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CLINICAL RESEARCH

Long-Term Renal Survival in Antineutrophil Cytoplasmic Antibody–Associated Glomerulonephritis With Complement C3 Deposition

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Introduction: Recent studies have revealed the pivotal role of complement activation in the pathogenesis of antineutrophil cytoplasmic antibody–associated glomerulonephritis (ANCA-GN). This study investigated the clinicopathologic and prognostic significance of glomerular C3 deposition in the renal histopathology of patients with ANCA-GN.

Methods: We retrospectively identified 142 patients with ANCA-GN from 6 hospitals in Japan (2004–2020). C3 deposition was defined as C3 staining ≥1+ on a scale of 0 to 2+ using direct immunofluorescence (IF). The primary composite end points included a 30% reduction in estimated glomerular filtration rate (eGFR), end-stage kidney disease (ESKD), and death. We compared clinicopathologic features and long-term outcomes between patients with and without C3 deposition.

Results: C3 deposition was observed in 56 of 142 kidney biopsy samples (39.4%). Patients with C3 deposition had a lower serum C3 level ($P = 0.002$). During a median follow-up of 2.9 (interquartile range: 0.2–5.7) years, 69 events occurred and the cumulative event-free survival rate at 5 years was significantly lower in the C3-positive group than in the C3-negative group (log-rank: $P = 0.002$). In multivariable analysis, C3 deposition was significantly associated with the composite end points after adjusting for age, sex, baseline eGFR, serum C3 level, treatment, and the percentage of normal glomerulus, cellular crescents, global sclerosis, and interstitial damage (adjusted hazard ratio [HR] = 2.02, 95% confidence interval: 1.20–3.40, $P = 0.008$).

Conclusion: This study revealed that ANCA-GN patients with glomerular C3 deposition on IF had worse renal and overall survival rates.

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ANCA-associated vasculitis (AAV) is a group of life-threating systemic autoimmune diseases which includes microscopic polyangiitis, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, and renal-limited vasculitis.[1] Kidney involvement is characterized by rapidly progressive glomerulonephritis; rapidly progressive glomerulonephritis is common in AAV and is the most important predictor of mortality.[2] In general, ANCA-GN is characterized by pauci-immune necrotizing crescentic glomerulonephritis with little Ig and complement deposition in the glomerular capillary walls.[1,3] Historically, it has long been considered that the complement played a limited role in ANCA-GN owing to the paucity of the immune deposits observed in kidney biopsy specimens.[4,5]

Since the 2000s, several studies from animal models have implied that complement activation, particularly the activation of the alternative complement pathway,

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played an important role in the pathogenesis of ANCA-GN.[6–9] The common pathway component C5-deficient and the alternative pathway component factor-B–deficient mice did not develop necrotizing crescentic glomerulonephritis in a myeloperoxidase (MPO)-ANCA mouse model.[6] Moreover, several clinical observations and accumulating evidence have found a certain degree of immune complex and complement deposition in the renal histopathology of human ANCA-GN.[10–13] C3 deposition was found in approximately 30% to 40% in ANCA-GN, which is associated with a higher amount of cellular crescents and a worse onset renal function.[11,13] Likewise, recent studies have reported that patients with AAV with low serum C3 level at diagnosis had poorer renal and overall survival rates.[14–16]

Although data from humans have supported the complement activation involvement in human ANCA-GN, the clinicopathologic significance of complement deposition in patients with ANCA-GN is yet unclear. Most clinical studies[11,12,14–16] investigating complement deposition in renal histopathology and/or serum complement level were limited because of small sample size or fewer kidney biopsy specimens. Moreover, studies analyzing complement deposition in renal histopathology[10–13] were cross-sectional, and the long-term outcomes of ANCA-GN patients with complement deposition on IF remain unclear.

In this study, we investigated the clinicopathologic and prognostic significance of glomerular C3 deposition in the renal histopathology of Japanese patients with ANCA-GN from several institutions. We also compared long-term outcomes between patients with and without C3 deposition.

METHODS

Subjects

Patients newly diagnosed with ANCA-GN by kidney biopsy from 2004 to 2020 at The Jikei University Hospital, Tokyo, Japan, and its affiliated hospitals (The Jikei University Daisan Hospital, The Jikei University Katsushika Medical Center, The Jikei University Kashiwa Hospital, Japanese Red Cross Ashikaga Hospital, and Atsugi City Hospital) were included in this retrospective observational study. Kidney biopsy was performed at the time of diagnosis; in the case of repetition, only the first diagnostic biopsy was considered for inclusion. Relapsing disease was excluded from the study.

