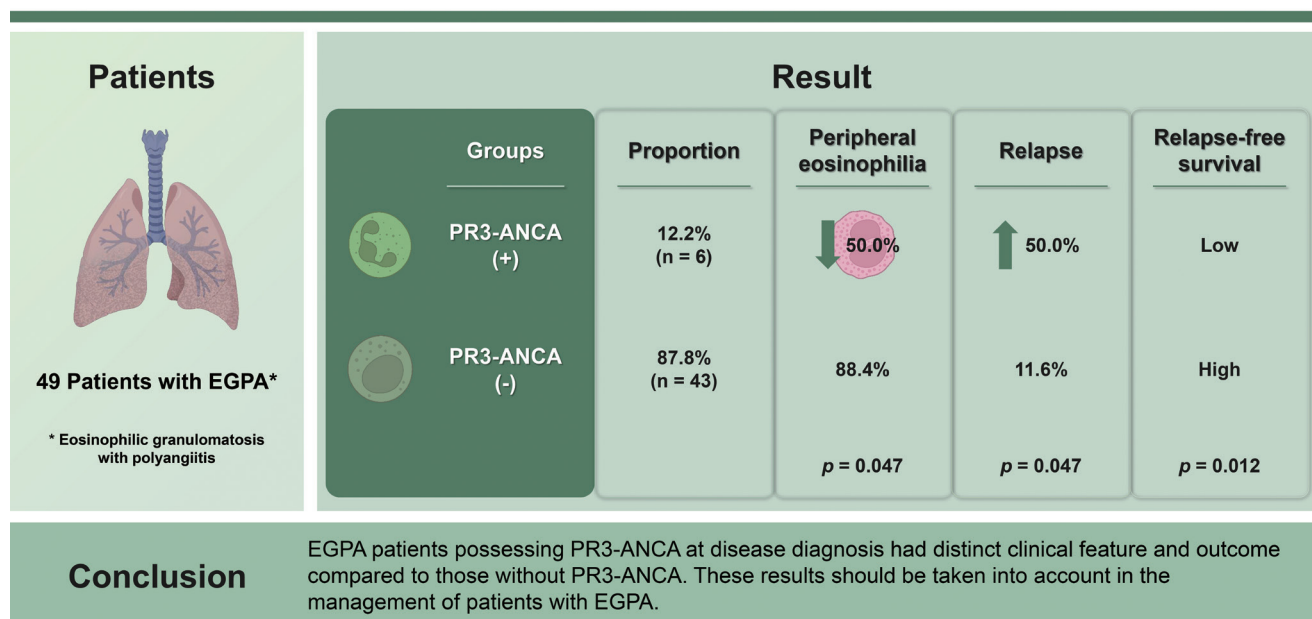# Clinical impact of proteinase 3-antineutrophil cytoplasmic antibody positivity in eosinophilic granulomatosis with polyangiitis

Sung Soo Ahn[1], Jason Jungsik Song[1,2], Yong-Beom Park[1,2], and Sang-Won Lee[1,2]

[1]Division of Rheumatology, Department of Internal Medicine, [2]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, Korea

Clinical impact of proteinase 3-antineutrophil cytoplasmic antibody positivity in eosinophilic granulomatosis with polyangiitis

Patients	Result				
	Groups	Proportion	Peripheral eosinophilia	Relapse	Relapse-free survival
	PR3-ANCA (+)	12.2% (n = 6)	50.0%	50.0%	Low
49 Patients with EGPA*	PR3-ANCA (−)	87.8% (n = 43)	88.4%	11.6%	High
*Eosinophilic granulomatosis with polyangiitis			p = 0.047	p = 0.047	p = 0.012

Conclusion	EGPA patients possessing PR3-ANCA at disease diagnosis had distinct clinical feature and outcome compared to those without PR3-ANCA. These results should be taken into account in the management of patients with EGPA.

Background/Aims: This study investigated the clinical implication of proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) in Korean patients with eosinophilic granulomatosis with polyangiitis (EGPA).
Methods: Among the 242 patients with ANCA-associated vasculitis identified from the hospital database, 49 patients with EGPA were selected and analysed in this study. Demographic, clinical, and laboratory data at diagnosis were reviewed to compare the features of patients with PR3-ANCA and without, as well as the clinical outcomes of relapse and end-stage renal disease (ESRD) during the follow-up period. The outcomes of patients with PR3-ANCA and without were compared by using the Kaplan-Meier survival analysis.
Results: The median age of the patients was 54 years, 17 (34.7%) were male, and six (12.2%) patients had PR3-ANCA at baseline. The most common items of the 1990 American College of Rheumatology criteria for EGPA were sinusitis (95.9%) and asthma (or asthmatic history) (93.9%). During the follow-up, none died, eight experienced relapse and two progressed to ESRD. EGPA patients with PR3-ANCA exhibited peripheral eosinophilia less frequently than those without (50.0% vs. 88.4%, $p = 0.047$). On the other hand, EGPA patients with PR3-ANCA experienced relapse more often compared to those without (50.0% vs. 11.6%, $p = 0.047$), and the cumulative relapse-free survival rate was lower compared to those without PR3-ANCA ($p = 0.012$).
Conclusions: EGPA patients possessing PR3-ANCA at disease diagnosis had distinct clinical feature and outcome compared to those without PR3-ANCA. These results should be taken into account in the management of patients with EGPA.

