Letter to editor (Other)

Title: ANCA-MPO: is this a useful test?

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Key message: ANCA-MPO may not accurately predict ANCA-associated vasculitis and drug-induced vasculitis.

Dear Editor,

Anti-neutrophil cytoplasmic antibodies (ANCA) bind proteins expressed in neutrophils responsible for vascular inflammation [1]. ANCA-associated vasculitis (AAV) is a small vessel vasculitis characterized by necrotizing vasculitis, with few/absent immune deposits. This classification includes granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) [2].

Positive staining of ANCA has three main patterns by indirect immunofluorescence (IIF), perinuclear (p-ANCA), cytoplasmatic (c-ANCA), and atypical. The last includes all the other neutrophil or monocyte-specific immunofluorescent reactivity, resulting from a mixture of cytoplasmic and perinuclear staining that can be present in other inflammatory diseases [3]. The immunoassay detects antibodies against PR3 and MPO are strongly associated with c-ANCA and p-ANCA, respectively [2].

We revisited the question of the usefulness of the ANCA-MPO assay. In particular is it of value in diagnosing AAV and drug-induced vasculitis. We performed a retrospective analysis of patients whose ANCA-MPO test was performed from July to December 2022 at University College London Hospital. We defined the following secondary goals: a. assess which specialties most frequently request this test; b. identify the diagnoses made; c. determine the frequency of positive ANCA-MPO tests; d. evaluate if the ANCA screen test, including the atypical p-ANCA and p-ANCA patterns, correlated with the ANCA-MPO test.

ANCA-MPO test was performed on 614 patients, female (64.8%), males (35.2%). Of the patients whose ethnicity was confirmed, Caucasians constituted (54.4%), Asian (13.2%), and Afro-Caribbeans (13.2%). Rheumatology (38.1%), Neurology (17.4%), Respiratory (6.5%), Dermatology (6.4%), Nephrology (5.5%), and Ear, Nose, and Throat (ENT) (5.4%) were the specialties who most frequently requested the test. Forty patients had a diagnosis of AAV, or cocaine induced vasculitis. We also noted lupus nephritis (4.3%), rheumatoid arthritis (3.7%), systemic lupus erythematosus (3.5%), and interstitial lung disease (2.8%). No diagnosis had been reached in 18.6%. Clinical diagnosis was inconclusive in 2.9%. GPA was established in 2.4%, EGPA 0.9%, AAV in 1.6%, and cocaine-induced vasculitis 0.4%. No patients tested had a diagnosis of MPA.

ANCA screen test performed in 476 patients (77.5%) was positive in 43.2%. Among them, 25.7% and 23.4% showed a p-ANCA and atypical p-ANCA pattern, respectively. The most frequent pattern was atypical c-ANCA (29.8%). The ANCA-MPO test was positive in 2.0%.

The performance of the ANCA-MPO test as a predictor of AAV or drug-induced vasculitis was assessed through ROC curve analysis. The area under the curve (AUC) was 0.59 (95% CI 0.5-0.7). The Youden index was determined to establish the cut-off value with the highest validity of

the ANCA MPO test. The optimal cut-off was 0.3, with a sensitivity of 40.0% and a specificity of 79.0% (Youden index 0.2). Calibration was verified through the Hosmer-Lemeshow test. The ANCA screen result did not correlate with ANCA-MPO result (OR 7,4; p-value 0.059; 95% CI 0.9-58.7). No correlation was also identified with p-ANCA (OR 3.8; p-value 0.05, 95% CI 0.1-14.7) or atypical p-ANCA (OR 0.9; p-value 0.933; 95% CI 0.2-4.6).

AAV is uncommon , with diverse clinical features [4]. *Guchelaar et al.* [5] compared the diagnostic value of ANCA IIF and immunoassay to diagnose AAV. The sensitivity obtained for ANCA-MPO with immunoassay was 58.1%, the specificity was 95.6%. Although ANCA test is considered relevant for AAV diagnosis, our data showed the discriminatory ability of the test is unsatisfactory, as the AUC is less than 0.6. With a Youden index close to zero, the ANCA-MPO test showed little ability to discriminate patients with AAV or drug-induced vasculitis. Given the low sensitivity of the test, the percentage of undetected cases is relatively high. Furthermore, the ANCA screening results were not correlated with the ANCA-MPO result, confirming the superior immunoassay sensitivity, and raising awareness about the uncertain clinical significance of an ANCA screen test.