All patients met the criteria of the Chapel Hill Consensus Conference definition of AAV[1] and were diagnosed with microscopic polyangiitis, granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis, and renal-limited vasculitis. The following were the exclusion criteria: patients aged <20 years, unavailable clinical data, presence of antiglomerular basement membrane antibodies, concurrent renal diseases causing secondary vasculitis (such as IgA nephropathy, postinfectious glomerulonephritis, Henoch–Schonlein purpura, systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, Behçet's disease, drug-induced vasculitis, and malignancy),[11,13,16] and/or <5 glomeruli in kidney biopsy specimens.[13,17] Biopsy specimens without fresh-frozen sections for IF staining were also excluded from the study (Figure 1).

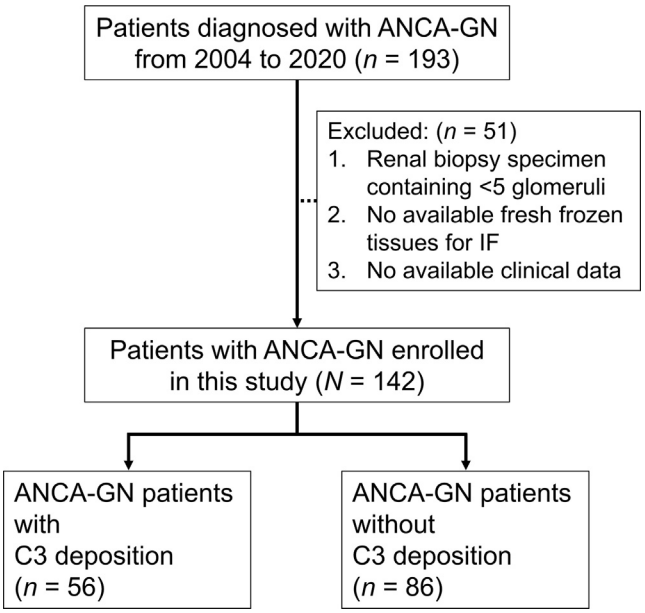

Figure 1. Patient selection. Of all patients who underwent kidney biopsy at our institutions from 2004 to 2020, those aged <20 years and with concurrent renal diseases were excluded and 193 patients were diagnosed with ANCA-GN. Of these patients, 51 were excluded from this study owing to missing clinical data, lack of fresh-frozen tissues for IF, or inadequate glomerular number. ANCA-GN, antineutrophil cytoplasmic antibody–associated glomerulonephritis; IF, immunofluorescence.

This study was approved by the ethics review board of The Jikei University School of Medicine (32-309, 10391) and performed in accordance with the Declaration of Helsinki. Because this was a retrospective cohort study, information on the research plan was proposed and an opportunity to opt-out was provided; therefore, individual informed consent was not required.

Clinical Measurements

Patients were treated with immunosuppressive therapy as per the Japanese clinical practice guidelines (first version, 2002) for rapidly progressive glomerulonephritis.[18] Demographic data, including age, gender, AAV type, height, body weight, blood pressure,

medical history, and hypertension frequencies, were obtained from medical records at the time of diagnostic kidney biopsy. Lung involvement, including pulmonary hemorrhage and interstitial pneumonitis, diagnosed clinically or by imaging was also recorded. Laboratory measurements included white blood cell (WBC) count, hemoglobin, albumin, urea nitrogen, creatinine, eGFR, C reactive protein (CRP), MPO-ANCA, proteinase-3-ANCA, Igs (IgG, IgA, and IgM), serum complements (C3, C4, and CH50), 24-hour proteinuria, and hematuria. ANCAs were measured by chemiluminescent enzyme immunoassay.

The mean arterial pressure was defined as the diastolic blood pressure plus the pulse pressure divided by 3, and hypertension was defined as the systolic blood pressure of >140 mm Hg and/or diastolic blood pressure of >90 mm Hg or the use of antihypertensive medications. Body mass index was defined as the body weight divided by the square of the height; eGFR was calculated from the following formula for Japanese subjects: eGFR (ml/min per 1.73 m^2) = 194 × $Cr^{-1.094}$ × $age^{-0.287}$ (× 0.7399 for women).[19]

Renal Histopathology

Kidney biopsy specimens were obtained using percutaneous needle biopsy. The tissues were embedded in paraffin; cut into 3-μm sections; and stained with hematoxylin and eosin, periodic acid–Schiff, Masson's trichrome, and periodic acid–silver methenamine. The specimens were then evaluated using light microscopy and electron microscopy by pathologists; the findings were derived from the central diagnostic pathologic reports.