Keywords: Eosinophilic granulomatous vasculitis; PR3; Anti-neutrophil cytoplasmic antibody; Prognosis; Recurrence

INTRODUCTION

Eosinophilic granulomatosis with polyangiitis (EGPA) is one of the three subtypes of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). The histological features of EGPA are characterised by necrotising vasculitis in small-sized vessels, including capillaries and their adjacent arterioles and venules, with evidence of eosinophil infiltration and granuloma formation [1,2]. However, even though EGPA is included as a subtype of AAV, ANCA is only found in approximately 40% of patients with EGPA [3]. Thus, ANCA positivity is not essential in classifying EGPA according to the 1990 American College of Rheumatology (ACR) criteria (the 1990 ACR criteria) [4] and the 2007 European Medicine Agency (EMA) algorithm [5]. The clinical features of EGPA patients with and without ANCA are different: EGPA patients with ANCA predominantly exhibit vasculitic features and those without ANCA preferentially have allergic features [6,7].

In patients with AAV, detection of ANCAs directed against myeloperoxidase (MPO) and proteinase 3 (PR3) (MPO-and PR3-ANCA) in the blood is a typical laboratory feature. Notably, there is accumulating evidence that suggest that different phenotypes and prognoses are expected based on ANCA types [8]. Similarly, we have previously shown that

AAV patients harbouring MPO-ANCA, PR3-ANCA, and ANCA-negativity revealed significantly distinct clinical features [9]. However, the clinical impact of ANCA types in AAV subtypes, especially EGPA, is not well understood. Recently, a study investigating the clinical characteristics of PR3-ANCA positivity in EGPA patients was published. The study analysed the data of 734 European patients with EGPA and revealed that different baseline characteristics were present among MPO-ANCA, PR3-ANCA, and ANCA-negative groups [10]. Given the ethnic and geographical differences in patients with AAV between Asia and Europe, it may be meaningful to evaluate the clinical features of Korean patients with EGPA according to the presence and absence of PR3-ANCA. Hence, in this study, we investigated the clinical implications of PR3-ANCA in Korean patients with EGPA.

METHODS

Study subjects

An electronic chart review was performed to identify patients that were diagnosed with AAV at Severance Hospital between October 2005 and October 2020. The inclusion criteria were as follows: (1) diagnosis of AAV according to the 2012 International Chapel Hill Consensus Conference

Ahn SS, et al. PR3-ANCA in Korean patients with EGPA

KJIM

definitions, 1990 ACR criteria and the 2007 EMA algorithm [1,4,5,11]; (2) patients with sufficient medical records available to collect clinical, laboratory, radiological and histological data, and to calculate AAV-specific indices including Birmingham vasculitis activity score (BVAS) version 3 [12] and five-factor score (FFS) [13]; and (3) patients who were initially tested for both MPO-ANCA or perinuclear (P)-ANCA and PR3-ANCA or cytoplasmic (C)-ANCA. Patients who had medical conditions that could affect the diagnosis of AAV and ANCA positivity, such as malignancies, infections, and other systemic autoimmune diseases except AAV, were excluded. Subsequently, 242 patients with AAV were identified, and 49 patients with EGPA were selected and analysed in this study. This study was approved by the Institutional Review Board of Severance Hospital (4-2020-1071), which waived the requirement for obtaining patients' written informed consent.

Baseline clinical and laboratory data

We collected clinical and laboratory data on the initial date of EGPA diagnosis. Age, gender, and body mass index at diagnosis were acquired as demographic data. Information regarding ANCA types, the items of the 1990 ACR criteria, AAV-specific indices and clinical manifestations were obtained. Laboratory results, as well as acute phase reactants such as erythrocyte sedimentation rate and C-reactive protein were also reviewed. Patients' comorbidities included chronic kidney disease without renal replacement therapy [14], diabetes, hypertension and dyslipidaemia prior to the diagnosis of AAV, in accordance with the 10th International Classification of Diseases.

Clinical outcomes and medications

As none of the EGPA patients died, clinical outcomes during follow-up included relapse and end-stage renal disease (ESRD). Relapse was defined as a recurrence or new onset of disease attributable to active vasculitis after remission, and remission as the absence of disease activity attributable to active disease qualified by the need for ongoing stable maintenance immunosuppressive therapy [15]. ESRD was defined as a status that require renal replacement therapy due to a decline in renal function. For patients with relapse or ESRD, the follow-up duration was calculated as the period from the diagnosis of EGPA until the date of first relapse or initiation of renal replacement therapy. Meanwhile, for patients without relapse or ESRD, the follow-up duration

was defined as the period from the day of diagnosis to the day of the last visit. Furthermore, the administration of glucocorticoid and immunosuppressive drugs to treat EGPA during follow-up was counted [16].

