Antimyeloperoxidase antibody in Chinese patients in Hong Kong: a five-year review

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An analysis was made of 51 Chinese patients in Hong Kong who tested positive for antimyeloperoxidase antibody in the five-year period from January 1989 to December 1993. Nineteen could clearly be classified as having an identifiable vasculitic illness, and the features of the remainder were suggestive of vasculitic syndromes in most cases. Renal disease was present in 84% of all cases and was the most common manifestation, followed by pulmonary diseases. Acute or acute-on-chronic renal failure developed in 20 patients (39%). Of those who had a renal biopsy performed, more than half had histological evidence of crescentic glomerulonephritis. Antimyeloperoxidase antibody-associated disease carries a grave prognosis with significant morbidity and mortality. Steroid and immunosuppressive therapy was given to most patients and in those who responded to immunosuppressive therapy, antimyeloperoxidase antibody levels provided a useful marker for monitoring disease activity. Twenty patients died, either from complications or as a result of disease activity.

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Introduction

In 1982, a new autoantibody was described which targets neutrophil cytoplasm in some cases of segmental necrotising glomerulonephritis.[1] This was followed in 1984 by reports of four cases of systemic vasculitis involving the same autoantibody.[2] It was not until 1985 that the association of the new class of antibody—now referred to as antineutrophil cytoplasm antibody (ANCA)—with Wegener's granulomatosis was established.[3] The observation is supported by other groups in the United States and Europe.[4-8] Using indirect immunofluorescence, two distinct forms of ANCA have been differentiated—the cytoplasmic form (C-ANCA) and the perinuclear form (P-ANCA).[9] The major antigen for C-ANCA is proteinase 3[10] whereas that for P-ANCA is myeloperoxidase (MPO).[9]

Whereas C-ANCA is often associated with Wegener's granulomatosis, P-ANCA–associated diseases form a more heterogeneous group. Antimyeloperoxidase antibody (anti-MPO), the most common subtype of P-ANCA, is reported to be closely related to limited forms of vasculitis and idiopathic necrotising crescentic glomerulonephritis (CG).[11,12] The latter is frequently thought to be a kidney-limited form of vasculitis. Anti-MPO is associated with the syndromes of acute renal failure and pulmonary haemorrhage.[13] Other disease associations include microscopic polyarteritis (MPA) and polyarteritis nodosa (PAN).

The discovery of ANCA has stimulated extensive research in the field of vasculitis over the past decade. As the spectrum of vasculitic or other autoimmune diseases varies from place to place, our understanding of the autoantibody's prevalence and clinical significance is important in a local context. In a preliminary survey of 1000 unselected sera sent to our laboratory for ANCA testing for the first time, 2% had elevated anti-MPO levels. This study reports our experience in the clinical application and significance of the anti-MPO test in Hong Kong.

Subjects and methods

The clinical immunology laboratory at the Queen Mary Hospital, Hong Kong, also serves other public and private institutions. The anti-MPO enzyme-linked immunosorbent assay (ELISA) test became available

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in early 1989. All ANCA screening tests are performed using indirect immunofluorescence (IIF) and serum samples positive for P-ANCA are tested for anti-MPO by ELISA. Physicians managing the care of anti-MPO–positive patients were asked to complete a standard clinical questionnaire detailing age, sex, presenting symptomatology, past medical history, clinical diagnosis, organ/system involvement, treatment, clinical responses, outcome, and anti-nuclear antibody (ANA) results (if available). The study examined all anti-MPO positive cases recruited in the five-year period from January 1989 to December 1993. Patients with systemic lupus erythematosus (SLE) or related diseases, such as scleroderma, dermatomyositis/polymyositis, overlap syndrome, and mixed connective tissue disease were excluded.

Indirect immunofluorescence

Sera were tested for ANCA by IIF in accordance with the method recommended at the First International Workshop on ANCA.[14] Briefly, normal human leucocytes were sedimented with 2% methylcellulose, washed and deposited on glass slides by cytocentrifugation prior to fixation in 95% ethanol at 4°C for five minutes. This substrate was incubated for 30 minutes with test serum, diluted 1:20 in phosphate-buffered saline and washed. Incubation with fluorescein isothiocyanate-conjugated antigen-binding fragment of rabbit antihuman immunoglobulin G for 30 minutes was followed by washing, application of diazo-bicyclooctane (DABCO) and cover slip, before viewing under blue light (490 nm) using a fluorescent microscope. Positive sera which gave a perinuclear pattern were reported as P-ANCA.

