International Consensus on ANCA Testing in Eosinophilic Granulomatosis with Polyangiitis

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Abstract

An international consensus on anti-neutrophil cytoplasm antibodies (ANCA) testing in eosinophilic granulomatosis with polyangiitis (EGPA) is presented. ANCA, specific for myeloperoxidase (MPO), can be detected in 30-35% of EGPA patients. 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 work-up for EGPA. Patients with MPO-ANCA associated EGPA have more frequently vasculitis features, such as glomerulonephritis, neuropathy, and skin manifestations 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.
Introduction

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, fluoro-enzyme immunoassay, chemiluminescence immunoassay, laser bead immunoassay or dot/line blot. In patients with AAV, IIF reveals two major patterns, either cytoplasmic ANCA (C-ANCA) or perinuclear ANCA (P-ANCA), whereas immunoassays reveals 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 multicentre European Vasculitis Society (EUVAS) study that 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 email 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 Lickert scale (strongly disagree, disagree, uncertain, agree, and strongly agree). The definition for consensus included percentage agreement at least 80% and the median score greater or equal to 4.

**Prevalence of ANCA in EGPA patients**

In 1989, Harrison et al. 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 et al. studied the clinical associations of MPO-ANCA in 53 patients and found that six 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 either GPA, MPA and/or EGPA. Although the majority (>90%) of GPA and MPA patients are ANCA-positive, in EGPA this is less than 50%. Even EGPA patients 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 EGPA patients, 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 EGPA patients, respectively [32].

The studies usually reported a higher occurrence of ANCA by IIF compared to that by ELISA, since solid-phase immunoassays could not confirm the 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 et al. compared the performance of IIF on ethanol-fixed granulocytes and antigen-specific ELISA in 93 EGPA patients [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 6 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 multicentre EUVAS evaluation of IIF versus antigen-specific immunoassays in GPA and MPA patients, 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 ANCA-positive EGPA patients ranged from 71.4% to 100% with a median of 93.3%. In the two largest studies that included 348 and 534 EGPA patients, 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 not only found in patients with MPO-ANCA but also in patients with antibodies to lactoferrin, elastase, cathepsin G, lysozyme and other antigens [33]. Recently, anti-lactoferrin antibodies were detected in EGPA patients (4/19, 21.1%) but not in patients with GPA or MPA. Anti-lactoferrin antibodies were associated with disease activity [34]. However, since anti-lactoferrin antibodies can be found in a wide variety of autoimmune disorders, further studies are needed to clarify their clinical value in EGPA.

Mukherjee et al. reported polyclonal ANCA reactivity in sputum samples of EGPA patients, 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 EGPA patients. Of the EGPA patients that test positive for ANCA, most (up to 90-100%) have MPO-ANCA. The results of IIF may be inconsistent in a proportion of EGPA patients when tested in different laboratories or on different substrates.
Clinical value of ANCA in EGPA

ANCA status defines two subsets of EGPA patients who have different clinical disease phenotypes (fig. 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 to ANCA-negative patients (table 1). For example, in the study from Lyons et al. (534 EGPA patients, including 159 MPO-ANCA-positive), the presence of MPO-ANCA was associated with a higher occurrence of peripheral neuropathy (78.6% versus 57.1%) and glomerulonephritis (28.9% versus 9.4%), and a lower frequency of lung infiltrates (45.3% versus 61.4%) and cardiomyopathy (14.5% versus 30.4%) [32]. These divergent clinical associations remained statistically significant after adjustment for country of origin.

Similar associations were reported by Comarmond et al. in 348 EGPA patients [20]. In this study, biopsy-proven vasculitis was more frequent in ANCA-positive patients than in ANCA-negative patients (77.4% versus 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 EGPA patients on multivariable analysis. However, most other studies did not confirm that ANCA-negative status affects overall 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 EGPA patients can occur both in the presence and absence of ANCA [7]. In the study from Cottin et al., 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 (GWAS) that tested 9.2 million genetic variants in 534 cases and 6688 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, while 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 et al. speculated that vasculitis phenotype of EGPA may be mediated by neutrophils, neutrophil extracellular traps (NETs) and B lymphocytes, whereas eosinophils may drive the non-vasculitis phenotype [42]. Specific data on NETs 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 both by clinical data and efficacy of rituximab in a proportion of EGPA patients.

