Anti-myeloperoxidase antibody positivity in patients without primary systemic vasculitis

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Abstract

Objectives: We aimed to elucidate the frequency and associations of MPO-ANCA positivity in patients without ANCA-associated vasculitis (AAV), in a large urban, multi-ethnic teaching hospital.

Methods: Retrospective review of 200 patients identified as MPO-ANCA positive over a five year period at Royal Free Hospital, London, UK.

Results: The incidence of anti-MPO positivity in patients without AAV was 39.5%. Gastrointestinal tract disorders, infections and other connective tissue disorders made up the majority of diagnoses, and there was a higher incidence of other concomitant autoantibodies compared to the group with known AAV. Renal disease was common in non-vasculitic patients with anti-MPO antibody positivity (occurring in 48%), the majority of which went on to renal biopsy to exclude vasculitic involvement.

Conclusions: The high incidence of MPO-ANCA positivity in patients with non-vasculitic conditions highlights the need for careful clinical correlation and confirmatory tissue diagnosis.

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Introduction

Advances in, and modifications of therapy in patients with ANCA-associated vasculitidies (AAV) have led to improved patient outcomes over the last decades (1). Historically, significant delays in diagnosis were common, as many symptoms are non-specific or may mimic infectious diseases. Increased awareness of these conditions and their milder variants, along with more widespread availability of ANCA testing, has potentially led to more rapid establishment of diagnosis.

ANCA are autoantibodies directed against cytoplasmic constituents of neutrophils and monocytes, the two main specificities being for proteinase 3 (PR3) and myeloperoxidase (MPO). Two patterns of staining are found on indirect immunofluorescence: cytoplasmic (C-ANCA), which usually occurs with PR3 specificity, and perinuclear (P-ANCA) that occurs with MPO (2). The combination of a pANCA immunofluorescence pattern with antibodies specific for MPO has been reported to have up to 99% specificity for the diagnosis of AAV (3). However, recent proposals, based on excellent performance of immunoassays, suggest that screening with immunofluorescence may be unnecessary (4). The increasing reliance on serological testing to establish a diagnosis has led to more widespread ANCA testing. With lower pre-test probabilities of underlying vasculitis in populations tested, there are increasing false positive tests which physicians have to be aware of. We have previously reported that up to 10% of positive PR3-ANCA serology may be found in patients without systemic vasculitis, but with infectious diseases, malignancies, other systemic autoimmune conditions or without obvious provoking factors (5). In this study we report on the frequency and associations of MPO-ANCA positivity in patients without primary vasculitis, and find it to be significantly higher (39.5%), and highlight the predominance of other autoimmune phenomena in these patients, as well as a high incidence of renal dysfunction which necessitates careful evaluation, with histological assessment in the majority, to exclude renal limited vasculitic disease.

Materials and Methods

A computerised search of all patients identified as MPO-ANCA positive in our local laboratory was performed between September 2010 and 2015. ANCA serology was examined by immunofluorescence on fixed neutrophil substrate and, if positive, by fluorescence enzyme immunoassays for the detection of antibodies to myeloperoxidase (EliA MPOs fluoroenzyeme immunoassay for anti-MPO antibodies, ThermoFisher). Upper limits of the normal range were MPO >10 IU/mL before 2014, MPO >5.0 IU/mL after 2014.

Case notes and laboratory results were reviewed subsequently to establish if patients that tested positive for MPO-ANCA had a diagnosis of AAV or alternative diagnoses.

Statistical analysis was conducted using GraphPad Prism (GraphPad Software, San Diego, CA). A p value <0.05 was considered to be statistically significant. Ethics board approval was not obtained as per institutional policy.

