Detection Rate and Antigenic Specificities of Antineutrophil Cytoplasmic Antibodies in Chinese Patients with Clinically Suspected Vasculitis

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The detection rate of antineutrophil cytoplasmic antibodies (ANCA) in Chinese patients with clinically suspected small vessel vasculitis was investigated, and their antigen specificity and demographic features were analyzed. A number of sera (n = 5,604) sent to our referral laboratory for ANCA screening were tested by indirect immunofluorescence (IIF), enzyme-linked immunosorbent assays (ELISAs) for myeloperoxidase (MPO)- and proteinase 3 (PR3)-ANCA. Then the IF-ANCA-positive sera that were negative for MPO- and PR3-ANCA were further tested by antigen-specific ELISA by using other five highly purified known ANCA antigens as solid-phase ligands. The known antigens included bactericidal/permeability-increasing protein (BPI), human leukocyte elastase (HLE), lactoferrin, cathepsin G, and azurocidins. Of the 5,604 sera, 267 (4.76%) sera were IIF-ANCA positive and 390 (7%) were antinuclear antibody (ANA) positive in the IIF assay. Of the IIF-positive samples, 213 were anti-MPO positive, 32 were anti-PR3 positive, and five cases were positive for both. Of the 48 sera positive for IIF-ANCA but negative for MPO- and PR3-ANCA, 13 sera (27%) recognized other target antigens, 7 sera recognized BPI, 5 recognized HLE, 1 recognize cathepsin G, and 1 recognized azurocidin. None of the sera recognized lactoferrin, and one serum sample recognized both BPI and HLE. The majority of ANCA-positive patients presented in summer or winter. There was no difference in gender (male/female ratio, 1:1.12) in ANCA-positive patients with a mean age of 53.1 years. The male/female ratio was 1.17:1 for patients over 60 years of age; however, it was 1:4 for patients under 20 years of age. We conclude that ANCA-related diseases are not rare in China, and the major antigens are MPO and PR3. When the IIF technique is used to detect ANCA, ANA should be carefully distinguished.

The systemic vasculitides comprise a spectrum of clinical syndromes united by a common pathology consisting of a primary inflammation and necrosis of blood vessels. Antineutrophil cytoplasmic antibodies (ANCA) are serological markers for certain primary vasculitic diseases, including Wegener’s granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and idiopathic pauci-immune necrotizing glomerulonephritis. These diseases were called ANCA-associated systemic vasculitis (AASV) (3, 7). ANCA can be identified by using indirect immunofluorescence (IIF) techniques and by overlay serum from patients with suspected vasculitis on alcohol-fixed human polymorphonuclear leukocytes (PMN). This procedure produces two staining patterns: a cytoplasmic pattern (C-ANCA) and a perinuclear pattern (P-ANCA) (2, 9). Although proteinase 3 (PR3) and myeloperoxidase (MPO) are the major C-ANCA and P-ANCA antigens (2, 9), several other neutrophil granule constituents can also be recognized by ANCA-positive sera, including bactericidal/permeability-increasing protein (BPI), cathepsin G (CG), lactoferrin, human leukocyte elastase (HLE), and azurocidin (16, 17, 18).

AASV are common autoimmune disorders in the Caucasian population but were not recognized in China until ANCA was available a decade ago (19). This study investigates the prevalence of ANCA and the antigen specificities, as well as demographic features of Chinese patients with AASV from a diagnostic referral ANCA screening center in the Institute of Nephrology, Peking University.

MATERIALS AND METHODS

Patients and sera. A number of sera (n = 5,604), sent for screening ANCA from January 1996 to December 2001, were collected from patients with clinical suspected vasculitis in our referral laboratory in the Institute of Nephrology, the First Hospital, Peking University. These sera came from all around China, including Beijing and other provinces. Clinical and demographic data of all ANCA-positive patients were collected and analyzed further.

Detecting strategy. All the serum samples were screened by IIF and enzyme-linked immunosorbent assays (ELISAs) for MPO-ANCA and PR3-ANCA. The IIF-ANCA-positive samples that were negative for MPO-ANCA and PR3-ANCA were further screened by ELISA for BPI, HLE, CG, lactoferrin, and azurocidin.

IIF assay for detecting ANCA. Standard IIF assays were performed according to the manufacturer’s instructions (EUROIMMUN, Lübeck, Germany). Ethanol-fixed human PMN were used to detect ANCA, and monkey liver sections were used to exclude antinuclear antibodies (ANA). By use of primate liver as an additional antigen substrate, P-ANCA and ANA can be differentiated, because the PMN in the sinuses lie in immediate proximity to the nuclei of the hepatocytes and can thus be identified optically together with them. If the PMN in the sinuses were positive, whereas the nuclei of the hepatocytes were negative, the antibody was ANCA; whereas if the PMN in the sinuses and the nuclei of the hepatocytes were all positive, the antibody perhaps was ANA. Cooccurrence of ANCA and ANA is not excluded without antigen-specific ELISAs, however.

Antigen-specific ELISAs. Seven highly purified known ANCA antigens, purified as detailed in previous reports (17, 18, 20), were used as solid-phase ligands in ELISA. PR3, MPO, HLE, CG, lactoferrin, and azurocidin were diluted to 1 to 2 μg/ml with 0.05 M bicarbonate buffer, pH 9.6, and BPI was diluted to 1 μg/ml with 0.01 M phosphate-buffered saline (PBS); the wells of one half of a Costar...
TABLE 1. Relationship between IIF-ANCA and ELISAs for MPO- and PR3-ANCAa

| ELISA sample type | No. of samples with: | Total no. of samples per type |
|-------------------|----------------------|------------------------------|
|                   | pANCA | cANCA | ANA  |
| Anti-MPO positive  | 179   | 3     | 31   | 213  |
| Anti-PR3 positive  | 0     | 32    | 0    | 32   |
| Double positive    | 5     | 0     | 0    | 5    |
| Double negative    | 48    | 0     | 0    | 48   |
| Total              | 232   | 35    | 31   | 298  |

a Double positive, both MPO- and PR3-ANCA positive; double negative, both MPO- and PR3-ANCA negative.

