

Clinical and pathological features of patients with antineutrophil cytoplasmic antibody-associated vasculitides concomitant with IgG4-related disease

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Abstract

Aim: The characteristics and the pathogenesis of the concomitant antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) and immunoglobulin G4-related disease (IgG4-RD) have not been elucidated.

Method: We included 92 AAV patients with renal biopsy results. Among them, 10 patients met both AAV and IgG4-RD criteria (concomitant group). The IgG subclasses of myeloperoxidase (MPO)-ANCA in both serum and renal tissue were measured and complement activation components were detected in serum.

Results: Patients in the concomitant group had both elevated serum IgG4 levels and positive MPO-ANCA. They had higher levels of eosinophil counts, serum globulin, IgG, IgE and C-reactive protein than patients in the AAV alone group. All 10 patients had glomerulonephritis with crescents and seven patients also had segmental necrosis of the glomerular capillary wall. Most of them also presented with storiform fibrosis and lymphoplasmacytic infiltration in renal interstitium with IgG4 positive plasma cells more than 10/high-power field. Eight patients achieved remission with improved renal function, the other two patients were on maintenance dialysis. The IgG4 subclass of MPO-ANCA was higher in the concomitant group than that in AAV alone group. A merge of IgG4 and MPO immunofluorescence was observed in parts of the mesangium of concomitant AAV and IgG4-RD patients. For complement components, Bb and mannose-binding lectin were elevated in serum of concomitant AAV and IgG4-RD patients.

Conclusion: We showed a new overlap syndrome of AAV and IgG4-RD, in which the IgG4 subclass of ANCA may be a pathogenic factor.

KEYWORDS

antineutrophil cytoplasmic antibody, complement, IgG4, IgG4-related disease, vasculitides

1 | INTRODUCTION

Immunoglobulin G4-related disease (IgG4-RD) is a systemic fibro-inflammatory disorder of unknown origin.¹ It is characterized by features including storiform fibrosis, a tendency to form tumefactive

lesions at multiple sites, a dense lymphoplasmacytic infiltrate with abundant IgG4-positive plasma cells, and often but not always, elevated serum IgG4 concentrations.^{2,3} Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) is a group of systemic vasculitides characterized by small-vessel pauci-immune vasculitides

and the presence of circulating pathogenic ANCA, including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA).⁴ ANCA is a group of autoantibodies against constituents of neutrophil's primary granules and lysosomes of monocytes.⁵ The commonly characterized ANCA targets myeloperoxidase (MPO) or proteinase 3 (PR3).⁶⁻⁸

Substantial overlap between IgG4-RD and AAV exists in terms of clinical manifestations and histopathological features. Both IgG4-RD and AAV can affect the orbital regions, sinuses, lungs, kidneys, meninges, the pituitary gland and so on.^{9,10} High serum IgG4 level, which is a biomarker of IgG4-RD, is also detected in AAV patients.^{11,12} Meanwhile, a growing number of IgG4-RD patients have been reported as ANCA positive. Histologically, some biopsies from AAV patients may be rich in plasma cells and accompanied by fibrosis and/or obliterated blood vessels as in IgG4-RD.¹³⁻¹⁵ Increased IgG4 positive plasmacytes can be seen in involved organs of AAV patients.^{11,16} Further, there have been cases suggestive that AAV and IgG4-RD may be concomitant.^{17,18} Here we retrospectively analyzed the manifestations of concomitant AAV and IgG4-RD patients, and detected the MPO-ANCA subclasses and complement activation components to investigate the pathogenesis.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

We conducted a computer-assisted search of the electronic medical record database for patients with AAV and IgG4-RD encountered at the First Affiliated Hospital, College of Medicine, Zhejiang University, from January 2014 to December 2018. AAV definitions were adopted from the 2012 Chapel Hill Consensus Conference on nomenclature of vasculitides⁴ and a previous European Union study.¹⁹ IgG4-RD was diagnosed according to the comprehensive diagnostic criteria²⁰ and the international consensus diagnostic criteria (ICDC) for autoimmune pancreatitis.²¹

Patients diagnosed with AAV alone were included in the AAV group and patients who met both AAV and IgG4-RD criteria were included in the concomitant group. The patients' characteristics included age, sex, clinical parameters and laboratory results. Laboratory results included complete blood counts, liver and renal function tests, and serological tests for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complement levels, ANCA and serum IgG4 levels. All the renal biopsy specimens were re-evaluated by the same pathologist.

