Original Article

A Novel Glomerular C4d Scoring System: A Tool to Prognosticate Proliferative Exudative Pattern of Glomerular Injury

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Abstract

Aim: Proliferative exudative pattern of glomerular injury is usually a manifestation of an infection related or a post-infectious glomerulonephritis (PIGN). Rarely, it may represent a C3 glomerulopathy, which is a dysfunction of the alternative pathway of complement activation, and is then termed an atypical PIGN (aPIGN). C4d deposits in the glomerulus are footprints of the classical and/or lectin pathway of complement activation and hence is expected to be positive in immune-mediated glomerulonephritis (GN) like classical infection-related GN, and could be used to differentiate classical PIGN from atypical PIGN. **Materials and Methods:** We report a novel C4d scoring system based on the intensity and the proportion of glomerular tuft staining, in a series of 104 biopsies with the proliferative exudative pattern of glomerular injury. Using a statistically derived cut-off score of 1.45, the cases were divided into C4d positive and C4d negative groups and compared to IF findings and the follow-up, available in 36 cases. **Results:** The C4d positive group had a significantly greater proportion of cases with immune complexes compared to the group with C3 deposits alone. In the follow-up, C4d negative group had also a greater number with partial/incomplete response compared to the C4d positive group. **Conclusions:** We recommend that the C4d stain be done in all cases with a proliferative exudative pattern of glomerular injury to identify patients who would need a close follow up and further assays of complement function.

Keywords: *Alternate complement pathway, atypical PIGN, C4d staining, infection-related glomerulonephritis*

Introduction

A proliferative and exudative pattern of injury is the characteristic light microscopic feature of infection-related glomerulonephritis (IRGN), better known as post-infectious glomerulonephritis (PIGN). Direct immunofluorescence (DIF) shows bright C3 deposits with or without immunoglobulins (Igs), while subepithelial humps are often seen in ultrastructure.[1-3] The diagnosis of PIGN is often made even in the absence of any clinical, bacteriological, or serological evidence of preceding infection and it has a typical benign self-limiting clinical course, and most patients show complete recovery of renal function within few days to weeks. A small subset of patients in the spectrum of C3 glomerulopathy has overlapping clinical, light microscopic, IF as well as ultrastructural features with PIGN and have been termed atypical PIGN (aPIGN).[4] The distinction of typical PIGN from aPIGN or C3GN is important in management and prognostication, as the patients with aPIGN and C3GN usually take longer time to resolve, resulting in persistent proteinuria and hematuria and may even progress to end-stage kidney disease.[5,6]

Patients with C3 glomerulopathy have an underlying defect in the regulation of the alternative pathway of complement activation.[7] As C4d is a by-product of classical and lectin pathways of complement activation, demonstration of C4d has been reported to be useful in distinguishing immune-complex mediated and C3 glomerulopathy in biopsies with a membranoproliferative glomerulonephritis (MPGN) pattern of glomerular injury.[8-12]

C4d stain is robust by immunofluorescence and immunohistochemical methods. However, minimal mesangial and a little peripheral stain can occur even in normal glomeruli. There may thus be a dilemma of when to call the stain positive and when negative. In the present study, we have

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devised a novel glomerular C4d scoring system in cases with a proliferative exudative pattern of injury presumed to be PIGN. We have then compared this score with the findings on DIF (complement and Igs versus complement alone). We have further correlated the C4d scores with the findings on follow-up wherever available.

Materials and Methods

Study design

A total of 104 renal biopsies received by the department of Histopathology with a proliferative and exudative pattern of glomerular injury were identified in a period of 10 months. Clinical, laboratory data, histological, and IF findings were reviewed in all. The available clinical and laboratory records are summarized in Table 1. The cases were then separated into two groups based on the DIF findings in the original reports. Group I included 70 patients with glomerular C3c deposits ≥2+ and IgG/C1q ≥2+, group II included 34 patients with C3c ≥2+, and IgG/C1q ≤1+ in the glomeruli. Cases of lupus nephritis were excluded from the study. The ultrastructural study was not done in any of the cases.

