

Antineutrophil cytoplasmic antibodies in Chinese patients with tuberculosis

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Abstract

Introduction: Based on reports, infection with *Mycobacterium tuberculosis* is believed to induce the development of antibodies that are considered to be biological indicators for the diagnosis of some other diseases. However, conflicting results have been published regarding the presence of antineutrophil cytoplasmic antibodies (ANCA) in patients with tuberculosis. We aim to study the seroprevalence of ANCA in a population of Chinese patients with tuberculosis, which may lead to the misdiagnosis of vasculitic disorders. **Methods:** The study was conducted from January 2016 to May 2017 to evaluate the presence of ANCA in 103 Chinese patients using indirect immunofluorescent assay. An enzyme-linked immunosorbent assay was performed for anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) detection. **Results:** Perinuclear ANCA (p-ANCA) was detected in 4.8% (5/103) of patients, whereas cytoplasmic ANCA (c-ANCA) was not detected; 1.9% (2/103) of patients with tuberculosis was positive for anti-MPO antibodies, and none had anti-PR3 antibodies. Both anti-MPO-positive patients were diagnosed with ANCA-associated vasculitides. **Conclusions:** ANCA positivity may be more related to vasculitis and immunological disorders than to a *M. tuberculosis* infection. Therefore, to improve diagnostic accuracy, patients with *M. tuberculosis* who are ANCA positive should be investigated for concurrent diseases, including the effects of drugs. Therefore, even in tuberculosis epidemic area, ANCA seropositivity, detected by ELISA, is still more suggestive of ANCA-associated vasculitides.

Keywords: Antineutrophil cytoplasmic antibodies. Tuberculosis. Proteinase 3. Myeloperoxidase. Seroprevalence.

INTRODUCTION

Mycobacterium tuberculosis infection is one of the major global public health threats because more than two billion people are estimated to be infected with tuberculosis¹. Tuberculosis is associated with autoimmune diseases, including rheumatoid arthritis, multiple sclerosis, and vasculitis, possibly through molecular mimicry². Antineutrophil cytoplasmic antibodies (ANCA) are directed against cytoplasmic ANCA (c-ANCA) and perinuclear ANCA (p-ANCA) antigens and are associated with Wegener's granulomatosis, polyangiitis, microscopic polyangiitis, and other autoimmune disorders, which are also considered to be clinical markers for systemic vasculitic disorders. Some studies reported that ANCAs could be positive in infectious diseases, such as tuberculosis; however, conflicting results have also been reported^{3,4}. In clinical practice, immunological diseases, such as granulomatosis with polyangiitis, can present with clinical features that overlap

with those of tuberculosis. Therefore, an ANCA test may help with differential diagnosis. This study aims to investigate the prevalence of serum ANCA positivity in Chinese patients with tuberculosis.

METHODS

This single-center retrospective study was conducted at the First Hospital of Jilin University in Northeast China between January 2016 and May 2017 in accordance with the Declaration of Helsinki, and the local Ethics Committee approved this study. All patients who participated in this study signed an informed consent form. Patients who were either untreated or were within 30 days of beginning their treatment for *M. tuberculosis* infection were included. Tuberculosis infection was diagnosed through sputum microbiology testing, clinical and radiological signs, and symptoms. The study participants underwent a detailed clinical history, including questions about musculoskeletal symptoms, duration of symptoms, and a history of medication use.

The presence of ANCA was determined via indirect immunofluorescence (IIF) using a commercially available kit (Cell Signaling Co.). Testing was performed according to the manufacturer's protocol. Serum samples were diluted 1:100 in

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phosphate buffered saline (PBS) and incubated in microplates coated with the specific antigen. Antigen-antibody binding was detected using an anti-human immunoglobulin conjugated with peroxidase and a 3,3',5,5'-tetramethylbenzidine (TMB) chromogenic substrate. After washing with PBS, the microplates were examined, using a fluorescence microscope. Serum samples were also tested for the presence of antibodies to proteinase 3 (PR3) and myeloperoxidase (MPO), using standardized kits (Fitzgerald Industries International). Therefore, 10µL of sample was combined with 990µL of sample buffer in a polystyrene tube and mixed well. Controls were ready to use and did not need to be diluted. PR3 and MPO were bound separately to the microwells. We added 100µL of each sample into the wells and incubated for 30 minutes at 20-28°C. After discarding the supernatant, we washed it 3 times with 300µL wash solution; and then TMB substrate solution was added into each well. A stop solution was used to quench the reaction. Finally, horseradish peroxidase (HRP)-conjugated anti-human immunoglobulin G (IgG) was used to immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. The microplates were examined at 450nm using a fluorescence microscope.

Statistical analysis of the data included the presentation of quantitative variables as means [95% confidence interval (95% CI)] or median, and interquartile range; qualitative variables were expressed as percentages. Statistical Package for the Social Sciences (SPSS) software (SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) was used for the statistical analysis.