Biopsies were classified according to the ANCA-GN histopathologic classification[20] into the following 4 categories: focal (≥50% normal glomeruli), crescentic (≥50% glomeruli with cellular crescents), mixed (<50% normal, <50% crescentic, and <50% globally sclerotic glomeruli), and sclerotic (≥50% globally sclerotic glomeruli). Cellular crescents were included either pure or partial cellular crescents (containing cellular components >10%), and global glomerulosclerosis was referred to as sclerotic changes in >80% of the glomerular tuft, as previously described.[20] Interstitial fibrosis and/or tubular atrophy was based on the affected tubulointerstitial compartment and scored semiquantitatively on a 0 to 3 scale (score 0 for <10%, 1 for 10%–25%, 2 for 26%–50%, and 3 for >50%) according to the Mayo Clinic/Renal Pathology Society Chronicity Score.[21]

Immunofluorescence

For direct IF, the specimens collected at the center where kidney biopsies were performed were embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA), snap frozen in an acetone–dry ice mixture, and stored at −80 °C until use. The specimens were then transported to the central facility (The Jikei University Hospital) for further experiments. The specimens were cut into 2-μm sections on a cryostat and fixed in absolute acetone for 10 minutes, rinsed thrice in 0.01 mol/l phosphate-buffered saline (pH 7.4), and blocked for 60 minutes at room temperature with 5% nonfat dry milk (Cell Signaling Technology, Danvers, MA). After 3 phosphate-buffered saline washes, the sections were incubated at 37 °C for 30 minutes with fluorescein isothiocyanate-conjugated polyclonal rabbit antihuman IgA, IgG, IgM, C3c, and C1q antibodies (Dako, Copenhagen, Denmark); they were then washed 3 times and mounted and examined under a fluorescence microscope (Olympus, Tokyo, Japan). The intensity of immunostaining was scored as negative (0), weak (±), moderate (1+), or strong (2+) by 2 nephrologists (RO and GK) independently without any patient clinical information. Differences in the scoring of the 2 nephrologists were resolved by re-reviewing the biopsies and subsequently reaching a consensus. Kidney biopsies were defined as positive for IF either when the stained Ig or the complement component scored ≥1+ in terms of glomerular staining on the abovementioned scale of 0 to 2+,[22] having excluded nonspecific staining found in segmental and/or global sclerotic lesions. The IF microscopy results were obtained using a separate research protocol from a diagnostic pathology laboratory; the protocol and the nephrologists were consistent throughout the study period.

Definition of the Follow-Up Period and End Points

We conducted patient follow-up for 5 years starting from the diagnostic kidney biopsy until their last available follow-up or their death; in this period, eGFR was recorded at a minimum of 6-month intervals and more frequently during the first year. The primary composite end points were a 30% reduction in eGFR, ESKD, and death. ESKD was defined as the maintenance dialysis therapy or kidney transplantation. The requirement for renal replacement therapy for acute kidney injury was also recorded, but it was not counted for the category related to ESKD events.

Statistical Analyses

Continuous variables were presented as medians and interquartile ranges or numbers with percentages in parentheses, and differences in continuous and categorical variables were evaluated using the Mann–

Table 1. Comparison of clinical features of patients with and without C3 deposition

