International Consensus on Antineutrophil Cytoplasm Antibodies Testing in Eosinophilic Granulomatosis with Polyangiitis

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International Consensus on Antineutrophil Cytoplasm Antibodies Testing in Eosinophilic Granulomatosis with Polyangiitis

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Abstract

An international consensus on antineutrophil cytoplasm antibodies (ANCA) testing in eosinophilic granulomatosis with polyangiitis (EGPA) is presented. ANCA, specific for myeloperoxidase (MPO), can be detected in 30–35% of patients with EGPA. MPO-ANCA should be tested with antigen-specific immunoassays in any patient with eosinophilic asthma and clinical features suggesting EGPA, including constitutional symptoms; purpura; polyneuropathy; unexplained heart, gastrointestinal, or kidney disease; and/or pulmonary infiltrates or hemorrhage. A positive MPO-ANCA result contributes to the diagnostic workup for EGPA. Patients with MPO-ANCA-associated EGPA have vasculitis features,

such as glomerulonephritis, neuropathy, and skin manifestations, more frequently than patients with ANCA-negative EGPA. However, the presence of MPO-ANCA is neither sensitive nor specific enough to identify whether a patient should be subclassified as having “vasculitic” or “eosinophilic” EGPA. At present, ANCA status cannot guide treatment decisions, that is, whether cyclophosphamide, rituximab, or mepolizumab should be added to conventional glucocorticoid treatment. In EGPA, monitoring of ANCA is only useful when MPO-ANCA was tested positive at disease onset.

Keywords: eosinophilic granulomatosis with polyangiitis; ANCA; vasculitis; consensus

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A list of European EGPA Study Group members not included in the list of authors may be found before the beginning of the REFERENCES.

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Antineutrophil cytoplasm antibodies (ANCA) were first described more than 50 years ago. During the last four decades, it was discovered that these antibodies are a distinctive laboratory feature of glomerulonephritis and vasculitis (1–3). ANCA are detected in most patients with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA), and in some patients with eosinophilic granulomatosis with polyangiitis (EGPA). ANCA can be detected in sera by indirect immunofluorescence (IIF) and/or solid-phase immunoassays, such as ELISA, fluoroenzyme immunoassay, chemiluminescence immunoassay, laser bead immunoassay, or dot/line blot. In patients with ANCA-associated vasculitis (AAV), IIF reveals two major patterns: either cytoplasmic ANCA (C-ANCA) or perinuclear ANCA (P-ANCA), whereas immunoassays reveal antibodies specific for proteinase-3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA).

In 2017, a revised international consensus on ANCA testing proposed that high-quality immunoassays can be used as the primary screening method for patients suspected of having GPA and MPA (1). A multicenter European Vasculitis Society (EUVAS) study showed a large variability between two IIF methods and a good diagnostic performance of PR3-ANCA and MPO-ANCA immunoassays (2, 3). The 2017 revised international consensus did not include EGPA. The current document is a follow-up on the revised consensus statement (1) and focuses on the clinical and diagnostic value of ANCA testing in patients suspected of having EGPA.

Methods

This consensus statement was prepared by a group of experts. References for this consensus statement were identified

through searches of PubMed, Embase, and Scopus for articles published from January 1951 to January 2020 by use of the terms “eosinophilic granulomatosis with polyangiitis,” “Churg-Strauss syndrome,” and “eosinophilic vasculitis.” Additional publications were identified in the references of the available articles. Articles resulting from these searches and relevant references cited in those articles were reviewed. Articles published in English, French, and German were included. The resulting manuscript was distributed by e-mail to experts from four continents, including rheumatologists, pulmonologists, immunologists, nephrologists, and specialists in laboratory medicine, selected based on their expertise and knowledge in clinical and laboratory aspects of ANCA-associated vasculitides and ANCA testing. All contributors approved the final document and voted for each statement using a 5-point Likert scale (strongly disagree, disagree, uncertain, agree, and strongly agree). The definition for consensus included percentage agreement of at least 80% and the median score ≥ 4 .

Prevalence of ANCA in Patients with EGPA

In 1989, Harrison and colleagues studied different cytoplasmic patterns when testing for ANCA by IIF in patients with vasculitis. Three patients with Churg-Strauss syndrome (or EGPA according to the current nomenclature) had atypical cytoplasmic staining (4). Cohen Tervaert and colleagues studied the clinical associations of MPO-ANCA in 53 patients and found that 6 patients (11%) had biopsy-proven EGPA (5). In addition, many patients that tested positive for MPO-ANCA had symptoms suggestive of EGPA without fulfilling the classification criteria for EGPA (5, 6). PR3-ANCA and MPO-ANCA were found

to be specific for GPA, MPA, and/or EGPA. Although the majority (>90%) of patients with GPA and MPA are ANCA positive, in EGPA this is <50%. Even patients with EGPA with biopsy-proven necrotizing arteritis could test ANCA negative (5, 7). The occurrence of MPO-ANCA in patients with EGPA suggested that EGPA belongs to the group of vasculitides encompassing GPA and MPA (8).