RESULTS

The clinical characteristics of the patients with tuberculosis and confirmed infection with *M. tuberculosis* are summarized in **Table 1**. The demographic, clinical, and radiological characteristics of the 103 patients with *M. tuberculosis* were expressed in numbers and percentages, unless otherwise stated.

Of the 103 patients with *M. tuberculosis* included in this study, 54% (56/103) were women with a mean age of 51 years. Thirty-four patients were in the early stages of their treatment for tuberculosis; treatment included rifampicin (RFP), isoniazid, and pyrazinamide. The median treatment duration was 8 days (interquartile range, 2-19 days).

Of the 103 individuals included in the study, six had tuberculosis involving more than two sites, including tuberculous peritonitis, pleuritis and pericarditis, maxillofacial tuberculosis, urologic tuberculosis, and spinal tuberculosis.

TABLE 1: Characteristics of 103 patients with tuberculosis.

Clinical characteristics	Number (%)
Women: men	56:47
Age in years (95% CI)	51 (30-72)
Clinical presentation of TB	
pulmonary	98
extrapulmonary	5
multiple (>2) sites	6
Symptoms	
fever, weight loss, cough	45
TB treatment	
rifampicin, isoniazid, and pyrazinamide	34
Diagnosis of TB	
abnormal chest X-ray	84
positive microbiology	4+18-
T-SPOT.TB	38+21-
Co-morbidities	
diabetes	6
hypertension	30
renal disease	19

95% CI: 95% confidence intervals; **TB:** tuberculosis; T-SPOT.TB: a T cell-based assay for the detection of infection with *Mycobacterium tuberculosis*.

A previous episode of tuberculosis, which had preceded the current infection by 2-31 years, was noted in 20 patients.

The serology testing for ANCA showed that p-ANCA was detected in 4.8% (5/103) of patients, and c-ANCA was not observed in any patients (**Table 2**). MPO was detected in 2/103 patients, who were diagnosed with tuberculosis and ANCA associated vasculitides. There were 3/103 patients without anti-MPO or PR3 who had a low serum titer of p-ANCA (1:10), among which, two patients had diabetes complicated by infection, and one patient had anaphylactoid purpura [Immunoglobulin A/Henoch-Schönlein purpura (IgA/HSP)], which is a form of vasculitis.

DISCUSSION

Although the most common clinical presentation of *Mycobacterium tuberculosis* infection is pulmonary tuberculosis, with more than 10% of patients developing extra-pulmonary manifestations, which often delay the diagnosis and allow the chronic inflammation to progress. Patients with pulmonary tuberculosis present with typical clinical symptoms, including

TABLE 2: Antineutrophil cytoplasmic antibody test results for 103 patients with culture-positive tuberculosis.

ELISA	Indirect immunofluorescence assay		
	negative	c-ANCA	p-ANCA
Anti-PR3/anti-MPO negative	98	0	3
Anti-PR3 positive	0	0	0
Anti-MPO positive	0	0	2
Total number	98	0	5

ELISA: enzyme-linked immunosorbent assay; **ANCA:** antineutrophil cytoplasmic antibody; **PR3:** proteinase 3; **MPO:** myeloperoxidase; **c-ANCA:** cytoplasmic ANCA; **p-ANCA:** perinuclear ANCA.

fever, cough, and hemoptysis, and with typical diagnostic findings that include nodular and cavitating lesions on chest imaging; chronic inflammation with necrotizing granuloma formation is histologically observed on lung biopsy. However, patients with autoimmune disorders, including systemic lupus erythematosus, and patients with systemic vasculitis, including Wegener's granulomatosis, share similar clinical and histological features with tuberculosis⁵⁻⁸.

The detection of serum antibodies against MPO can be associated with microscopic periarteritis (MPA) and necrotic and crescentic glomerulonephritis (NCGN). The anti-MPO antibody titer is associated with the disease activity and can be used for early diagnosis, prediction of disease recurrence, and guidance of patient response to clinical treatment. Anti-MPO antibody positivity is highly suggestive of MPA and NCGN. The presence of serum PR3 antibodies are indicative of Wegener's granulomatosis, which can be considered as a primary vasculitis.

Previously published studies showed that patients with tuberculosis may have serum ANCA, as well as anti-MPO, and anti-PR3. Clinical studies on the presence of ANCA in patients with tuberculosis have been controversial. As shown in **Table 3**, p-ANCA was the predominant pattern in four studies that included ANCA-positive patients. However, Florez-Suarez et al. reported that 80% of ANCA-positive patients had c-ANCA, and 44.4% of patients with tuberculosis showed ANCA positivity, and that 90% of ANCA-positive patients had anti-PR3 and anti-MPO⁹. The proportion of serum-positive tuberculosis patients has been shown to decrease during tuberculosis therapy¹⁰. In another study, p-ANCA was detected

in 25% of Iranian patients with tuberculosis, and c-ANCA was detected in 3.1%; ELISA results showed that 75% of cases had anti-MPO, and 12.5% had anti-PR3, indicating a high ANCA seropositivity rate in tuberculosis³. However, Teixeira et al. showed that tuberculosis was associated with low ANCA (10%) seroprevalence, including 4% of c-ANCA and 6% of p-ANCA, but only one IIF-negative specimen was anti-PR3 positive¹¹.