A novel C4d glomerular scoring system: C4d stain was done by immunohistochemistry using a rabbit monoclonal antibody (CellMarque, ready to use) and the automated Ventana Benchmark detection system. All the glomeruli in each case were assessed for the intensity of staining and the percentage of stained glomerular tuft. The intensity was a subjective score ranging from 0 to 3 (0 for no staining and 3 for dark intense stain). The proportion of stained glomerular tuft was also scored from 0 to 3 (0 for no stain, 1 for <25% glomerular circumference positivity, 2 for 25 to 50% glomerular circumference positivity and 3 when >50% of the glomerular circumference was stained). The average scores for intensity and proportion were then obtained (every available glomerulus in each case was scored) and the final score was a sum of these two average scores, which ranged from 0 (completely negative) to 6 (intense positive) [Figure 1].

Statistical analysis

The statistical difference between the two groups was analyzed by the Student t test. Receiver operating characteristic (ROC) analysis of subjects on the basis of parameter C4d was done and a cut-off value with maximum sensitivity and specificity was determined by the Youden Index [J]. This score was then correlated with a follow up (over periods between 3 months to a year) wherever available. The follow-up was recorded as a complete recovery when urine protein, serum complement, and creatinine returned to normal range. If otherwise, it was recorded as partial or no recovery.

Results

Of the 104 biopsies studied, 70 were classified as group I (C3c ≥ 2+ and IgG/C1q ≥ 2+) and 34 as group II (C3c ≥ 2+, and IgG/C1q ≤ 1+). Of the 18 cases with recurrent hematuria, 14 were in group II and 4 in group I. Low C3 and low C4 were seen in 27 of 29 in which the values were available and all were in group I [Table 1]. The number of males (60%) in group I was greater than that in group II (38.24%). The mean age and the distribution across age groups were similar in the two groups [Table 2].

According to the proposed C4d scoring system, the mean score in group I was 3.39 ± 1.83 and that in group II was 1.72 ± 1.87 and this difference was statistically significant ($P < 0.0001$) [Table 3].

Table 4 shows that the area under the ROC curve (AUC) is 0.735, indicating that the test parameter C4d had fair accuracy in separating the two groups. A cut-off value of 1.45 had a sensitivity of 75.38% and specificity of 64.71%, which was maximum as determined by the Youden Index J. The ROC curve obtained is shown in Figure 2.

Table 1: Clinical parameters in groups I and II

Clinical Parameters	Group I	Group II
Fever, nephritic syndrome	45	13
Recurrent hematuria	4	14
History of past infection	8	2
Low C3 and C4 levels*	27	0
Low C3 and normal C4 levels*	0	2
No. available for follow-up	27	9

*Complement levels available in a total of 29 cases in this cohort

Table 2: Descriptive statistics for age and gender

Characteristics	Levels	Groups	
		Group I (*n*=70)	Group II (*n*=34)
		C3 ≥2+, IgG ≥2+	C3 ≥2+, IgG ≤1+
Age (Mean±SD)		38.42±20.545	34.765±21.073
Gender [No (%)]	Male	42 (60)	13 (38.24)
	Female	28 (40)	21 (61.76)

Table 3: Descriptive statistics for C4d according to groups

Parameter	Groups (Mean±SD)		*P**
	Group I	Group II	
	C3 ≥2+, IgG ≥2+	C3 ≥2+, IgG ≤1+	
C4d	3.39±1.83	1.72±1.87	<0.0001

**P* are estimated using independent samples *t*-test

Table 4: ROC analysis of subjects on the basis of parameter C4d

Statistical index	Value
Area under the ROC curve (AUC)	0.735
Youden index J	0.4009
Associated criterion	1.45
Sensitivity	75.38
Specificity	64.71