To evaluate the prevalence of ANCA in patients with EGPA, we selected 24 studies that included at least 30 patients and were published in peer-reviewed journals (Table 1) (9–32). In most studies, sera from patients were initially screened by IIF. MPO-ANCA and PR3-ANCA were subsequently assessed by antigen-specific immunoassays only in patients in whom ANCA had been detected by IIF. The reported prevalence of ANCA positivity was variable, ranging from 14.6% to 73.0% as assessed by IIF and from 14.6% to 60.6% as assessed by ELISA. The median frequencies were 33.0% and 30.6% for IIF and ELISA, respectively. The reported high variability of ANCA prevalence is probably due to the small number of patients in some studies, to selection bias, to the different classification criteria used for EGPA, and to the various methods used to test for ANCA (12). For example, in one study, the prevalence of ANCA positivity depended largely on the different organ involvement of patients and ranged from 0–12.5% in pulmonary patients to 90–100% in patients with renal disease (12). However, the median frequency of ANCA positivity in the selected studies was similar to that in the largest study, in which ANCA were detected by IIF and specific immunoassays in 33.0% and 30.7% of 534 patients with EGPA, respectively (32).

The studies usually reported a higher occurrence of ANCA by IIF compared with that by ELISA, because solid-phase immunoassays could not confirm the

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Table 1. The Prevalence and Clinical Value of ANCA Positivity as Determined by IIF or Antigen-Specific Assays in Patients with EGPA

Studies	N	IIF		Antigen-Specific Assays				Comments
		Pos. [n (%)]	P-ANCA	C-ANCA	Pos. [n (%)]	MPO	PR3	
Guillevin <i>et al.</i> , 1999 (9)	42	20 (47.6)	15	1	—	—	—	Central nervous system involvement was more common in ANCA-positive patients, whereas other clinical manifestations did not differ by ANCA status. Serial measurements indicated a correlation of ANCA levels with disease activity.
Keogh and Specks, 2003 (10)	73	22 (73.0)*	21	1	—	—	—	
Sablé-Fourtassou <i>et al.</i> , 2005 (11)	112	43 (38.4)	39	4	34 (30.3)	34	0	Positive ANCA status at diagnosis was associated with renal involvement, peripheral neuropathy, and biopsy-proven vasculitis, whereas negative ANCA status was associated with heart disease and fever. The percentages of remissions, relapses, and deaths did not differ between the ANCA-positive and ANCA-negative groups.
Sinico <i>et al.</i> , 2005 (12)	93	35 (37.6)	26	3	27 (29.0)	24	3	ANCA positivity was associated with higher prevalences of renal disease (51.4% vs. 12.1%; $P < 0.001$), pulmonary hemorrhage (20.0% vs. 0.0%; $P = 0.001$), mononeuritis multiplex (51.4% vs. 24.1%; $P = 0.013$), and purpura (25.7% vs. 6.9%; $P = 0.015$) but lower frequencies of lung disease (34.3% vs. 60.3%; $P = 0.019$) and heart disease (5.7% vs. 22.4%; $P = 0.042$). ANCA-positive patients were more likely to be treated with cyclophosphamide (65.7% vs. 32.7%; $P = 0.003$).
Cohen <i>et al.</i> , 2007 (13)	46	19 (41.3)	16 (34.8)	3 (6.5)	15 (32.6)	15	0	There were relapses in 73.6% of ANCA-positive patients vs. 59.3% of ANCA-negative patients (ns).
Ribi <i>et al.</i> , 2008 (14)	72	28 (38.9)	20 (27.8)	8 (11.1)	20 (27.8)	18	2	—
Baldini <i>et al.</i> , 2009 (15)	38	—	—	—	—	15 (39.5)	—	MPO-ANCA positivity was associated with peripheral neuropathy ($P = 0.0006$) and a higher incidence of relapses ($P = 0.01$), whereas negative ANCA status was associated with lung involvement ($P = 0.002$).
Neumann <i>et al.</i> , 2009 (16)	48	7 (14.6)	5	2	7 (14.6)	5	2	All patients with cardiac involvement were ANCA negative ($P < 0.05$).
Dennert <i>et al.</i> , 2010 (17)	32	—	—	—	13 (40.6)	13	0	Cardiac involvement was 23% in MPO-ANCA-positive patients and 74% in ANCA-negative patients.

(Continued)

Table 1. (Continued)