The clinical distinction between tuberculosis and Wegener's granulomatosis can be difficult at disease onset. According to some studies, ANCA testing has an important role in the differential diagnosis. However, there have been conflicting results regarding the presence of ANCA in tuberculosis. Although reasons for the conflicting findings from these studies are not clear, it is likely that geographical factors and ethnic factors may have accounted for the differences. Additionally, drugs, such as hydralazine and propylthiouracil are associated with ANCA. Ori Elkayam¹² have shown that most patients with serum anti-MPO normalized following the treatment, whereas anti-PR3 usually increased in some treated patients, which is probably due to drug-related autoimmune phenomena^{2,12}. Esquivel-Valerio et al. found that in 68 cases, tuberculosis patients with ANCA positivity detected by an indirect immunofluorescent assay (IIF) was increased from 4.4% (one c-ANCA and two p-ANCA) to 28.8% (3 c-ANCA and 12 p-ANCA) after treatment, while anti-PR3 and anti-MPO were negative in all serum samples from patients with TB¹⁰. A published case report has shown that following treatment with RFP and ethambutol, serum anti-MPO and ANCA titers of the patient were high and a drug-induced nephritis was found¹³. Another case report showed that the serum

TABLE 3: Clinical studies on ANCA, anti-MPO, and anti-PR3 in patients with tuberculosis.

Author	Patients with TB	ANCA-positive rate (%)	p-ANCA (%)*	c-ANCA (%)*	Anti-MPO (%)*	Anti-PR3 (%)*
Pradhan, et al ¹	30	30.0	52.4	38.1	47.6	28.6
Sherkat, et al ³	32	28.1	89.0	11.0	75.0	12.5
Lima, et al ⁴	50	0.0	0.0	0.0	0.0	0.0
Esquivel, et al ¹⁰	68	4.4	66.7	33.3	0.0	0.0
Teixeira, et al ¹¹	67	10.0	60.0	40.0	0.0	14.3
Flores, et al ¹⁵	45	44.4	20.0	80.0	15.0	75.0
Ghosh, et al ¹⁶	70	30.0	52.4	47.6	47.6	--

ANCA: antineutrophil cytoplasmic antibody; **anti-MPO:** anti-myeloperoxidase; **anti-PR3:** anti-proteinase 3; **MPO:** myeloperoxidase; **PR3:** proteinase 3; **TB:** tuberculosis; **p-ANCA:** perinuclear ANCA; **c-ANCA:** cytoplasmic ANCA. *The percentage of p-ANCA, c-ANCA, anti-MPO and anti-PR3 = positive number/ANCA positive number × 100%.

anti-PR3 was positive after anti-tuberculosis treatment with RFP, which indicated a strong correlation between RFP and ANCA¹⁴. We consider that the presence of ANCA might be due to drug-related autoimmune phenomena and, in some cases, might be affected by anti-tuberculosis treatment to a certain degree, with an increase in antibody titers to MPO and PR3¹². Our study, which included 103 patients with a confirmed diagnosis of TB on chest X-ray and sputum microbiology, showed that the seroprevalence of ANCA was 4.9% (5/103), which was low. The patients with positive ANCA, especially with anti-MPO, tended to have other additional diseases, which included ANCA-associated vasculitis in two patients. In our study, the positive rate of serum p-ANCA was 40% in the ANCA positive patients. Although anti-MPO positivity was found, no anti-PR3 was detected. Based on these findings, we conclude that anti-PR3/c-ANCA is a rare occurrence in tuberculosis, and that autoimmune disease or vasculitis should be considered in the differential diagnosis, and that other diseases should be excluded, especially in tuberculosis endemic areas.

Compared with previous studies^{1,3,4,10,11,15-17}, this study recruited patients with many types of tuberculosis, including spinal tuberculosis, peritoneal tuberculosis, and tuberculous meningitis. Based on the findings of this study, patients who were ANCA-positive (especially for MPO or PR3) were more likely to have other ANCA-related disorders. Therefore, concurrent diseases, including the effects of drugs should initially be considered when ANCA positivity is found, even in countries with a high prevalence of tuberculosis.

This study had several limitations. It had a small study population and was performed in China. Therefore, the findings of this study in a Chinese population with tuberculosis may not be applicable to other geographical areas or other ethnic groups, although it indicates a low seroprevalence of ANCA in China. However, the study findings, when considered together with previously published studies, support that ANCA positivity without microbiological confirmation of tuberculosis is more likely to be due to systemic vasculitis.

Ethical considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments, or comparable ethical standards. Formal consent was not required for this type of study.

Conflict of interest

The authors declare that there is no conflict of interest.

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