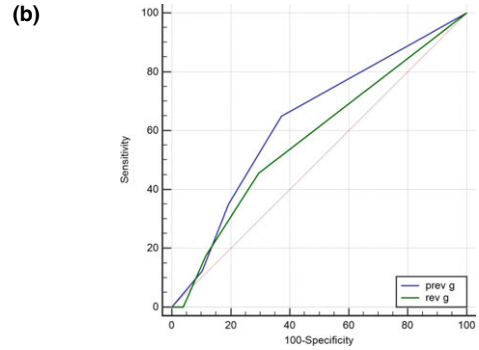
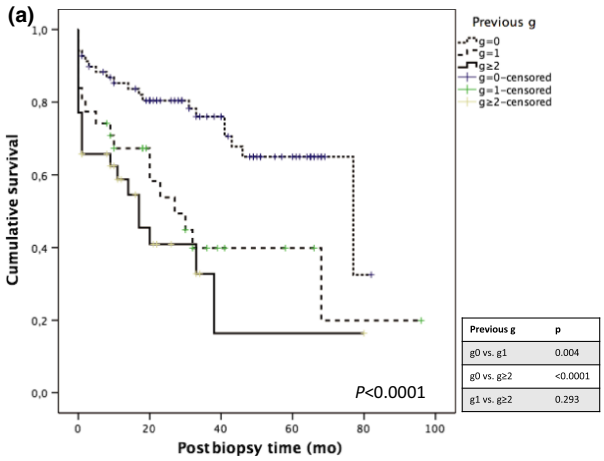


	AUC	SE	p	Comparison of two AUCs*
Previous g score	0.773	0.042	<0.0001	z = 2.671; P = 0.0076
Revised g score	0.693	0.051	<0.0001	

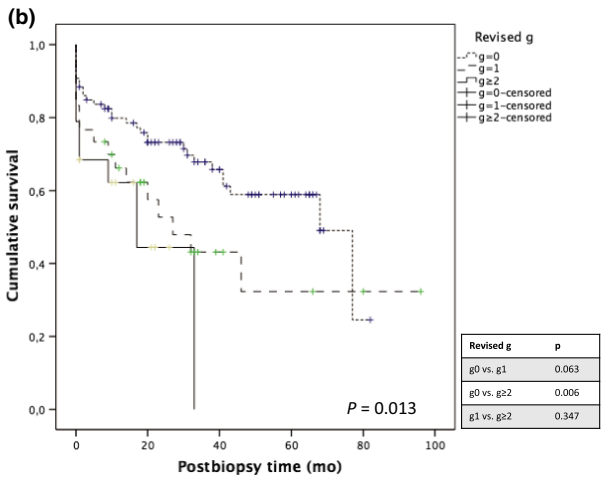


	AUC	SE	p	Comparison of two AUCs*
Previous g score	0.635	0.045	0.007	z = 1.889; P = 0.0589
Revised g score	0.577	0.043	0.128	

Figure 3 ROC analysis for previous and revised glomerulitis scoring regarding donor-specific antibody (DSA) (a) and graft loss (b). AUC, area under the curve; SE, standard error, *Comparison was made between AUCs of previous and revised g scores using DeLong *et al.*'s method [9].



Previous g	p
g0 vs. g1	0.004
g0 vs. g≥2	<0.0001
g1 vs. g≥2	0.293



Revised g	p
g0 vs. g1	0.063
g0 vs. g≥2	0.006
g1 vs. g≥2	0.347

Figure 4 Kaplan–Meier survival curves for previous (a) and revised (b) glomerulitis scoring (N = 135).

that this revised definition of g resulted in underscoring in 25.9% of cases and missed ABMR components (definite or suggestive) in five cases. Therefore, the revised definition of glomerulitis showed lower sensitivity but higher specificity than the original description for DSA prediction. We also observed that previous g scores predicted graft failure better than the revised g scores.