Studies	N	IIF			Antigen-Specific Assays			Comments
		Pos. [n (%)]	P-ANCA	C-ANCA	Pos. [n (%)]	MPO	PR3	
Healy <i>et al.</i> , 2012 (18)	93	28 (30.1)	18	4	15 (16.1)	15	0	ANCA positivity was associated with a higher frequency of peripheral neuropathy ($P = 0.023$) and a lower frequency of heart disease ($P = 0.003$), gastrointestinal involvement ($P = 0.03$), pulmonary infiltrates ($P = 0.009$), and the outcome of a life-threatening event or death ($P = 0.015$).
Samson <i>et al.</i> , 2013 (19)	118	48 (40.7)	—	—	36 (30.5)	34	2	MPO-ANCA positivity carried a higher risk of relapse ($P = 0.009$) but did not affect overall survival.
Comarmond <i>et al.</i> , 2013 (20)	348	108 (31.0)	72	68	72 (20.7)	68	4	ANCA-positive patients had significantly more frequent ENT manifestations, peripheral neuropathy, and/or renal involvement but less frequent heart disease. Vasculitis relapses occurred in 35.2% of the ANCA-positive vs. 22.5% of the ANCA-negative patients ($P < 0.01$), and 5.6% vs. 12.5%, respectively, died ($P < 0.05$).
Sada <i>et al.</i> , 2014 (21)	315	—	—	—	—	139/277 (50.2)*	6/238 (2.5)*	In MPO-ANCA/P-ANCA-positive patients, renal involvement ($P < 0.0001$), mucous membranes or ophthalmological symptoms ($P = 0.017$), and ENT disease ($P = 0.0092$) were more frequent, whereas skin lesions ($P = 0.0354$) and heart involvement ($P = 0.0418$) occurred less frequently than in ANCA-negative patients.
Moosig <i>et al.</i> , 2012 (22)	150	45 (30.0)	38	7	37 (24.7)	34	3	ANCA positivity was associated with a higher prevalence of kidney involvement ($P < 0.001$) and peripheral neuropathy ($P = 0.021$) but did not affect mortality.
Sokolowska <i>et al.</i> , 2014 (23)	50	15 (30.0)	10	4	15 (30.0)	14	1	ANCA-positive patients had a higher incidence of renal involvement (53% vs. 7.7%; $P < 0.001$), skin involvement (93.3% vs. 57.1%; $P = 0.03$), and mononeuritis multiplex (60% vs. 25.7%; $P = 0.021$) and a lower occurrence of cardiac disease (68.6% vs. 33.3%; $P = 0.021$). ANCA-positive patients required a higher steroid dose to maintain remission ($P = 0.023$), whereas the presence of ANCA did not affect the frequency of relapses.
Dunogu�� <i>et al.</i> , 2015 (24)	42	11 (26.8)	—	—	9 (21.4)	9	0	The incidence of alveolar hemorrhage was significantly higher in the ANCA-positive group (27.3% vs. 3.2%; $P = 0.048$), whereas cardiomyopathy tended to be more frequent in ANCA-negative patients (48.4% vs. 18.2%; $P = 0.15$).
Hazebroek <i>et al.</i> , 2015 (25)	50	—	—	—	16 (32.0)	16	0	Cardiac involvement was observed in 62% of patients with EGPA; 79% in ANCA-negative vs. 65% in ANCA-positive patients.

(Continued)

Table 1. (Continued)

Studies	N	IIF			Antigen-Specific Assays			Comments
		Pos. [n (%)]	P-ANCA	C-ANCA	Pos. [n (%)]	MPO	PR3	
Durel <i>et al.</i> , 2015 (26)	101	—	—	—	43 (42.6)	37	nd	ANCA-positive patients did not relapse more frequently but exhibited mononeuritis ($P = 0.0004$) and renal involvement ($P = 0.02$) more frequently. There was no difference in survival between ANCA-positive and ANCA-negative patients.
Cottin <i>et al.</i> , 2017 (27)	157	—	—	—	48 (30.6)	46	2	ANCA-positive patients more frequently had biopsy-proven vasculitis ($P = 0.004$), biopsy-proven glomerulonephritis ($P < 0.001$), and mononeuritis multiplex ($P < 0.001$) and less frequently had myocarditis ($P < 0.05$).
Solans-Laqué <i>et al.</i> , 2017 (28)	99	—	—	—	60 (60.6) [†]	50	10	—
Tsurikisawa <i>et al.</i> , 2017 (29)	121	—	—	—	41 (36.6) [‡]	34/115 (29.6)	7/113 (6.2)	MPO-ANCA positivity was not associated with the relapse rate or survival.
Saku <i>et al.</i> , 2018 (30)	188	—	—	—	88 (46.8)	78	10	ANCA-positive patients had more frequent kidney involvement (32.1% vs. 6.9%; $P < 0.01$) and less frequent heart disease (5.9% vs. 15.8%; $P = 0.03$). Multivariate analysis did not confirm an association between ANCA positivity and the risk of relapse.
Schroeder <i>et al.</i> , 2019 (31)	134	—	—	—	41 (30.6)	34	7	ANCA positivity was associated with peripheral and renal involvement (Fisher's test: $P < 0.0005$ and $P = 0.002$, respectively), whereas there was no association between cardiac involvement and ANCA negativity. Treatment with anti-leukotriene receptor antagonists was associated with an increased risk of ANCA positivity.
Lyons <i>et al.</i> , 2019 (32)	534	176 (33.0)	—	—	164 (30.7)	159	5	Glomerulonephritis and peripheral neuropathy were more prevalent in the MPO-ANCA-positive patients, whereas lung infiltrates and cardiac involvement were more common in the ANCA-negative subgroup.

Definition of abbreviations: ANCA = antineutrophil cytoplasm antibodies; C = cytoplasmic; EGPA = eosinophilic granulomatosis with polyangiitis; ENT = ear, nose, and throat; IF = indirect immunofluorescence; MPO = myeloperoxidase; nd = no data; ns = nonsignificant; P = perinuclear; Pos. = positive results of test; PR3 = proteinase-3.

The available data are presented.

*Among those tested prior to treatment.

[†]The authors did not indicate the number of patients who were tested by IIF and/or ELISA. Therefore, the numbers in the table include MPO-ANCA/P-ANCA-positive and PR3-ANCA/C-ANCA-positive patients.

