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Pilot Study of sC5b-9 and Bb Fragment Plasma Levels in Crescentic Immunoglobulin A Nephropathy

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Received 28 September 2023; revised 27 January 2024; accepted 13 February 2024; published online 23 February 2024

Kidney Int Rep (2024) **9,** 1517–1520; https://doi.org/10.1016/j.ekir.2024.02.1400
KEYWORDS: Bb fragment; complement activation; crescent; IgA nephropathy; sC5b-9

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common glomerulonephritis worldwide and an important cause of end-stage kidney disease.[1] The hallmark is the mesangial deposition of IgA1 immunoglobulins with an aberrant glycosylation that triggers mesangial cell hyperplasia with the release of inflammatory cytokines and chemokines.[2] IgAN reflects a spectrum of pathologies with similar pathological mechanisms. Clinical presentation is variable ranging from mild urinary abnormalities, typically microhematuria and proteinuria to severe forms leading to chronic kidney disease and end-stage kidney disease. Severe forms of IgAN are frequently associated with the presence of crescents at the time of kidney biopsy (KB) and the risk of end-stage kidney disease increases as the percentage of glomeruli with crescents increases.[3]

The complement system consists of several circulating and membrane-bound proteins which appear to play a significant role in the pathogenesis of IgAN.[4] The following 3 different pathways activate complement system: the classical pathway, the lectin pathway (LP), and the alternative pathway (AP). The classical pathway and the LP may be initiated by the recognition of damaged or nonhost cell surfaces. The AP is continuously activated in a nonspecific manner, through spontaneous C3 hydrolysis. Activated C3 binds to complement factor B, which is then cleaved by factor D, with the generation of C3bBb, that is, the AP-C3 convertase. All 3 pathways converge into the formation of C3 and then C5 convertases, leading to the generation of the membrane attack complex sC5b-9 and the release of C3a and C5a, which are 2 potent anaphylatoxins with multiple immunomodulatory activities.[5] The AP amplification loop is strictly regulated by membrane-bound and circulating inhibitors, such as factor H, to prevent unnecessary and deleterious AP activation. Factor H accelerates the degradation of the C3bBb complex, and thus plasma levels of Bb fragment reflect AP activation. Complement activation (CA) in IgAN seems to occur dominantly through the LP and the AP.[6] Patients with IgAN with crescents in more than 50% of glomeruli show higher urinary complement levels and markers of the LP, which correlate with the proportion of crescents.[7] Moreover, higher levels of circulating Bb fragment are associated with worst vascular lesions and outcomes of IgAN.[8]

Plasma level of split products from the AP, such as the Bb fragment, may be increased similarly to the terminal effector of CA, sC5b-9, in severe crescentic forms of IgAN. The aim of this preliminary study was to compare plasma levels of sC5b-9 and Bb fragment in patients with biopsy-proven IgAN with active glomerular crescents versus patients without crescents.

We retrospectively collected plasma sC5b-9 and Bb fragment levels measured from September 1, 2018 to

September 30, 2021 in a cohort of patients with biopsy-proven primary IgAN referred for acute or chronic glomerulonephritis to the Service of Nephrology of the HVS (Hôpital du Valais). Full Methods are available in the supplementary materials.

RESULTS

Twenty-seven patients with biopsy-proven primary IgAN underwent complement protein measurements, including sC5b-9 and Bb factor in plasma, from September 1, 2018 to September 30, 2021. The median time interval between KB and complement measurements was 6 days. Ten patients were excluded, including 3 patients with IgA vasculitis and Schoenlein-Henoch purpura, 3 patients with IgAN associated with diabetic nephropathy, 1 patient with IgAN secondary to liver disease, 1 patient with IgAN associated with postinfectious glomerulonephritis, 1 patient with IgAN nephropathy associated with anti-neutrophil cytoplasmic autoantibody, and 1 patient who did not give consent to participate. Thus, 17 patients were finally included in the study, of whom 6 patients were with active crescents (35%) and 11 patients were without active crescent in the KB. They all gave informed consent to participate (Supplementary Figure S1). In Table 1, we summarize and compare the main clinical, histological, and laboratory characteristics of the 2 groups.