RESULTS

Rate of detection of ANCA and ANA. Of the 5,604 sera, 267 (4.76%) were IIF-ANCA positive, 232 (4.14%) were P-ANCA positive, and 35 (0.62%) were C-ANCA positive. However, 390 of the 5,604 (7%) sera were ANA positive.

ANCA antigen specificity. Of the 5,604 sera, 213 recognized MPO, 32 recognized PR3, and 5 sera recognized both. Of the 232 P-ANCA-positive sera, 179 recognized MPO, while 5 recognized both MPO and PR3. Of the 35 C-ANCA-positive sera, 32 recognized PR3 and 3 recognized MPO. However, 31 of the 390 ANA-positive sera also recognized MPO and 2 sera recognized both MPO and PR3 (Table 1).

For the 48 P-ANCA-positive sera yet not MPO-ANCA and PR3-ANCA, only seven sera recognized BPI, five sera recognized HLE, one serum recognized CG, and one recognized azurocidins, while none of the sera recognized lactoferrin and one serum recognized both BPI and HLE. Titers of anti-MPO versus anti-PR3 of the five double-positive sera were 94 versus 25%, 85 versus 32%, 98 versus 29%, 78 versus 35%, and 25 versus 25%, respectively. The five patients were diagnosed as having propylthiouracil-induced ASSV.

Etiology of ANCA-positive patients. According to the request forms and/or questionnaires, 254 (85%) ANCA-positive patients had microscopic polyangiitis or Wegener’s granulomatosis; four patients had ulcerative colitis, and two patients had hemolytic anemia, while the others had unknown etiology.

Epidemiologic and demographic features. ANCA prevalence increased chronologically. The rate of detection of ANCA in 2000 and 2001 was significantly (P < 0.05) higher than that in 1998 and before (Table 2). Most patients presented from June to December with a peak in July (Fig. 1).

Out of the 254 patients with ANCA-associated vasculitides, 209 had complete clinical data. They had an average age of 53.1 (7 to 79) years, and the male/female ratio was 1:1.12. However, the male/female ratio was 1.17:1 in patients over 60 years of age and was 1:4 in patients below 20 years of age (Fig. 2). The gender ratio for patients under 20 years of age is

![FIG. 1. Distribution of ANCA-positive samples in different months.](image)

![FIG. 2. C-ANCA-positive sera.](image)
years of age, the females are clearly predominant (4:1), a

equally affected; however, for the Chinese patients under 20

tabled that Caucasian patients were male predominant (12, 13), while for the Chinese patients, males and females are

discussed features compared with Caucasian patients. The first
difference concerned sexual predominance. Many reports re-

20 years of age, the females are clearly predominant (4:1), a

antigenic predominance. MPO and PR3 are the two major

FIG. 2. Distribution by gender of patients of different ages having

significantly different from that for patients over 60. The aver-
age duration from onset of the disease to final diagnosis was


discussion

DISCUSSION

Although AASV are common in Caucasians, the clinical
diagnosis was still difficult until the discovery and clinical ap-
lication of ANCA (8, 10). In the 1980s, the incidence of

some serum samples contain other cytoplasmic fluorescence patterns and/or ANA, which results in homog-

eous fluorescence alone is not specific for the diagnosis of Wegener’s granulomatosis or microscopic poly-

vascular or peripheral nuclear fluorescence. Many reports in-

dicate that positive fluorescence alone is not specific for the
diagnosis of Wegener’s granulomatosis or microscopic poly-

In China systemic lupus erythematosus is the most

common autoimmune disease, and in the present study, the

prevalence of ANA was much higher than that of ANCA;

therefore, IIF-ANCA, especially P-ANCA, should be clearly
distinguished from ANA. To distinguish P-ANCA and ANA,

formaldehyde-fixed neutrophils, mammal liver sections and

Hep2 cells are good substrates for excluding ANA. On form-

aldehyde-fixed neutrophils, ANA were not detected, whereas

P-ANCA diffusely labeled the cytoplasm. Mammal liver can

also be used to distinguish ANCA and ANA. When ANA and

ANCA coexist, however, these techniques could not distin-

guish ANCA from ANA clearly; therefore, just using the IIF

assay as serum markers to diagnose AASV is not good enough.

It has been suggested that IIF-ANCA combined with anti-

gen-specific ELISAs, for MPO-ANCA and PR3-ANCA, had good sensitivity and specificity for diagnosing AASV (2, 9), and

our results supported this conclusion. Although many ANCA

antigens have been identified, only MPO and PR3 are of con-

firmed clinical significance. In our study, 84% of the IIF-

ANCA-positive serum samples recognized MPO and PR3, in-

dicating that MPO and PR3 are indeed the two major ANCA

antigens as well for Chinese patients with AASV. Although 16%
of our samples did not recognize MPO and PR3, only a few of them (13 of 48) recognized the other known ANCA

antigens, such as BPI, HLE, cathepsin G, and azurocidins. The

specific target antigens of the other serum samples still need to

be further characterized. The sera of the four patients with

ulcerative colitis showed P-ANCA but were negative for MPO-

and PR3-ANCA; instead, they recognized BPI. Some of the

patients diagnosed as having drug-induced ASSV had more
than one ANCA specificity. All these results need to be further
investigated.

In conclusion, ANCA-related diseases are not rare in China
and MPO is the most common ANCA antigen.

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