[‡]Not reported. Calculated as a sum of MPO-ANCA-positive and PR3-ANCA-positive patients.

presence of MPO-ANCA or PR3-ANCA in a small proportion of IIF-positive patients showing P-ANCA, C-ANCA, or atypical ANCA patterns. Sinico and colleagues compared the performance of IIF on ethanol-fixed granulocytes and antigen-specific ELISA in 93 patients with EGPA (12). ANCA were detected by IIF in 35 of 93 patients (37.6%) tested at the time of diagnosis. Two of 26 P-ANCA-positive samples were negative for MPO-ANCA by ELISA, whereas MPO-ANCA was detected in 6 patients with C-ANCA or atypical ANCA patterns. These latter six samples showed an atypical cytoplasmic pattern when tested in the central laboratory, which contrasted with the reported finding in the local laboratory. These data are in accordance with the results of a multicenter EUVAS evaluation of IIF versus antigen-specific immunoassays in patients with GPA and MPA, underscoring the high variability in results obtained by IIF in different laboratories (2, 3).

When ANCA was found positive in EGPA, the antibodies are in most cases directed against MPO rather than against PR-3. The proportion of MPO-ANCA among all patients with ANCA-positive EGPA ranged from 71.4% to 100% with a median of 93.3%. In the two largest studies that included 348 and 534 patients with EGPA, MPO-ANCA were detected in 94.4% and 97.0%, respectively, of the patients that tested positive for ANCA by ELISA (20, 32). The remaining patients tested positive for PR3-ANCA.

P-ANCA is found not only in patients with MPO-ANCA but also in patients with antibodies to lactoferrin, elastase, cathepsin G, lysozyme, and other antigens (33). Recently, antilactoferrin antibodies were detected in patients with EGPA (4/19, 21.1%) but not in patients with GPA or MPA. Antilactoferrin antibodies were associated with disease activity (34). However, because antilactoferrin antibodies can be found in a wide variety of autoimmune disorders, further studies are needed to clarify their clinical value in EGPA.

Mukherjee and colleagues reported polyclonal ANCA reactivity in sputum samples of patients with EGPA, irrespective of the presence of MPO-ANCA in the sera of these patients (35). Sputum ANCA positivity was associated with severe respiratory symptoms, and immunoglobulins from ANCA-positive sputum triggered extensive extracellular trap formations from both neutrophils and

eosinophils *in vitro*, indicating possible pathogenicity of detected IgG autoantibodies. Detection of ANCA in sputum probably precedes serum ANCA positivity and may identify a subset of patients with eosinophilic asthma who are at increased risk of developing EGPA in the future.

In conclusion, ANCA can be detected by IIF or antigen-specific immunoassays in approximately 30–35% of patients with EGPA. Of the patients with EGPA that test positive for ANCA, most (up to 90–100%) have MPO-ANCA. The results of IIF may be inconsistent in a proportion of patients with EGPA when tested in different laboratories or on different substrates.

Clinical Value of ANCA in EGPA

ANCA status defines two subsets of patients with EGPA who have different clinical disease phenotypes (Figure 1). Most studies that evaluated an association between ANCA positivity and predominant clinical features of EGPA showed that ANCA-positive patients were more likely to have vasculitis manifestations, such as glomerulonephritis, peripheral neuropathy, alveolar hemorrhage, or purpura, and less frequently had heart disease or granulomatous lung involvement compared

with ANCA-negative patients (Table 1). For example, in the study by Lyons and colleagues (534 patients with EGPA, including 159 MPO-ANCA positive), the presence of MPO-ANCA was associated with a higher occurrence of peripheral neuropathy (78.6% vs. 57.1%) and glomerulonephritis (28.9% vs. 9.4%) and a lower frequency of lung infiltrates (45.3% vs. 61.4%) and cardiomyopathy (14.5% vs. 30.4%) (32). These divergent clinical associations remained statistically significant after adjustment for country of origin.

Similar associations were reported by Comarmond and colleagues in 348 patients with EGPA (20). In this study, biopsy-proven vasculitis was more frequent in ANCA-positive patients than in ANCA-negative patients (77.4% vs. 48.8%, $P=0.01$), whereas granulomatous and eosinophilic infiltrates occurred with a similar frequency in the two groups. ANCA-positive patients were more likely to develop vasculitis relapses and less likely to die than ANCA-negative patients. The higher mortality rate associated with ANCA-negative status was probably related to a higher occurrence of cardiomyopathy (25) that was the main independent predictor of death in patients with EGPA on multivariable analysis. However, most other studies did not confirm that ANCA-negative status affects overall

Figure 1. Clinical phenotypes and genetic features of eosinophilic granulomatosis with polyangiitis. MPO-ANCA = myeloperoxidase–antineutrophil cytoplasm antibodies.

survival (11, 19, 22, 26, 29). These differences may reflect differences in methods used to perform cardiac screening in these patients (17) and time from disease onset to diagnosis in patients with severe cardiac involvement. Also, several studies reported no association between ANCA positivity and a higher relapse rate in EGPA (23, 26, 29, 30). PR3-ANCA are found in only 1–2% of patients with EGPA (12, 20), and little is known about their association with clinical manifestations and outcome.