Age; gender; the degree of microhematuria; macrohematuria; diastolic blood pressure; and scoring data from the Oxford classification examining mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental glomerulosclerosis (S), and tubular atrophy and interstitial fibrosis (T) did not differ between the 2 groups.

The median plasma creatinine, proteinuria in g/d and albumin-to-creatinine ratio in urine were significantly higher in the group with active crescents than in the group without active crescent ($P = 0.027$, 0.005, and 0.012, respectively). Immunofluorescence for IgA, C3, and C5b-9 in KB did not differ between the 2 groups, nor did C3 and C4 levels or CH50, MBL, and AP50 complement activity. The median plasma concentration of sC5b-9 was 162 ng/ml in the group without active crescent and 561 ng/ml in the crescentic group, which was out of ranges of normal values and markedly higher ($P = 0.0036$), as shown in Figure 1a. Plasma level of Bb was 1.06 μg/ml into the group without active crescent versus 1.615 μg/ml in the crescentic group (reference values in our laboratory <1.65 μg/ml). The difference was not statistically significant ($P = 0.083$), as shown in Figure 1b.

A strong correlation between sC5b-9 plasma levels and the percentage of crescents was found ($rs = 0.73$; $P = 0.0009$; Figure 1c), and this marker showed a remarkable performance as a diagnostic test by its excellent discrimination according to the receiver operating characteristic curve obtained (area under the receiver operating characteristic curve = 0.94; 95% confidence interval: 0.83–1.00; Figure 1d).

DISCUSSION

In this pilot study, patients with crescentic IgAN show a markedly higher terminal CA fragments than those without active crescent. In addition, a strong positive correlation between sC5b-9 levels and the percentage of crescents was observed. Bb fragment levels were higher in patients with IgAN with active crescents than in those without crescents, although they remained in the reference values from our laboratory. The trend toward higher levels of Bb fragment in patients with crescents suggests that the AP of complement mediates, at least in part, CA in crescentic forms of IgAN. We suggest that sC5b-9 and perhaps Bb fragment may represent novel plasma biomarkers, predictive of crescentic IgAN, and that sC5b-9 may appear as an excellent diagnostic test to discriminate patients with crescentic IgAN from those without crescents. The following studies are in line with our findings. Urinary levels of C3a, C5a, sC5b-9, Bb, MBL, and C4d, the latter 2 markers of the LP activation, were significantly higher in patients with IgAN with more than 50% of crescents in glomeruli.[7] Avacopan, a C5a receptor antagonist, improved the slope of proteinuria in patients with IgAN.[9] Iptacopan, an inhibitor of factor B activation, reduced proteinuria in patients with IgAN.[S5] Finally, eculizumab, an anti-C5 inhibitor, was successfully used as a therapeutic alternative for severe crescentic recurrence of IgAN after kidney transplantation.[S6]

However, this study has 2 limitations. Our cohort of patients with IgAN is a relatively small sample size. The second limitation is the real-life nature of our study in which complement analysis was not always performed the same day of KB. However, the latter limitation may also be interpreted as a strength because it reflects real-life clinical management in patients with IgAN. Despite these limitations, our data suggest that plasma sC5b-9 and probably Bb fragment may represent novel biomarkers of crescentic IgAN. The measurements of complement fragments in the plasma from a larger cohort of patients, recruited prospectively and into a multicenter study initiative is needed to extend these findings. If confirmed, our results may contribute to opening new avenues to complement blockade in crescentic form of IgAN.

