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Granulomatosis With Polyangiitis Induced by Infection

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INTRODUCTION

Granulomatosis with polyangiitis (GPA) is a small-vessel vasculitis associated with antineutrophil cytoplasmic antibody (ANCA), especially proteinase 3−ANCA. Granulomatosis with polyangiitis typically demonstrates kidney lesions after involvement of the nasal passages and lungs, and less than 20% of patients with GPA have nephritis at the time of presentation.[1] Therefore, clinical presentation of rapid progressive glomerulonephritis is considered less likely in GPA than in microscopic polyangiitis.

In general, GPA occurs in patients between 45 and 60 years of age[2]; however, our report presents a rare case of a 17-year-old man with multiple lung nodules who developed diffuse cellular crescentic glomerulonephritis with C3-dominant staining. Furthermore, the patient showed positive results for nephritis-associated plasmin receptor (NAPlr) and plasmin activity, possible histological markers of infection-related glomerulonephritis, in the glomeruli by immunofluorescence staining and *in situ* zymography with plasmin-sensitive synthetic substrate, respectively. The present case indicates that infection can also be involved in the development of GPA in addition to ANCA.

CASE PRESENTATION

A 17-year-old Japanese man without any past medical history was admitted to an outside hospital due to an elevated serum creatinine level with a 2-week history of upper respiratory infection and a 3-day history of gross hematuria. His serum creatinine level progressively increased after admission. He was referred to our hospital for further intensive treatment. We suspected a diagnosis of GPA because he had nasal bleeding, and multiple cavitary pulmonary nodules were observed on chest computed tomography (CT) at admission to an outside hospital. He was a senior high school student and did not have daily use of any drug or a recent history of traveling abroad.

On physical examination, his blood pressure was 120/60 mm Hg, temperature was 37.3°C, and SpO_2 was 98% on room air. Physical examination showed no saddle nose or cheek pain; his cardiovascular examination results were normal; and his lungs were clear to auscultation. His abdominal and neurological examination results were unremarkable, and no edema or skin lesions were observed.

Initial laboratory results are shown in Table 1. Serum creatinine was 7.43 mg/dl and estimated glomerular filtration rate was 14.8 ml/min per 1.73 m^2 on admission. Urinalysis revealed glomerular hematuria with red blood cell casts and proteinuria of 1.93 g/gCr. Myeloperoxidase−ANCA (MPO-ANCA; measured by enzyme-linked immunosorbent assay) was slightly elevated at 10.6 U/ml, and proteinase 3−ANCA was absent. Serum C3 was markedly decreased to 10 mg/dl, but the C4 level was normal at 26 mg/dl. Anti−glomerular basement membrane antibody was absent, and antistreptolysin O and antistreptokinase antibody were elevated at 250 IU/ml and 2560 IU/ml, respectively, which are close to the upper limits of the normal ranges. *Streptococcus pyogenes* was found on sputum culture. There was no bacterial growth on blood culture. Results for T-SPOT and βD-glucan were negative. CT performed at admission to our hospital showed multiple bilateral cavitary pulmonary nodules and bilateral renal enlargement (Figure 1a). In addition, paranasal sinus mucosal membrane swelling and fluid in the left sinus were identified; there was no evidence of bone destruction or

Table 1. Laboratory data

Complete blood count WBC	10,500/μl	Na	132 mEq/l	Microbiology
Seg	72%	K	4.2 mEq/l	Blood culture: negative
Eos	0.5%	Cl	100 mEq/l	Sputum culture:
Lym	15.5%	cCa	9.3 mg/dl	*Streptococcus pyogenes*
Mono	12.0%	IP	4.6 mg/dl	βD-glucan (−)
RBC	4.31×10^4 /μl	UA	8.6 mg/dl	*Asperugillus* antigen (−)
Hb	11.6 g/dl	CRP	6.57 mg/dl	TSPOT (−)
Ht	35.9%	Serology IgA	202 mg/dl	
MCV	83.2 fl	IgG	1539 mg/dl	Urinalysis
PLT	26.9万/μl	IgM	107 mg/dl	Occult blood (3+)
Biochemistry TP	6.2 g/dl	C3	10 mg/dl	Protein (2+)
Alb	2.9 g/dl	C4	26 mg/dl	WBC 30–49/HPF
AST	13 IU/l	RF	<2 IU/ml	RBC >100/HPF
ALT	11 IU/l	ANA	×40(homo)	Lleukocyte cast (+)
LDH	293 IU/l	dsDNA-IgG	4.5 IU/ml	Erythrocyte cast (+)
ALP	301 IU/l	MPO-ANCA	10.6 U/ml	Granular cast (2+)
γGTP	18 IU/l	PR3-ANCA	<1.0 U/ml	Waxy cast (+)
BUN	56.6 mg/dl	anti-GBM-Ab	<2.0 U/ml	Protein quantity 1.93g/gCr
Cr	7.53 mg/dl	ASO	250 IU/ml	NAG 52.0U/l
eGFR[a]	14.8 ml/min per 1.73 m^2	ASK	2560×	β_2MG 927 mg/dl

