doi: 10.53446/actamednicomedia.1227630

Acta Medica Nicomedia

Cilt: 6 Sayı: 1 Şubat 2023 / Vol: 6 Issue: 1 February 2023
https://dergipark.org.tr/tr/pub/actamednicomedia

Araştırma Makalesi | Research Article

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY SEROTYPES MAY AFFECT THE CLINICAL PRESENTATION OF PATIENTS WITH POLYANGIITIS: A SINGLE-CENTER, RETROSPECTIVE CROSS-SECTIONAL STUDY

ANTİNÖTROFİL SİTOPLAZMİK ANTİKOR SEROTİPLERİ GRANÜLOMATÖZ POLİANJİİTLİ HASTALARDA KLİNİK PREZANTASYONU ETKİLEYEBİLİR: TEK MERKEZ, RETROSPEKTİF KESİTSEL ÇALIŞMA

Ayten Yazici[1*], Oznur Sadioglu Cagdas[1], Ali Osman Arisal[2], Ozlem Ozdemir Isik[1], Neslihan Gokcen[2], Ayse Cefle[1]

[1]University of Kocaeli, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Kocaeli, Türkiye. [2]University of Kocaeli, School of Medicine, Department of Internal Medicine, Kocaeli, Türkiye.

ABSTRACT

Objective: Granulomatosis with polyangiitis (GPA) is one of the systemic vasculitis included in antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV). Clinical characteristics, ANCA serotypes, and their frequencies vary depending on the type of vasculitis. In this study, we aimed to investigate the link between ANCA serotypes and clinical variables documented at the time of admission.

Methods: This study was a retrospective cross-sectional study including 40 patients with GPA. The demographic, clinical, laboratory, and radiological findings were extracted from medical records. Logistic Regression Analysis was performed to estimate the relationship between ANCA serotypes and other variables.

Results: The mean age was 53.95±12.94 years, and there was a delay of 15.85±23.03 months in diagnosis. While constitutional symptoms were observed in 42% of patients, symptoms related to ear-nose-throat in 70%, lung in 87.5%, kidney in 62.5%, neurological findings in 30%, eye in 25%, skin in 25% and musculoskeletal system findings in 90% were noted. Male gender was seen more frequently in cANCA (60.7%) positive patients; however, pANCA-positive patients were mainly female (83.3%) (p=0.050). Delay in diagnosis was longer (p=0.001) and subglottic stenosis was more common (p<0.001) in pANCA-positive patients. However, the inflammatory response was more severe, and renal involvement (p=0.007) and arthralgia (p=0.048) were higher in cANCA-positive patients.

Conclusion: While renal involvement seemed to be related to cANCA, subglottic stenosis was mostly associated with pANCA in GPA. However, larger studies are needed to make a more accurate conclusion about the effect of ANCA serotypes on diversity in the clinical spectrum.

Keywords: Granulomatosis with polyangiitis, vasculitis, antineutrophil cytoplasmic antibody, renal involvement, subglottic

ÖZ

Amaç: Granülomatöz polianjiit (GPA) antinötrofil sitoplazmik antikor (ANCA) ilişkili vaskülitlerden (AİV) biridir. Vaskülitin tipine bağlı olarak klinik özellikler, ANCA serotipleri ve sıklıkları değişmektedir. Bu çalışmada, ANCA serotipleri ile başvuru anındaki klinik değişkenler arasındaki ilişkinin araştırılması amaçlandı.

Yöntem: Bu retrospektif kesitsel çalışmaya 40 GPA hastası dahil edildi. Demografik, klinik, laboratuar ve radyolojik bulgular hastaların dosyalarından toplandı. ANCA serotipleri ile diğer değişkenlerin ilişkisini değerlendirmek için Logistic Regresyon analizi kullanıldı.

Bulgular: Hastaların ortalama yaşları 53,95±12,94 yıl olup tanıda 15,85±23,03 aylık bir gecikme vardı. Konstitüsyonel semptomlar %42 hastada gözlenirken %70'inde kulak-burun-boğaz, %87,5'inde akciğer, %62,5'inde böbrek, %30'unda nörolojik, %25'inde göz, %25'inde cilt ve %90'ında kas-iskelet sistemi ile ilişkili semptomlar kaydedildi. cANCA pozitif olanlarda erkekler daha sık iken (%60,7) pANCA pozitiflerde kadınlar daha fazla (%83,3) idi (p=0.050). pANCA pozitif hastalarda tanıda gecikme daha uzun (p=0.001) ve subglottik stenoz daha sıktı (p<0.001). Bunun yanında cANCA pozitif hastalarda inflamatuar yanıt daha şiddetli ve böbrek tutulumu (p=0.007) ile artralji (p=0.048) daha fazlaydı.