C4d-negative ABMR was introduced using the criteria of g + ptc ≥ 2, which indicates endothelial injury due to antibodies. Therefore, it is important to detect cases with g + ptc ≥ 2 in C4d-negative biopsies. The most challenging group is biopsies with ptc0-1 in which g scores would change the final sum score and subsequent diagnosis. Furthermore, regarding the lack of specificity of ptc for ABMR [21,22], ptc > 2 alone is not sufficient for ABMR in the presence of acute TCMR, borderline changes or infection and g must be >1 [23]. Therefore, we need to recognize and grade g correctly. In our study, among 35 cases that had lower scores of g based on the revised definition, the final diagnosis did not differ in 30 (85.7%), as these cases also had ptc and/or C4d immunoexpression. Eleven cases changed from g + ptc ≥ 2 to g + ptc < 2 and ABMR components were missed in only five of these.

The current definition of g is based on the percentage of glomeruli involved as <25%, 25–75%, and >75% for g1, g2, and g3, respectively [1,2,23]. There is no description for a cutoff limit or for the minimum number of inflammatory cells required. The methodology in glomerulitis scoring regarding the percentage of involved glomeruli was shown to be superior to the method using the count of leukocytes per glomerulus [2]. This finding was in accordance with previous data showing a high association of this grading with clinical parameters and prognosis [15]. When only glomeruli showing occlusion, along with intraglomerular inflammatory cells and endothelial enlargement (Banff 2013 criteria) were taken into account for g grading, the percentage of affected glomeruli was assumed to be

Table 4. Univariate and multivariate Cox regression analysis regarding death-censored allograft loss (N = 135).

	Univariate		Multivariate Model 1 with previous g		Multivariate Model 2 with revised g	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Previous g	1.883 (1.382–2.568)	<0.001			Not included	
g0	Reference		Reference			
g1	2.519 (1.312–4.839)	0.006	1.828 (0.835–3.985)	0.129		
g ≥ 2	3.547 (1.865–6.745)	0.001	3.079 (1.343–7.061)	0.008		
Revised g	1.642 (1.161–2.323)	0.005	Not included			
g0	Reference				Reference	
g1	1.763 (0.959–3.241)	0.068			1.116 (0.540–2.305)	0.767
g ≥ 2	2.616 (1.251–5.470)	0.011			1.327 (0.505–3.486)	0.566
ptc	1.653 (1.188–2.299)	0.003				
ptc0	Reference		Reference		Reference	
ptc1	1.718 (0.905–3.262)	0.098	1.274 (0.605–2.686)	0.524	1.728 (0.851–3.511)	0.130
ptc ≥ 2	2.734 (1.407–5.312)	0.003	1.252 (0.502–3.119)	0.630	2.049 (0.803–5.229)	0.134
C4d (negative–positive)	1.699 (1.006–2.868)	0.047	0.906 (0.479–1.714)	0.762	0.930 (0.481–1.795)	0.828
cg (absent–present)	2.180 (1.255–3.788)	0.006	0.926 (0.537–1.981)	0.926	1.178 (0.603–2.302)	0.632
Post-transplant biopsy time (days, log-transformed)	2.096 (1.334–3.293)	0.001	2.074 (1.263–3.408)	0.004	2.076 (1.254–3.436)	0.005

HR, hazard ratio; CI, confidence interval; g, glomerulitis; ptc, peritubular capillaritis; cg, transplant glomerulopathy.

changed. This new percentage might result in lower scores of g. In a very recent retrospective study, in which 73 indication biopsies were reclassified in accordance with the Banff 2013 definition of g, a conversion from a higher grade to a lower grade was demonstrated in only nine cases with initial scores of g1 [24]. Among these nine cases, diffuse C4d and DSA positivity was present in three and five, respectively. The diagnosis of ABMR with previous g scoring was lost with the revised g score in five cases in that study. Similarly, regarding the presence of occlusion, higher g scores turned into lower scores in our study also. Eighteen cases (13%) changed from g1–3 to g0 depending on the percentage of glomeruli with intraglomerular inflammatory cells, endothelial enlargement, and occlusion/near-occlusion. Although transformation into a lower g score missed ABMR in only a few cases in these two studies, the consequences may vary in different clinical settings.