Table 1. Participant's population characteristics

Characteristics	Patients without crescents (n = 11)	Patients with active crescents (n = 6)	P-value
Age at diagnosis, (yr, range)	41 (34–57)	48 (36–57)	0.92
Gender (male, %)	10 (91%)	6 (100%)	0.45
Creatinine (μmol/l, range)	164 (107–201)	328.5 (171–392)	0.027[a]
eGFR (ml/min per 1.73 m^2, range)	43 (30–73)	22 (14–38)	0.049[a]
Proteinuria (g/d, range)	0.8 (0.2–1.2)	2.6 (1.7–5.3)	0.005[a]
ACR (mg/mmol, range)	76 (19–120)	188 (134–400)	0.012[a]
Microhematuria			0.171
1+	4 (36.36%)	0 (0.0%)	
2+	5 (45.45%)	3 (50.0%)	
3+	2 (18.18%)	3 (50.0%)	
Macrohematuria	2 (18.18%)	0 (0%)	0.266
SBP (mm Hg, range)	134 (130–136)	150 (140–180)	0.030[a]
DBP (mm Hg, range)	86 (77–93)	97 (88–110)	0.078
M			0.446
0	1 (9.09%)	0 (0.0%)	
1	10 (90.91%)	6 (100.0%)	
E			0.169
0	9 (81.82%)	3 (50%)	
1	2 (18.18%)	3 (50%)	
S			0.627
0	5 (45.45%)	2 (33.33%)	
1	6 (54.55%)	4 (66.67%)	
T			0.370
0	4 (36.36%)	1 (16.67%)	
1	5 (45.45%)	2 (33.33%)	
2	2 (18.18%)	3 (50%)	
IF IgA			0.539
+	0 (0.0%)	0 (0.0%)	
++	1 (9.09%)	0 (0.0%)	
+++	3 (27.27%)	3 (50.0%)	
++++	7 (63.64%)	3 (50.0%)	
IF C3			0.433
+	2 (18.18%)	1 (16.67%)	
++	5 (45.45%)	1 (16.67%)	
+++	4 (36.36%)	4 (66.67%)	
IF C5b-9			0.645
-	8 (72.73%)	3 (50.0%)	
+	2 (18.18%)	2 (33.33%)	
++	1 (9.09%)	1 (16.67%)	
C3 (mg/dl, range)	1.145 (1.05–1.37)	1.105 (0.91–1.21)	0.587
C4 (mg/dl, range)	0.27 (0.22–0.31)	0.28 (0.24–0.38)	0.462
CH50 (70%–140%, range)	99.5 (97–140)	102.5 (100–110)	0.704
AP50 (>71%, range)	109 (106–111)	94.5 (84–106)	0.071
MBL (>49%, range)	17 (0–45)	71.5 (18–117)	0.104
sC5b-9 (127–303 ng/ml, range)	162 (113–204)	561 (246–734)	0.0036[a]
Bb fragment (<1.65 μg/ml, range)	1.06 (0.94–1.34)	1.615 (1–1.79)	0.083

ACR, albumin-to-creatinine ratio; AP50, measurement of the activity of alternative pathway; Bb, marker of complement alternative pathway activation; C3, C3 complement protein; C4, C4 complement protein; CH50, measurement of the activity of classical pathway; CKD-EPI, chronic kidney disease-epidemiology collaboration; DBP, diastolic blood pressure; E, endocapillary proliferation; eGFR, estimated glomerular filtration rate, CKD-EPI formula; HPF, high-power field; IF, immunofluorescence; IQR, interquartile range; M, mesangial cellularity; MBL, mannose binding lectin; RBC, red blood cells; S, segmental glomerulosclerosis; SBP, systolic blood pressure; sC5b-9, soluble complement C5b-9 complex; T, tubular atrophy and interstitial fibrosis,

[a]Statistically significant different.

Microhematuria: the presence of >3 RBCs per HPF on microscopic evaluation of a single, properly collected urine specimen. Grade 1 microhematuria is in case of more than 3 RBCs per HPF but less than 11; grade 2 microhematuria is between 11 and 25 RBCs per HPF; and grade 3 microhematuria is in case of more than 25 RBCs per HPF.

M, mesangial cellularity: defined as more than 4 mesangial cells in any mesangial area of a glomerulus. M0 is mesangial hypercellularity in < 50% of glomeruli; M1 ≥ 50%.

E, endocapillary proliferation: defined as hypercellularity due to an increased number of cells within glomerular capillary lumina. E0 is absence of hypercellularity, and E1 is hypercellularity in any glomeruli.