The serum samples of the patients were collected on the day of renal biopsy before starting immunosuppressive treatment. Normal control samples were collected from 20 healthy individuals matched by age and gender. The cryosections which were left over from the renal biopsy specimens were used for detecting the deposition of MPO-ANCA IgG subclasses in kidneys. The study protocols conformed to the provisions of the Declaration of Helsinki and were approved by the Ethic Committee of our hospital (reference number: 2018010). Informed consents were obtained from all the patients.

2.2 | Serum MPO-ANCA subclasses measured by ELISA

We measured serum levels of MPO-ANCA IgG subclasses using horseradish peroxidase (HRP)-conjugated monoclonal mouse antihuman IgG subclass antibodies, clone 4E3 for IgG1, HP6014 for IgG2, HP6050 for IgG3 and HP6025 for IgG4 (Southern Biotech). The antibodies were diluted at 1:8000, 1:2000, 1:4000, 1:4000 in phosphate-buffered saline-0.05% Tween 20 (PBS-T). Enzyme-linked immunosorbent assay (ELISA) plates pre-coated with MPO (Abcam) were incubated with MPO-ANCA positive serum at dilutions of 1:100-1:800 in Sample Diluent. All samples were first tested at dilution of 1:100. If the optical density at 450 nm (OD450) value was passed, higher dilutions were tested. After a 1-hour incubation at room temperature, the plates were washed three times with 300 μ L of the kit wash buffer. Then, 100 μ L of antihuman IgG1, IgG2, IgG3 or IgG4 HRP-conjugated subclass-specific antibody diluted as described or MPO anti-IgG HRP conjugate (Abcam) was added. After a 30-minute incubation at room temperature, the plates were washed three times with 300 μ L of the kit wash buffer again and 100 μ L 3,3',5,5'-tetramethylbenzidine substrate solution was added into all wells. After incubating for exactly 15 minutes at room temperature in the dark, 100 μ L stop solution was added into all wells. The OD450 of the plate was read within 5 minutes and the mean value was calculated.

2.3 | Renal MPO-ANCA subclasses measured by double immunofluorescence staining

The cryosections were incubated with rabbit polyclonal antihuman MPO antibody (Abcam) at 37°C for 1 hour. After three washes with phosphate-buffered saline, Alexa Fluor 555-conjugated goat anti-rabbit IgG antibody (Abcam) was incubated with samples at 37°C for 1 hour. The cryosections were then incubated with fluorescein isothiocyanate (FITC)-labeled goat antihuman IgG1, IgG2, IgG3 and IgG4 (Zhongshan) respectively for another 1 hour and then observed with a Leica Fluorescence Microscope DM4000 (Frankfurt, Germany).

2.4 | Detection of the complement activation components

The commercial ELISA kits of human complement components Bb (Quidel Corporation), C1q (Abcam) and mannose-binding lectin (MBL) (R&D Systems) were used. The serum Bb, MBL, C1q levels of 10 concomitant patients, 20 AAV patients and 20 normal controls were all determined by the assays, which were conducted according to the manufacturer's instructions.

2.5 | Statistical analysis

Statistical analysis was performed by SPSS 23.0 (IBM, Armonk, NY, USA). Numerical data were expressed as mean $\pm$ SD.