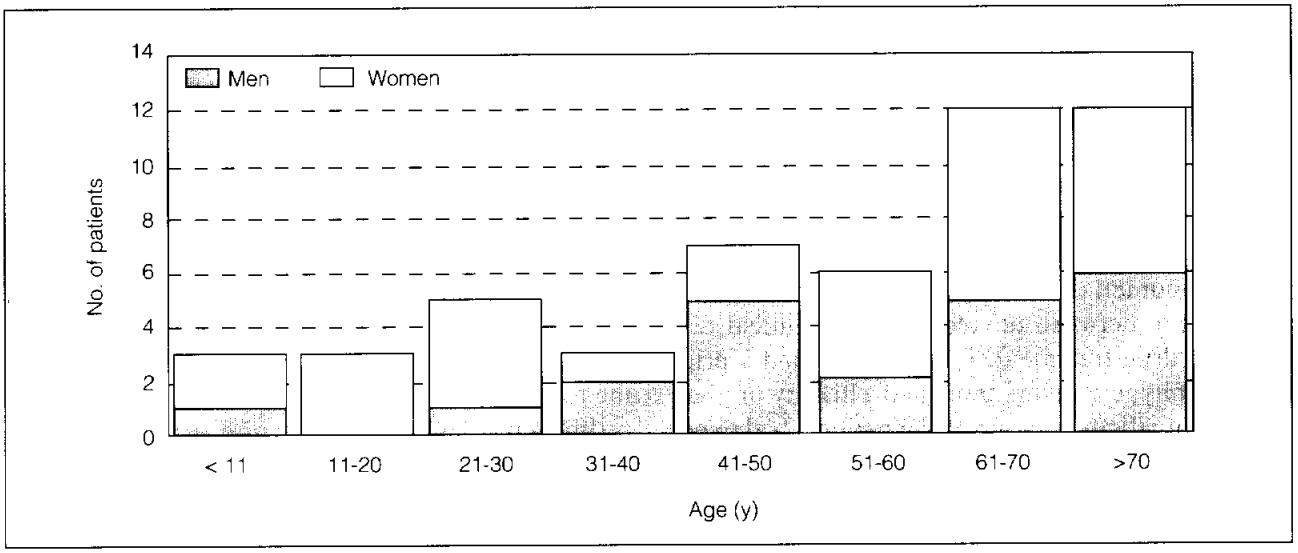
Enzyme-linked immunosorbent assay for antimyeloperoxidase antibody

Human neutrophil MPO (Calbiochem, La Jolla, Ca, US) was used at a concentration of 1.25 mg/ml in 0.05 mol/L bicarbonate buffer (pH 9.5) to coat the micro-titreplate (Immulon 2, Dynatech, Va, US) overnight at 4°C. After blocking with 1% bismuth sulphite agar in phosphate-buffered saline with 0.05% Tween-20 at 37°C for one hour, serum diluted 1:25 in the same buffer was applied for another hour at the same temperature, followed by washing. The bound antibody was detected by alkaline phosphatase-conjugated goat anti-human IgG (Binding Site, Birmingham, UK) followed by the addition of the substrate p-nitrophenyl phosphate (Sigma, St Louis, Mo, US). The absorbance was read at optical density 405 nm using a microtitreplate reader. Results were expressed as a percentage of a known positive serum sample which was serially diluted to give a standard curve. The positive reference serum was obtained from a patient who died of systemic vasculitis with CG. Serum samples from 44 healthy volunteers were taken to form the control group for establishing the normal reference range. The cutoff point (3.9%) was defined as three standard deviations above the mean of the 44 normal samples.

Results

In the five-year period from January 1989 to December 1993, a total of 318 samples tested positive for anti-MPO, after preliminary screening for P-ANCA. These belonged to 94 patients, of which 30 were diagnosed with SLE and were thus excluded from the study. Details of only 51 of the remaining 64 patients were available. The following analysis is based on the clinical results of these individuals.

Fig 1. Age and sex distribution of patients with antimyeloperoxidase antibody present (n=51)



The male to female ratio was 1:1.3 (Fig1). The mean age was 52 ± 22 years (range, 1 month to 86 years). Approximately three-fifths of the subjects were older than 50 years. The anti-MPO activities ranged from 4.2% to more than 100%, with a mean of 49%.

The clinical diagnoses of the 51 patients are listed in Table 1. Twenty-two patients were diagnosed with renal disease only, 12 having necrotising CG. Nine patients presented with acute or chronic renal failure without pathologic evidence of CG, and one with proteinuria. Eighteen patients were classified as having vasculitis, namely, PAN (3), Wegener's granulomatosis (1), and other forms of systemic vasculitis (14). In the latter group, physicians had made a diagnosis of MPA in nine patients.[15] Of the remainder, various clinical diagnoses were noted.

