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SHORT COMMUNICATION

Anti-PR3 and anti-MPO antibodies are not present in sera of patients with pulmonary tuberculosis

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Abstract Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed to intracellular components of neutrophils and are present in several vasculitic syndromes. Recently, these autoantibodies have been described in other autoimmune disorders as well as in infectious diseases such as tuberculosis (TB). As there are some clinical similarities between TB and granulomatosis with polyangiitis, we searched for ANCA in a group of patients with proven TB. Patients with TB confirmed by chest X-ray and sputum bacilloscopy either before or within 30 days after beginning treatment were included in this study. Anti-MPO and anti-PR3 antibodies were studied using well-standardized ELISA kits (INOVA Diagnostics, Inc.). ANCA were also investigated by indirect immunofluorescence (IIF). Fifty TB patients (26 females, mean age 47.34 ± 17 years) were enrolled in the present study. No patient tested positive for ANCA by IIF, or anti-MPO or anti-PR3 antibodies by ELISA. Although previous studies have shown the presence of ANCA in some infectious diseases, the findings of the present study demonstrated the absence of such antibodies in TB. The discrepancy in the prevalence of ANCA in TB among different studies may be attributed to methodological factors and/or the genetic background of the studied populations.

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Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed to intracellular components of neutrophils, usually described in small vessel systemic vasculitis such as granulomatosis with polyangiitis (GPA), microscopic Polyangiitis and Churg–Strauss syndrome. In GPA, their specificity is to proteinase 3 (PR3) whereas in the other two types of vasculitis, the most common antigenic target is myeloperoxidase (MPO) [1].

These autoantibodies have also been described in other autoimmune disorders [2–4] as well as in infectious diseases [5–10] particularly tuberculosis (TB) [11–13]. As GPA shares some clinical features with TB, in areas with high prevalence of this infectious disease, the positivity of these serologic tests may lead to a misdiagnosis and consequently wrong treatment. The aim of the present study was to investigate the frequency of these antibodies in a group of patients with proven pulmonary TB from an endemic area in Brazil.

Patients and methods

Patients either untreated or within 30 days after beginning treatment for pulmonary TB confirmed by chest X-ray and sputum bacilloscopy were included in this study. All patients were older than 18 years, voluntarily agreed to participate in this study and signed a consent form. The Ethics Committee of our institution approved the project. The included patients were submitted to a complete clinical evaluation including muscle skeletal manifestations, time of the diagnosis and medicine in use.

ANCA were determined by indirect immunofluorescence (IIF) using a commercially available kit (EUROIMMUN) following the protocol summarized below: The sera to be tested were diluted 1:10 in phosphate buffered saline (PBS) and incubated on the slides for 30 min in a moist chamber; after this period, the slides were washed with PBS. Again, the slides were incubated in the moist chamber for 30 min, this time with an antihuman IgG conjugate (goat). After washing with PBS, the slides were examined with a fluorescence microscope. Serum samples were also tested for the presence of antibodies to PR3 and MPO utilizing well-standardized kits according to the manufacturer's (INOVA Diagnostics Inc.) recommendations. Values above 20 U are considered positive.

Quantitative variables were presented as mean ± SD or median and interquartile range, and qualitative variables were expressed as percentages. For statistical analysis, a package program (SSPS for Windows version 18.0) was used.

Results

Fifty pulmonary TB patients [twenty-six (52 %) female, mean age 47.34 (±17 years)] were enrolled in the present study. Forty-six patients were in the beginning of TB treatment with rifampicin, isoniazid and pyrazinamide and had a median treatment time of 8 days (interquartile range 2–19). The majority of the patients (31) had no joint symptoms, whereas the others described some unspecific muscle skeletal pain without evidence of arthritis. The clinical and epidemiological characteristics of the patients are presented in Table 1.

None of the tested samples was positive for ANCA by IIF or had antibodies to MPO or PR3 by ELISA. The statistical power of 50 patients to detect at least a 10 % prevalence of antibodies to MPO or PR3 is 94 %.

