

JOURNAL HIGHLIGHT: SPUTUM ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES IN SERUM ANTINEUTROPHIL CYTOPLASMIC ANTIBODY-NEGATIVE EOSINOPHIL GRANULOMATOSIS WITH POLYANGIITIS

Reviewed by Alane Blythe Dy
PhD Candidate

Asthma and Airway Disease Research Center, University of Arizona Health Sciences, Tucson AZ, USA

Eosinophilic granulomatosis with polyangiitis (EGPA), also known as Churg-Strauss syndrome, is considered a rare disease that has multiple overlapping symptoms with other conditions that may confound diagnosis. Variable clinical manifestations and severity observed from patient to patient likewise contributes to the difficulty in establishing diagnosis. According to the American College of Rheumatology, EGPA is assumed when 4 of 6 of their designated criteria are met, which includes: 1) asthma, 2) greater than 10% eosinophils on a differential leukocyte count, 3) neuropathy, 4) pulmonary infiltrates, 5) abnormal paranasal sinuses and 6) extravascular eosinophils. This method has a sensitivity of 85% and a specificity of 99.7%. It is intriguing that an eosinophilic disease is associated with anti-neutrophil cytoplasmic antibodies (ANCA), often directed against myeloperoxidase, and visualized in a peri-nuclear distribution. Further, ANCA is detected in serum of less than 50% of patients with EGPA and does not always correlate with disease activity. These may be due to several factors, such as poor detection techniques directed against non-pathogenic epitopes of the antigen, molecular mimicry with other peroxidases like eosinophil peroxidases or compartmentalization of immune responses.

In this study by Mukherjee et al (2019), the authors investigate the latter hypothesis and the relevance of testing for sputum ANCA in the context of EGPA. They obtained sputum and blood samples from patients diagnosed with EGPA (n = 23), eosinophilic asthma (n = 19) and healthy controls (n = 13). Of the 23 patients with EGPA, 10 were serum ANCA⁺ and 17 were sputum ANCA⁺. Sputum ANCA positivity was significantly increased in patients with EGPA compared to patients with eosinophilic asthma and healthy controls, regardless of serum ANCA results, and was heavily associated with severe asthma symptoms. Further analysis showed that sputum ANCA⁺ samples had evidence of neutrophil and eosinophil extracellular trap release and elevated levels of cytokines relevant to inflammatory cell recruitment, such as CXCL8, CCL24 and CXCL12. Taken together, this group reports novel findings on sputum ANCA reactivity in patients with EGPA whose symptoms are of the respiratory type, which may be a useful biomarker for diagnosis and treatment.

Questions and Answers with Parameswaran Nair, MD, PhD (senior author):

1. My understanding is EGPA is a rare disease and from my limited knowledge, there is currently no animal model that can aptly capture it. Can you speak to the challenges of being able to obtain enough patient volunteers and also to the barriers of having no adequate animal model to study disease pathogenesis?

Yes, EGPA is generally considered a rare disease. However, with increasing awareness of the condition and more specialty clinics being set up, more patients are likely to be diagnosed with this condition. It is important to set up registries and network to facilitate the recruitment of these patients into protocols that would provide them with better care, help to understand mechanisms of disease, and to evaluate new therapeutic strategies in clinical trials. I have very little knowledge of animal models. I believe that setting up an animal model may indeed be difficult for a number of reasons. We still do not know the precise mechanism of this condition, We also do not know if ANCA is pathogenic or not. Our study (and others) would suggest that indeed they may contribute to both eosinophil and neutrophil trap release. Finally, the relationship between eosinophil and neutrophil infiltration in this disease is not known, nor is it clear whether the eosinophil is directly contributing to the vasculitis or not.

2. Can you elaborate on how these current findings can be applied to clinical diagnosis and management of EGPA?

I think these observations provide two new clinically relevant messages and one related to mechanism. First, clinicians should perhaps examine ANCA in sputum when ANCA is negative in serum

in those patients who are clinically suspected to have EGPA, particularly in those who have predominantly severe respiratory symptoms such as alveolar haemorrhage, pulmonary infiltrates, and airway eosinophilia. Secondly, the observation that sputum ANCA may not necessarily be p-ANCA but could also be c-ANCA raise questions about mechanisms. Finally, the identification of ANCA in sputum to induce both eosinophil and neutrophil trap release would suggest that they are directly contributing to the pathobiology of the disease.

3. Eosinophil-targeted therapies were mentioned in the paper as being beneficial in patients that have eosinophil-associated symptoms. Since there is now evidence that eosinophils have roles in homeostasis, what is your opinion on systemic depletion of eosinophils?

Although there is evidence in animal models of eosinophils contributing to defence against bacterial infection (Yousefi et al, Nature Medicine, 2008) and viral infections (Percopo et al, Blood, 2014), there is very little to no human data to suggest that decreasing eosinophil numbers may lead to airway infections other than perhaps parasitosis. Indeed there may be a population of resident interstitial eosinophils that may have some homeostatic roles as demonstrated by Mesnil and colleagues, but we do not have evidence that they are critically involved in host defence mechanisms in humans, when there are other professional cells to carry out this function. Having said that, there may be an increased risk of infections in some patients on eosinophil depleting treatment strategies (as opposed to eosinophil normalization treatment strategies) and this may be related to the effect of these molecules on other cells in addition to eosinophils. We are currently investigating this.

4. How do you plan to move forward with this area of your research?

We are looking at four different aspects of eosinophil biology related to EGPA. First, it would be helpful for clinicians and clinical labs if we could establish a protocol to measure ANCA in bronchial wash and lavage fluids as well. Second, we are examining other autoimmune targets in EGPA in addition to PR3 and MPO and the nature of the antibody responses. Third, we are trying to find predictive markers in patients with severe asthma at risk for progressing to EGPA. Finally, in the context of a clinical trial directly comparing an anti-IL5 mAb with an anti-IL5R mAb for EGPA, we hope to identify baseline predictors of sub-optimal response to one strategy or other, that may be partially driven by the presence of ANCA responses in the airway, and other aspects of eosinophil and NK cell biology.



Paper: Mukherjee et al. (2019). Sputum antineutrophil cytoplasmic antibodies in serum antineutrophil cytoplasmic antibody-negative eosinophil granulomatosis with polyangiitis. American Journal of Respiratory and Critical Care Medicine, 199, 2; 158-170.

Associated reference: King, Talmadge Jr. (2018). Clinical features and diagnosis of eosinophilic granulomatosis with polyangiitis (Churg-Strauss). Hollingsword, H (Ed.), UpToDate, Retrieved December 31, 2019, from <https://www.uptodate.com/contents/clinical-features-and-diagnosis-of-eosinophilic-granulomatosis-with-polyangiitis-churg-strauss>.