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 epineurial vessels, whereas in sural nerve biopsies of MPO-ANCA associated EGPA patients necrotizing vasculitis of epineurial vessels was more frequently found [45]. Based on these results, Nishi et al. 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 non-vasculitis 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 ANCA-negative and ANCA-positive EGPA patients. 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 et al. 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 (ACR) classification criteria included asthma, blood eosinophilia greater than 10%, neuropathy, non-fixed pulmonary infiltrates, paranasal sinus abnormality, and biopsy containing a blood vessel with extravascular eosinophils. The presence of at least 4 of these 6 criteria yielded a sensitivity of 85% and a specificity of 99.7% [48]. In 2007, the European Medicines Agency (EMA) algorithm based on ACR 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. Since 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 EGPA patients 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 EGPA patients) 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 extra-thoracic non-ENT manifestation of disease [27]. The Task Force proposed a new terminology for this eosinophilic tissue phenotype, i.e., hypereosinophilic asthma with systemic (non vasculitic) manifestations (HASM). Patients with HASM 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, randomised, 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 Diagnostic and Classification of the Systemic Vasculitides (DCVAS) 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 work-up of patients with suspected EGPA. The sensitivity of MPO-ANCA detection as a classification criterion for EGPA is low (≈30-35%). The presence of MPO-ANCA as a vasculitis surrogate should, however, be used only in the right clinical context, since 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 ESR and CRP) 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 (FFS≥1) or not (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, ANCA-positive EGPA patients 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 humanised monoclonal antibody that targets interleukin (IL)-5. Currently, rituximab is frequently used both for remission induction and maintenance therapy for GPA and MPA, based on the results of randomised 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 (though 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 et al. presented data on 41 patients with refractory, relapsing or new-onset EGPA (44% ANCA-positive) treated with rituximab in four vasculitis centres [62]. Rituximab
administration resulted in the improvement of disease activity in 90% of patients at 12 months and the reduction of prednisolone dose, though 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% versus 38%; p=0.013).

In a retrospective study, Teixeira et al. evaluated the longer-term efficacy and safety of rituximab in 69 EGPA patients (34.8% ANCA-positive) treated in a single tertiary centre [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/day. 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% versus 13.3%, and 34.8% versus 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 versus 24 months; p=0.02). Moreover, ANCA-positive patients had a longer asthma/ENT relapse-free survival time than ANCA-negative patients (p=0.04). While 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 et al. also found no statistically significant differences in relapse rates or time-to-relapse between ANCA-positive and ANCA-negative EGPA patients (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 ANCA-positive EGPA patients 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 both as 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 to 52, or no EGPA relapses) in mepolizumab and placebo treated patients respectively [67]. Analyses of outcomes according to ANCA status were not performed because less than 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 neutralising antibody currently approved for the treatment of severe eosinophilic asthma. In nine EGPA patients with severe steroid-dependent eosinophilic asthma, 48 week 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 EGPA patients may signal a requirement for more intensive immunosuppressive therapy, e.g., addition of cyclophosphamide or rituximab, since 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.

**ANCA monitoring**

The utility of serial ANCA measurements for predicting relapse of AAV remains controversial due 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 EULAR, the European Renal Association—European Dialysis and Transplant Association (ERA-EDTA) and EUVAS concluded that neither persistence, fourfold rise, or 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 EGPA patients did not provide data on repeat ANCA testing following immunosuppressive therapy. In the study of 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 of Sinico et al., 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 et al. 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 MPO-ANCA-positive EGPA patients, since either persistence, rise or reappearance of ANCA may justify more frequent clinical assessment.

**Conclusion**

ANCA can be detected in approximately 30-35% of EGPA patients 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 a 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 high level of agreement. Only one expert voted against statements 5 and 6.

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References


Fig. 1. Clinical phenotypes and genetic features of EGPA
Table 1. The prevalence and clinical value of ANCA-positivity as determined by IIF or antigen-specific assays in EGPA patients.