Sonuç: GPA'da cANCA böbrek tutulumu ile ilişkili görünürken subglottik stenoz daha çok pANCA ile ilişkiliydi. Bununla birlikte ANCA serotiplerinin klinik spektrumdaki çeşitlilik üzerindeki etkisi hakkında daha doğru bir sonuca varmak için daha büyük çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Granülomatöz polyanjiit, vaskulit, antinötrofil sitoplazmik antikor, böbrek tutulumu, subglottic stenoz

[*]**İletişim kurulacak yazar/Corresponding author:** Ayten Yazici; University of Kocaeli, School of Medicine, Department of Internal Medicine, Division of Rheumatology, Baki Komsuoğlu Bulvarı, No:515, Umuttepe Campus, İzmit, Kocaeli, 41001, Türkiye.
Telefon/Phone: +90 (262) 303 75 25 **e-posta/e-mail:** burakdefy@hotmail.com
Başvuru/Submitted: 1.01.2023 • **Kabul/Accepted:** 1.02.2023 • **Online Yayın/Published Online:** 28.02.2023

Introduction

Granulomatosis with polyangiitis (GPA), also known as Wegener granulomatosis, is an antineutrophil cytoplasmic antibody (ANCA) associated necrotizing vasculitis involving small and medium-sized vessels. The etiology of GPA is not fully understood, but it is thought to be a complex multifactorial process in which genetic predisposition, environmental and immunological factors have a unique role in the etiology of the disease. Although GPA mainly targets the upper and lower respiratory tract and kidney, it can affect other organs and systems.[1,2] The prevalence and annual incidence have been reported as 2.3-146 and 0.4-14.4 cases per one million, respectively.[2] Studies have shown that GPA, which affects men and women equally, is more common in Northern European countries than in other countries.[2-6] It is well known that clinical characteristics, organ involvement, ANCA types and their frequencies vary through ANCA associated vasculitis (AAV) types including GPA, microscopic polyangiitis (MPA), and eosinophilic granulomatous polyangiitis (EGPA). Furthermore, some authors proposed a classification based on types of ANCA as anti-proteinase 3 (PR3) associated vasculitis and anti-myeloperoxidase (MPO) associated vasculitis due to the close relationship between ANCA serotype and disease characteristics, but it is not widely accepted in current literature.[5] Although anti-PR3 (65-75%) is the most frequently detected ANCA serotype in GPA, anti-MPO positivity and ANCA negativity have been reported in 20-30% and 5% of the patients, respectively.[5,7] The aim of the study was to reveal the clinical and laboratory characteristics of GPA patients and to investigate the possible relationship of those with ANCA serotypes.

Methods

Study Design and Patients

The study was designed as a retrospective cross-sectional study. All patients with GPA aged 18-80 years who were followed up in the rheumatology outpatient clinic between 2004 and 2021, and fulfilled the 2012 revised Chapel-Hill consensus criteria were included in the study.[8] Patients with both ANCA serotypes positive or negative, and patients with other vasculitis were excluded. Study protocol was approved by the local ethics committee (ethics approval number: GOKAEK-2021/9.11).

Clinical and Laboratory Assessment

The demographic, clinical, laboratory, and radiological data of the patients at the time of admission to the clinic were retrospectively screened from medical records. In our tertiary university hospital laboratory, ANCA detection is made by indirect immunofluorescence (IIF) while ANCA serotypes are determined by enzyme-linked immunosorbent assay (ELISA). Since PR3 and MPO were not assessed in all patients, the ANCA serotypes were defined as cANCA and/or PR3 and pANCA and/or MPO. Three patients with positive for both ANCA serotypes and three patients with negative ANCA were excluded from the comparisons. Systemic involvement was recorded from patients' database. Accordingly, an otolaryngologist evaluated patients in terms of ear-nose-throat (ENT) involvement with clinical examination. In some patients, head and neck magnetic resonance imaging was performed to evaluate and/or confirm ENT involvement. Lung involvement was assessed by computed tomography. Renal involvement was defined as abnormal urine test including hematuria and/or urine protein greater than 0.5g/24 hours. Neurological involvement was defined as peripheral and central nervous system findings that were assessed by clinical examination, electromyography, and brain magnetic resonance imaging. Similarly, cutaneous and eye involvement were assessed by dermatologist and ophthalmologist. Musculoskeletal system involvement was defined as arthralgia, arthritis, and myalgia. Patients underwent electrocardiography to assess cardiac involvement. Relapse was defined as re-occurrence or new onset of the organ or life-threatening disease.[1]