**TABLE 1** The clinical and pathological parameters of the AAV and concomitant groups

	AAV group	Concomitant group	P value
Case number	82	10	
Course, median (range), mo	2 (0.3-48)	2 (0.5-48)	.828
Age, y	58.0 ± 12.7	62.9 ± 11.0	.251
Male, n, %	27, 32.9	7, 70.0	.052
WBC, 10 ⁹ /L	7.9 ± 4.1	8.2 ± 2.7	.815
Eosinophils, median (IQR),/μL	70.3 (0.0-200.1)	349.7 (58.5-739.8)	.007
Hemoglobin, g/L	89.7 ± 19.4	82.4 ± 15.6	.256
Platelets, 10 ⁹ /L	243.3 ± 104.8	266.4 ± 61.6	.498
Serum albumin, g/L	33.7 (30.4-37.3)	31.6 (23.1-36.8)	.190
Serum globulin, g/L	30.1 ± 6.1	34.9 ± 5.4	.020
Alanine transaminase, μ/L	13.9 ± 16.1	14.5 ± 9.6	.903
Serum creatinine, μmol/L	319.4 ± 244.0	289.5 ± 234.3	.714
eGFR, mL/min/1.73 m ²	30.4 ± 29.2	29.2 ± 20.9	.895
Urine protein, g/24 h	2.6 ± 2.3	1.9 ± 1.1	.349
C3, mg/dL	101.1 ± 25.8	103.1 ± 37.0	.826
C3 reduced, n, %	2, 2.6	1, 10.0	.768
C4, mg/dL	27.8 ± 12.5	23.4 ± 10.2	.293
C4 reduced, n, %	0, 0	1, 10.0	.114
IgG, mg/dL	1331.5 ± 468.5	1765.6 ± 447.1	.007
IgE, median (IQR), IU/mL	64.4 (24.7-155.8)	595.0 (299.2-1046.3)	.006
IgE positive, cases, %	18, 36.0	5, 83.3	.074
ESR, mm/h	65.0 ± 36.9	85.7 ± 36.2	.098
CRP, median (IQR), mg/L	7.4 (3.6-21.2)	50.5 (7.2-87.3)	.013
IgG4, mg/dL	151.1 ± 199.0	368.4 ± 144.2	.014
MPO, U/mL	55.3 ± 38.4	59.4 ± 35.7	.747
MPO-ANCA positive, cases, %	70, 86.4	10, 100.0	.603
BVAS	15.5 ± 3.9	14.7 ± 3.3	.536
Renal pathology			
Glomerular sclerosis, %	32.3 ± 25.4	36.6 ± 25.7	.616
Glomerular crescent, %	28.5 ± 21.3	23.2 ± 15.4	.448
Fibrinoid necrosis, cases, %	33, 40.2	7, 70.0	.146
Mesangial proliferation 1/2/3, cases	2/75/5	0/10/0	.682
Interstitial infiltrates 0/1/2/3, cases	18/25/39	0/3/7	.103
Therapy			
Methylprednisolone pulse, cases, %	47, 57.3	6, 60.0	1.000
P/P + IVC/P + MMF/P + others, cases	22/28/28/2/2	1/8/0/1/0	.026
Outcome			
Dialysis independence, cases, %	64, 78.0	8, 80.0	1.000

Abbreviations: AAV, ANCA-associated vasculitides; ANCA, anti-neutrophils cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Score; Concomitant, AAV concomitant with IgG4-related disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (calculated by modification of diet in renal disease equations for Chinese patients); ESR, erythrocyte sedimentation rate; IQR, interquartile range; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil; MPO, myeloperoxidase; P, prednisone; PR3, proteinase 3; WBC, white blood cells.

Differences of numerical data with normal distribution were tested by Student's *t* test. Semi-quantitative scores were compared by Mann-Whitney *U* test. The categorical data were

expressed as counts or percentages and were compared using Fisher's exact test. A *P* value of <.05 was considered statistically significant.