Statistical analyses

All statistical analyses were performed using SPSS software version 23 for Windows (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as median (interquartile range), and categorical variables are expressed as numbers and percentages. Significant differences in categorical variables were analysed using the chi-square test or the Fisher's exact test for cases that the number of patients was less than 5, whereas differences in continuous variables were compared using the Mann-Whitney U test. The cumulative relapse-free and ESRD-free survival rates were analysed using the Kaplan-Meier survival analysis. p values < 0.05 were considered statistically significant.

RESULTS

Baseline characteristics of patients with EGPA

The clinical characteristics at diagnosis and during follow-up in 49 patients with EGPA are shown in Table 1. In terms of variables at diagnosis, the median age was 54 years, 17 (34.7%) were male, and six (12.2%) patients had PR3-ANCA at baseline. The median absolute eosinophil count was 1,090/mm^3. The most common items of the 1990 ACR criteria for EGPA were sinusitis (95.9%) and asthma (or a history of asthma) (93.9%). The most common clinical presentations were otorhinolaryngologic manifestation (81.6%), followed by pulmonary (65.3%) and nervous systemic (59.2%) manifestation. During the follow-up period, no patients died, eight patients experienced relapse and two progressed to ESRD. Glucocorticoids were administered to 47 patients (95.9%). The most common induction and maintenance therapeutic regimens were cyclophosphamide (55.1%) and azathioprine (55.1%).

Comparison of variables between patients with PR3-ANCA and without

Regarding variables at diagnosis, EGPA patients with PR3-ANCA exhibited peripheral eosinophilia (≥ 10% of total white blood cell count) less frequently than those without (50.0% vs. 88.4%, p = 0.047), whereas there was no sig-

Table 1. Clinical and laboratory data at diagnosis and during follow-up (n = 49)

Variable	Value
At EGPA diagnosis	
Demographic data	
Age, yr	54.0 (41.0–62.0)
Male gender	17 (34.7)
Body mass index, kg/m^2	21.4 (19.3–23.8)
ANCA types	
MPO-ANCA positivity	19 (38.8)
PR3-ANCA positivity	6 (12.2)
1990 ACR criteria for EGPA	
Asthma or asthmatic history	46 (93.9)
Peripheral eosinophilia (≥ 10%)	41 (83.7)
Absolute eosinophil count, /mm^3	1,090.0 (255.0–6,490.0)
Sinusitis	47 (95.9)
Non-fixed lung lesions	20 (40.8)
Peripheral neuropathy	32 (65.3)
Eosinophil infiltration on histology	27 (55.1)
Clinical manifestations based on BVAS	
General manifestations	15 (30.6)
Cutaneous manifestations	17 (34.7)
Mucous and ocular manifestations	1 (2.0)
Otorhinolaryngologic manifestations	40 (81.6)
Pulmonary manifestations	32 (65.3)
Cardiovascular manifestations	9 (18.4)
Gastrointestinal manifestations	5 (10.2)
Renal manifestations	13 (26.5)
Nervous systemic manifestations	29 (59.2)
AAV-specific indices	
BVAS	12.0 (8.0–17.5)
FFS	1.0 (0–1.0)
Comorbidities	
Chronic kidney disease without renal replacement	11 (22.4)
Diabetes mellitus	8 (16.3)
Hypertension	20 (40.8)
Dyslipidaemia	9 (18.4)

Table 1. Continued

Variable	Value
Laboratory results	
WBC count, /mm^3	11,250.0 (7,050.0–14,715.0)
Haemoglobin, g/dL	13.2 (11.5–14.3)
PLT count, × 1,000/mm^3	312.0 (245.5–379.5)
Fasting glucose, mg/dL	105.0 (89.0–114.5)
Blood urea nitrogen, mg/dL	12.6 (9.8–18.9)
Creatinine, mg/dL	0.8 (0.7–1.0)
Total protein, g/dL	6.6 (5.9–7.5)
Serum albumin, g/dL	3.8 (3.2–4.3)
Acute phase reactants	
ESR, mm/hr	44.0 (11.0–73.0)
CRP, mg/L	5.7 (1.6–38.4)
During the follow-up duration	
Clinical outcome	
All-cause mortality	0
Relapse	8 (16.3)
ESRD	2 (4.1)
Follow-up duration based on each clinical outcome, mo	
Follow-up duration based on relapse	32.7 (15.4–86.2)
Follow-up duration based on ESRD	33.3 (18.6–33.3)
Immunosuppressive drugs administered	
Glucocorticoids	47 (95.9)
Cyclophosphamide	27 (55.1)
Rituximab	5 (10.2)
Azathioprine	27 (55.1)
Mycophenolate mofetil	4 (8.2)
Calcineurin inhibitor	1 (2.0)

Values are presented as median (interquartile range) or number (%).