Statistical Analysis

SPSS for Windows 20.0 version was used for statistical analysis. The normal distribution of numerical variables was evaluated with the Kolmogorov-Smirnov Test. Besides descriptive statistical methods (mean, standard deviation, median and 25-75th percentile, min and max), the independent groups T test or Mann Whitney test were used for continuous variables, in comparison of paired groups. For categorical variables chi-square test and Fisher exact test were used. The relationship between ANCA serotypes and clinical and laboratory findings was determined by Spearman Correlation Analysis, and Logistic Regression Analysis was used as advanced statistics. For the testing of two-sided hypotheses, $p < 0.05$ was considered as sufficient for statistical significance.

Results

The mean age of GPA patients (female/male= 22/18) was 53.95±12.94 (median, min-max=55, 25-75) years. There was 15.85±23.03 (median, min-max=4, 1-180)-month delay in the diagnosis. During a mean follow-up period of 95.03±61.36 (median, min-max=82, 2-360) months, two patients died; one of them due to disease activation and concomitant infection, another one due to COVID-19 infection (5%). 37.5% of patients suffered from at least one relapse during follow-up. At the time of admission, 42% of the patients had constitutional findings. Most patients had several types of involvement. Accordingly, ear-nose-throat involvement, lung involvement, renal involvement, neurological involvement, skin involvement, and musculoskeletal system involvement were detected in 70%, 87.5%, 62.5%, 30%, 25%, 25%, and 90% of patients, respectively. None of patients had cardiac involvement (Table 1).

The number and percentages of patients according to the presence of ANCA positivity and its subtypes are given as following; 3 patients (7.5%) had neither pANCA nor cANCA positivity, 3 patients (7.5%) had positive test results for both pANCA and cANCA, 28 patients (70%) had cANCA/anti-PR3 positivity, finally, 6 patients (15%) had pANCA/anti-MPO positivity (Table 2).

The comparison of only pANCA/anti-MPO positive and only cANCA/anti-PR3 positive groups revealed that female gender was seen more frequently in pANCA/anti-MPO positive patients and male gender was seen more frequently in cANCA/anti-PR3 positive patients (p=0.050). While patients in pANCA/anti-MPO positive group had prolonged time to diagnosis (p=0.001), cANCA/anti-PR3 positive group had higher inflammatory markers including erythrocyte sedimentation rate, C-reactive protein, and thrombocyte count (p<0.001, p=0.006, and p=0.034, respectively). In addition, anemia was more common in cANCA/anti-PR3 positive group than in pANCA/anti-MPO positive group (75% vs 33.3%, p=0.048) (Table 2). Among

Table 1. The comparison of demographic and clinical findings according to ANCA serotypes