TABLE 2 Detailed data of patients with concomitant IgG4-RD and AAV in our center

Case	Age, y	Sex	Organ involvement	ANCA (U/mL)	IgG4 (mg/dL)	ESR (mm/h)	CRP (mg/L)	Complement (mg/dL)	IgE (IU/mL)	Kidney pathology	Therapy	Outcome
Case 1	69	Male	Kidney, lacrimal glands, lymph glands	MPO 146.6	347	>140	85.3	Normal	/	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC > 10/HPF	MP pulses, GC, CTX, IVIG	Maintaining dialysis
Case 2	75	Male	Kidney, lung, lymph glands	MPO 78.4	412	27	101.4	C3, 33; C4, 3	1032	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC = 50/HPF	MP pulses, GC, CTX	Remission
Case 3	70	Male	Kidney, lung pancreas, lacrimal glands	MPO 78.1	177	30	89.3	Normal	/	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC > 10/HPF	GC, CTX	Remission
Case 4	66	Male	Kidney, lacrimal glands	MPO 50.4	178	96	63	C3, 155; C4, 41	/	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC > 20/HPF	GC, CTX	Remission
Case 5	61	Female	Kidney, lung	MPO 16.0	630	90	21.1	C3, 69; C4, 18	395	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC > 10/HPF	MP pulses, GC, CTX	Remission
Case 6	66	Male	Kidney, lung, lymph glands	MPO 100	527	90	4.3	C3, 116; C4, 27	415	GN with crescents and sclerosis, SF, LP, IgG4 PC > 30/HPF	MP pulses, GC, CTX	Maintaining dialysis
Case 7	57	Female	Kidney	MPO 5.5	272	54	38	C3, 137; C4, 34	11.9	GN with crescents, SF, LP, IgG4 PC > 10/HPF	MP pulses, GC, CTX	Remission
Case 8	35	Female	Kidney	MPO 30.8	307	58	3.8	C3, 114; C4, 28	1089	GN with crescents and sclerosis, SF, LP, IgG4 PC = 8/HPF	GC, RTX	Remission
Case 9	62	Male	Kidney, lung	MPO 38.7	402	92	8.2	C3, 130; C4, 23	775	GN with crescents and sclerosis, SN, SF, LP, IgG4 PC = 7/HPF	GC	Remission
Case 10	68	Male	Kidney, lymph glands	MPO 96	432	140	83.6	C3, 118; C4, 18	/	GN with crescents and sclerosis, SN, LP, IgG4 PC > 20/HPF	MP pulses, GC, CTX	Remission

Abbreviations: AAV, ANCA-associated vasculitides; ANCA, anti-neutrophils cytoplasmic antibody; CRP, C-reactive protein; CTX, cyclophosphamide; ESR, erythrocyte sedimentation rate; GC, glucocorticoids; GN, glomerulonephritis; HPF, high-power field; IgG4-RD, immunoglobulin G4-related disease; IVIG, intravenous immunoglobulins; LP, lymphoplasmacytic infiltrate; MP, methylprednisolone, RTX, rituximab; MPO, myeloperoxidase; PC, plasma cells; SF, storiform fibrosis; SN, segmental necrosis of glomerular capillary wall.



TABLE 3 The IgG subclasses of MPO-ANCA in AAV group and concomitant group

	AAV group	Concomitant group	P value
Case number	20	6	
IgG1 (OD450 value)	4.38 ± 4.25	6.16 ± 3.89	.370
IgG2 (OD450 value)	0.97 ± 1.54	0.48 ± 0.38	.450
IgG3 (OD450 value)	1.58 ± 2.67	1.34 ± 2.33	.844
IgG4 (OD450 value)	3.58 ± 4.60	12.42 ± 6.63	.001
Total IgG (OD450 value)	3.44 ± 3.23	5.52 ± 3.37	.182

Abbreviations: AAV, antineutrophil cytoplasmic antibody-associated vasculitides; ANCA, anti-neutrophils cytoplasmic antibody; concomitant, AAV concomitant with IgG-related disease; IgG, immunoglobulin G; MPO, myeloperoxidase; OD450, optical density at 450 nm.