<table>
<thead>
<tr>
<th>Studies</th>
<th>n</th>
<th>IIF Pos., n (%)</th>
<th>MPO Pos., n (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guillevin et al., 1999</td>
<td>42</td>
<td>20 (47.6)</td>
<td>-</td>
<td>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.</td>
</tr>
<tr>
<td>Keogh, Specks, 2003</td>
<td>73</td>
<td>22 (73.0)*</td>
<td>-</td>
<td>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.</td>
</tr>
<tr>
<td>Sablé-Fourtassou et al., 2005</td>
<td>112</td>
<td>43 (38.4)</td>
<td>34 (30.3)</td>
<td>ANCA positivity was associated with higher prevalences of renal disease (51.4% vs. 12.1%; P &lt; 0.001), pulmonary hemorrhage (20.0% vs 0.0%; P = 0.001), mononeuritis multiplex (51.4% vs 24.1%; P = 0.013), purpura (25.7% vs 6.9%; P = 0.015), but with 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).</td>
</tr>
<tr>
<td>Sinico et al., 2005</td>
<td>93</td>
<td>35 (37.6)</td>
<td>27 (29.0)</td>
<td>Relapses in 73.6% of ANCA-positive pts vs. 59.3% of ANCA-negative pts (ns)</td>
</tr>
<tr>
<td>Cohen et al., 2007</td>
<td>46</td>
<td>19 (41.3)</td>
<td>15 (32.6)</td>
<td>All patients with cardiac involvement were ANCA-negative (p &lt; 0.05).</td>
</tr>
<tr>
<td>Ribi et al., 2008</td>
<td>72</td>
<td>28 (38.9)</td>
<td>20 (27.8)</td>
<td>Cardiac involvement was 23% in MPO-ANCA positive patients and 74% in ANCA negative patients</td>
</tr>
<tr>
<td>Baldini et al., 2009</td>
<td>38</td>
<td>-</td>
<td>-</td>
<td>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).</td>
</tr>
<tr>
<td>Neumann et al., 2009</td>
<td>48</td>
<td>7 (14.6)</td>
<td>7 (14.6)</td>
<td>Cardiac involvement was 23% in MPO-ANCA positive patients and 74% in ANCA negative patients</td>
</tr>
<tr>
<td>Dennert et al., 2010</td>
<td>32</td>
<td>-</td>
<td>13 (40.6)</td>
<td>ANCA positivity was associated with a higher frequency of peripheral neuropathy (p =0.023), a lower frequency of heart disease (p = 0.003), gastrointestinal involvement (p = 0.03), pulmonary infiltrates (p=0.009), and the outcome of a</td>
</tr>
<tr>
<td>Healey et al., 2012</td>
<td>93</td>
<td>28 (30.1)</td>
<td>15 (16.1)</td>
<td>ANCA positivity was associated with a higher frequency of peripheral neuropathy (p =0.023), a lower frequency of heart disease (p = 0.003), gastrointestinal involvement (p = 0.03), pulmonary infiltrates (p=0.009), and the outcome of a</td>
</tr>
<tr>
<td>Study</td>
<td>N</td>
<td>MPO-ANCA-Pos</td>
<td>ANCA-Pos</td>
<td>N</td>
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<td>-------------------------------------</td>
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<tr>
<td>Samson et al., 2013</td>
<td>118</td>
<td>48 (40.7)</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td>Comarmond et al., 2013</td>
<td>348</td>
<td>108 (31.0)</td>
<td>72</td>
<td>68</td>
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<tr>
<td>Sada K-E et al., 2014</td>
<td>315</td>
<td>-</td>
<td>-</td>
<td>139/27</td>
</tr>
<tr>
<td>Moosig et al., 2012</td>
<td>150</td>
<td>45 (30.0)</td>
<td>38</td>
<td>37</td>
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<tr>
<td>Sokolowska et al., 2014</td>
<td>50</td>
<td>15 (30.0)</td>
<td>10</td>
<td>15</td>
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<tr>
<td>Dunogué et al., 2015</td>
<td>42</td>
<td>11 (26.8)</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Hazebroek et al., 2015</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>16</td>
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<tr>
<td>Durel et al., 2015</td>
<td>101</td>
<td>-</td>
<td>-</td>
<td>43</td>
</tr>
<tr>
<td>Cottin et al., 2016</td>
<td>157</td>
<td>-</td>
<td>-</td>
<td>48</td>
</tr>
<tr>
<td>Solans-Laqué et al., 2017</td>
<td>99</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Patients Tested</td>
<td>ANCA-Positive</td>
<td>ANCA-Negative</td>
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<td>---------------</td>
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<tr>
<td>Tsukisawa et al., 2017</td>
<td>121</td>
<td>-</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td>Saku et al., 2018</td>
<td>188</td>
<td>-</td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td>Schroeder et al., 2019</td>
<td>134</td>
<td>-</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td>Lyons et al., 2019</td>
<td>534</td>
<td>176</td>
<td>-</td>
<td>164</td>
</tr>
</tbody>
</table>

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

**Box 1. Indications for ANCA testing in suspected EGPA**

In order 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, 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
- Ischaemic abdominal pain and otherwise unexplained gastrointestinal disorder
- Purpura or other skin rashes
Fig. 1. Clinical phenotypes and genetic features of EGPA

80x80mm (300 x 300 DPI)