The major presenting symptomatology is given in Table 2. Irrespective of the final diagnosis, renal manifestations were the most common initial presenting feature, accounting for half (26/51) of the patients, followed by neurological (8) and pulmonary (8) symptoms. Acute or acute-on-chronic renal failure developed in 20 (39%) patients. The overall organ/system involvement in the 51 patients is given in Fig 2. Among those diagnosed with systemic vasculitis (including MPA), the majority (13/14) had renal involvement presenting as either acute or acute-on-chronic renal failure. The remaining patient had cutaneous vasculitis, fever, arthritis, and respiratory failure. Overall, renal biopsy was performed in 37 patients (Table 3), and 21 (57%) had histological evidence of CG. Even though none could be classified as having SLE, ANA was performed (by IIF using Hep-2 cells) in 45 patients and was positive in 19 (42%) patients. Approximately one-third of the positive results were only weakly positive, with a titre of 1:40 (screening dilution). There was no correlation between the anti-MPO and ANA titres (data not shown).

Most patients had no other significant medical illness. Four had a history of thyroid disease—thyrotoxicosis (3) and hypothyroidism (1). The three patients with hyperthyroidism had been previously treated with either neomercazole (2) or propylthiouracil (PTU) [1]. There was no temporal relationship between development of clinical disease and drug treatment. Five patients had a prior history of hypertension, three had a history of gout, and two of silicosis. None of the patients had experienced other forms of autoimmune disease before the current episode of vasculitic illness. A 23-year-old woman with Wilson's disease developed penicillamine-induced lupus with CG associated with a high anti-MPO titre. However, her sister with the same hereditary disease tolerated penicillamine well.

The majority of patients received some form of immunosuppressive treatment except when the diagnosis was made after death, or when end-stage renal failure had already developed. Thirty-five patients (69%) received oral steroids and 23 (45%) were given oral cyclophosphamide. Twenty-two patients were given steroids and cyclophosphamide, and three were given steroids and azathioprine. Plasma exchange was performed in 15 patients.

Twenty-two patients received some form of dialysis during the follow up period. Seven were given peritoneal dialysis, 10 were given haemodialysis, and five received both. End-stage renal failure developed in 12 patients. Physicians noted clinical responses in only 25 of the 51 patients. Twenty patients died, eight from active disease and five from complications—pulmonary oedema (1), fungal septicaemia (1), fungal pneumonia (1), intracerebral haemorrhage (1) and septicaemia (1). The information on the remaining seven was insufficient for thorough analysis. The diagnoses of the 20 patients who died included systemic vasculitis (7), CG (3), end-stage renal failure (3), PAN (2), pulmonary haemorrhage (2), fibrosing alveolitis (1), Wegener's granulomatosis (1), and Goodpasture's syndrome (1).

Discussion

Antimyeloperoxidase antibody has now become an important autoantibody in the study of vasculitic diseases. This study demonstrated the strong association of anti-MPO with vasculitis among Chinese patients in Hong Kong. Of the 51 cases reported, 19 could be classified as having a vasculitic illness, and the features of the remainder were strongly suggestive of vasculitic syndromes. For instance, the 12 patients with CG had no evidence of other systemic illnesses and could be considered as suffering from a kidney-limited form of vasculitis. In addition, 10 other patients had various forms of renal failure but without histological proof of glomerulonephritis or vasculitis; in these a vasculitic cause could not be excluded. Similarly, the four patients with pulmonary diseases (two with pulmonary haemorrhage, one with fibrosing alveolitis, one with atypical pneumonia) and the patient with retinal artery occlusion might have been affected by a vasculitic process.

Fig 2. Organ/system involvement of patients with antimyeloperoxidase antibody present (n=51)