Variable	All (N = 142)	C3 positive (n = 56)	C3 negative (n = 86)	P value
Age, yr	73.0 (65.0–80.0)	72.5 (64.3–80.0)	73.0 (66.5–79.3)	0.87
Male, n (%)	55 (38.7)	26 (46.4)	29 (33.7)	0.13
AAV subtype (MPA/GPA/EGPA/RLV, *n*)	124/14/1/3	50/4/1/1	74/10/0/2	0.51
MPO-ANCA positive, *n* (%)[a]	128 (90.1)	53 (94.6)	75 (87.2)	0.15
PR3-ANCA positive, *n* (%)[a]	20 (14.1)	4 (7.1)	16 (18.6)	0.06
Lung involvement, *n* (%)	85 (59.9)	32 (57.1)	53 (61.6)	0.59
Dialysis at onset, *n* (%)	34 (23.9)	17 (30.4)	17 (19.8)	0.15
BMI (kg/m^2)	21.7 (19.3–23.6)	22.1 (19.8–23.8)	21.7 (19.1–23.4)	0.56
MAP (mm Hg)	96.0 (86.7–105.3)	96.7 (87.4–108.8)	95.2 (86.5–104.7)	0.31
Hypertension, *n* (%)	85 (59.9)	34 (60.7)	51 (59.3)	0.87
Immunosuppressors, *n* (%)				0.11
PSL	84 (59.2)	34 (60.7)	50 (58.1)	—
PSL + RTX	25 (17.6)	12 (21.4)	13 (15.1)	—
PSL + IVCY	16 (11.3)	2 (3.6)	14 (16.3)	—
Others	17 (12.0)	8 (14.3)	9 (10.5)	—
eGFR (ml/min per 1.73 m^2)	17.9 (8.0–39.0)	13.6 (8.0–35.7)	21.4 (8.8–42.6)	0.24
1-year eGFR (ml/min per 1.73 m^2)[b]	23.5 (0.0–40.3)	22.9 (0.0–33.9)	25.0 (10.4–43.8)	0.17
UPE (g/d)	1.2 (0.5–2.2)	1.3 (0.5–2.7)	1.2 (0.5–2.0)	0.37
U-RBC (/HPF), *n* (%)				0.28
<0 to 5	11 (7.7)	2 (3.6)	9 (10.5)	—
<50	50 (35.2)	19 (33.9)	31 (36.0)	—
≥50	27 (19.0)	14 (25.0)	13 (15.1)	—
≥100	54 (38.0)	21 (37.5)	33 (38.4)	—
WBC (μl)	8600 (6650–11,725)	7600 (5375–11,675)	9600 (7375–11,950)	0.01
Hb (mg/dl)	9.6 (8.4–11.1)	9.5 (8.2–10.9)	9.7 (8.5–11.3)	0.51
Alb (mg/dl)	2.8 (2.3–3.3)	2.9 (2.1–3.5)	2.8 (2.5–3.2)	0.93
CRP (mg/dl)	3.9 (0.5–11.3)	1.5 (0.2–10.9)	5.0 (1.2–11.6)	0.049
IgG (mg/dl)	1574.0 (1284.5–1858.0)	1531.0 (1283.0–1845.0)	1601.0 (1295.5–1867.3)	0.80
IgA (mg/dl)	315.0 (231.8–389.0)	319.0 (243.0–389.0)	310.5 (226.3–396.5)	0.91
IgM (mg/dl)	80.0 (54.5–132.0)	74.0 (51.3–138.5)	86.5 (58.8–126.0)	0.50
C3 (mg/dl)	108.0 (94.0–126.0)	101.0 (89.0–118.0)	115.0 (96.5–137.5)	0.002
C4 (mg/dl)	30.0 (23.0–36.8)	28.0 (22.0–35.0)	31.0 (23.5–38.5)	0.09
CH50 (U/ml)	50.5 (41.4–57.4)	48.6 (39.4–54.6)	53.3 (43.4–58.3)	0.04

AAV, antineutrophil cytoplasmic antibody–associated vasculitis; Alb, albumin; ANCA, antineutrophil cytoplasmic antibody; BMI, body mass index; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; Hb, hemoglobin; HPF, high-power field; IQR, interquartile range; IVCY, i.v. cyclophosphamide; MAP, mean arterial pressure; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase-3; PSL, prednisolone; RLV, renal-limited vasculitis; RTX, rituximab; UPE, urinary protein excretion; U-RBC, urinary red blood cell; WBC, white blood cell.
[a]A total of 8 dual-positive (both MPO-ANCA and PR3-ANCA) patients were included, and 2 patients were dual negative.
[b]The 1-year eGFR for dialysis-dependent patients was defined as 0 ml/min per 1.73 m^2.[25]
Values are presented as medians and IQRs or numbers with percentages in parentheses.

Whitney U and χ^2 tests, respectively. Event-free survival at 1 year and 5 years of follow-up was measured and compared using the Kaplan–Meier method and log-rank tests. A Cox regression model was used to compare the HRs of composite end points between the C3 positive and negative groups. The prognostic factors of renal outcomes in ANCA-GN[16,20,23–26] were included in multivariable analyses. The treatments were divided into the following 3 categories: none, prednisolone only, and prednisolone + other immunosuppressors. All covariates, except sex and treatment, were treated as continuous variables in the multivariable analyses. Censored cases were regarded as patients remaining under observation at the end of the 5-year follow-up period and those who were lost to follow-up during the surveillance period. Sensitivity analyses were performed by changing the definition of outcomes and exposure (grouped according to C3 staining intensity) and by analyzing the subject subpopulations. Statistical significance was defined according to a two-sided $P < 0.05$. All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY).

RESULTS

Baseline Demographic and Clinical Characteristics

Table 1 lists the demographic, clinical, and laboratory data of 142 patients enrolled in this study. The median age was 73.0 years (interquartile range: 65.0–80.0), 38.7% of which were male; the median eGFR at diagnosis was 17.9 (8.0–39.0) ml/min per 1.73 m^2. A total of 34 patients (23.9%) required hemodialysis at onset, and 85 patients (59.9%) presented with lung involvement.