3 | RESULTS

The computer-assisted search identified 92 AAV patients with renal biopsy results. Among them, 10 patients met both AAV and IgG4-RD criteria. Demographic characteristics, clinical presentations and laboratory parameters of the patients in the AAV and concomitant (diagnosed of AAV and IgG4-RD) groups are shown in Table 1. Patients in the concomitant group had both elevated serum IgG4 levels and positive MPO-ANCA. They had higher levels of eosinophil counts, serum globulin, IgG, IgE and CRP than patients in the AAV group. The detailed data of individual patients in the concomitant group are shown in Table 2. All patients had glomerulonephritis with crescents and seven patients also had segmental necrosis of the glomerular capillary wall. Most of them also presented with storiform fibrosis and lymphoplasmacytic infiltration in renal interstitium with IgG4 positive plasma cells more than 10/high-power field. The percentages of glomerular global sclerosis and crescents were $36.6\% \pm 25.7\%$ and $23.2\% \pm 15.4\%$, respectively. Eight patients received combined therapy of glucocorticoids and cyclophosphamide, with or without methylprednisolone pulses. One patient received glucocorticoids only and another patient received glucocorticoids and rituximab. Eight patients achieved remission with improved renal function, the other two patients were on maintaining dialysis.

Serum samples from 20 AAV patients and six patients in the concomitant group were measured to determine the IgG subclasses of serum MPO-ANCA. The OD450 values of MPO-ANCA IgG subclasses are shown in Table 3. The IgG4 subclass of MPO-ANCA was significantly higher in the concomitant group than that in the AAV group (12.42 ± 6.63 vs 3.58 ± 4.60 , $P = .001$), whereas the other 3 subclasses (IgG1, IgG2 and IgG3) of MPO-ANCA were parallel between these two groups.

To reveal the IgG subclasses of MPO-ANCA in renal biopsies, we applied double immunofluorescence using antihuman IgG subclass antibodies (IgG1, IgG2, IgG3 and IgG4) labeled with FITC and anti-MPO antibody labeled with Alexa Fluor 555. MPO staining was

observed in the mesangium in 10 AAV patients and 10 concomitant patients. A merge of IgG4 and MPO immunofluorescence was observed in some parts of the mesangium, indicating colocalization of MPO and IgG4 in these areas (Figure 1). In contrast, the cases of AAV did not show IgG4 staining and colocalization of MPO and IgG1 was not evident (Figure 1).

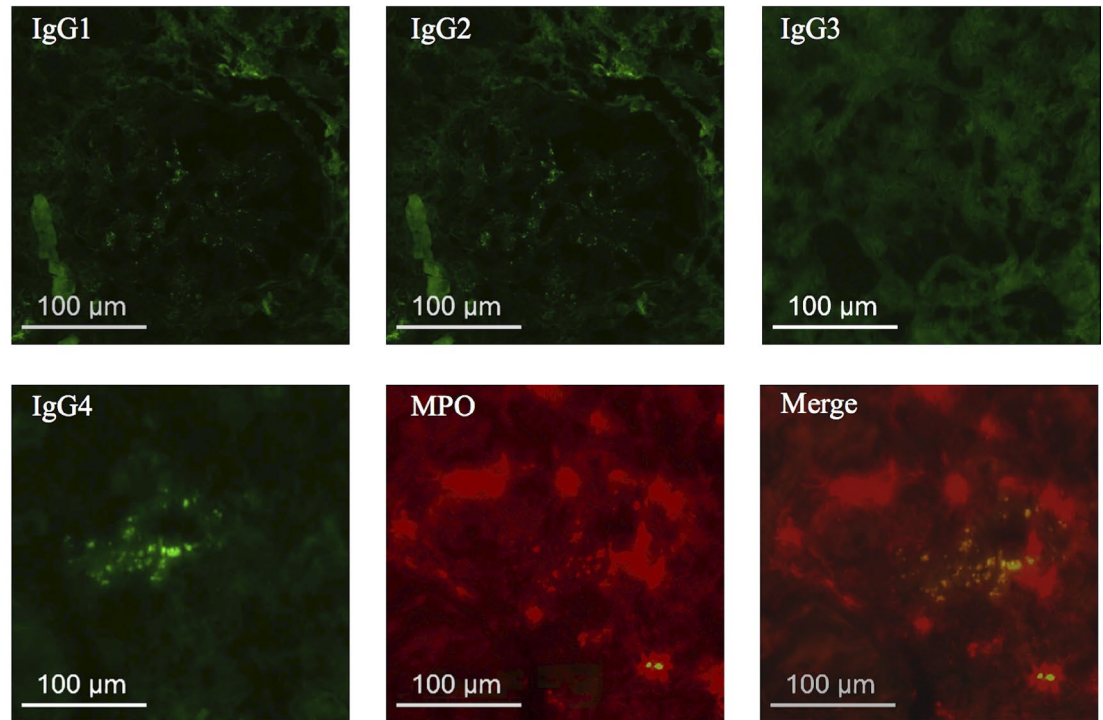
To investigate the complement role in the development of AAV concomitant with IgG4-RD, the serum levels of Bb, C1q, and MBL were detected among the 20 AAV patients, 20 healthy control people and 10 concomitant patients, as shown in Figure 2. The serum levels of MBL in concomitant patients were significantly higher than those of AAV patients and normal controls (3820 ± 1854 ng/mL vs 2170 ± 1339 ng/mL, $P = .009$; 3820 ± 1854 ng/mL vs 1677 ± 1512 ng/mL, $P = .002$, respectively) (Figure 2A). There was no significant difference in serum levels of C1q among the three groups (Figure 2B). The levels of serum Bb were higher in concomitant patients and AAV patients than normal controls (11.5 ± 4.4 µg/mL vs 6.5 ± 6.5 µg/mL, $P = .024$; 12.4 ± 5.4 µg/mL vs 6.5 ± 6.5 µg/mL, $P = .005$, respectively).