The pathogenic role of MPO-ANCA in vasculitis was shown in experimental studies. In MPO-immunized rats, pulmonary artery perfusion with polymorphonuclear leukocytes lysosomal enzymes resulted in extensive lung injury with granuloma-like lesions and giant cell formation (36), whereas the transfer of splenocytes from MPO-deficient mice immunized with murine MPO led to the development of glomerulonephritis and pulmonary capillaritis (37).

However, available evidence suggests that vasculitis in patients with EGPA can occur in both the presence and absence of ANCA (7). In the study by Cottin and colleagues, definite clinical or pathologic features of vasculitis were found in 28% of ANCA-negative patients and were absent in 29% of MPO-ANCA-positive patients. These data suggest that MPO-ANCA cannot be used as a unique surrogate of systemic vasculitis (27).

A recent genome-wide association study that tested 9.2 million genetic variants in 534 cases and 6,688 controls provided additional evidence for genetic distinctions between MPO-ANCA-positive and ANCA-negative EGPA (32). Analysis of MPO-ANCA-positive versus ANCA-negative cases revealed a genome-wide significant association at *rs17212014* in the *HLA-DQ* region for MPO-ANCA-positive status, whereas ANCA-negative status alone was associated with variants at the *GPA33* and *IL5/IRF1* loci. ANCA-negative EGPA was more genetically similar to asthma than MPO-ANCA-positive EGPA. The authors suggested that MPO-ANCA-positive EGPA is an eosinophilic autoimmune disease sharing clinical features and an *HLA-DQ* association with MPO-ANCA-associated vasculitis, whereas ANCA-negative EGPA may instead have a mucosal barrier dysfunction origin.

Despite the clinical, histological, and genetic differences between ANCA-positive

and ANCA-negative EGPA, few studies have reported whether distinct pathophysiological mechanisms or molecular pathways operate in these two subsets. Reports investigating the clinical or prognostic significance of circulating biomarkers (e.g., eosinophil counts, eotaxin-3, IgG4) either did not assess or failed to detect associations between such parameters and the ANCA status (38–41). In a recent review, Chaigne and colleagues speculated that vasculitis phenotype of EGPA may be mediated by neutrophils, neutrophil extracellular traps, and B lymphocytes, whereas eosinophils may drive the nonvasculitis phenotype (42). Specific data on neutrophil extracellular traps in EGPA are lacking, although a link between enhanced netosis and MPO-ANCA-associated vasculitis was shown in animal models (43, 44). A potential role for B lymphocytes in the pathogenesis of vasculitis in EGPA is suggested by both clinical data and efficacy of rituximab in a proportion of patients with EGPA.

Eosinophils release toxic products such as eosinophilic cationic protein and eosinophil-derived neurotoxin that may directly damage nerve fibers or endomyocardial tissue. Moreover, eosinophils may induce ischemic damage through occlusion of vessels or activation of the clotting cascade.

In MPO-ANCA-negative patients, sural nerve biopsy findings showed that large numbers of eosinophils occluded epineural vessels, whereas in sural nerve biopsies of patients with MPO-ANCA-associated EGPA, necrotizing vasculitis of epineural vessels was more frequently found (45). On the basis of these results, Nishi and colleagues recently postulated two distinct pathophysiological mechanisms in the two different phenotypes: in the vasculitis subtype, ischemia and tissue damage is due to necrotizing vasculitis, whereas in the nonvasculitis phenotype, intraluminal eosinophils occlude blood vessels, resulting in ischemia and eosinophil-associated tissue damage.

In summary, clinical and genetic evidence supports the use of ANCA testing to define two distinctive subsets of EGPA. However, the clinical value of ANCA positivity should not be overestimated, given a significant overlap between two clinical phenotypes. Clinical and/or pathologic features of vasculitis, such as

glomerulonephritis or peripheral neuropathy, can be observed in both patients with ANCA-negative EGPA and those with ANCA-positive EGPA. Moreover, some disease manifestations including peripheral neuropathy or cardiomyopathy could be due to both vasculitis and eosinophilic infiltration (46). Given the primacy of the association of genetic polymorphisms with ANCA serotype, future disease classifications based on serotype will have an influence on the subclassification of EGPA, with the possibility that PR3-ANCA and MPO-ANCA-positive EGPA will become eosinophilic variants of PR3-ANCA and MPO-ANCA-positive AAV.

MPO-ANCA as a Classification Criterion for EGPA

In 1984, Lanham and colleagues proposed the first criteria for EGPA, which included asthma, blood eosinophilia, and evidence for vasculitis involving two or more organs (47). The 1990 American College of Rheumatology classification criteria included asthma, blood eosinophilia >10%, neuropathy, nonfixed pulmonary infiltrates, paranasal sinus abnormality, and biopsy containing a blood vessel with extravascular eosinophils. The presence of at least four of these six criteria yielded a sensitivity of 85% and a specificity of 99.7% (48). In 2007, the European Medicines Agency algorithm based on American College of Rheumatology criteria and Chapel Hill Consensus Conference (CHCC) definitions provided a stepwise approach for classifying patients with AAV into the disease types for the purposes of epidemiological studies (49). The presence of ANCA was included in this algorithm.