Alb, albumin; ALT, alanine aminotransferase; ANCA, antineutrophil cytoplasmic antibody; ANA, antinuclear antibody; ASK, antistreptokinase antibody; ASO, antistreptolysin O; AST, aspartate aminotransferase; BUN, blood urea nitrogen; cCa, corrected calcium; Cr, creatinine; Cl, chloride; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; GBM, glomerular basement membrane; γGTP, γ-glutamyl transpeptidase; IP, inorganic phosphorus; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; NAG, *N*-acetyl-glutamate; PLT, platelet; RBC, red blood cell; RF, rheumatoid factor; TP, transpeptidase; UA, urinary albumin; WBC, white blood cell.
[a]Estimated equation from the Japanese Society for Pediatric Nephrology.

nasal mass (Figure 1c). Paranasal sinus biopsy showed no evidence of granulomatous lesion.

Based on the clinical findings of rapidly progressive nephritic syndrome, upper respiratory lesions (nasal bleeding, paranasal sinusitis), lower respiratory lesions (cavitary pulmonary nodules), and low grade MPO-ANCA positivity, we diagnosed GPA with high activity, with a Birmingham Vasculitis Activity Score of 21 points. This was considered to be severe GPA according to the European League Against Rheumatism guidelines. We initiated treatment with steroids (i.v. methylprednisolone pulses for 3 days, followed by 1.0 mg/kg per day) and 5 rounds of plasma exchange with hemodialysis. Initially, due to the possibility of bacterial infection, we also administered ceftriaxone. We added rituximab (375 mg/m^2 every week for 4 times) for remission induction therapy; rituximab was selected because it does not require dose change due to renal function and has no infertility risk, as seen with cyclophosphamide.

Renal biopsy performed on the fourth day showed 34 glomeruli, with 30 demonstrating cellular crescents; there was no global sclerosis. Light microscopy showed diffuse cellular crescents with fibrinoid necrosis of the capillary tuft without fibro-cellular or fibrous crescents (Figure 2a). Immunofluorescence microscopy revealed strong positive C3 staining in the mesangial area; IgG, IgA, IgM, C1q, and C4d showed no staining (Figure 2b, c). There was no electron-dense deposit including hump on electron microscopy (Figure 2f). The final histopathological diagnosis was diffuse crescentic glomerulonephritis; the etiology was thought to be associated with an infectious process, due to positive staining for NAPlr and plasmin activity (Figure 2d, e).

During treatment, the patient's serum creatinine level increased to 10.41 mg/dl and he developed oliguria; hemodialysis was started on the sixth day. After remission induction therapy, the urine volume was gradually increased and the patient could stop hemodialysis on the 15th day. The results for MPO-ANCA became negative, and the serum C3 level showed a relative increase for the first week after induction therapy. Computed tomography demonstrated resolution of the pulmonary nodules on the 13th day. The patient's serum creatinine level improved to 0.93 mg/dl and his C3 level improved to 80 to 100 mg/dl, within the normal range; his Birmingham Vasculitis Activity Score also improved to 6 points. He was discharged on the 36th day with a prescription for 30 mg prednisolone (PSL). Both anti−streptolysin O antibody and anti-streptokinase antibody were checked on the 10th and 31st days. The patient's ASO was 301 IU/ml on the 10th day and 268 IU/ml on the 31st day, showing no remarkable improvement from admission. His anti-streptokinase antibody decreased to 1280 IU/ml on the 10th day and was 640 IU/ml on the 31st day.

During outpatient follow-up, treatment with losartan was started; the patient's serum creatinine level was 0.6 to 0.7 mg/dl, and his C3 level remained within the normal range (86–160 mg/dl). Azathioprine was added for maintenance immunosuppression when PSL was tapered to 15 mg, 3 months after discharge. After approximately 6 months, microscopic hematuria was resolved, and the patient's proteinuria was less than 0.3 g/gCr; at that time, he was being treated with 7.5 mg PSL and 100 mg azathioprine.

DISCUSSION

Here, we present a case of a young man with MPO-ANCA−positive GPA who had diffuse cellular crescentic glomerulonephritis with strong C3 staining, an absence of any Igs, and no deposits on electron microscopy.