4 | DISCUSSION

We analyzed the clinical and pathological characteristics of 10 patients with concomitant IgG4-RD and AAV. The kidney biopsies of these patients revealed that they had typical changes of IgG4-RD, including renal interstitial lymphoplasmacytic infiltration, storiform fibrosis and IgG4 positive plasmacytes infiltration. All patients presented with glomerulonephritis with crescents and 7 had segmental necrosis of the glomerular capillary wall, which showed typical changes of AAV-associated renal injury. Clinically, the patients with concomitant IgG4-RD and AAV shared similar manifestations with AAV patients in most aspects. Nevertheless, they had higher inflammatory parameters than AAV patients. Their involved organs were mainly kidneys and lungs. These observations suggested that patients with IgG4-RD and AAV were more likely to show the clinical features of AAV. They received therapy targeting AAV and eight patients achieved remission with improved renal function. Thus, we suggest that these concomitant patients should be treated as AAV. Meanwhile, patients with IgG4-RD and AAV had enlargement of lacrimal glands, which is not common in AAV, and higher serum globulin, absolute eosinophil counts and higher IgE levels which were comparable with the characteristic of IgG4-RD.²² Both IgG4 and IgE were produced as a result of Th2 cytokines, revealing the existence of a similar pathological foundation involving the function of Th2 cells in these patients.^{23,24}

In the mechanism of AAV onset, ANCA has been proven to be pathogenic. In vitro studies have shown that ANCA activates cytokine-primed neutrophils and monocytes through both direct Fab'2 binding and Fc receptor engagement, then the neutrophils activated by ANCA release oxygen radicals, lytic enzymes, and inflammatory cytokines and adhere to and kill endothelial cells.²⁵ ANCA were predominantly of the IgG isotype, and IgG1, IgG3 and IgG4 subclasses were particularly represented. Different IgG subclasses may have