The presence of ANCA was first indicated in the definition of EGPA at the 2012 CHCC, which stated that ANCA is more frequent when glomerulonephritis is present (50). However, CHCC was a nomenclature system specifying the name that should be used for a defined disease process. CHCC definitions were not intended as classification criteria and cannot be used for diagnostic purposes. Because histological data may not be available for all patients with suspected vasculitis, surrogates for vasculitis, such as clinical and laboratory findings suggestive

of vasculitis in the absence of histologic evidence, may be used (50).

Over the last few years, classification schemes for EGPA continued to evolve and incorporated ANCA testing as a valuable diagnostic aid. In 2015, the EGPA Consensus Task Force issued recommendations for evaluation and management of patients with EGPA stating that, in the clinical context of asthma and eosinophilia, MPO-ANCA positivity is highly suggestive for EGPA, but ANCA negativity does not rule out this diagnosis (51). Therefore, ANCA testing with IIF and ELISA was recommended for patients with suspected EGPA. The Task Force also stated that the presence of a C-ANCA pattern or PR3-ANCA is unusual for EGPA, and diagnosis should be critically reviewed in this setting.

Recently, the “Groupe d’Etudes et de Recherche sur les Maladies Orphelines Pulmonaires” and the “European Respiratory Society Task Force” suggested that EGPA with genuine vasculitis features (absent in at least 40% of patients with EGPA) should be differentiated from an eosinophilic tissue infiltrate phenotype based on the presence of definite vasculitis features (e.g., biopsy-proven necrotizing vasculitis of any organ), strong surrogate markers of vasculitis, such as mononeuritis multiplex, and/or the presence of ANCA as determined by antigen-specific assay with at least one extrathoracic non-ear, nose, and throat (ENT) manifestation of disease (27). The Task Force proposed a new terminology for this eosinophilic tissue phenotype, that is, hypereosinophilic asthma with systemic (nonvasculitic) manifestations. Patients with hypereosinophilic asthma with systemic (nonvasculitic) manifestations typically present with asthma, blood eosinophilia $>1.5 \times 10^9/L$, and systemic manifestations but without biopsy-proven vasculitis and/or surrogate markers of vasculitis. Of note, the Task Force acknowledged the clinical value of ANCA as a surrogate of systemic vasculitis when associated with systemic manifestations and recommended testing for ANCA using an antigen-specific assay.

In the MIRRA study (a double-blind randomized placebo-controlled study to investigate the efficacy and safety of mepolizumab in the treatment of EGPA in subjects receiving standard of care therapy), a positive result for antigen-specific ANCA was listed as one of at least two additional

features necessary to define eosinophilic asthma as EGPA (52).

New classification criteria for ANCA-associated vasculitides including EGPA have been drafted based on the data from the DCVAS (Diagnostic and Classification of the Systemic Vasculitides) study and are currently undergoing formal review by the American College of Rheumatology and the European League Against Rheumatism (53).

Over the last decade, ANCA testing has become a part of the routine workup of patients with suspected EGPA. The sensitivity of MPO-ANCA detection as a classification criterion for EGPA is low ($\sim 30\text{--}35\%$). The presence of MPO-ANCA as a vasculitis surrogate should, however, be used only in the right clinical context because antigen-specific ANCA testing can give positive results in various inflammatory disorders mimicking ANCA-associated vasculitis (54).

Importantly, MPO-ANCA in combination with blood eosinophilia and vasculitic symptoms may occur in the setting of cholesterol emboli syndrome. This syndrome occurs in patients with severe atherosclerosis and is associated with acrocyanosis, livedo reticularis, progressive renal failure, and other signs and symptoms (e.g., fever, weight loss, myalgia, leukocytosis, eosinophilia, raised erythrocyte sedimentation rate and C-reactive protein) mimicking vasculitis (55, 56).

MPO-ANCA as a Guide for Treatment Decisions

Glucocorticoids are the cornerstone of therapy for EGPA. Additional immunosuppressive agents (e.g., cyclophosphamide) should be prescribed for patients with life- and/or organ-threatening manifestations, such as heart disease, glomerulonephritis, alveolar hemorrhage, or mononeuritis multiplex, and can be considered for selected patients with glucocorticoid dependence or recurrent disease (51). The Five-Factor Score (FFS) is frequently used to decide whether cytotoxic drugs are indicated ($\text{FFS} \geq 1$) or not ($\text{FFS} = 0$). Importantly, however, this index does not include alveolar hemorrhage and/or mononeuritis multiplex, conditions that may have a severe impact on function in patients with EGPA (57). In several studies, patients with ANCA-positive EGPA were more likely to

be treated with cyclophosphamide (12) or required a higher glucocorticoid dose to maintain remission (53). However, MPO-ANCA positivity alone does not justify more intensive immunosuppressive treatment.

Other treatment options for EGPA include rituximab, an anti-CD20 monoclonal antibody directed against B cells, and mepolizumab, a humanized monoclonal antibody that targets IL-5. Currently, rituximab is frequently used for both remission induction and maintenance therapy for GPA and MPA, based on the results of randomized controlled clinical trials (58–60). The efficacy of rituximab in EGPA was not evaluated in clinical trials but was shown in case reports and case series (61–64). The rationale for using rituximab in EGPA comes from the known overlap between various ANCA-associated vasculitides, that is, ANCA positivity and vasculitis features (although less common in EGPA than in GPA/MPA) (63), and the ability of rituximab to reduce T-cell-derived production of IL-5 that induces stimulation and maturation of eosinophils (65).