EGPA, eosinophilic granulomatosis with polyangiitis; ANCA, antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; PR3, proteinase 3; ACR, American College of Rheumatology; BVAS, Birmingham vasculitis activity score; AAV, ANCA-associated vasculitis; FFS, five-factor score; WBC, white blood cell; PLT, platelet; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ESRD, end-stage renal disease.

Ahn SS, et al. PR3-ANCA in Korean patients with EGPA

KJIM

Table 2. Comparison of variables at diagnosis and during follow-up according to baseline PR3-ANCA status

Variable	EGPA patients without PR3-ANCA (n = 43)	EGPA patients with PR3-ANCA (n = 6)	p value
At EGPA diagnosis			
Demographic data			
Age, yr	54.0 (42.0–63.0)	43.5 (32.5–58.5)	0.228
Male gender	15 (34.9)	2 (33.3)	1.000
Body mass index, kg/m^2	21.9 (19.3–24.0)	20.5 (19.0–24.2)	0.647
1990 ACR criteria for EGPA			
Asthma or asthmatic history	40 (93.0)	6 (100)	1.000
Peripheral eosinophilia (≥ 10%)	38 (88.4)	3 (50.0)	0.047
Absolute eosinophil count, /mm^3	1,110.0 (290.0–6,520.0)	710.0 (125.0–7,346.5)	0.615
Sinusitis	41 (95.3)	6 (100)	1.000
Non-fixed lung lesions	18 (41.9)	2 (33.3)	1.000
Peripheral neuropathy	28 (65.1)	4 (66.7)	1.000
Eosinophil infiltration on histology	24 (55.8)	3 (50.0)	1.000
Clinical manifestations based on BVAS			
General manifestations	15 (34.9)	0	0.159
Cutaneous manifestations	14 (32.6)	3 (50.0)	0.405
Mucous and ocular manifestations	1 (2.3)	0	1.000
Otorhinolaryngologic manifestations	36 (83.7)	4 (66.7)	0.302
Pulmonary manifestations	27 (62.8)	5 (83.3)	0.650
Cardiovascular manifestations	6 (14.0)	3 (50.0)	0.067
Gastrointestinal manifestations	3 (7.0)	2 (33.3)	0.107
Renal manifestations	12 (27.9)	1 (16.7)	1.000
Nervous systemic manifestations	25 (58.1)	4 (66.7)	1.000
AAV-specific indices			
BVAS	13.0 (8.0–17.0)	8.0 (5.0–20.0)	0.463
FFS	1.0 (0–1.0)	1.5 (0–2.5)	0.290
Comorbidities			
Chronic kidney disease without renal replacement	9 (20.9)	2 (33.3)	0.605
Diabetes mellitus	6 (14.0)	2 (33.3)	0.250
Hypertension	17 (39.5)	3 (50.0)	0.677
Dyslipidaemia	9 (20.9)	0	0.577
Laboratory results			
WBC count, /mm^3	11,750.0 (7,100.0–14,630.0)	10,995.0 (5,137.5–25,722–5)	0.976
Haemoglobin, g/dL	13.2 (11.7–14.2)	12.8 (10.6–15.0)	0.867
PLT count, × 1,000/mm^3	312.0 (243.0–384.0)	297.0 (262.0–367.0)	0.737
Fasting glucose, mg/dL	103.0 (88.0–114.0)	111.0 (102.0–123.3)	0.228
Blood urea nitrogen, mg/dL	12.6 (10.1–20.5)	10.8 (7.5–13.7)	0.100
Creatinine, mg/dL	0.8 (0.7–1.0)	0.8 (0.5–1.1)	0.714
Total protein, g/dL	6.6 (5.9–7.5)	7.0 (5.6–7.8)	0.511
Serum albumin, g/dL	3.7 (1.1)	4.1 (3.3–4.5)	0.445

Table 2. Continued

Variable	EGPA patients without PR3-ANCA (n = 43)	EGPA patients with PR3-ANCA (n = 6)	p value
Acute phase reactants			
ESR, mm/hr	54.0 (11.0–77.0)	33.5 (8.3–66.8)	0.473
CRP, mg/L	8.2 (1.7–38.0)	4.7 (0.7–92.8)	0.680
During the follow-up duration			
Clinical outcome			
All-cause mortality	0	0	NA
Relapse	5 (11.6)	3 (50.0)	0.047
ESRD	2 (4.7)	0	NA
Follow-up duration based on each clinical outcome, mo			
Follow-up duration based on relapse	33.9 (15.3–87.2)	28.4 (12.7–54.4)	NA
Follow-up duration based on ESRD	32.5 (16.9–102.0)	75.5 (27.6–159.9)	NA
Immunosuppressive drugs administered			
Glucocorticoids	42 (97.7)	5 (83.3)	0.232
Cumulative dose, mg	3,701.3 (1,788.3–9,810.0)	3,735.0 (1,029.4–17,991.6)	0.807
Cyclophosphamide	24 (55.8)	3 (50.0)	1.000
Cumulative dose, mg	3,395.0 (0–6,157.0)	1,901.5 (0–4,517.5)	0.362
Rituximab	5 (11.6)	0	1.000
Azathioprine	22 (51.2)	5 (83.3)	0.204
Mycophenolate mofetil	3 (7.0)	1 (16.7)	0.418
Calcineurin inhibitor	1 (2.3)	0	1.000

Values are presented as median (interquartile range) or number (%).