Table 2. Comparison of histologic features of patients with and without C3 deposition

Variable	All (N = 142)	C3 positive (n = 56)	C3 negative (n = 86)	P value
No. glomeruli	21 (14–33)	20 (16–32)	22 (14–33)	0.79
% Normal glomeruli	37.5 (17.2–61.2)	37.1 (17.3–57.1)	38.9 (17.1–64.4)	0.45
% Global sclerosis	23.1 (9.8–43.9)	23.2 (11.1–49.7)	22.6 (8.3–41.6)	0.45
% Cellular crescents	23.6 (8.3–43.6)	21.7 (7.4–43.0)	24.7 (8.9–44.2)	0.73
% Fibrous crescents	0.0 (0.0–5.3)	0.0 (0.0–5.5)	0.0 (0.0–5.5)	0.65
IF/TA, *n* (%)				0.82
<10%	8 (5.6)	3 (5.4)	5 (5.8)	—
10%–25%	33 (23.2)	14 (25.0)	19 (22.1)	—
26%–50%	57 (40.1)	22 (39.3)	35 (40.7)	—
>50%	44 (31.0)	17 (30.4)	27 (31.4)	—
ANCA-GN classification, *n* (%)				0.52
Focal	50 (35.2)	19 (33.9)	31 (36.0)	—
Crescentic	25 (17.6)	9 (16.1)	16 (18.6)	—
Mixed	40 (28.2)	14 (25.0)	26 (30.2)	—
Sclerotic	27 (19.0)	14 (25.0)	13 (15.1)	—
Immunofluorescence, *n* (%)				
IgM	46 (32.4)	31 (55.4)	15 (17.4)	<0.001
IgG	22 (15.5)	15 (26.8)	7 (8.1)	0.003
IgA	20 (14.1)	10 (17.9)	10 (11.6)	0.30
C1q	18 (12.7)	12 (21.4)	6 (7.0)	0.01
EDD, *n*/*N* (%)[a]	15/127 (11.8)	9/48 (18.8)	6/79 (7.6)	0.06

ANCA-GN, antineutrophil cytoplasmic antibody–associated glomerulonephritis; EDD, electron-dense deposit; IF/TA, interstitial fibrosis and/or tubular atrophy; IQR, interquartile range.
[a]A total of 15 missing values.
Values are presented as medians and IQRs or numbers with percentages in parentheses.

A total of 120 patients (84.5%) were MPO-ANCA single-positive, 12 (8.5%) were proteinase-3-ANCA single-positive, and 8 (5.6%) were double-positive for both MPO-ANCA and proteinase-3-ANCA. Although these levels remained within the normal range (reference range: 73–138 mg/dl and 30–50 mg/dl, respectively), patients with C3 deposition had lower serum C3 (101.0 mg/dl, interquartile range: 89.0–118.0 mg/dl) and CH50 levels (P = 0.002 and P = 0.04, respectively), lower WBC counts, and lower CRP levels than those without. Of the 34 patients who needed acute hemodialysis at onset, 7 got off dialysis. Of the 7 patients, 4 were C3 positive and the remaining 3 were C3 negative. Other clinical and laboratory characteristics, including age, sex, AAV subtype, baseline kidney function, hemodialysis dependence at onset, urinalysis, and treatments, were not statistically different between the 2 groups.

Histopathologic Characteristics

Table 2 and Supplementary Table S1 list the renal histopathologic characteristics. The kidney biopsies contained a median of 21 (14–33) glomeruli, of which 37.5% (17.2–61.2) were normal, 23.6% (8.3–43.6) contained cellular crescents, and 23.1% (9.8–43.9) were sclerosed. Most patients had interstitial fibrosis and/or tubular atrophy.

Glomerular C3 deposition was detected in the specimens of 56 of 142 patients (39.4%); 47 of 56 patients (83.9%) had C3 deposits in the mesangium, 12 of 56 (21.4%) had C3 deposits in the glomerular capillary wall; and 3 had deposits in both the mesangium and glomerular capillary wall (Supplementary Table S1). Those with kidney biopsy specimens positive for C3 had more IgM, IgG, and C1q deposition than those with specimens negative for C3 ($P < 0.001$, $P = 0.003$, and $P = 0.01$, respectively). Electron-dense deposit was detected in 15 of 127 patients (11.8%). Patients with C3 deposition had more electron-dense deposit (18.8%) than those without C3 deposition (7.6%), which was statistically not significant ($P = 0.06$). No other significant histopathologic differences were found between the C3 positive and negative groups.