Mohammad and colleagues presented data on 41 patients with refractory, relapsing, or new-onset EGPA (44% ANCA positive) treated with rituximab in four vasculitis centers (62). Rituximab administration resulted in the improvement of disease activity in 90% of patients at 12 months and the reduction of prednisolone dose, although only 6% of patients were able to discontinue glucocorticoids completely. ANCA-positive patients were more likely to achieve remission when on rituximab than ANCA-negative patients (80% vs. 38%; $P = 0.013$).

In a retrospective study, Teixeira and colleagues evaluated the longer-term efficacy and safety of rituximab in 69 patients with EGPA (34.8% ANCA positive) treated in a single tertiary center (63). By 24 months, rituximab administration was associated with a complete or partial response in 93% of patients and a reduction in the median prednisolone dose from 12.5 to 5 mg/d. Nevertheless, asthma and ENT relapse rates were high despite repeat rituximab dosing. At 6 and 12 months, more ANCA-positive patients were in remission compared with ANCA-negative patients (29.2% vs. 13.3% and 34.8% vs. 23.1%, respectively), but the differences between the two groups did not reach

statistical significance. The median time to remission was shorter in the ANCA-positive group than in the ANCA-negative group (15 vs. 24 mo; $P=0.02$). Moreover, ANCA-positive patients had a longer asthma/ENT relapse-free survival time than ANCA-negative patients ($P=0.04$). Although there was no difference in the vasculitis relapse rate between the two subsets, none of the 11 patients with glomerulonephritis relapsed during treatment with rituximab, and skin flares were usually described as erythematous and not vasculitic with purpura, suggesting that rituximab treatment may be better in preventing vasculitis relapses.

Emmi and colleagues also found no statistically significant differences in relapse rates or time-to-relapse between patients with ANCA-positive EGPA and those with

ANCA-negative EGPA ($n=15$) who achieved remission following rituximab induction. Scheduled maintenance with rituximab significantly reduced relapse rate compared with rituximab given “on demand” for relapse (66).

According to the EGPA Consensus Task Force recommendations, rituximab administration may be reasonable for patients with ANCA-positive EGPA with renal involvement or severe refractory disease, despite conventional therapy, for whom traditional cytotoxic agents are contraindicated or undesirable (51). Two prospective trials by the French Vasculitis Study Group are currently investigating rituximab as both induction (ClinicalTrials NCT02807103) and maintenance (ClinicalTrials NCT03164473) therapy of EGPA.

The efficacy and safety of mepolizumab in EGPA were established in the MIRRA trial that recruited 136 patients with relapsing/refractory disease receiving stable oral glucocorticoids for 4 or more weeks (52). A total of 78% versus 32% of patients ($P<0.001$) experienced clinical benefit (remission at any time, 50% or greater glucocorticoid dose reduction during weeks 48–52, or no EGPA relapses) in mepolizumab- and placebo-treated patients, respectively (67). Analyses of outcomes according to ANCA status were not performed because <10% of the participants were ANCA positive at baseline. Mepolizumab seems to be a promising agent to control the disease activity and to spare glucocorticoids, although its ability to control vasculitis manifestations remains unclear (46).

Reslizumab is another IL-5–neutralizing antibody currently approved for the treatment of severe eosinophilic asthma. In nine patients with EGPA with severe steroid-dependent eosinophilic asthma, 48 weeks of treatment with intravenous reslizumab was associated with a significant reduction in oral corticosteroid use. However, the authors suggested that reslizumab may be less effective in controlling extrapulmonary manifestations of EGPA, such as neuropathy (68). A therapeutic antibody to the IL-5 receptor benralizumab is the subject of a current clinical trial in EGPA (MANDARA) (ClinicalTrials.gov Identifier: NCT04157348).

In summary, ANCA positivity in patients with EGPA may signal a requirement for more intensive immunosuppressive therapy (e.g., addition of cyclophosphamide or rituximab) because vasculitis features may be present that are usually not sufficiently treated with glucocorticoids as monotherapy. However, ANCA positivity alone does not indicate a need for intensification of treatment. The efficacy and long-term safety of mepolizumab in MPO-ANCA–associated EGPA remains to be established. In the MIRRA trial that showed clear clinical benefit of IL-5 inhibition in EGPA, most patients were ANCA negative at baseline. At present, the presence or absence of MPO-ANCA should not be regarded as a barrier to administration of rituximab or mepolizumab, respectively, if considered necessary in the clinical context.

