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Case Report

Myeloperoxidase-antineutrophil Cytoplasmic Antibodies with Cytoplasmic Fluorescence Pattern

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ABSTRACT

We report here two rare cases of myeloperoxidase–antineutrophil cytoplasmic antibody (MPO-ANCA)-positive Wegener's granulomatosis (limited variant) which deceptively produced a cytoplasmic (C-ANCA) pattern on indirect immunofluorescence.

Keywords: Indirect immunofluorescence, enzyme linked immunosorbent assay, Wegener's granulomatosis

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INTRODUCTION

Autoantibodies causing a C-ANCA (cytoplasmic) pattern are generally directed against proteinase-3 (PR-3) and can be specifically detected with the anti-PR-3 enzyme-linked immunosorbent assay (ELISA).[1] In contrast, P-ANCA antibodies can be produced by a variety of different autoantibody specificities. Originally, it was suspected that myeloperoxidase (MPO) was the target antigen of P-ANCA, but later it became evident that a P-ANCA staining pattern can also be produced by other antibodies.[2]

C-ANCA with MPO specificity accounts for fewer than 10% of all patients with Wegener's granulomatosis (WG).[3] P-ANCA with PR3 specificity is even rarer. The two cases that we are presenting here demonstrate rare findings with the C-ANCA fluorescence pattern seen with ELISA-proven MPO-ANCA positivity.

CASE REPORT

Case 1

A 42-year-old hypertensive female presented with cough, joint pains with morning stiffness involving small and large joints, gangrene of right toe, and right upper motor neuron facial palsy. History of oral ulcers, blueing of fingers, redness and drying of eyes was also given by the patient. She has received full course of antitubercular therapy (ATT) 3 years ago but did not show any improvement in the symptomatology. CT chest at presentation revealed bilateral lung nodules with one of them showing cavitation and mediastinal lymphadenopathy. Fine needle aspiration cytology from mediastinal lymph nodes showed AFB (acid-fast bacilli)-negative non-caseating necrotizing granulomatous lesions. Histopathology of the skin lesion showed leukocytoclastic vasculitis. Rheumatoid factor and C-reactive protein (12 mg/L) were positive.

Case 2

A 41-year-old female presented with high grade fever non-responsive to antibiotics, redness of eyes, bilateral hearing loss, rhinorrhea, oral ulcers, and moderate distal painful parasthesias. Past history of sudden loss of consciousness was also elicited. Eyes showed congestion of bilateral bulbar conjunctiva and episcleritis. Complete ENT examination showed bilateral chronic suppurative otitis media, mastoiditis, and pansinusitis. MRI of paranasal sinuses showed maxillary and sphenoid sinusitis. A tongue biopsy demonstrated necrotizing vasculitis. Skin biopsy showed erythema nodosum. Lung biopsy showed interstitial fibrosis. CT chest revealed mediastinal lymphadenopathy and pulmonary alveolar hemorrhage.

Autoimmune work-up in both patients revealed 4+ C-ANCA pattern by IIF on ethanol-fixed neutrophil

preparation. IIF was performed on two different patients' samples and also with two different sets of neutrophil preparations, and every time the ANCA pattern was cytoplasmic only. Antinuclear antibodies (ANA) as well as dsDNA ELISA were negative. PR3 ELISA (VARELISA) was negative in both with a value less than 6 U/ml. MPO ELISA (VARELISA) was positive with results more than 9 U/ml in both the cases. All the ELISAs were put in duplicate. Finally, ANCA results were reported as 4+ C-ANCA with MPO-ANCA. In both, serum creatinine levels were within normal limits and 24-h urinary protein was nil.

According to American College of Rheumatology (ACR) criteria, based on clinical features and organ manifestations, a diagnosis of WG (without renal involvement) was considered in both the cases. They were put on pulsed cyclophosphamide therapy and showed symptomatic improvement.

DISCUSSION

MPO-ANCA-positive sera are known to produce a characteristic perinuclear pattern on IIF of ethanol fixed neutrophil preparations.[2] In isolated cases only MPO-ANCA may be detected even in sera showing atypical ANCA and C-ANCA patterns.

False positive MPO-ANCA may occur which can be due to the presence of dsDNA antibodies resulting in binding via DNA to MPO. Both of our patients were ANA negative as well as dsDNA negative thereby excluding the possibility of cross reactivity. We have also shown that sera from these patients did not react at all with proteinase 3 in ELISA, thereby also ruling out the possibility of cross reactivity with PR3.

Segelmark *et al*. have previously reported that antibodies to certain epitopes on MPO produce a cytoplasmic pattern.[4] They have clearly shown in their experiments that all myeloperoxidase do not relocate toward the nucleus after ethanol fixation (some MPO has to remain in the granules or in the cytoplasm) and that C-ANCA and P-ANCA epitopes exist simultaneously on the same MPO molecule. They also proposed that two immunofluorescence patterns arise due to different availabilities of the epitopes in the microenvironment where myeloperoxidase is present.[4]

It has been previously shown that patients with MPO-ANCA have a tendency towards more frequent renal involvement than patients with PR3-ANCA, thereby substantiating a stronger renal pathogenic effect of MPO-ANCA compared to PR3-ANCA.[5] Both the cases that we have presented here showed MPO-ANCA in ELISA with normal renal function tests. However, in the absence of a renal biopsy, we cannot exclude confidently the absence of renal pathology in these cases.

It further needs to be reinforced that the diagnosis of WG should be based on ACR criteria and not on ANCA positivity. In a country like India, where tuberculosis (TB) is endemic and also in view of potential phenotypic similarities of TB and WG, it is necessary that TB should be ruled out by confirming AFB negativity from sputum, body fluids or aspirates and biopsy material or even giving a trial of ATT to check for response to therapy. Though ANCA is an important serologic tool to assist in the diagnosis of WG and other small vessel ANCA-associated vasculitis (SVAAV), it should always be interpreted in conjunction with clinical features. Moreover, clinical definitions of WG, microscopic polyangiitis, and Churg-Strauss syndrome do not include the demonstration of ANCA or target antigen specificity. A number of previous reports confirm the presence of this autoantibody in known cases of tuberculosis and leprosy.[6-8] A medical rarity known to exist is Wgener's tuberculosis described by Gordon *et al*.[9]

The two cases that we have shown here present a rare phenomenon of MPO-ANCA presenting as a C-ANCA pattern on IIF. It is important to be aware of such a situation while reporting and assessing the clinical relevance of ANCA positivity.

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