S, segmental glomerulosclerosis: defined as adhesion or sclerosis (obliteration of capillary lumina by matrix) in part of but not the whole glomerular tuft. S0 is absence of segmental glomerulosclerosis, and S1 is presence of segmental glomerulosclerosis in any glomerulus.

T, tubular atrophy and interstitial fibrosis: defined as the estimated percentage of cortical area showing tubular atrophy or interstitial fibrosis, whichever is greater. T0 is 0% to 25%, T1 is 25% to 50%, and T2 is >50%.

IF IgA, C3 and C5b-9: immunofluorescence for IgA, C3, and C5b-9 is categorized on the basis of the intensity of immunofluorescence. Negative IF: -, very light IF: +, light IF: ++, moderate IF: +++, and strong IF: ++++.

Continuous variables are summarized as median (IQR)

Categorical variables as counts (percentages).

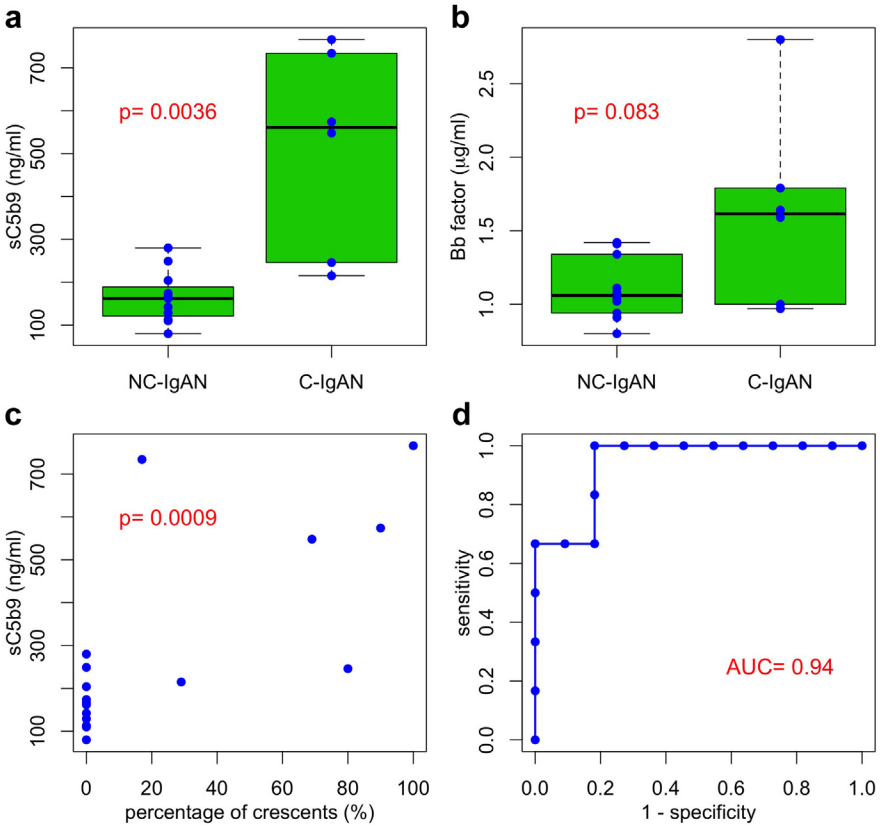

Figure 1. Key study findings: (a) sC5b-9 with/without crescents in glomeruli, (b) Bb fragment with/without crescents in glomeruli, (c) Spearman correlation sC5b-9 and percentage of crescents, and (d) AUC for sC5b-9. AUC, area under ROC curve; C-IgAN, IgA nephropathy with crescents; NC-IgAN, IgA nephropathy with no crescents; ROC, receiver operating characteristic.

DISCLOSURE

GG's institution has received honoraria from Alexion Pharmaceuticals and Vifor Fresenius Medical Care Renal Pharma for giving lectures. All the other authors declared no competing interests.

DATA AVAILABILITY STATEMENT

The data collected for the study, including individual patient data and a data dictionary that defines each field in the data set, will be made available as deidentified participant data to researchers who propose to use the data for individual patient data meta-analysis. Data will be shared following approval of the proposal by the corresponding author and a signed data access agreement.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. Flow chart of the patients included in the study.

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