The 1- and 5-Year Cumulative Event-Free Survival

During the median follow-up of 2.9 (0.2–5.7) years, 69 patients reached the primary composite end point of 30% reduction in eGFR, ESKD, and death; 36 and 33 end point events occurred in the C3 positive and negative groups, respectively. Of note, the cumulative event-free survival rates at 1 year and 5 years were significantly lower in the C3-positive group than in the C3-negative group (log-rank: $P = 0.008$ and $P = 0.002$, respectively). Moreover, the 1-year and 5-year cumulative event-free survival rates were 44% and 31% in the C3-positive group and 67% and 54% in the C3-negative group, respectively (Figure 2a). The omission of the 13 cases with (2+) Ig staining (11 cases with IgM and 2 cases with IgG) did not substantially change the association of C3 deposition and the primary composite

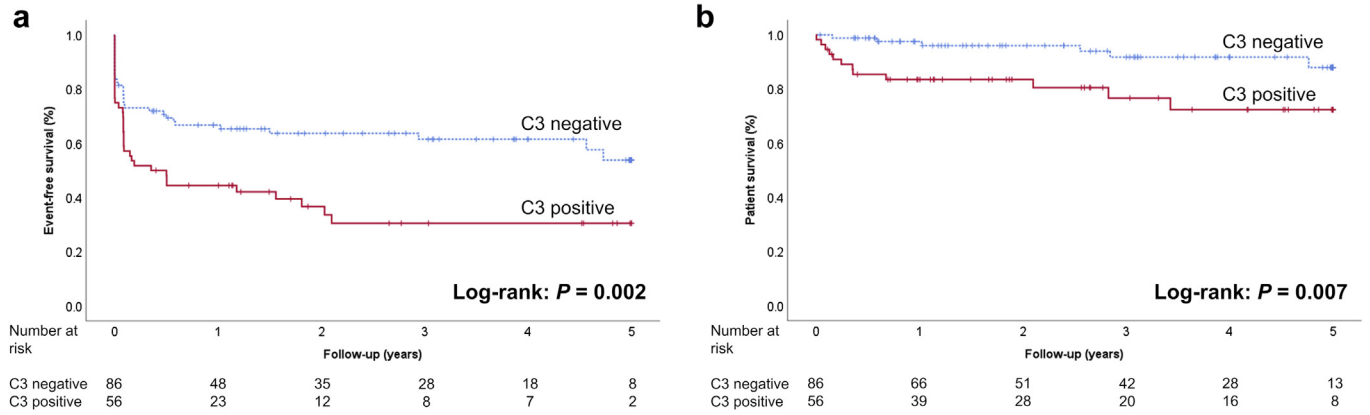

Figure 2. Kaplan–Meier plots of event-free survival stratified by C3 deposition (with and without). (a) 5-year cumulative event-free survival; (b) 5-year cumulative patient survival. The solid red line represents patients with C3 deposition, whereas the dashed blue line represents patients without C3 deposition. (a) C3-positive patients had a significantly lower cumulative event-free survival at 5 years after the diagnostic kidney biopsy. (b) C3-positive patients had a significantly worse patient survival at 5 years after the diagnostic kidney biopsy.

end point (Supplementary Figure S1). With respect to the composite end point of renal survival, that is, 30% reduction in eGFR and ESKD, patients with C3 deposition had a poorer renal survival rate (Supplementary Figure S2). Moreover, after classifying patients into groups as per no (−), low (±), medium (1+), or high (2+) level of C3 staining, patients with stronger C3 deposition had a worse 1-year cumulative event-free survival rate than those with weaker C3 deposition (Supplementary Figure S3).

During the total follow-up period, 38 of 142 patients (26.8%) progressed to ESKD and 18 of 142 patients (12.7%) died. The causes of death included serious infection (9 of 18, 50.0%); intestinal or brain hemorrhage (3 of 18, 16.7%); and others (6 of 18, 33.3%), including lung cancer, heart failure, suicide, and unknown causes. Of the 38 patients who progressed to ESKD, 17 of 56 (30.4%) were in the C3-positive group and 21 of 86 (24.4%) were in the C3-negative group. Of the 18 deaths, 6 of 56 (10.7%) were in the C3-positive group and 12 of 86 (14.0%) were in the C3-negative group. Among the 9 deaths caused by serious infection, 6 were C3 positive. The 1-year and 5-year patient survival rates were worse in the C3-positive group than in the C3-negative group (log-rank: $P = 0.003$ and $P = 0.007$, respectively). While the 5-year cumulative patient survival rate was 72% in patients with C3 deposition and 88% in those without C3 deposition (Figure 2b), the 1-year cumulative patient survival rate was 84% in patients with C3 deposition and 98% in those without C3 deposition.

Multivariable Cox Proportional Hazard Analyses for Renal and Overall Survival

Table 3 reveals the unadjusted and multivariable-adjusted HRs for predicting the primary composite end point in the comparison between the C3 positive and negative groups. As per the multivariable analysis for the 5-year period after kidney biopsy, the C3-positive group had a significantly higher risk of kidney function deterioration, ESKD, and death (HR = 2.01, 95% confidence interval: 1.25–3.23, $P = 0.004$). After adjusting for age, sex, baseline eGFR, serum C3 level, treatment, normal glomerulus percentage, global sclerosis percentage, cellular crescent percentage, and interstitial fibrosis and/or tubular atrophy percentage, the C3-positive group was significantly associated with poor renal and overall survival (adjusted HR = 2.02, 95% confidence interval: 1.20–3.40, $P = 0.008$).