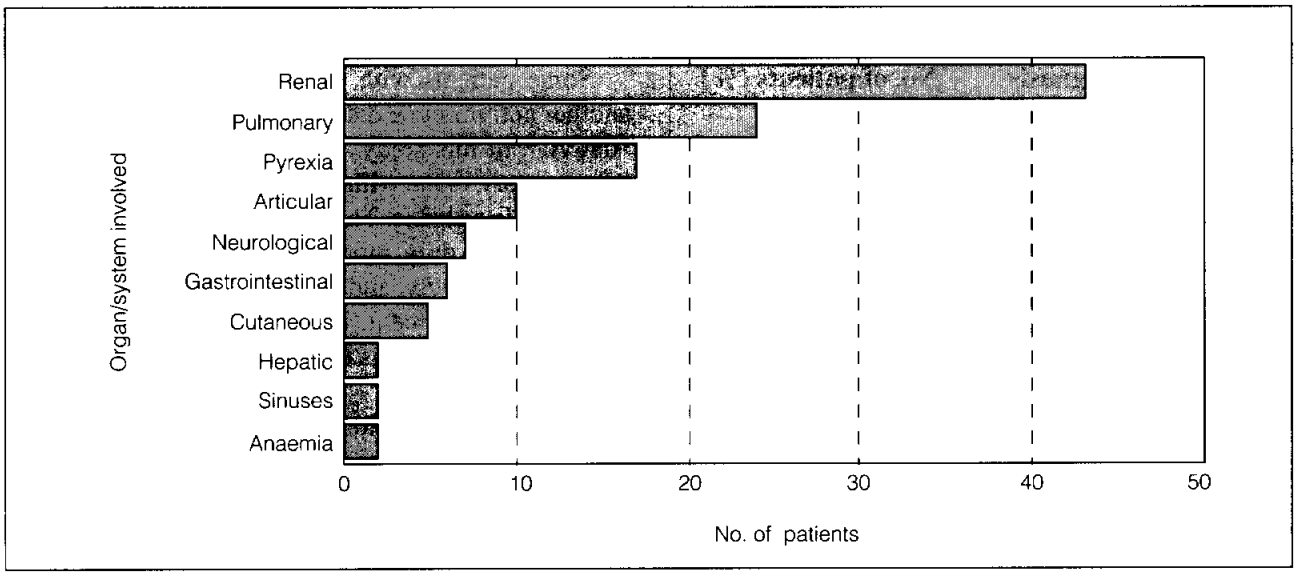


Table 1. Clinical diagnoses of antimyeloperoxidase antibody-positive patients (n=51)

Diagnosis	No. of patients (%)	
Crescentic glomerulonephritis	12	(23.5)
Renal failure acute renal failure (3) acute-on-chronic renal failure (1) chronic renal failure (5)	10	(19.6)
Microscopic polyarteritis	9	(17.6)
Other systemic vasculitis	5	(9.8)
Pulmonary diseases pulmonary haemorrhage (2) fibrosing alveolitis (1)	3	(5.8)
Polyarteritis nodosa	3	(5.8)
Wegener's granulomatosis	1	(2.0)
Kawasaki's disease	1	(2.0)
Renal artery occlusion	1	(2.0)
Ulcerative colitis	1	(2.0)
Goodpasture's syndrome	1	(2.0)
Juvenile chronic arthritis	1	(2.0)
Penicillamine-induced renal failure	1	(2.0)
Rheumatoid arthritis	1	(2.0)
Pneumonia	1	(2.0)
Total	51	(100)

Table 2. Major presenting symptomatology of antimyeloperoxidase antibody-positive patients (n=51)

Symptoms	No. of patients (%)	
Renal acute/acute-on-chronic renal failure (20) chronic or end-stage renal failure (2) proteinuria (2) hypertension (1) haematuria (1)	26	(51.0)
Neurological peripheral neuropathy (6) headache (1) visual blurring (1)	8	(15.6)
Pulmonary pulmonary haemorrhage (4) respiratory distress (2) respiratory failure (1) sinusitis (1)	8	(15.6)
Gastrointestinal bloody diarrhoea (1) bowel ischaemia (1)	2	(4.0)
Skin rash	2	(4.0)
Unexplained fever	5	(9.8)
Total	51	(100)

Table 3. Renal histopathology of biopsy patients (n=37)

Pathological diagnosis	No. of patients	(%)
Crescentic glomerulonephritis	21	(56.8)
Other glomerulonephritis focal glomerulosclerosis (2) mesangial glomerulonephritis (2) Goodpasture's disease (1)	5	(13.5)
End-stage renal disease	3	(8.1)
Interstitial nephritis	2	(5.4)
Sclerosis	2	(5.4)
Vasculitic changes	1	(2.7)
Granulomatosis	1	(2.7)
Ischaemia	1	(2.7)
No pathological diagnosis	1	(2.7)
Total	37	(100)

Anti-MPO carries a high predictive value for small vessel vasculitis only if SLE is excluded from the analysis. In this study, we deliberately excluded all cases of SLE, which approximated one-third (30/94) of all anti-MPO positive cases collected during the study period. The presence of anti-MPO in an SLE patient may carry a significance very different from that in other diseases. An earlier study reports that 9% of a group of SLE patients tested positive for anti-MPO, and there is positive correlation between the presence of anti-MPO and serositis.[16] However, anti-MPO titres were generally lower among lupus patients. Its presence could be attributed to polyclonal antibody production resulting from a flare of lupus activity, although a direct immunopathogenic mechanism cannot be excluded.