Table 1 Clinical and epidemiological features of the tuberculosis patients ($n = 50$)

Characteristics	*N* (%)
Female	26 (52)
Positive bacilloscopy	50 (100)
Abnormal chest X-ray	49 (98)[a]
Tabagism	21 (42)
Alcoholism	13 (26)
Illicit drug use	1 (2)
Tuberculosis symptoms	
Fever	18 (36)
Weight loss	17 (34)
Cough	50 (100)
Hemoptysis	5 (10)
Chest pain	6 (12)
Dyspnoea	9 (18)
Tuberculosis treatment	
R/I/P[c]	46 (92)[b]
Co-Morbidities	
HIV	3 (6)
Diabetes	8 (16)
Hypertension	10 (20)

[a] One patient did not have a chest X-ray

[b] Three patients had not started treatment, and one patient discontinued it due to intolerance

[c] *R* rifampicin/*I* isoniazid/*P* pyrazinamide

Discussion

Historically antibodies to cytoplasmic components of neutrophils detected by immunofluorescence were initially described in patients supposedly infected by *Ross River virus* in 1982 [14]. These patients had several clinical features of autoimmune disorders such as fever, arthralgia and myalgia, and some of them had glomerulonephritis. Later on, such antibodies were associated with GPA [15], and presently, it is well known that patients with GPA have antibodies with specificity to PR3 that gives the cytoplasmatic pattern of ANCA (C-ANCA), whereas antibodies to MPO with the perinuclear pattern of ANCA (P-ANCA) are seen frequently, although not exclusively in patients with Chung Strauss syndrome and microscopic polyangiitis [1].

Curiously, in the last few years, studies have presented conflicting results regarding ANCA positivity in infectious diseases, particularly pulmonary TB. As this condition bears some clinical and histopathological features of GPA, such as cough, fever, hemoptysis, nodular and cavitation lesions on chest imaging and chronic inflammation and granuloma at lung biopsy, it is mandatory to clarify this point, specially in endemic areas of TB, otherwise it would lead to misdiagnosis with dangerous consequences.

In this context, Flores-Suárez et al. [16] studying 45 TB patients in Mexico found a prevalence of ANCA of 44 % by IIF, mainly of the cytoplasmic pattern. These authors also found an astonishing positivity of 40 % in their cohort, for anti-PR3 and anti-MPO antibodies by ELISA. Pradhan et al. [11] demonstrated a positivity of ANCA by IIF, anti-MPO and anti-PR3 by ELISA in 30, 47.6 and 28.6 %, respectively, in their cohort of patients with TB. Ghosh et al. [13] gathered the findings obtained by Pradhan et al. with those retrieved from two other studies summing up a total of 318 TB patients. ANCA were positive in 30 % of the entire studied population. Recently, Sherkat et al. evaluated the prevalence of ANCA by IIF, anti-MPO and anti-PR3 antibodies in 32 subjects with active TB comparing with 32 healthy controls. They found P-ANCA and C-ANCA in 25 and 3.1 % of the cases, respectively, mainly due to the presence of anti-MPO antibodies [12]. Another recent study showed

very intriguing results with most TB patients having high baseline anti-PR3, anti-MPO and anti-lactoferrin levels. After treatment, most anti-lactoferrin and anti-MPO levels decreased, while anti-PR3 increased. Some patients had *de novo* anti-PR3 and anti-MPO positivity after treatment [17].

On the other hand, other studies, apart from the present one, have demonstrated an absence or low frequency of positivity of these antibodies in TB. Teixeira et al. [18] found only 10 % of ANCA positivity by IIF in 67 TB patients, and only one had antibodies to PR3. Esquivel-Valerio et al. searched ANCA by IIF, anti-PR3 and anti-MPO in 68 TB patients before treatment and in 52 of them, 60–90 days after initiation of anti-TB therapy. In the pre-treatment samples, the positivity of ANCA by IIF found was 3/68 (4.4 %), being one C-ANCA and two P-ANCA. None of the samples had anti-PR3 or anti-MPO antibodies. Whereas after initiation of treatment, ANCA were identified in 15/52 (28.8 %) being twelve P-ANCA and three C-ANCA. In 11 of these 15 samples (73.3 %), there was a specificity of the antibodies to bactericidal/permeability-increasing protein. Anti-PR3 and anti-MPO antibodies were negative in all tested samples [19].

The findings of the present study, demonstrating the negativity of ANCA by IIF, anti-PR3 and anti-MPO antibodies by ELISA in TB, confirm the high specificity of these tests for the identification of vasculitic syndromes. This observation has been corroborated by the results of a previous study from our institution demonstrating very low prevalence of these antibodies in Hansen's disease [20].

The discrepancy in the prevalence of ANCA in TB observed in different studies may be attributed to methodological factors such as inclusion of patients with bacteriologically unproved TB, or at a later stage of treatment and use of diagnostic kits from unreliable sources. As addressed in a recently published review, lack of standardization of the current assays is one of the problems with the use of ANCA testing in the daily clinical practice [21]. Moreover, the possibility of associated chronic bacterial infections and/or difference in the genetic background of the studied populations cannot be excluded.

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Conflict of interest The authors have no conflict of interest that is directly relevant to the content of this manuscript.

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