Results

Between September 2010 and 2015, 208 patients tested positive for MPO-ANCA. Eight patients did not have adequate clinical data available and were subsequently excluded from the analysis. Of the 200 patients analysed, 39% were men and the median (IQR) age of the cohort was 59 years (44-74). The majority of patients, 121 (60.5%) had an established or new diagnosis of AAV, while the remaining 79 (39.5%) did not have a diagnosis or evidence of primary systemic vasculitis. The median MPO-ANCA titre in the group with known AAV was 66(24-100) units/ml and the median serum creatinine 115(77-252.5) µmol/L compared with 16(12-30) units/ml and 81.5 (63-133.5) µmol/L respectively in the group without AAV (p<0.001) (Figure 1). The most common indications for testing ANCA status were joint pain and swelling (21.5%), persistent respiratory symptoms (12.7%) and acute kidney injury (8.9%).

Of the 79 patients with positive MPO-ANCA without a diagnosis of AAV, 60 (75.9%) patients had an established alternative diagnosis including infections (n = 14, 17.7%), inflammatory bowel disease (n =10, 12.7%), autoimmune hepatitis (n = 9, 11.4%), systemic lupus erythematosous (n = 7, 8.8%), scleroderma (n=6,7.6%) and malignancy (n = 6, 7.6%). Nineteen patients had various symptoms that did not fulfil a single diagnosis (Figure 2). Sixty of the 79 patients (75.9%) also had other autoantibodies detected, the commonest of which was antinuclear antibody (ANA)(n=45, 56.7%), as compared with the MPO-ANCA AAV group, in whom only 21(17.3%) patients had other autoantibodies (p<0.0001).

Thirty eight of the 79 patients without a known diagnosis of AAV had renal involvement as defined by an estimated glomerular filtration rate (eGFR) of less than 60 ml/min (n=28) and/or a urinary protein creatinine ratio greater or equal to 100mg/mmol (n=13) and/ or the presence of microscopic haematuria (n=10). Twenty two of these patients underwent a renal biopsy, and demonstrated various histological findings most commonly including lupus nephritis (n=8, 36.4%), chronic tubulointerstitial scarring (n=5, 22.8%) and diabetes (n=4, 18.2%).

Sixteen patients did not have a renal biopsy. Eleven (69%) of these patients had coexistent autoantibodies, mainly ANA (n=6), Rheumatoid factor (n=3) and anti-double stranded DNA (n=2). The majority (n=10, 62.5%) had autoimmune conditions known to be associated with anti-MPO antibodies. In two patients, MPO positivity was associated with positive hepatitis and HIV serology respectively.

In the remaining four patients, no associations were found to explain the presence of MPOANCA. The median (IQR) follow-up of these four patients was 4(2-7) years. Their median MPO ANCA titre was 11(10-39) U/ml, eGFR 40(15-76) ml/min and age 69(51-86) years. One patient died at two year follow-up from unknown cause and the remaining three patients did not have signs or symptoms of primary vasculitis at latest follow-up.

Discussion

The combination of pANCA- and anti-MPO antibodies in patients with suspected small vessel vasculitis carries a specificity of 99% for MPA(3). However, in this cohort we report a 39.5% incidence of unselected MPO-ANCA positive patients without primary vasculitis. Disorders of the gastrointestinal tract and liver (24%), infections (17.7%) and other connective tissue disorders made up the majority of diagnoses in the non-AAV group followed by several other potential associations including, medications and malignancy. The non-AAV group had a much higher incidence of other concomitant autoantibodies. This may suggest that the MPO-ANCA in this group of patients could be secondary to a polyclonal B-cell response in the presence of systemic inflammation seen in autoimmunity, infection or malignancy. Previous reports have also shown that pANCA can occur in a variety of medical conditions apart from AAV including chronic inflammatory processes such as tuberculosis, inflammatory bowel disease, malignancies, connective tissue diseases and following introduction of certain medications (6,7), and highlighted that anti-MPO positivity was less common in those patients without AAV.