In general, GPA occurs in patients between 45 and 60 years of age[2]; serum complement levels are typically normal, and renal biopsy usually shows pauci-immune necrotizing glomerulonephritis. The present case is atypical for GPA in regard to several features, including the disease onset at a very young age, low titer of MPO-ANCA, and histopathological findings of diffuse cellular crescents with C3-dominant staining. Although

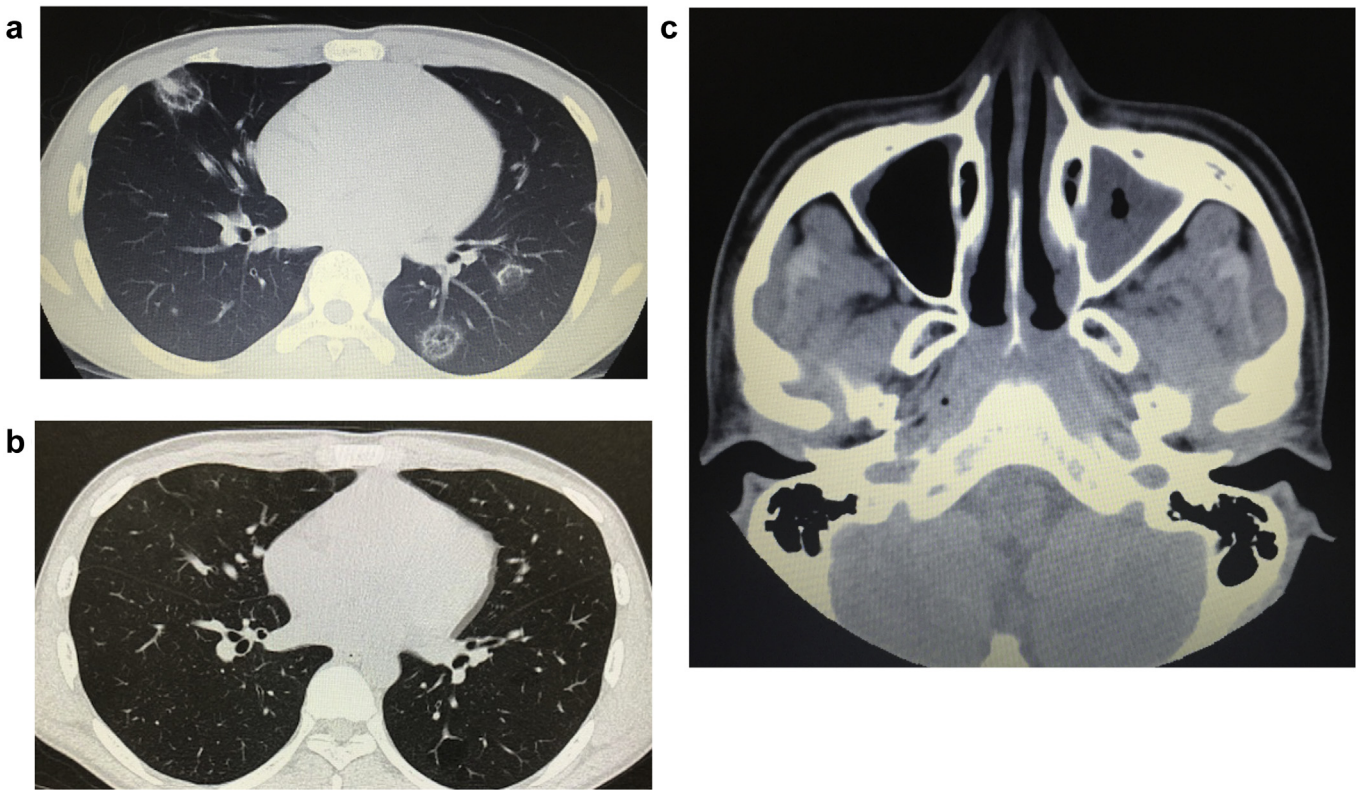

Figure 1. (a) Chest abdominal computed tomogram at admission showing multiple bilateral cavitary pulmonary nodules. (b) Computed tomogram after treatment on the 13th hospital day showing resolution of the lung nodules. (c) Computed tomogram of facial bone showing paranasal sinus mucosal membrane swelling and fluid in the left sinus. There was no evidence of bone destruction or mass lesion.

anti−glomerular basement membrane nephritis shows similar clinical manifestations and histopathological findings,[3] it was considered an unlikely diagnosis because of the absence of IgG on immunofluorescence microscopy and serum anti−glomerular basement membrane antibody.