	All Patients (n=40)	**cANCA/PR3 (+) Patients (n=28)**	**pANCA/MPO (+) Patients (n=6)**	**p***
Age of onset	46.10±15.02	44.8±17.7	38.2±15.7	0.326[a]
Delay in diagnosis	15.85±23.03	11.4±16.5	45.5±36.4	**0.001**[a]
Gender (Female)	22 (55)	11 (39.3)	5 (83.3)	0.050[b]
Constitutional findings	34 (42)	25 (89.3)	5 (83.3)	0.681[b]
Fever	20 (50)	16 (57.1)	3 (50)	0.749
Weight loss	17 (42.5)	14 (50)	1 (16.7)	0.136
Weakness	34 (85)	25 (89.3)	5 (83.3)	0.681
Anorexia	20 (50)	16 (57.1)	2 (33.3)	0.289
Musculoskeletal involvement	36 (90)	26 (92.9)	4 (66.7)	0.071[b]
Myalgia	32 (80)	24 (85.7)	4 (66.7)	0.267
Arthralgia	27 (67.5)	21 (75)	2 (33.3)	**0.048**
Arthritis	7 (17.5)	6 (21.4)	1 (16.7)	0.793
Ear-nose-throat involvement	28 (70)	19 (67.9)	5 (83.3)	0.450[b]
Mastoiditis	7 (17.5)	6 (21.4)	0	0.211
Otitis	7 (17.5)	6 (21.4)	0	0.211
Sinusitis	11 (27.5)	7 (25)	2 (33.3)	0.675
Septum perforation	2 (5)	1 (3.6)	0	0.638
Subglottic stenosis	6 (15)	1 (3.6)	4 (66.7)	**<0.000**
Pulmonary involvement	35 (87.5)	26 (92.9)	4 (66.7)	0.071
Nodule	27 (67.5)	19 (67.9)	3 (50)	0.406
Cavity	15 (37.5)	11 (39.3)	1 (16.7)	0.293
ILD	14 (35)	13 (46.4)	1 (16.7)	0.179
Alveolar hemorrhage	8 (20)	7 (25)	1 (16.7)	0.662
Pleurisy	1 (2.5)	1 (3.6)	0	0.638
Lymphadenopathy	11 (27.5)	8 (24.2)	1 (16.7)	0.549
Renal involvement	25 (62.5)	21 (75)	1 (16.7)	**0.007**[b]
Neurological involvement	12 (30)	8 (28.6)	1 (16.7)	0.549[b]
Mononeuritis multiplex	1 (2.5)	0	0	NA
Peripheral nervous system	8 (20)	5 (17.9)	0	0.262
Central nervous system	4 (10)	3 (10.7)	1 (16.7)	0.681
Skin involvement	10 (25)	9 (32.1)	0	0.105[b]
Erythema nodosum/nodule	4 (10)	3 (10.7)	0	0.401
Palpable purpura	2 (5)	1 (3.6)	0	0.638
Leukocytoclastic vasculitis	2 (5)	2 (7.1)	0	0.500
Skin ulcer	2 (5)	2 (7.1)	0	0.500
Urticaria	1 (2.5)	1 (3.6)	0	0.638
Eye involvement	10 (25)	7 (25)	1 (16.7)	0.662[b]
Episcleritis/scleritis	6 (15)	5 (17.9)	0	0.324
Uveitis	2 (5)	2 (7.1)	0	0.500
Conjunctivitis	3 (7.5)	3 (10.7)	0	0.401
Gastrointestinal involvement	5 (12.5)	2 (7.1)	2 (33.3)	0.071[b]
Urogenital involvement (orchitis)	1 (2.5)	1 (3.6)	0	0.638[b]
Comorbid Disease	28 (70)	18 (64.3)	4 (66.7)	0.912[b]
Relapse	15 (37.5)	10 (35.7)	3 (50)	0.513[b]
Mortality	2 (5)	1 (3.6)	1 (16.7)	0.216[b]

[a]Values given as mean±standard deviation, Independent Samples T test and One way ANOVA

[b]Values given as n (%), Fisher's Exact test

*The comparisons were made between cANCA/PR3 (+) patients and pANCA/MPO (+) patients

ILD: Interstitial lung disease, ANCA: Antineutrophil cytoplasmic antibody, PR3: Anti-proteinase 3 antibody, MPO: Anti-myeloperoxidase antibody

Table 2. The comparison of laboratory findings at the time of diagnosis according to ANCA serotypes

	All Patients n=40	cANCA/PR3 (+) Patients n=28	pANCA/MPO (+) Patients n=6	p*
Increased ESR	28 (70)	25 (89.3)	0	**0.000**
Increased CRP	30 (75)	24 (85.7)	2 (33.3)	**0.006**
Leukocytosis	26 (65)	19 (67.9)	3 (50)	0.406
Neutrophil	21 (52.5)	15 (53.6)	2 (33.3)	0.325
Lymphopenia	12 (30)	10 (35.7)	1 (16.7)	0.338
Anemia	25 (62.5)	21 (75)	2 (33.3)	**0.048**
Increased RDW	9 (22.5)	7 (25)	0	0.150
Thrombocytosis	15 (37.5)	13 (46.4)	0	**0.034**
Increased Creatinine	10 (25)	7 (25)	1 (16.7)	0.632
Increased LDH	10 (25)	6 (21.4)	2 (33.3)	0.566
RF positivity	13 (32.5)	11 (39.3)	0	0.056
Anti-CCP positivity	2 (5)	2 (0.7)	0	0.458
ANA positivity	4 (10)	3 (10.7)	0	0.392
ANCA positivity	37 (92.5)	28 (100)	6 (100)	1.000
c-ANCA	28 (70)	26 (92.9)	0	
p-ANCA	9 (22.5)	0	6 (100)	
anti-PR3	29 (72.5)	25 (89.3)	1 (16.7)	
anti-MPO	3 (7.5)	0	2 (33.3)	

Values given as n (%), Fisher's Exact test

*The comparisons were made between cANCA/PR3 (+) patients and pANCA/MPO (+) patients