Specialties who requested the test most frequently were those managing the common symptoms of AAV, renal, pulmonary, including upper respiratory tract, and dermatological features.

In addition to AAV and drug-induced vasculitis, 200 additional diagnoses were noted among those in whom the test was requested (see supplementary material – Table 1). Our study supports previous data describing a positive ANCA test in non-vasculitic conditions. This is due to indiscriminate testing in patients with low pre-test probability of AAV and drug-induced vasculitis, increasing the frequency of false positive results obtained. Although the 2017 consensus statement [7] recommends testing with high quality MPO-ANCA assays rather than screening with indirect immunofluorescence this has serious cost implications. We suggest that internal policies should guide the use of this test so that it is only requested when clinically appropriate [6].

The main limitations of our study are that it is single-center and a retrospective study, but the detailed assessment of the clinical diagnosis in >600 patients in whom the test was performed does provide significant compensation.

In conclusion, our results indicate that the ability of ANCA-MPO to predict AAV or drug-induced vasculitis is limited.

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Characteristics	Total (n= 614)
Age (year)	48.98 ± 18.04
Gender	
Female	398 (64.8)
Ethnicity	
Caucasian	230 (37.5)
Asian	56 (9.1)
African	56 (9.1)
Mixed Ethnicity	8 (1.3)
Other Ethnicity	73 (11.9)
Unknown	191 (31.1)
Specialties that required the test	
Rheumatology	234 (38.1)
Neurology	107 (17.4)
Respiratory Medicine	40 (6.5)
Dermatology	39 (6.4)
Nephrology	34 (5.5)
Ear, nose and throat	33 (5.4)
Haematology	27 (4.4)
Gastroenterology	26 (4.2)
Accident and Emergency	10 (1.6)
Geriatric Medicine	7 (1.1)
Acute Medicine	6 (1.0)
Oncology	6 (1.0)
Infectious Diseases	6 (1.0)
Pharmacology	5 (0.8)
Obstetrics	4 (0.7)
Audiovestibular Medicine	3 (0.5)
Tropical Medicine	3 (0.5)
Critical Care Medicine	3 (0.5)
General (internal) Medicine	2 (0.3)
General Practitioner	2 (0.3)
Orthopaedics	2 (0.3)
Allergy and Clinical Immunology	1 (0.2)
Cardiology	1 (0.2)
Endocrinology	1 (0.2)
Ginecology	1 (0.2)
Neurogenetic	1 (0.2)
Neurosurgery	1 (0.2)
Oral Medicine	1 (0.2)
Sport and Exercise Medicine	1 (0.2)
Vascular Surgery	1 (0.2)
Unknown	6 (1.0)
ANCA screen	
Positive	265 (43.2)
Negative	211 (34.4)
Not requested	138 (22.5)
ANCA screen pattern	
Atypical c-ANCA	79/265 (29.8)
p-ANCA pattern	68/265 (25.7)
Atypical p-ANCA	62/265 (23.4)
c-ANCA pattern	56/265 (21.1)
Diagnosis	

Rheumatologic diseases	223 (29.2)
Neurologic diseases	72 (9.4)
Respiratory medicine diseases	51 (6.7)
Nephrologic diseases	49 (6.4)
Gastroenterologic diseases	40 (5.2)
Haematologic diseases	37 (4.9)
Allergy and Clinical Immunologic diseases	21 (2.8)
Infectious diseases	20 (2.6)
Ear, nose and throat diseases	20 (2.6)
Oncologic diseases	14 (1.8)
Pharmacologic diseases	13 (1.7)
Dermatologic diseases	13 (1.7)
Orthopedic diseases	5 (0.7)
Ophthalmologic diseases	7 (0.9)
Cardiologic diseases	7 (0.9)
Vascular Surgery diseases	3 (0.4)
Endocrinologic diseases	2 (0.3)
Obstetric diseases	1 (0.1)
Psychiatric diseases	1 (0.1)
Under investigation	142 (18.6)
Unknown	22 (2.9)
Mortality	13 (0.02)

Table 1. Patients' demographic, clinical, and laboratory data. Data are shown as number (%) for categorical variables and median ± interquartile range for continuous variables. The denominators of patients who were included in the analysis are provided if they differed from the overall numbers within the group.