Several studies have reported that ANCA-associated vasculitis correlates with various infections and shows frequent occurrence in the winter and spring.[4] Due to the presence of positive NAPlr and plasmin staining, the present case was considered to be associated with infection. Nephritis-associated plasmin receptor is a nephritogenic protein isolated from group A *Streptococcus*, and is recognized to be the same molecule as streptococcal glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[5] Because glomerular deposition of NAPlr is usually strong and is observed with high frequency in the early phase of poststreptococcal acute glomerulonephritis, NAPlr staining was originally considered useful for the diagnosis of poststreptococcal acute glomerulonephritis.[6] However, further investigation of histological staining for NAPlr and plasmin activity revealed that this staining was not specific for poststreptococcal acute glomerulonephritis. These were positively stained in other types of glomerulonephritis after streptococcal infection, and even after other bacterial infections. We have summarized these cases with both C3 and NAPlr staining in Table 2. In addition to the cases described in Table 2, infection related glomerulonephritis (IRGN) caused by *Staphylococcus* infection has also been reported (unpublished data).

The reason for such broad specificity of the staining could be explained by homology of the GAPDH molecule among microorganisms and its universal plasmin-binding capacity. Based on these findings, we now regard the positive staining for NAPlr and plasmin activity as the markers for IRGN in general. Thus, it is unclear what infection was associated with this case, *Streptococcus* or another organism.

The immunofluorescent staining results demonstrating strongly positive C3 staining but negative C4 staining, and the history of preceding infectious symptoms, suggest the possible role of an infectious process in the development of glomerulonephritis in this case. On the other hand, recent studies have reported low serum C3 levels in patients with ANCA-associated vasculitis which were associated with poor renal outcome and patient survival.[7] It was also suggested that activation of the alternative complement pathway plays a crucial role in the pathogenesis of adeno-associated virus with low C3.[7]

This raises the question as to whether low serum C3 level and strong C3 staining in this case resulted from