Concomitant case



AAV case

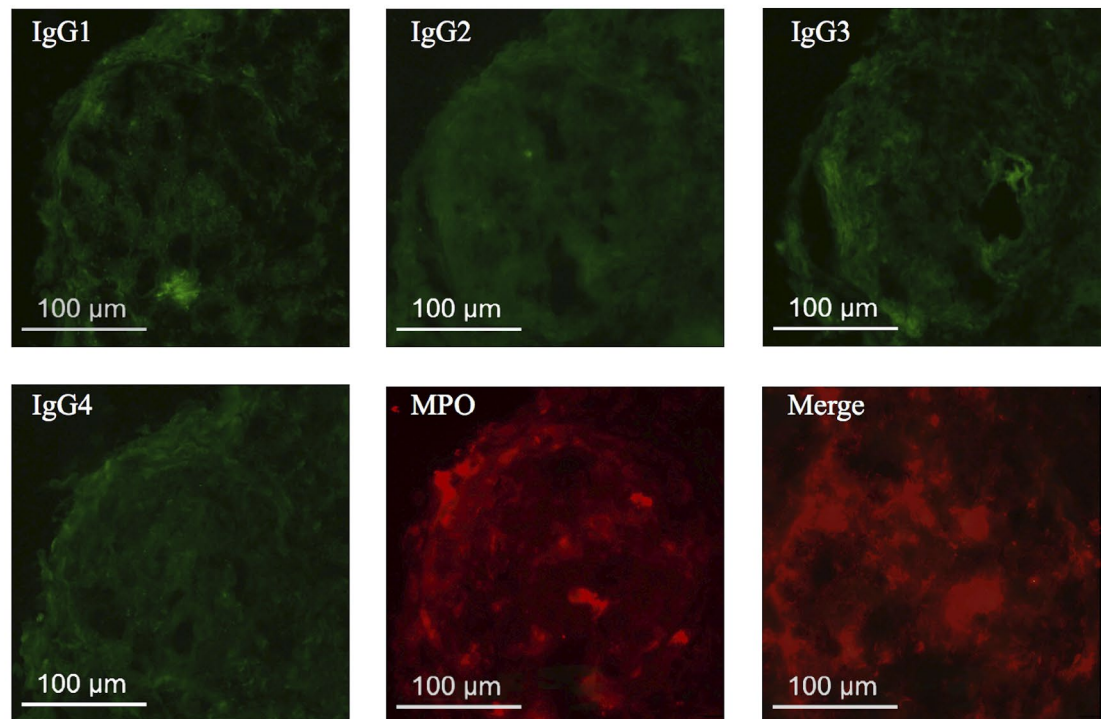


FIGURE 1 Glomerular deposition of myeloperoxidase (MPO) and immunoglobulin G (IgG) subclasses in antineutrophil cytoplasmic antibody-associated vasculitides (AAV) patients and AAV concomitant with IgG-related disease (Concomitant) patients. Double staining with anti-IgG4 antibody and anti-MPO antibody was performed on biopsy specimens from patients with AAV and concomitant disease. IgG4 was clearly localized predominantly in the mesangium with MPO. (Bars 100 µm; original magnification ×400)

different immunological functions. IgG4 was thought to induce less severe inflammation than other subclasses due to its inability to activate complement or engage Fcγ receptors on proinflammatory phagocytes. However, Pankhurst et al²⁶ compared adhesion

of neutrophils treated with different subclasses of soluble ANCA IgG to P-selectin surfaces or endothelial cells and found neutrophils treated with ANCA IgG subclasses 1, 3 and 4 showed stabilization of adhesion to P-selectin surfaces and endothelial cells, but the