Glomerulitis with the previous definition was known to be associated with the presence of DSA, C4d positivity, and endothelial- and antibody-associated transcripts [25,26]. At the Banff 2013 meeting, the Glomerulitis Working Group presented that g definition including occlusion/near-occlusion of capillaries was better correlated with C4d scores and gene transcripts [2]. We demonstrated a similar correlation with C4d for both definitions of g. However, revised g scores showed a lower correlation with DSA. Chronic lesions that result from cumulative antibody-associated endothelial damage (cg) were better correlated with the revised g scores. Furthermore, we showed that the revised g scoring was more specific but less sensitive for ABMR features. Likewise, both definitions of glomerulitis regarding the percentage of involved glomeruli and the presence of occlusion was previously shown to be correlated with allograft dysfunction and cg [15]. Contrary to this, another study found no correlation of g with cg using the Banff 2013 criteria [4]. The conflicting results of these studies are most probably related to the variance in post-transplant time of the biopsy and follow-up time, along with the baseline characteristics of the study populations and definition criteria used.

Glomerulitis was shown as an independent predictor for graft loss [3,8,15,16,27]. In accordance with the literature, we also demonstrated a significant association between g scoring, both with previous and revised criteria, and poor graft survival. However, in contrast to the previous g score and post-transplant biopsy time, g with the Banff 2013 criteria was not an independent prognostic predictor after adjusted for ptc, C4d, cg. In a previous study, g was analyzed for the association with graft survival in three ways in terms of the percentage of glomeruli involved, inflammation in the most severely affected glomerulus, and the presence of occlusion [15]. Similar to our results, endocapillary occlusion, which is the defining criteria of g in the recent definition, had no impact on graft survival in this study cohort. In another recent study, no association was seen

between graft survival and g score using Banff 2013 criteria [4]. However, using time-dependent ROC analysis the authors demonstrated that early g scoring with macrophages could be used as a predictor for death-censored graft survival, independent of other features of ABMR. The prognostic importance of g with macrophages was also previously shown [3]. All these studies with different results regarding the association between g and graft survival highlighted that the prognostic impact of g changes according to the definition and methodology (immunohistochemistry) used. We did not perform immunohistochemistry in the current study, because this was beyond the scope of this article and might be the subject of another study.

Like all histopathology-based criteria, the analysis of occlusion using light microscopy is highly subjective, which results in low reproducibility [28–30]. The agreement for glomerulitis was shown between poor to moderate in previous studies (κ values 0.195–0.50) [2,28–30]. The Glomerulitis Working Group demonstrated better agreement with the revised definition based on the presence of occlusion [2]. Our findings supported this finding by a higher kappa value for the revised definition than the original description. We want to emphasize that although the two observers in this single-centered retrospective study work and teach together, the interobserver agreement still was not perfect. Additional tools in the interpretation of glomerulitis are needed to lower interobserver variation.

There are some limitations of this study. The retrospective study design provoked therapeutic variance among the patient group. Electron microscopic evaluation and molecular data were missing unfortunately; however, this is what we experience in daily routine. DSA measurements were not available for all patients, but performed in a significant number of cases. On the other hand, this study has several strengths. This is the first study to compare the two recent Banff definitions of glomerulitis in terms of the clinical perspective including survival data. Furthermore, although performed in a small subset of cases, an interobserver reproducibility analysis was also included. A decrease in g scores, higher specificity, and better agreement between observers with the occlusion criteria for ABMR components were well demonstrated.

In conclusion, the present study highlights that the revised Banff 2013 criteria for g scoring, including occlusion or near-occlusion of glomerular capillaries, have lower sensitivity but higher specificity for DSA and the prediction of graft loss than the previous definition. However, the revised criteria missed ABMR components and changed the clinical picture in a few cases. Additionally, the revised g scoring was not demonstrated as an independent prognostic factor for graft loss in contrast to the previous g scoring. This article documents a single institution's observations with small sample size, and there is a need to widen this to larger series with multiple centers.

Authorship

YO: designed the study, performed research, collected and analyzed the data and wrote the manuscript. YC: performed research, collected and analysed the data and wrote the manuscript. MS, AB and EA: performed research and collected the data. AT, FOS, MSS and IK: Performed and supervised research and contributed to the discussion. SA: collected the data and wrote the manuscript.

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Conflict of interest

None of the authors have any conflict of interests to disclose.

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