ESH: erythrocyte sedimentation rate, CRP: C-reactive protein, RDW: Red cell distribution width, LDH: Lactate dehydrogenase, RF: Rheumatoid factor, CCP: cyclic citrullinate peptide, ANA: Anti-nuclear antibody, ANCA: Antineutrophil cytoplasmic antibody, PR3: Anti-proteinase 3 antibody, MPO: Anti-myeloperoxidase antibody

Table 3. Logistic regression analysis for ANCA serotypes in patients with GPA

	Univariable Analysis			Multivariable Analysis		
	OR	95% CI	p	OR	95% CI	p
Delay in diagnosis	1.056	1.010-1.105	**0.017**			
Subglottic stenosis	54.000	3.931-741.79	**0.003**	54.000	0.061-0.928	**0.039**
Renal involvement	0.067	0.007-0.672	**0.022**			
Increased ESR	0.000	0.000	0.998			
Increased CRP	0.083	0.011-0.615	**0.015**			

ANCA: antineutrophil cytoplasmic antibody, GPA: Granulomatosis with polyangiitis, CI: Confidence interval, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

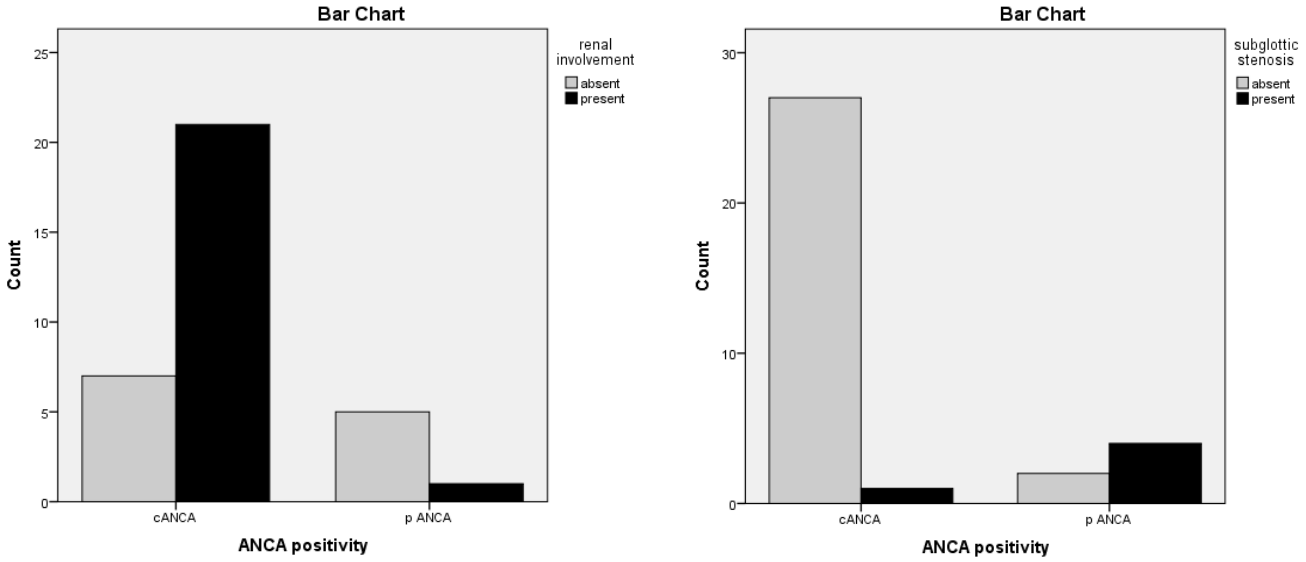

Figure: Frequency of renal involvement and subglottic stenosis according to ANCA serotypes

the clinical findings, while the frequency of subglottic stenosis (66.7% vs 3.6%; p<0.001) was seen higher in pANCA/anti-MPO positive patients, renal involvement (75% vs 16.7%; p=0.007) and arthralgia (75% vs 33.3%; p=0.048) were seen more frequently in those with cANCA/anti-PR3 positivity (Table 1) (Figure). Additionally, there were no correlations between ANCA serotypes and clinical/laboratory findings except delay in diagnosis (r=0.539, p= 0.001), renal involvement (r=-0.465, p=0.006), subglottic stenosis (r=0.679, p<0.001) and elevation of ESR (r=-0.772, p<0.001) and CRP (r=-0.471, p=0.005). However, ANCA serotypes were only associated with subglottic stenosis in logistic regression analysis (Table 3).