Table 2. Recommendations for ANCA Testing in EGPA

Recommendations	LoA (%)	Median Score
Recommendation 1 A gating policy for requesting an ANCA test and adherence to clinical indications for ANCA testing (Box 1) is advisable.	96.3	5
Recommendation 2 ANCA testing in EGPA should be conducted, as with GPA and MPA, according to a revised 2017 international consensus: high-quality antigen-specific assays for MPO-ANCA and PR3-ANCA should be used as the primary screening method for ANCA.	100	5
Recommendation 3 A diagnosis of EGPA cannot be excluded on the basis of negative MPO-ANCA results.	100	5
Recommendation 4 A positive MPO-ANCA result only contributes to the diagnostic workup for EGPA and is not diagnostic by itself.	100	5
Recommendation 5 A positive MPO-ANCA result is neither sensitive nor specific enough to identify the vasculitis phenotype of EGPA, although MPO-ANCA positivity is associated with a higher occurrence of vasculitis features.	92.6	5
Recommendation 6 ANCA status by itself cannot guide treatment decisions, such as addition of cyclophosphamide, rituximab, or mepolizumab to conventional treatment with glucocorticoids.	92.6	5
Recommendation 7 The result of serial ANCA measurements in patients with MPO-ANCA–associated EGPA (persistence, rise, or reappearance of MPO-ANCA) justifies more frequent clinical assessment.	88.9	5

Definition of abbreviations: ANCA=antineutrophil cytoplasm antibodies; EGPA=eosinophilic granulomatosis with polyangiitis; GPA=granulomatosis with polyangiitis; LoA=level of agreement; MPA=microscopic polyangiitis; MPO=myeloperoxidase; PR3=proteinase-3.
Scores: 1 = strongly disagree, 2 = disagree, 3 = undecided, 4 = agree, 5 = strongly agree.

Box 1. Indications for ANCA testing in suspected EGPA

To assure appropriate ANCA-test usage to support the diagnosis of EGPA, ANCA should be requested for patients with asthma or rhinosinusitis and blood eosinophilia if one or more of the following clinical features are present:

- Fever, weight loss, arthralgia, and/or myalgia in the presence of laboratory signs of inflammation
- Pulmonary infiltrates
- Sensory peripheral or motor neuropathy (including mononeuritis multiplex)
- Unexplained heart disease (e.g., arrhythmias or decreased left ventricular function)
- Urine nephritic sediment, a rising creatinine combined with hematuria or new-onset hematuria
- Alveolar hemorrhage
- Ischemic abdominal pain and otherwise unexplained gastrointestinal disorder
- Purpura or other skin rashes

ANCA Monitoring

The utility of serial ANCA measurements for predicting relapse of AAV remains controversial owing to contradictory results of clinical studies (69–71).

In three recent studies, serial ANCA testing in AAV was found to be useful in prediction of relapse in patients with renal and/or pulmonary vasculitis but not in patients with more limited disease (72–74). In 2015, an international task force representing the European League Against Rheumatism, the European Renal Association—European Dialysis and Transplant Association, and EUVAS concluded that neither persistence nor fourfold rise nor reappearance of ANCA should lead to a change in therapy but more frequent clinical assessment should be considered (70).

Of note, all studies that evaluated the clinical value of ANCA monitoring were restricted to patients with GPA and MPA, and it is possible that these results cannot be extrapolated to EGPA. Most studies in patients with EGPA did not provide data on repeat ANCA testing following immunosuppressive therapy. In the study by Keogh and Specks, 76% of patients with active EGPA were P-ANCA positive, whereas most of those tested during remission or after treatment initiation were ANCA negative (10). Serial data, although limited, indicated that ANCA status appeared to correlate with disease activity. In the study by Sinico and colleagues, ANCA were detected in only 10.8% of initially positive patients tested at the end

of the follow-up period, and in 18.8% of patients tested at the time of a relapse (12), whereas Saku and colleagues showed that the first EGPA relapse was associated with ANCA positivity in 61.9% of patients who were ANCA positive at onset and in 12.9% of patients who initially were ANCA negative (30). It is postulated that immunosuppressive therapy usually results in seroconversion in MPO-ANCA-positive patients, and relapse of EGPA is usually associated with reoccurrence of MPO-ANCA (75, 76). However, limited evidence precludes firm conclusions.

Serial ANCA measurements can have some value in predicting relapses of GPA and MPA in patients with renal vasculitis and/or alveolar hemorrhage. In EGPA, monitoring of ANCA is only useful when MPO-ANCA was tested positive at disease onset. Repeat ANCA testing is recommended in patients with MPO-ANCA-positive EGPA because persistence, rise, or reappearance of ANCA may justify more frequent clinical assessment.

Conclusions

ANCA can be detected in approximately 30–35% of patients with EGPA and support a diagnosis of EGPA established on the clinical grounds. Given inconsistent results of IIF and established specificity of autoantibodies (MPO-ANCA in most cases), ANCA testing in EGPA should be performed

as with GPA and MPA according to the revised 2017 consensus. Antigen-specific immunoassays are the preferred approach to detect ANCA for diagnosis of EGPA. ANCA status defines two distinctive but overlapping subsets of EGPA (“vasculitic” and “eosinophilic”), which may differ in outcomes and response to therapy. MPO-ANCA positivity in EGPA is associated with *HLA-DQ* and a higher occurrence of vasculitis features, such as renal involvement, neuropathy, and skin vasculitis. MPO-ANCA negativity in EGPA lacks an HLA association and is associated with *IRF1/IL5* and *GPA33* and a higher occurrence of cardiomyopathy and lung manifestations. However, ANCA status taken on its own is neither sensitive nor specific enough to identify the vasculitis phenotype of EGPA and at present cannot guide treatment decisions.

All recommendations stated in Table 2 reached a high level of agreement. Only one expert voted against statements 5 and 6. ■

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