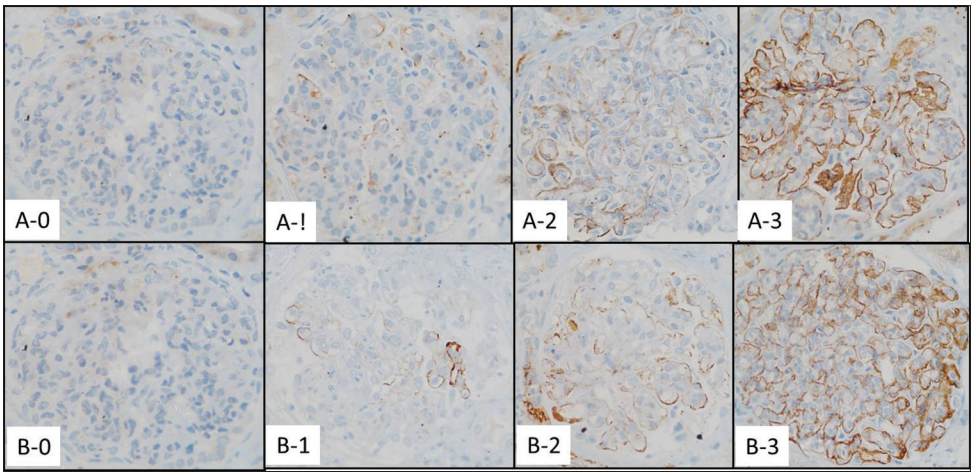

Figure 1: IHC stain for C4d: The top four panels show intensity scoring from 0 to 3+ (left to right). The bottom four panels show scoring for circumferential positivity from 0 to 3+ (left to right)

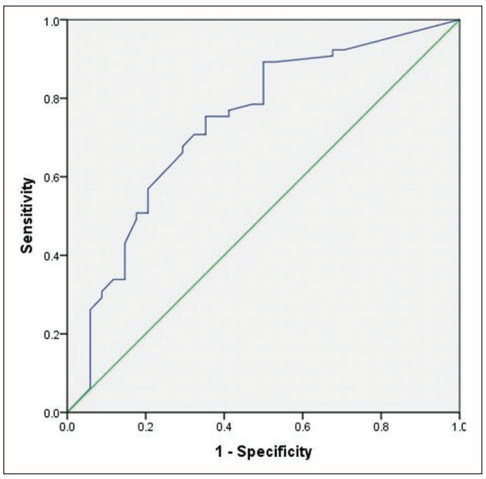

Figure 2: ROC curve

Using this C4d score of 1.45 obtained by the above analysis, we reclassified the cases into C4d positive C4d positive, probable immune complex (IC) mediated GN and C4d negative, probable complement-mediated GN. Thus, 15 cases of the original group II were reclassified as IC- mediated GN and 13 cases of group I were reclassified as complement-mediated GN [Table 5]. This can be explained by the fact that Igs can be absent in resolving PIGN and C3GN can also have minimal Igs. Follow-up was available in 36 patients and this was correlated with the grouping done based on the IF and that done based on the cut-off value of 1.45 for the C4d score.

IF-based groups

Complete recovery was seen in 27 patients, of which 21 patients were in group I and 6 in group II. Nine patients showed persistence or partial recovery of symptoms of whom 6 were in group I and 3 were in group II.

C4d-based groups

Out of those 27 patients who showed complete recovery, 25 were C4d positive and 2 were C4d negative. Seven of 9 patients with partial recovery were C4d negative and 2 were positive. *P* value was calculated using Pearson's Chi-square test for correlation of clinical findings with IF and C4d and it was found to be 0.6434 and 0.0012, respectively [Table 6].

Table 5: Re-classification of the group on the basis of C4d score of 1.45

C4d status	Positive Score >1.45	Negative Score ≤1.45	Total
Group 1 C3 ≥2+, IgG ≥2+	57	13	70
Group 2 C3 ≥2+, IgG ≤1+	15	19	34
Total	72	32	104

Discussion

C4d is a surface-bound spilt product of inactive C4b, which is obtained from the classical and/or lectin pathways of complement activation but is absent in the alternate pathway. As C4d remains at the site of activation for up to 2 weeks, recognition of C4d by DIF or immunohistochemistry (IHC) has become a reliable marker to identify the sites of complement activation by the classical or lectin pathway. The novel semi-quantitative scoring system of C4d, which has not been done earlier, we feel gives more objectivity to the process of C4d reporting, especially as it is increasingly being shown to be of diagnostic importance as discussed below.