In this study, 70% of the patients had at least one comorbid disease (Diabetes Mellitus 15.5%, Hypertension 33.3%) and there was no difference between ANCA serotypes in terms of comorbid disease frequency. Although relapse and mortality rate were seemed to be higher in pANCA/anti-MPO positive patients, comparison of two groups did not reveal statistically significant differences (Table 1). The treatments received by the patients during the follow-up are given in Table 4.

Table 4. Previous and current treatments of patients

	n (%)
Previous treatment	
Cyclophosphamide	22 (55)
Corticosteroid	39 (97.5)
Azathioprine	23 (57.5)
Methotrexate	14 (35)
Rituximab	22 (55)
IVIG	10 (25)
Plasmapheresis	5 (12.5)
Current treatment	
Cyclophosphamide	3 (7.5)
Azathioprine	13 (32.5)
Methotrexate	8 (20)
Rituximab	17 (42.5)

IVIG: Intravenous immunoglobulin

Discussion

GPA is an ANCA-related necrotizing vasculitis with unknown etiology. This study investigated whether there is a relationship between ANCA serotypes and clinical and laboratory findings in patients with GPA. Similar to other systemic autoimmune diseases, GPA frequently causes nonspecific constitutional symptoms (in 60% of the patients), and lesser degree fever (48%), weight loss (28%), and joint findings (38%).[9] Arthralgia and myalgia have been reported in 70% of the patients, and arthritis in 23%.[10] In our study, constitutional findings, fever and arthritis were found in 42% of the patients, similar to previous studies, while weight loss and arthralgia/ myalgia ratio were found to be higher.

GPA is a multisystemic disease, however mostly targets the upper (75-90%) and lower respiratory tracts (60-80%), and kidney (56-80%).[7,9-12] In a cohort study including 502 patients with ANCA associated vasculitis, organ predilection was studied based on ANCA serotypes. Accordingly, the majority of patients with crescentic glomerulonephritis (above 80% of patients) were strongly related anti-MPO ANCA while most of patients with lung cavities and destructive ear-nose-throat involvement (above 80% of patients) were closely associated with anti-PR3 ANCA.[12] In a review article, authors searched the literature to show the differences of clinical, histopathologic, and pathophysiologic variables between anti-PR3 positive and anti-MPO positive patients. As a result, extrarenal organ manifestations and respiratory tracts involvement were frequently observed in anti-PR3 positive patients.[13] Among the upper respiratory tracts, the nasal cavity, and paranasal sinuses are the most commonly involved organs, followed by otologic (35%) and subglottic involvement (16-23%).[14,15] A study conducted by Schirmer et al. showed that anti-MPO positive patients had limited disease and had an increased frequency of subglottic stenosis.[16] Other organs and systems affected by GPA in decreasing fashion are given as following; eyes in 24-33%, mucocutaneous involvement in 25-50%, neurological involvement in 35%, and gastrointestinal involvement in 15%.[6,9-11] In our study, ear-nose-throat organs (70%), lungs (87.5%), renal (62.5%), neurologic (30%), eyes (25%), skin (25%) and gastrointestinal (12.5%) involvement were seen in similar rate compared to previous reports. Moreover, in the present study, we found that arthralgia and renal involvement were more common in patients with anti-PR3 ANCA while subglottic stenosis was more seen in patients with anti-MPO ANCA. Our results were conflicting with the study conducted by Cornec et al.[12] Retrospective cross-sectional study design and small sample size of our study may be the reasons for the conflicting results.

ANCA positivity is associated with disease activity and seen in 80-90% of patients with GPA. Studies have reported about 80% positivity for cANCA and 10% for pANCA subtype.[9,11,17] Although 65-75% of anti-PR3 positivity and 20-30% of anti-MPO positivity have been reported in GPA, anti-PR3 positivity has been reported up to %90 of patients in previous papers, especially during active periods.[5,7,18] In the current study, ANCA positivity was observed in 92.5% of patients while the rates of its serotypes positivity including cANCA, pANCA, anti-PR3, and anti-MPO were found in 70%, 22.5%, 72.5%, and 7.5% of those patients, respectively.