PR3, proteinase 3; ANCA, antineutrophil cytoplasmic antibody; EGPA, eosinophilic granulomatosis with polyangiitis; ACR, American College of Rheumatology; BVAS, Birmingham vasculitis activity score; AAV, ANCA-associated vasculitis; FFS, five-factor score; WBC, white blood cell; PLT, platelet; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NA, not applicable; ESRD, end-stage renal disease.

nificant difference in absolute eosinophil count between the two groups (Table 2). Cardiovascular manifestations were more common in EGPA patients with PR3-ANCA than in those without, but this difference was not statistically significant (50.0% vs. 14.0%, p = 0.067). The other variables did not differ between the two groups. During follow-up, EGPA patients with PR3-ANCA experienced relapse more often compared to those without PR3-ANCA (50.0% for 28.4 months vs. 11.6% for 33.9 months, p = 0.047). Glucocorticoids and immunosuppressive drugs were administration evenly to the two groups. In particular, there were no differences in the cumulative doses of glucocorticoid and cyclophosphamide between the two groups (Table 2).

Cumulative survival rates between patients with PR3-ANCA and those without PR3-ANCA

In a Kaplan-Meier analysis, EGPA patients that had PR3-ANCA exhibited a lower cumulative relapse-free survival rate than those without PR3-ANCA (p = 0.012) (Fig. 1). Since no EGPA patients with PR3-ANCA had experienced ESRD during the follow-up period, a comparison analysis regarding ESRD occurrence according to the presence of PR3-ANCA was not performed (Table 2).

DISCUSSION

In this study, we analysed the differences in clinical features

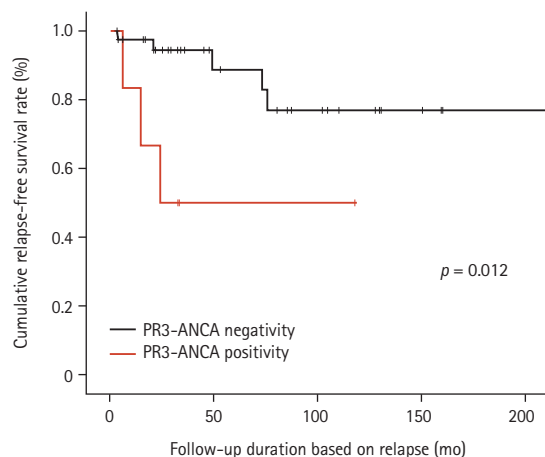Ahn SS, et al. PR3-ANCA in Korean patients with EGPA

KJIM

Figure 1. Cumulative survival rates of patients according to proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) status. Eosinophilic granulomatosis with polyangiitis patients with PR3-ANCA exhibited a lower cumulative relapse-free survival rate than those without (p = 0.012); however, no difference was found in the cumulative end-stage renal disease-free survival rate between the groups.

at diagnosis and prognoses during follow-up between EGPA patients with PR3-ANCA and those without PR3-ANCA. We first compared the major clinical characteristics between EGPA patients that were included in our study and those included in previous studies [6,10]. Age at diagnosis (54 years vs. 40–60 years), pulmonary involvement (65.3% vs. 34%–76%), heart involvement (18.4% vs. 12%–49%), kidney involvement (26.5% vs. 12%–49%), and peripheral nerve involvement (59.2% vs. 43–84%) did not differ between the two groups. However, EGPA patients included in our study had higher rates of PR3-ANCA positivity (12.2% vs. 0%–3.2%) and ear, nose, and throat involvement (81.6% vs. 53%–78%) than those in previous studies. However, skin involvement was less often observed in EGPA patients that were included in our study compared to those in previous studies [6,10].

Our results demonstrated that patients with PR3-ANCA exhibited less frequent peripheral eosinophilia, while disease relapse was more common. Importantly, differences and similarities were found between our study and a previous publication by Papo et al. [10]. First, the report by Papo et al. [10] showed that EGPA patients with PR3-ANCA had active asthma and peripheral neuropathy less often than those without PR3-ANCA. Meanwhile, it has been shown that EGPA patients that had PR3-ANCA had cutaneous manifestations, pulmonary nodules, and reduced eosinophil counts

more frequently than those without PR3-ANCA [10]. One important finding was that reduced eosinophil count was more common in EGPA patients with PR3-ANCA, and this was demonstrated in our study; however, there was no significant difference in absolute eosinophil count between the two groups. The frequencies of peripheral neuropathy and cutaneous manifestations did not differ in the present study, and pulmonary nodules were not found in our study population. A previous study demonstrated that EGPA patients with PR3-ANCA and MPO-ANCA showed lower cumulative relapse-free survival rates than those in the ANCA-negative group [10]. In line with this observation, we also found that EGPA patients with PR3-ANCA exhibited a lower cumulative relapse-free survival rate than those without PR3-ANCA (p = 0.012), but not in ESRD-free survival rate.