DISCUSSION

The major novel findings of this study, which included 142 Japanese patients with ANCA-GN, are as follows: glomerular complement deposition was detected in

Table 3. Univariable and multivariable Cox proportional hazard analyses for the composite end points of renal and overall survival

	Unadjusted			Adjusted model 1[a]			Adjusted model 2[b]		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
C3 positive	2.01	1.25–3.23	0.004	2.05	1.22–3.45	0.007	2.02	1.20–3.40	0.008

CI, confidence interval; HR, hazard ratio.
[a]Model 1: adjusted for age, sex, baseline estimated glomerular filtration rate, serum C3 level, and treatment.
[b]Model 2: adjusted for model 1 + % normal glomeruli, % global sclerosis, % cellular crescents, and % interstitial fibrosis and/or tubular atrophy.

approximately 40% of the ANCA-GN kidney biopsy specimens by routine IF and C3-positive patients with ANCA-GN had both worse renal and overall survival at 5 years. The association between C3 deposition and poor renal and overall survival persisted after considering the previously reported predictors of renal outcomes in ANCA-GN[16,20,23–26] and after changing the definition of outcomes or analyzing the patient subgroups. To the best of our knowledge, this is the largest study to date that investigated the glomerular deposition of Igs and complement using IF and the first study that evaluated the prognostic significance of C3 deposition.

The results revealed that C3 deposition was associated with an aggressive clinical course. This is consistent with a study from Spain involving 85 patients with biopsy-proven pauci-immune necrotizing crescentic glomerulonephritis revealing glomerular complement (C3d) staining by immunohistochemistry was an independent risk factor for ESKD development.[27] Although we hypothesized that these patients may have potential predisposing factors that cause the sustained activation of complement systems, the reason why this subgroup of ANCA-GN patients with C3 deposition exhibited worse renal prognosis is yet unclear. Nevertheless, several studies have recently revealed the significance of complement activation and glomerular C3 deposition in various other glomerular diseases, including focal segmental glomerulosclerosis,[28] IgA nephropathy,[29] membranous nephropathy,[30] lupus nephritis,[31] C3 glomerulonephritis,[32] and transplant glomerulopathy.[33] These studies support the hypothesis that C3 deposition in renal histopathology indicates complement activation in human glomerular diseases.

Although no significant histopathologic differences were observed between patients with and without complement deposition in this study and in the study conducted by Villacorta *et al.*,[27] previous cross-sectional studies focusing on complement C3 deposition in human ANCA-GN[11,13] concluded that patients with C3 deposition had a lower percentage of normal glomeruli, higher percentage of cellular crescents, and higher percentage of interstitial fibrosis and/or tubular atrophy. A possible reason for these discrepancies is that C3-positive patients in our study might be in the active inflammatory phase, which occurs before crescent formations or global sclerosis. In an MPO-ANCA rat model, C3 and IgG deposits were detectable in the acute phase of the disease, but they disappeared once the chronic inflammatory lesions had developed.[34,35] Persistent glomerular C3 positivity supports our hypothesis that sustained complement activation may be associated with C3-positive patients. A pathologic study that included 20 patients with MPO-ANCA–positive ANCA-GN revealed a glomerular colocalization of MPO, IgG, and C3 with a decreased staining intensity of CD34.[36] Glomerular C3 localization might thus serve as a sensitive marker of an active glomerular capillary injury by the complement system in human ANCA-GN.

In this study, a larger proportion of C3 deposition was found by IF in the mesangium than in the glomerular capillary wall, which is consistent with previous studies.[3,13] Although it was difficult to clearly distinguish the location of C3 deposition on IF specimens, we did not observe any clinicopathologic differences between patients with mesangial C3 deposition and those with glomerular capillary wall C3 deposition. This made it difficult to ascertain and appropriately discuss the location of C3 deposition.

Consistent with previous results,[10,22,37] we observed that patients with C3 deposition had more IgM, IgG, and C1q deposition on IF and electron-dense deposit on electron microscopy; the relatively small presence of electron-dense deposit was most often observed within the mesangium. Although the precise mechanism is unclear, the immune complex and complement activation through a classical pathway may contribute to ANCA-GN pathogenesis. Nevertheless, our findings were not influenced by excluding 13 cases with a strong Ig staining (IgM, IgG) who might have a different pathophysiology from pauci-immune glomerulonephritis.