The significance of ANCA positivity in patients without AAV is unclear. Evidence from animal studies, which is supported by a case report of transplacental transfer of MPO-ANCA resulting in neonatal pulmono-renal syndrome, suggest that MPO-ANCA are pathogenic (8,9). It has also been shown that naturally occurring anti-MPO autoantibodies can exist in healthy individuals (10). However, these anti-MPO autoantibodies have epitope specificities that are different from those present in ANCA disease implicating immunodominant epitopes in the pathology of AAV (11). It is therefore possible that anti-MPO positivity in patients without AAV, like healthy controls, are directed against non-vasculitic disease-causing epitopes, but specifically testing for these remains outside standard clinical care. In four of our patients with MPO-ANCA but without AAV, who had renal involvement and in whom we found no clinical association to explain the MPO-ANCA positivity, none developed AAV during a median four year follow-up. However, in studies using stored samples from US military recruits it was shown that MPOANCA can predate onset of the disease for up to 5 years, which supports the need of long-term follow-up for these patients (12).

Our incidence of 39.5% of MPO-ANCA positive patients with a variety of non-vasculitic conditions is significantly higher than the previously reported incidence of 9.7% for PR3-ANCA in patients without AAV (5). In a retrospective review of 236 ANCA-positive patients, patients with an alternative diagnosis to AAV were more often anti-MPO positive than anti-PR3 positive (58% vs. 42%) (6). interestingly, the main difference in these cohorts is that in non-AAV PR3- ANCA patients the commonest disease association was infection whereas in non-AAV MPOANCA patients were other autoimmune conditions. This is in keeping with dual positivity/overlap conditions that have shown a higher incidence of MPO, rather than PR3- ANCA in patients expressing other autoantibodies or with other immune mediated conditions, such as anti-GBM disease and scleroderma (13). It is possible that the HLA requirements for successful MPO presentation may be less restricted compared with PR3 presentation, explaining the finding in various autoimmune diseases (14). The association of MPO-ANCA with renal limited vasculitis, in which extra-renal manifestations are absent, means that many MPOANCA patients with vasculitis present late with more advanced renal failure, often following routine screening blood tests (15). The high incidence of renal

disease in the MPO-ANCA patients without vasculitis (48%) means that careful evaluation is required (often with renal biopsy) to secure the correct diagnosis and exclude a renal-limited vasculitic process.

In conclusion, we demonstrated a high incidence of MPO-ANCA positivity in patients with a variety of non-vasculitic conditions, significantly higher than the previously reported incidence of PR3-ANCA in patients without AAV. As detectable antibodies may predate disease onset, unexplained ANCA positivity requires long-term follow-up. In cases of diagnostic uncertainty confirmatory tissue diagnosis should be pursued as serology alone may be misleading.