Disease severity, which varies from extensive organ involvement to limited form, affects ANCA positivity ratio. In the study of Stone et al., ANCA (90.6% vs 78.4%) and anti-PR3 (78.1% vs 58.8%) positivity have been found to be associated with severe disease, but not anti-MPO positivity (13.4% vs 8%).[11] In our study, we found that there were significant differences between cANCA and pANCA positive patients in terms of demographic, clinical, and laboratory findings. While cANCA/anti-PR3 positivity was related with male gender, pANCA/anti-MPO positivity was related with female gender. Patients with pANCA/anti-MPO positivity had a higher delay in

diagnosis. Elevated inflammatory markers, anemia, arthralgia and renal involvement were more prominent in the cANCA/anti-PR3 positive group, while subglottic stenosis was more common in pANCA/anti-MPO positive group.

In addition to the different clinical presentation in GPA, MPA and EGPA, the positivity rates of ANCA serotypes are also varies.[5] It has been shown that anti-MPO and anti-PR3 are associated with different organ involvement in AAV. While anti-PR3 positive AAV patients had more upper respiratory tract involvement, kidney is affected more frequently in anti-MPO positive AAV patients. In a previous study, anti-MPO have been found in 80% of AAV patients limited to the kidneys and anti-PR3 have been found in 90% of those with destructive lesions in the nasal septum.[7] On the basis of abovementioned literature, it can be assumed that similar differences in clinical presentation and organ involvement might be observed in GPA patients. Surprisingly, we observed an inverse relationship with ANCA serotypes and organ involvement compared to previous reports regarding AAV patients. Although ANCA serotypes were associated with only subglottic stenosis in logistic regression analysis, cANCA/anti-PR3 positivity was more common in patients with renal involvement, and pANCA/anti-MPO positivity was more common in patients with subglottic stenosis. However, our study has limited number of patients to generalize the present observation to GPA population, and needs to be validated by larger cohorts.

Anti-PR3 and anti-MPO are important prognostic indicators in AAV. Studies have shown that patients with positive anti-PR3 have increased relapse risk than those with positive anti-MPO. In addition, the frequency of relapse has been reported to be lower in those with ANCA negative compared to positive ones.[5,7] During the median 82 months follow-up, 37.5% of our patients had at least one relapse. A trend was seen regarding association of pANCA/anti-MPO positivity with increased risk of relapse but this did not reach statistical significance. Possible explanation for this might be limited numbers of patients in pANCA/anti-MPO positive group.

In GPA, 5-year survival has been reported to be 70-80%, and 10-year survival has been reported as 40% in patients with renal involvement and 60-70% in those without renal involvement.[5,15] In our study, the mortality rate was 5% in the median 82 months follow-up and one of these patients was lost due to the COVID-19 pandemic. The patient who died due to disease activity had pANCA/anti-MPO positivity, and renal lung and neu-rological involvement. These data are not sufficient to mention the effect of the ANCA serotype on prognosis.

The study has some limitations. First, the retrospective study design and small sample size might be the important reasons why we could not show other possible links between ANCA serotypes and clinical presentations in patients with GPA. Second, the lack of uniformity in serotype selection (ELISA or IIF) might cause heterogeneity. The other limitation was that we could not obtain more information about disease activity of patients such as the Birmingham vasculitis activity score, vasculitis damage index, and five factors score. Last but not least, the short follow-up period prevented us from investigating the impact of ANCA serotypes on prognosis. However, this study has also some strengths. First, even though an international consensus recommends that immunoassays can be used as the primary screening method for patients suspected of having ANCA-associated vasculitis, these methods might still not be available in some clinics. Indirect immunofluorescence is a slightly more used method. Thus, the advantage of our study is that we obtain the indirect immunofluorescence results and compare these results with the clinical parameters of patients with GPA. Second, we show the close relationship between pANCA/anti-MPO and subglottic stenosis, which can contribute to the literature due to the lack of data on this involvement.

Conclusions

The main findings of this study could be summarized as presence of association of renal involvement with cANCA /anti-PR3 and sublottic stenosis with pANCA/anti-MPO. However, we have to emphasize that large, multicenter studies are needed to achieve stronger evidence regarding ANCA serotypes and clinical diversity in GPA. Just as classification of GPA on the basis of limited vs multiorgan involvement which helps clinicians to determine prognosis and the best treatment, larger multicenter prospective studies might reveal the answer to the question of is there any prognostic benefit of dividing GPA patients according to ANCA serotypes. Thereby, defining new high-risk subgroups and treating them more aggressively can improve the prognosis in GPA.

Compliance with Ethical Standards

Study protocol was approved by Kocaeli University Local Ethics Committee (ethics approval number: GOKAEK-2021/9.11), and a written informed consent was obtained from each patient.

Acknowledgements

None

Conflict of Interest

The authors have declared no conflict of interest.