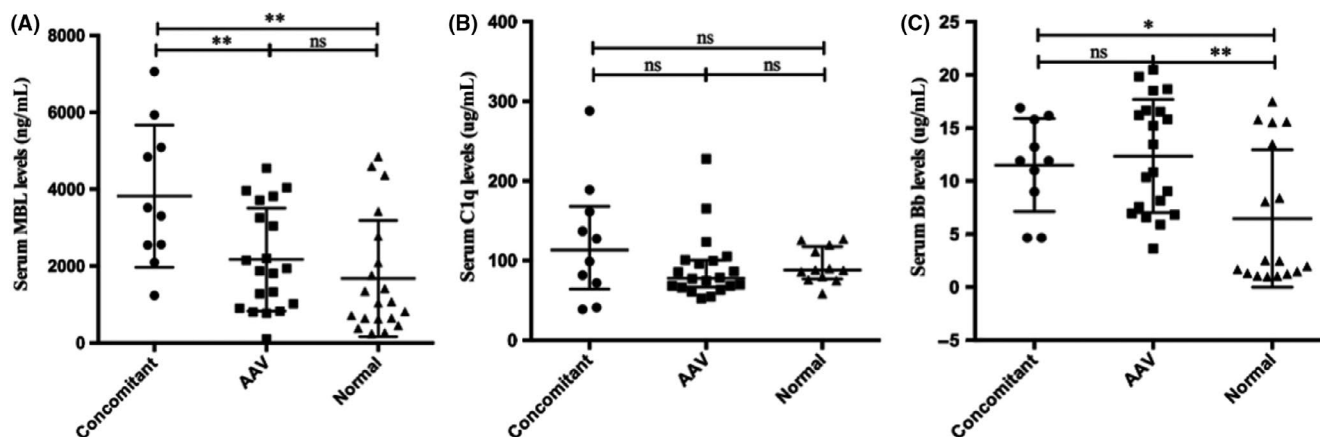


FIGURE 2 Serum mannose-binding lectin (MBL), C1q and Bb levels in patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV) concomitant with immunoglobulin G (IgG)-related disease (Concomitant), AAV and in normal controls. Horizontal solid lines of A and C indicate the mean level; horizontal solid lines of B indicated median level. A, Serum levels of MBL. B, Serum levels of C1q. C, Serum levels of Bb. * $P < .05$, ** $P < .01$, *** $P < .001$

potency of the subclasses followed the pattern IgG3 > IgG1 > IgG4. Holland et al²⁷ also showed that IgG4 subclass of PR3-ANCA can ligate FcγRIIa/IIIb receptors to activate neutrophils to produce a superoxide response. These results provided the possible pathogenic mechanism of IgG4 ANCA. In this study, the serum IgG4 subclass level of MPO-ANCA was much higher in concomitant patients than that in AAV patients, but the levels of the other three subtypes (IgG1, IgG2, IgG3) of MPO-ANCA were parallel between groups. In addition, the colocalization of MPO and IgG4 measured by immunofluorescence was observed in some parts of the mesangium in these patients. Given the diffuse infiltration of IgG4 positive plasma cells in the kidney, it is reasonable to hypothesize that IgG4-positive plasma cells may produce IgG4 subclass antibodies to activate neutrophils to induce a secondary inflammatory response. However, the mechanism by which IgG4 positive plasmacytes produce antibodies targeting MPO needs further study.

Since IgG4-RD and AAV are both immune-mediated diseases, closer attention was paid to complements to reveal the pathogenesis. The complement activation system is composed of three pathways: the classical pathway, the alternative pathway, and the MBL pathway. Evidence from in vivo and in vitro studies have suggested that the alternative pathway plays a crucial role in the development of AAV.²⁸⁻³⁰ Sugimoto et al³¹ illuminated that polyethylene glycol-precipitated immune complexes from IgG4-RD patients could activate both the classical and the MBL pathways, although the mechanism has not been clarified. Here, we observed that both Bb and MBL were elevated in sera of patients with concomitant IgG4-RD and AAV, suggesting activation of the alternative pathway and MBL pathway. So, these patients may have different complement activation compared with solely AAV patients.

To conclude, we showed an overlap of IgG4-RD and AAV in this study, and the IgG4 subclass of ANCA may be a pathogenic factor in this concomitant disease. Based on the limited cases of these patients, significant clinical presentation cannot be concluded yet; however, for patients presenting both elevated serum IgG4 levels

and positive MPO-ANCA, especially IgG4 subclass of MPO-ANCA, high index of suspicion is needed.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

FH, YM and LC came up with the research idea and designed the study. FH, YM, LC, YX, QH and BY collected and analyzed the data. FH, YM, LC, YY and JZ performed the experiments. FH, YY and JC supervised the research. YM wrote the first draft of the manuscript. FH reviewed and edited the manuscript. YM and LC contributed equally.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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