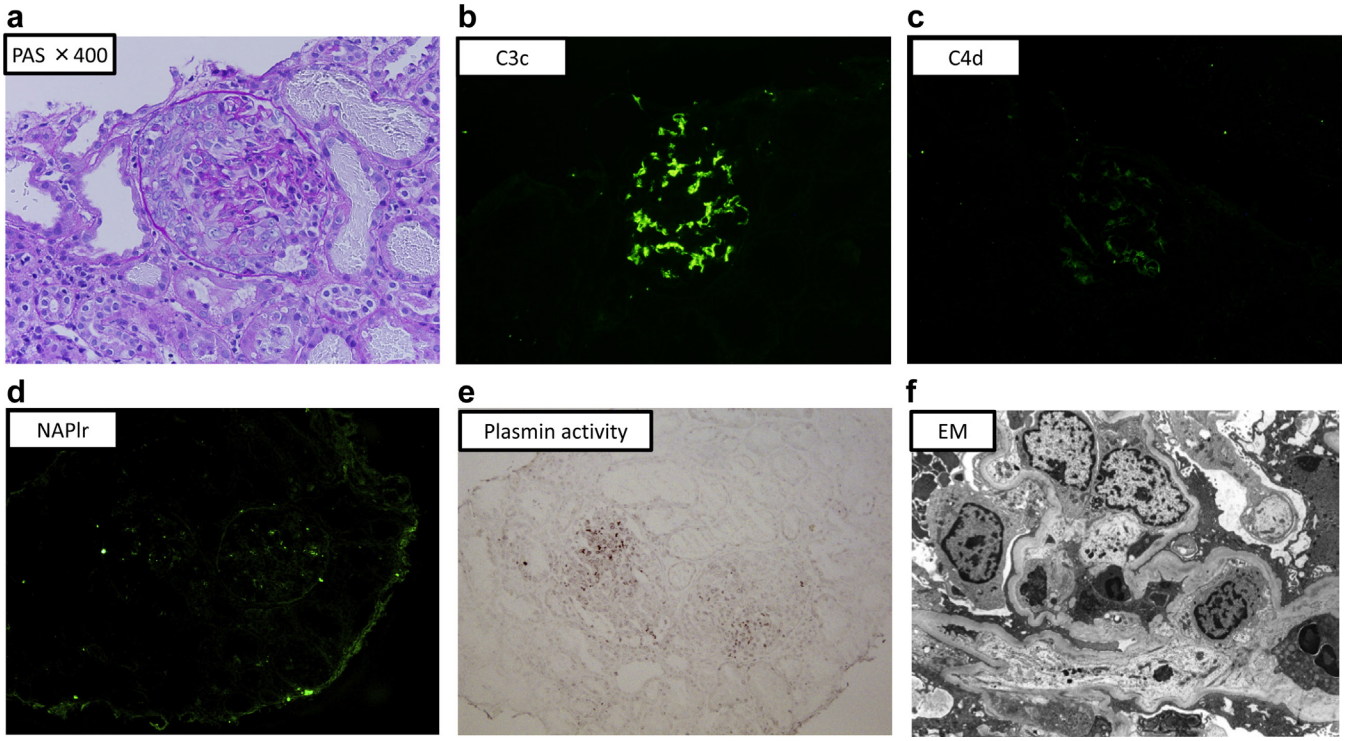

Figure 2. (a) Light microscopy showing diffuse cellular crescents with fibrinoid necrosis of the capillary tuft without fibro-cellular or fibrous crescents. PAS, periodic acid–Schiff. (b,c) Immunofluorescence microscopy showing strongly positive C3c staining in the mesangial area; C4d was negative. (d,e) Staining for nephritis-associated plasmin receptor (NAPlr) and plasmin activity were similarly positive in segmental areas of the glomeruli on immunofluorescence staining and *in situ* zymography with plasmin sensitive synthetic substrate, respectively.[5] (f) Electron microscopy (EM) showed no deposits. There was necrosis of the capillary tuft.

alternative complement pathway activation induced by GPA itself or by a postinfectious process. The good clinical course indicates that the postinfectious process is more likely; however, the possibility remains that the patient's very young age at diagnosis contributed to the better prognosis. Consequently, we cannot deny both possibilities, and we suspect that GPA was induced by infection.

In this case, staining for glomerular C4d, a known byproduct of activation of the classic and lectin pathways and a marker for immune complex–mediated glomerulonephritis was negative; C1q staining was also negative. These results are not incompatible with postinfectious glomerular nephritis because 1 study reported that 46% of biopsy specimens of postinfectious glomerular nephritis showed negative staining for C4d.[8]

Strong positive C3 staining and the absence of both C4d and C1q indicate an association with alternative complement pathway activation, as seen in C3 glomerulopathy. We cannot deny the possibility of C3 glomerulopathy, because we did not perform genetic testing in this case. Indeed, there is a report of severe crescentic and necrotizing glomerular nephritis with novel complement Factor H mutation showing extensive glomerular C3 staining on immunofluorescence.[9] However, it seemed less likely from the point of the absence of deposits on electron microscopy. We have shown the key points for diagnosing glomerular C3-dominant staining in Table 3.

Table 2. Overview of IRGN with both of C3 dominant and NAPlr staining[a]

		Infection			
GN type	Age, sex	Pathogen	Type	Duration	Duration of hypo-complementemia
DDD[S1,S2]	12 Male 14 Female	GAS GAS	Pharyngitis unknown	2 mo A few days	>7 yr unknown
MPGN type1[S3]	18 Male	GAS	Upper respiratory infection	2 wk	3 mo
AGN[S4]	12 Female	*Streptococcus pneumoniae*	pneumonia	1 wk	54 d
AGN[S5]	64 Male	*Aggregatibacter actinomycetemco-mitans*	Infectious endocarditis	Several mo	No hypo-complementemia

AGN, acute glomerulonephritis; DDD, dense deposit disease, GAS, group A streptococcus; IRGN, infection related glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; NAPlr, nephritis-associated plasmin receptor.
[a]See Supplementary References for Table 2.

Table 3. Key points of C3-dominant staining

• Differential diagnosis of C3-dominant staining are C3 glomerulopathy (C3 glomerulonephritis and dense deposit disease), masked IgG proliferative glomerulonephritis, and infection-related glomerulonephritis
• To distinguish this disease, C4d staining, electron-dense deposit, and nephritis-associated plasmin receptor (NAPlr) staining are important
• The concepts of C3 glomerulonephritis and IRGN may overlap

IRGN, infection related glomerulonephritis

In conclusion, we present a case of a young man with GPA showing a low serum C3 level, glomerular C3−positive staining, and negative C4d and C1q staining. The possibility of severe C3 glomerulopathy remains to be distinguished, in the absence of genetic testing. Based on the clinical and histological features as well as on the positive staining for NAPlr and plasmin activity, we suggest that this is a case of infection-induced GPA.

DISCLOSURE

All the authors declared no competing interests.

AUTHOR CONTRIBUTIONS

KK wrote the manuscript. TS, TO, and YS contributed by reviewing and revising the manuscript. MY and KY took clinical care of the patient. TS, DI, TO, and JK contributed histological interpretation.

SUPPLEMENTARY MATERIAL

Supplementary References for Table 2.

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

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