PR3-ANCA is generally regarded as a potential risk factor for relapse in AAV patients [8,17]. In particular, as demonstrated in a study by Fussner et al. [18], the increase in PR3-ANCA levels was closely related to the forthcoming relapse in AAV, and relapses occurred within 1 year after the elevation of PR3-ANCA titres. Nonetheless, it is still uncertain whether the presence of PR3-ANCA is predictive of relapse in patients with EGPA. However, in line with the results of the study of Papo et al. [10] and the results of our study, PR3-ANCA appears to potentially increase the risk of relapse in EGPA patients. Therefore, we carefully suggest that physicians should treat EGPA patients with PR3-ANCA, considering the possibility of relapse.

A previous study conducted by Papo et al. [10] included four EGPA patients with PR3-ANCA who had pulmonary nodules. Based on the 2007 EMA algorithm for the classification of AAV, if pulmonary nodules had been considered as one of the surrogate markers of granulomatosis with polyangiitis (GPA), such as fixed, nodular and cavitary lesions in the lower respiratory tract, these four EGPA patients with pulmonary nodules should have been classified as GPA [5]. According to the 2007 EMA algorithm, patients who did not undergo biopsy, can be classified as having GPA if they have both surrogate markers of GPA and ANCA positivity [5]. Furthermore, according to the ACR/European Alliance of Associations for Rheumatology (EULAR) 2017 provisional classification criteria for GPA, if they had sino-nasal symptoms such as sinusitis, they could be preferentially classified as having GPA (≥ 5 points) based on sino-nasal symptoms (3 points), PR3-ANCA positivity (5 points), pulmonary nodule (2 points), and peripheral eosinophilia (–3 points) [19].

Given this ambiguity in differentiating EGPA patients that have PR3-ANCA from GPA patients with PR3-ANCA, we wondered whether the clinical features of the two groups might differ (Supplementary Fig. 1). Of the 242 patients, we selected 29 GPA patients that had PR3-ANCA and compared their clinical features with six EGPA patients with PR3-ANCA. GPA patients with PR3-ANCA had the clinical features that were similar to those of EGPA patients with PR3-ANCA except for cutaneous and gastrointestinal manifestations that occurred more frequently in EGPA patients with PR3-ANCA than GPA patients with PR3-ANCA (Supplementary Table 1). In addition, there was no significant difference in the cumulative relapse-free survival rates between the two groups (Supplementary Fig. 2).

At the entry of the present study, we intended to investigate the differences in variables among PR3-ANCA positive, MPO-ANCA positive and ANCA-negative groups of EGPA patients. However, in the Kaplan-Meier analysis, the occurrence of relapse in EGPA patients was apparent in the PR3-ANCA group compared to the MPO-ANCA positive and ANCA-negative groups; furthermore, no difference in the cumulative relapse-free survival rates was found between EGPA patients with MPO-ANCA and those without any type of ANCAs. Meanwhile, EGPA patients with PR3-ANCA exhibited a significantly lower cumulative relapse-free survival rate than those without any type of ANCA. They also tended to show a lower cumulative relapse-free survival rate than those with MPO-ANCA even though not statistically significant (Supplementary Fig. 3). On the other hand, the main purpose of this study was to clarify the clinical significance of PR3-ANCA as a risk factor for relapse in EGPA patients. Therefore, we divided EGPA patients into two groups according to PR3-ANCA positivity, and we investigated the differences between EGPA patients with PR3-ANCA and those without PR3-ANCA.

Although the advantage of this study is that this was the first study to evaluate the features and outcomes of Korean patients with EGPA according to PR3-ANCA status, the small number of EGPA patients with PR3-ANCA and the retrospective study design should be clearly addressed as a critical limitation. Additionally, serial results of PR3-ANCA might have highlighted the clinical implication of PR3-ANCA in three EGPA patients who had PR3-ANCA but not relapse. However, since serial PR3-ANCA results during follow-up were not available in two of three EGPA patients with PR3-ANCA but without relapse, we could not analyse the significance of the follow-up result how PR3-ANCA affected EGPA relapse.

In conclusion, we showed that EGPA patients that had PR3-ANCA at diagnosis have distinct clinical features and outcomes compared to those without PR3-ANCA. Therefore, we believe that these results should be considered when managing EGPA patients.