Several studies have revealed that patients with AAV with low serum C3 levels have higher creatinine levels at diagnosis and worse renal and overall survival rates.[14–16,26,38] Our study revealed that patients with C3 deposition had lower serum C3 and CH50 levels than those without. Nevertheless, the multivariable Cox proportional hazard analyses revealed that patients with C3 deposition were independently associated with the composite outcome of renal and overall survival rates after adjusting for serum C3 level, which was also associated with renal and overall survival rates as found in model 3 (adjusted HR = 1.01, 95% confidence interval: 1.00–1.02, $P = 0.04$). According to the serum C3 level,[14] patients were classified into 3 tertiles; however, no significant differences were observed for the event-free survival between the lower serum C3 group (tertile 1) and the higher serum C3 groups (tertiles 2 and 3) (Supplementary Figure S4). These results suggest that glomerular C3 deposition by IF is a stronger prognostic factor than serum C3 level with respect to renal and overall survival rates. Likewise, serum C4 level was not different between the C3 positive and negative groups. Moreover, in accordance with previous reports,[26,38,39] serum C4 level was

not associated with AAV prognosis, which supports the activation of the alternative complement pathway.

Baseline characteristics between patients with and without C3 deposition did not include WBC counts and CRP level in previous studies.[11,13,27] Two studies with MPO-ANCA–positive ANCA-GN revealed that patients with ANCA-GN with immune complex deposits had lower CRP levels at diagnosis than those without immune complex deposits.[22,37] WBC count has not been reported as a prognostic factor in ANCA-GN, whereas CRP is a component of the Japanese rapidly progressive glomerulonephritis clinical grading system.[18] In this study, the laboratory clinical results revealed that C3-positive patients had lower systemic inflammatory marker levels, including WBC count and CRP level, than C3-negative patients. On including WBC and CRP levels in the multivariable analysis as continuous variables, the association between C3 deposition and the primary outcome did not change (Supplementary Table S2).

This study has several limitations. First, this was a retrospective observational study; therefore, the causal relationship between C3 deposition and poor renal survival could not be proven. Second, we did not perform additional specific staining for C3d, C4d, properdin, factor B, membrane attack complex, or mannose-binding lectin in glomeruli and did not evaluate other plasma complement components. Third, the intensity of IF was defined qualitatively by the nephrologists; the intensity could have been affected by the different conditions of the staining procedure, kidney biopsy specimens, storage time, and exposure time of the microscopy. Fourth, the Japanese clinical practice guidelines recommend corticosteroids alone in patients aged >70 years to avoid over-immunosuppression, which is different from the European and American guidelines. Finally, this study cohort included biopsy-proven Japanese patients with ANCA-GN, a large proportion of which was the MPO-ANCA–positive AAV subtype; this makes the generalization of these results difficult. Hence, further studies are needed.

Despite these limitations, the study followed the long-term renal survival of ANCA-GN patients with positive C3 deposition for the first time, which is a major strength. Several studies, including this study, revealed that 30% to 40% of patients with ANCA-GN have C3 deposition. Moreover, the results of this study suggest that a routine direct IF investigation in the clinical setting provides clinically important information regarding active inflammation and renal prognosis in ANCA-GN. Positive C3 staining may be useful for earlier detection of ANCA-GN disease activity at the time of biopsy compared with other clinical and histopathologic parameters. We speculate that complement-targeting therapy, such as avacopan (CCX168),[8,9,40–42] works satisfactorily in patients with C3 deposition in the persistent active inflammatory phase. Repeated biopsies of C3-positive patients with ANCA-GN who received C5a receptor inhibitor will provide interesting results in future studies.

CONCLUSION

In conclusion, patients with C3 deposition had worse renal and overall survival rates. The study results suggest that glomerular C3 deposition provides clinically important information regarding active inflammation and long-term renal prognosis in ANCA-GN.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, data curation, writing—original draft preparation: RO and GK; formal analysis: RO, TS, and GK; resources: KH, HO, and TY; writing—review and editing: RO, GK, TS, YO, KH, MO, SY, KK, KH, HO, NT, and TY; visualization: RO; supervision: TY; project administration: GK. All authors contributed to data interpretation and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Kaplan–Meier plot of event-free survival excluding patients with (2+) immunoglobulin staining.

Figure S2. Kaplan–Meier plot of renal survival stratified by C3 deposition (with and without).

Figure S3. Kaplan–Meier plot of event-free survival according to C3 staining intensity.

Figure S4. Kaplan–Meier plot of event-free survival according to baseline serum C3 levels.

Table S1. Immunofluorescence intensities and localization of immunoglobulin and complement deposits.

Table S2. Uni- and multivariable Cox proportional hazard analyses for the composite endpoint of renal and overall survival.

STROBE Statement (PDF).

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