The importance of C4d staining in peritubular capillary walls in acute and chronic antibody-mediated rejections in renal transplant biopsies is well established.[13,14] The role of C4d in native renal biopsies has been documented in membranous nephropathy, IgA nephropathy, and lupus nephritis.[15,16] Glomerular C4d staining in lupus nephritis has been associated with a higher risk of developing thrombotic microangiopathy and positive interstitial

peritubular capillary C4d staining indicates intense immunological disease activity.[17,18] In IgA nephropathy, Espinosa *et al*. have shown an association of C4d positivity with the development of end-stage renal disease and considered it as a new prognostic indicator.[19] Sethi *et al*. have very elegantly demonstrated that the C4d staining pattern in the MPGN pattern of glomerular injury can be used to differentiate immune complex-mediated GN from C3 glomerulopathy. This is important as the two groups have distinctly different etiologies and need to be managed differently.[11]

A proliferative and exudative pattern of glomerular injury, commonly seen in infection-related GN is also sometimes seen in other immune complex-mediated GN such as lupus nephritis and can also be a part of the spectrum of C3 glomerulopathy that is now referred to as aPIGN.[1-4] PIGN and aPIGN can have overlapping clinical, histological, and DIF findings. C4d staining could be one important method to differentiate the two and devising a C4d scoring system is one step in that direction.

A summary of the study is depicted in Table 7. Four permutations and combinations are possible with the results on IF and C4d and these four groups have been designated A to D. The pathogenesis and the clinical profile in each of these are different as discussed below.

Group A, comprising 57 cases had C4d, C3, and immunoglobulin (Ig) positivity. They represent the classical PIGN, due to activation of the classical pathway. Twenty of the 22 followed up in this group, recovered completely and this was as expected. Group B with 13 cases was C4d negative and C3 and Igs positive. Here, the underlying pathogenesis is assumed to be a dysfunction of the alternative complement pathway with infection being a possible trigger accounting for the deposition of the Igs. Also, the scoring of immune deposits on DIF is still subjective and it is also possible that a 1+ score of IgG could have been interpreted as 2+. In a follow-up of 7 patients in this group, 5 did not recover.

Group C with 19 cases was C4d and Igs negative and C3 positive. This group can be unequivocally classified as aPIGN or C3 glomerulopathy and probably represent cases with alternative pathway dysfunction. Two patients followed up in this group did not recover. Group D with 15 cases was C4d and C3 positive and Igs negative. Here, we presume that these are indeed immune complex-mediated GN with classical pathway activation but the Igs are either masked or it represents a resolving PIGN where the Igs disappear and the complements persist. All five patients in this group followed up had a complete recovery as expected.

We may add that clinically, C4d negativity in a proliferative exudative pattern of injury is more significant for it could indicate an underlying dysfunction of the alternative complement pathway. However, one should not be lulled into complacency in a C4d positive case, for rarely these may represent C3 glomerulopathies with poor prognosis.[20]

We conclude that in a proliferative exudative pattern of GN, C4d staining aids in the identification of that small proportion where a dysfunction of the alternative complement pathway is a likely etiology and where the patients are likely to have persistent, recurrent, or progressive disease. We would, therefore, recommend that C4d staining be done in all cases of a proliferative and

Table 6: Comparison of IF and C4d based groups with follow up

Follow up	IF		C4d	
	Group 1 C3 ≥2+, IgG ≥2+	Group 2 C3 ≥2+, IgG ≤1+	Positive Score >1.45	Negative Score <1.45
Completely resolved	21	6	25	2
Persistent/Partially resolved	6	3	2	7
Total number (*n*=36)	27	9	27	9
P	0.6434		0.0012	

Table 7: Summary of the study

Groups	A	B	C	D
C4d	Score ≥1.45	Score <1.45	Score <1.45	Score ≥1.45
C3 on IF (≥2+)	Positive	Positive	Positive	Positive
IgG/C1q on IF (≥2+)	Positive	Positive	Negative	Negative
No. of cases	57	13	19	15
Pathogenesis	IC mediated	Compl.mediated	Compl.mediated	IC mediated
Activation pathway	CP/LP	AP+CP/LP	AP	CP/LP
Diagnosis	Classical PIGN	aPIGN/C3GN triggered by infection	aPIGN/C3GN	Resolving PIGN/masked Igs
Follow-up	22	7	2	5
Complete response	20	2	-	5
Partial response/no response	2	5	2	-

IC mediated GN- immune complex mediated GN, CP- classical pathway, LP- lectin pathway, AP- alternate pathway

exudative pattern of glomerulonephritis to identify patients who would need a close follow-up, and further assays of complement function.

Disclosure

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Conflicts of interest

There are no conflicts of interest.

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