KEY MESSAGE

1. Eosinophilic granulomatosis with polyangiitis (EGPA) patients with proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) exhibited peripheral eosinophilia ($\geq$ 10% of the total white blood cell count) less frequently than those without PR3-ANCA.
2. EGPA patients with PR3-ANCA exhibited a lower cumulative relapse-free survival rate than those without PR3-ANCA.
3. Physicians should pay more attention to PR3-ANCA positivity when managing patients with EGPA.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, et al. 2012 Revised International Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheum 2013;65:1-11.
2. Greco A, Rizzo MI, De Virgilio A, et al. Churg-Strauss syndrome. Autoimmun Rev 2015;14:341-348.
3. Radice A, Bianchi L, Sinico RA. Anti-neutrophil cytoplasmic autoantibodies: methodological aspects and clinical significance in systemic vasculitis. Autoimmun Rev 2013;12:487-495.

4. Masi AT, Hunder GG, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990;33:1094-1100.

5. Watts R, Lane S, Hanslik T, et al. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. Ann Rheum Dis 2007;66:222-227.

6. Millet A, Pederzoli-Ribeil M, Guillevin L, Witko-Sarsat V, Mouthon L. Antineutrophil cytoplasmic antibody-associated vasculitides: is it time to split up the group? Ann Rheum Dis 2013;72:1273-1279.

7. Choi CB, Park YB, Lee SW. Eosinophilic granulomatosis with polyangiitis: experiences in Korean patients. Yonsei Med J 2019;60:705-712.

8. Cornec D, Cornec-Le Gall E, Fervenza FC, Specks U. ANCA-associated vasculitis: clinical utility of using ANCA specificity to classify patients. Nat Rev Rheumatol 2016;12:570-579.

9. Yoo J, Kim HJ, Ahn SS, et al. Clinical and prognostic features of Korean patients with MPO-ANCA, PR3-ANCA and ANCA-negative vasculitis. Clin Exp Rheumatol 2017;35 Suppl 103:111-118.

10. Papo M, Sinico RA, Teixeira V, et al. Significance of PR3-ANCA positivity in eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Rheumatology (Oxford) 2021;60:4355-4360.

11. Leavitt RY, Fauci AS, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. Arthritis Rheum 1990;33:1101-1107.

12. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis 2009;68:1827-1832.

13. Guillevin L, Pagnoux C, Seror R, et al. The Five-Factor Score revisited: assessment of prognoses of systemic necrotizing vasculitides based on the French Vasculitis Study Group (FVSG) cohort. Medicine (Baltimore) 2011;90:19-27.

14. Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. Lancet 2017;389:1238-1252.

15. Mukhtyar C, Flossmann O, Hellmich B, et al. Outcomes from studies of antineutrophil cytoplasm antibody associated vasculitis: a systematic review by the European League Against Rheumatism systemic vasculitis task force. Ann Rheum Dis 2008;67:1004-1010.

16. Yates M, Watts RA, Bajema IM, et al. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis 2016;75:1583-1594.

17. Kitching AR, Anders HJ, Basu N, et al. ANCA-associated vasculitis. Nat Rev Dis Primers 2020;6:71.

18. Fussner LA, Hummel AM, Schroeder DR, et al. Factors determining the clinical utility of serial measurements of antineutrophil cytoplasmic antibodies targeting proteinase 3. Arthritis Rheumatol 2016;68:1700-1710.

19. Yoo J, Kim HJ, Ahn SS, et al. The utility of the ACR/EULAR 2017 provisional classification criteria for granulomatosis with polyangiitis in Korean patients with antineutrophil cytoplasmic antibody-associated vasculitis. Clin Exp Rheumatol 2018;36 Suppl 111:85-87.

Ahn SS, et al. PR3-ANCA in Korean patients with EGPA

KJIM✕

Supplementary Table 1. Comparison of variables at diagnosis and during follow-up between GPA patients with PR3-ANCA and EGPA patients with PR3-ANCA

Variable	GPA patients with PR3-ANCA (n = 29)	EGPA patients with PR3-ANCA (n = 6)	p value
At diagnosis			
Demographic data			
Age, yr	54.0 (42.0–64.0)	43.5 (32.5–58.5)	0.283
Male gender	14 (48.3)	2	0.504
Clinical manifestations based on BVAS			
General manifestations	7 (24.1)	0	0.178
Cutaneous manifestations	1 (3.4)	3 (50.0)	0.001
Mucous and ocular manifestations	4 (13.8)	0	0.334
Otorhinolaryngologic manifestations	22 (75.9)	4 (66.7)	0.639
Pulmonary manifestations	15 (51.7)	5 (83.3)	0.154
Cardiovascular manifestations	5 (17.2)	3 (50.0)	0.082
Gastrointestinal manifestations	1 (3.4)	2 (33.3)	0.017
Renal manifestations	12 (41.4)	1 (16.7)	0.254
Nervous systemic manifestations	10 (34.5)	4 (66.7)	0.143
AAV-specific indices			
BVAS	10.0 (5.0–13.0)	8.0 (5.0–20.0)	0.693
FFS	1.0 (0.5–1.5)	1.5 (0–2.5)	0.560
Comorbidities			
Chronic kidney disease without renal replacement	10 (34.5)	2 (33.3)	0.957
Diabetes mellitus	6 (20.7)	2 (33.3)	0.502
Hypertension	10 (34.5)	3 (50.0)	0.474
Dyslipidaemia	5 (17.2)	0	0.272
Acute phase reactants			
ESR, mm/hr	59.0 (22.5–86.5)	33.5 (8.3–66.8)	0.405
CRP, mg/L	18.5 (1.7–60.7)	4.7 (0.7–92.8)	0.484
During the follow-up duration			
Clinical outcome			
All-cause mortality	4 (13.8)	0	NA
Relapse	16 (55.2)	3 (50.0)	0.817
ESRD	5 (17.2)	0	NA