Among the 51 non-lupus patients analysed in this study, there was one case of penicillamine-induced CG with high serum ANA. This patient could be classified as having SLE, or a case of vasculitis with positive ANA. Thirty-six per cent of the 51 patients in this study were ANA positive, and the phenomenon has been reported elsewhere among patients with other vasculitic diseases.[4,9] In our patients who were ANA positive, no correlation could be found between the levels of anti-MPO and ANA. As both SLE and systemic vasculitis are clinical syndromes, it is possible that a grey area exists in which some patients would be classified as having SLE or a lupus-like syndrome by one physician and as having vasculitis by another.

Some patients with rheumatoid arthritis (RA)—another autoimmune disease—have been positive for ANCA.[17] In this study, only two patients fitted into this category—one with RA and the other with juvenile chronic arthritis. The anti-MPO titres for the two patients were only moderately elevated, 6.6% and 11.0% respectively, and the levels fell when the disease became quiescent. The significance of anti-MPO in RA patients remains to be established.

The key manifestation of anti-MPO–associated disease reported in this study, irrespective of the clinical diagnosis, was renal involvement. It occurred in 84% of the 51 patients. The close association between anti-MPO and renal disease has been reported in other studies.[11-13,18,19] In the absence of features suggestive of other clinical entities, the anti-MPO test has become an important tool in the investigation of renal failure. The diagnostic yield of anti-MPO in pulmonary diseases is much lower, because of the lower incidence of vasculitic lung diseases and the inherent difficulty in arriving at a pathological diagnosis for pulmonary lesions. In Hong Kong, where Wegener's granulomatosis is uncommon compared with systemic vasculitis, the presence of strongly positive anti-MPO in a patient with renal or pulmonary diseases often prompts one to make a presumptive diagnosis of vasculitic illness, facilitating the initiation of immunosuppressive therapy, if SLE can be excluded.

The development of vasculitic diseases following drug treatment has received much attention in recent years.[20] Antineutrophil cytoplasm antibody production has been linked to the use of PTU in the treatment of thyrotoxicosis and it is worth noting that four of the patients in this series had a history of thyroid disease; three had received antithyroid treatment–neomercazole in two cases and PTU in another. There was no temporal relationship between taking the antithyroid drug and the development of clinical vasculitic disease, nor was there improvement of symptoms on the cessation of drug treatment. There have been suggestions that thyrotoxicosis and vasculitis are interrelated pathogenetically as both target the peroxidase enzymes—thyroid peroxidase (TPO) for thyrotoxicosis and MPO for systemic vasculitis.[21] Sequence homology between TPO and MPO has been demonstrated.[22] Hence, it is possible that thyroid disease and systemic vasculitis are related through their immunopathogenic mechanism and not by anti-thyroid treatment.

The prognosis of patients with anti-MPO–associated vasculitic disease is not favourable. Because of the rapid progression of the disease, mostly with renal failure, patients often died of the disease or developed end-stage renal failure before therapy could be instituted. Even when treatment was started without delay, infective complications resulting from immunosuppression were common. For those who responded to treatment, anti-MPO could be used for monitoring disease activity. However, its value as an activity marker remains to be established. Because of the relatively long time taken for the antibody level to change, this role would very likely be inferior to that of C-reactive protein. Nevertheless, anti-MPO is useful in monitoring the long term progression of the underlying disease.

Almost a decade has passed since ANCA was first described in association with Wegener's granulomatosis.[3] The increasing range of associated diseases and the delineation of the various sub-specificities of ANCA—including anti-MPO—have gradually changed our views of their significance. Comparison between studies is difficult because of the different criteria physicians use in diagnosing vasculitis—this is still largely a clinical or clinico-pathological diagnosis and autoantibodies such as anti-MPO play only a supplementary role in the diagnostic workup.[23]

Greater knowledge in recent years of the clinical associations of ANCA has gradually changed physician practice in diagnosing this interesting group of diseases. In the same way that organ non-specific antibodies are used to classify SLE and related rheumatic diseases, ANCA subspecifities should perhaps be included in the criteria for classifying vasculitic illnesses.[24] Not only would this help yield a clinical diagnosis and the treatment of vasculitic diseases, but it would also pave the way for comparative clinical studies to be conducted based on the relevant immunological disease markers.

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