Acknowledgements

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Figure 1. a) Individual and median (horizontal bar) values of MPO-ANCA titres (units/ml) in patients with MPO-ANCA and with (n = 121) or without (n = 79) a diagnosis of primary AAV (Mann Whitney U t-test p<0.0001) and b) serum creatinine levels in (μmol/L) patients with MPO-ANCA and with (n = 117) or without (n = 78) a diagnosis of primary AAV (Mann Whitney U t-test p<0.001).
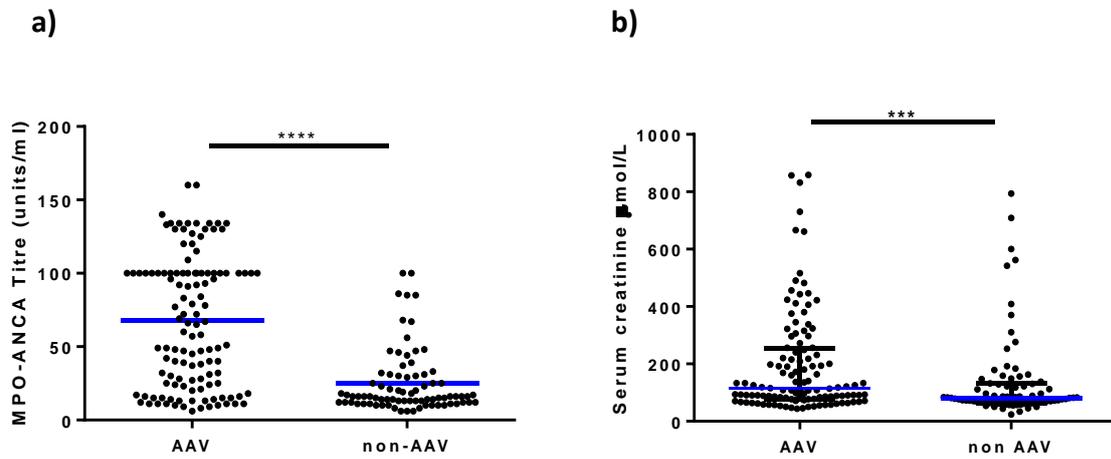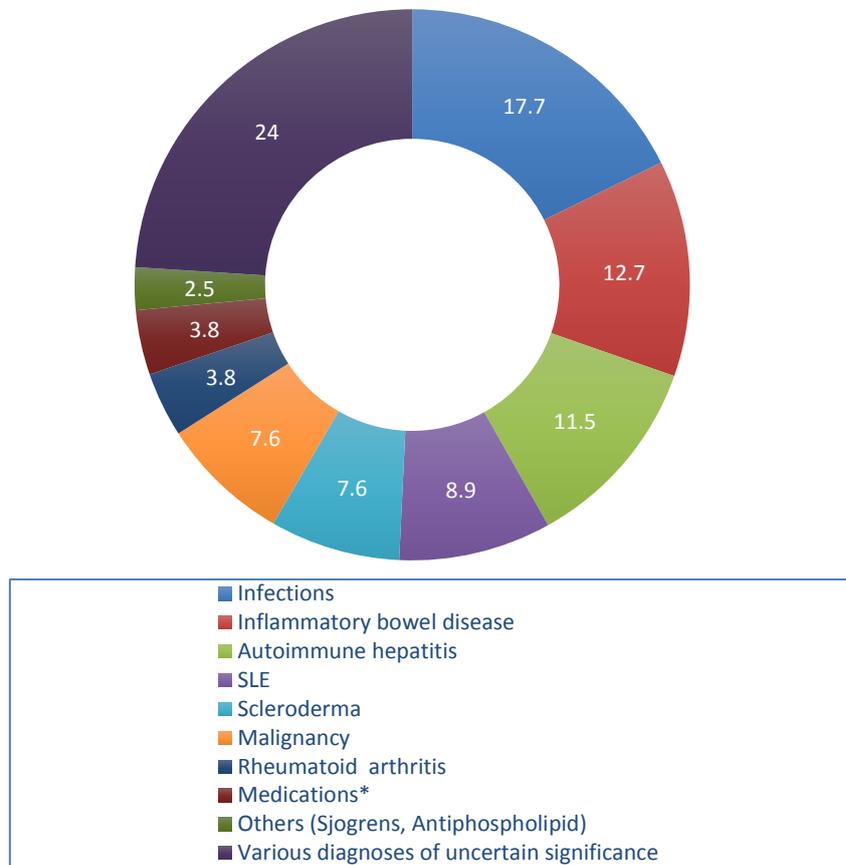
Figure 2. Associated diagnoses in MPO-ANCA positive patients without known AAV (n=79), showing percentages of each condition

Legend	
■ Infections	
■ Inflammatory bowel disease	
■ Autoimmune hepatitis	
■ SLE	
■ Scleroderma	
■ Malignancy	
■ Rheumatoid arthritis	
■ Medications*	
■ Others (Sjogrens, Antiphospholipid)	
■ Various diagnoses of uncertain significance	

*These include propylthiouracil, carbamezipine,and hydralazine