Values are presented as median (interquartile range) or number (%).
GPA, granulomatosis with polyangiitis; PR3, proteinase 3; ANCA, antineutrophil cytoplasmic antibody; EGPA, eosinophilic granulomatosis with polyangiitis; BVAS, Birmingham vasculitis activity score; AAV, ANCA-associated vasculitis; FFS, five-factor score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ESRD, end-stage renal disease.
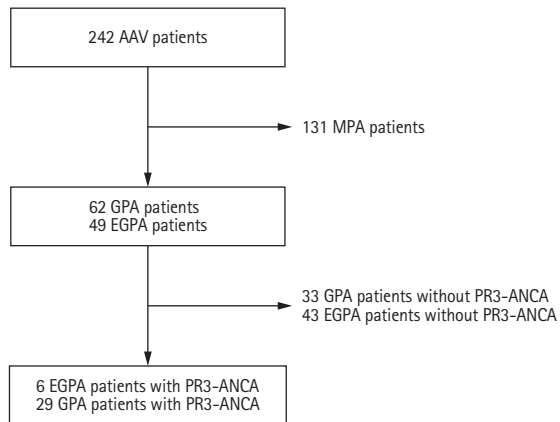
Supplementary Figure 1. Selection of the study population. AAV, ANCA-associated vasculitis; MPA, microscopic polyangiitis ; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; PR3, proteinase 3; ANCA, antineutrophil cytoplasmic antibody.

Ahn SS, et al. PR3-ANCA in Korean patients with EGPA

KJIM
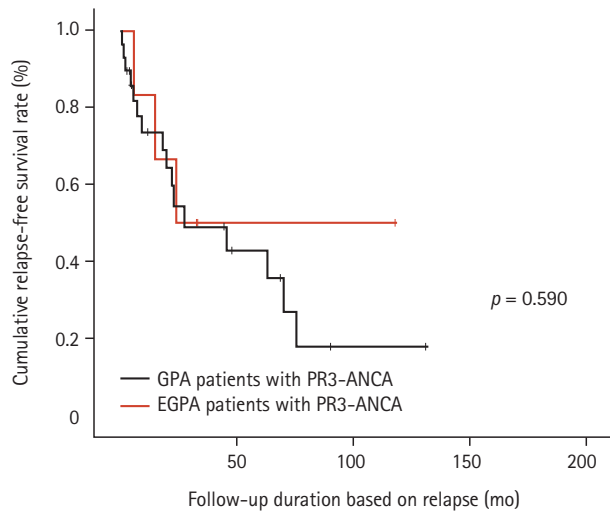
Supplementary Figure 2. Comparison of the cumulative relapse-free survival rates between granulomatosis with polyangiitis (GPA) patients with proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) and eosinophilic granulomatosis with polyangiitis (EGPA) patients with PR3-ANCA.
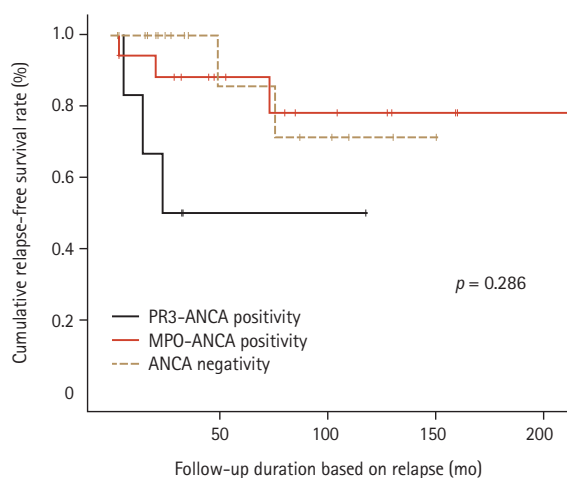
Supplementary Figure 3. Comparison of the cumulative relapse-free survival rates among proteinase 3 (PR3)-antineutrophil cytoplasmic antibody (ANCA) positivity, myeloperoxidase (MPO)-ANCA positivity and ANCA negativity.