Author contributions

All authors contributed to the study design and AY, OSC collected the data. AY and OOI analyzed the data and take responsibility for the accuracy of the data analysis. All authors interpreted the data, drafted the manuscript, and critically revised it for important contents. All authors read and approved the final manuscript.

Financial Disclosure

None

References

1. Salvador F. ANCA associated vasculitis. *Eur J Intern Med.* 2020;74:18-28. doi:10.1016/j.ejim.2020.01.011
2. Watts RA, Mahr A, Mohammad AJ, Gatenby P, Basu N, Flores-Suarez LF. Classifcation, epidemiology and clinical subgrouping of antineutrophil cytoplasmic antibody (ANCA)- associated vasculitis. *Nephrol Dial Transplant.* 2015;30 (suppl 1):i14-22. doi:10.1093/ndt/gfv022
3. Fujimoto S, Watts RA, Kobayashi S, et al. Comparison of the epidemiology of anti-neutrophil cytoplasmic antibody-associated vasculitis between Japan and the U.K. *Rheumatology (Oxford)* 2011;50(10):1916-1920. doi:rheumatology/ker205
4. Puechal X. Granulomatosis with polyangiitis (Wegener's). *Joint Bone Spine* 2020;87(6):572-578. doi:10.1016/j.jbspin.2020.06.005
5. Kitching AR, Anders HJ, Basu N, et al. ANCA-associated vasculitis. *Nat Rev Dis Primer.* 2020;6(1):71. doi:10.1038/s41572-020-0204-y
6. Grygiel-Gorniak B, Limphaibool N, Perkowska K, Puszczewicz. Clinical manifestations of granulomatosis with polyangiitis: key considerations and majör features. *Postgrad Med.* 2018;130(7):581-596. doi:00325481. 2018.1503920
7. Jennette JC, Nachman PH. ANCA glomerulonephritis and vasculitis. *Clin J Am Soc Nephrol*. 2017;12(10):1680-1691. doi:10.2215/CJN.02500317
8. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. *Arthritis Rheum*. 2013;65:1-11. doi:10.1007/s10157-013-0869-6
9. Ghorbel B, Belfeki N, Baouendi N, Salem TB, Houman M. Granulomatosis with polyangiitis in Tunisia. *Rheumatismo*. 2017;69(1):23-29. doi:10.4081/reumatismo.2017.935
10. Bajema IM, Hagen EC, van der Woude FJ, Bruijn JA. Wegener's granulomatosis: A meta-analysis of 349 literary case reports. *J Lab Clin Med.* 1997;129(1):17-22. doi:10.1016/S0022-2143(97)90157-8
11. Stone JH. Wegener's granulomatosis etanercept trial research group. Limited versus severe Wegener's granulomatosis: Baseline data on patients in the Wegener's granulomatosis etanercept trial. *Arthritis Rheum.* 2003;48(8):2299-2309. doi:10.1002/ art.11075
12. 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(10):570-579. doi:10.1038/nrrheum.2016.123
13. Franssen CF, Stegeman CA, Kallenberg CG, et al. Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int.* 2000;57(6):2195-2206. doi:10.1046/j.1523-1755.2000.00080.x
14. Quinn KA, Gelbard A, Sibley C, et al. Subglottic stenosis and endobronchial disease in granulomatosis with polyangiitis. *Rheumatology (Oxford).* 2019;58(12):2203-2211. doi:10.1093/rheumatology/kez217
15. Greco A, Marinelli C, Fusconi M, et al. Clinical manifestations in granulomatosis with polyangiitis. *Int J Immunopathol Pharmacol*. 2016;29(2):151-159. doi:10.1177/0394632015617063
16. Schirmer JH, Wright MN, Herrmann K, et al. Myeloperoxidase-Antineutrophil Cytoplasmic Antibody (ANCA)-Positive Granulomatosis with Polyangiitis (Wegener's) is a clinically distinct subset of ANCA-associated vasculitis: A retrospective analysis of 315 patients from a German Vasculitis Referral Center. *Arthritis Rheumatol*. 2016;68(12):2953-2963. doi:10.1002/art.39786
17. Romero-Gomez C, Aguliar-Garcia JA, Garcia-de-Lucas MD, et al. Epidemiological study of primary systemic vasculitidies among adults in southern Spain and review of the main epidemiological studies. *Clin Exp Rheumatol.* 2015;33 (Suppl 89):11-18.
18. Cuchacovich R. Immunopathogenesis of vasculitis. *Curr Rheumatol Rep*. 2002;4(1):9-17. doi:10.1007/s11926-002-0018-9