

## SHORT PAPER

# Antineutrophil Cytoplasmic Antibodies in Patients with Pulmonary Tuberculosis

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### ABSTRACT

**Background:** Mycobacterium tuberculosis is a major cause of mortality and morbidity worldwide. Infection with this bacterium is known to induce the development of autoantibodies of which a few are also known to be diagnostic markers for some other diseases. Antineutrophil Cytoplasmic Antibodies (ANCA's) are among those autoantibodies used in clinical setting for diagnosing systemic vasculitic syndromes. Multiple studies investigated ANCA positivity in diseases other than small vessel vasculitis. **Objective:** This study was performed to determine the prevalence of ANCA in pulmonary tuberculosis (TB) which may lead to the false diagnosis of Wegener's granulomatosis (WG) or vice versa. **Methods:** In a case-control study, 32 consecutive smear positive pulmonary TB patients and 32 normal individuals were studied. All cases and controls were screened for ANCA by indirect immunofluorescent assay (IIF), and MPO and PR3 were also tested by ELISA. **Results:** A prenuclear pattern (P-ANCA) was detected in 25% of the cases and 6.25% of the controls and a cytoplasmic pattern (C-ANCA) was observed in 3.1% of both the cases and the controls by IIF assay. ANCA specificities tested by ELISA in cases revealed that 75% of the cases had anti-MPO and 12.5% had anti-PR3, while in the controls, 3.12% had anti-MPO and none had anti-PR3. The positive ANCA significantly correlated with TB ( $p < 0.01$ ). **Conclusion:** ANCA's may be observed in both TB and systemic vasculitic syndromes such as WG. Tuberculosis and WG share some clinical features. Therefore, in countries with a high prevalence of TB, one has to distinguish between these two diseases especially when no sign of extrapulmonary involvement is observed.

**Keywords:** Antineutrophil Cytoplasmic Antibody, Myeloperoxidase Antibody

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## INTRODUCTION

Antineutrophil Cytoplasmic Antibodies (ANCA) are autoantibodies directed against antigens found in the cytoplasmic granules of neutrophils and monocytes (1), with two major immunofluorescence staining patterns i.e. cytoplasmic (C-ANCA) and perinuclear (P-ANCA) (2). ANCA has been closely related to the diagnosis of a subset of primary systemic vasculitides, namely Wegener's granulomatosis, microscopic polyangiitis and its renal limited form (idiopathic necrotizing and crescentic glomerulonephritis) and Churg-Strauss Syndrome (3,4). ANCA detection has become a major hallmark, in particular, those classifying the cytoplasm of neutrophils in indirect immunofluorescent (IIF) assays (C-ANCA) and targeting proteinase-3 (anti-PR3) in enzyme linked immunosorbent assay (ELISA) are considered highly specific for Wegener's Granulomatosis (WG) and those antibodies observed in the perinuclear region of neutrophils in indirect immunofluorescent (IIF) assays (P-ANCA) which target myeloperoxidase (anti-MPO) in patients with microscopic polyangiitis (MPA), Idiopathic Necrotizing and Crescentic Glomerulonephritis (1,3,4).

However, multiple research on ANCA provided evidence that various subtypes of these autoantibodies are also being produced in patients with non-vasculitic diseases and infections such as invasive amoebiasis, infective endocarditis, malaria, atypical pneumonias, respiratory tract infections, Poststreptococcal glomerulonephritis, onchocerciasis, blastomycosis, chromomycosis and leptospirosis (5-13).

Mycobacterial infections such as leprosy (14) and Tuberculosis are known to induce autoantibodies and some of these antibodies are also known to be diagnostic markers for some other diseases such as WG and Churg-Strauss syndrome and it is not certain if such autoantibodies play a role in the pathogenesis of autoimmune disorders (15). Some studies point out to a new clinical issue of the potential phenotypic similarities between TB and WG and emphasize the poor ability of ANCA to distinguish between these two diseases (16). Because of the high prevalence of tuberculosis in Iran as well as similarities in clinical presentations between TB and WG, we designed a study to measure the prevalence of ANCA in patients with pulmonary TB.

## MATERIALS AND METHODS

**Patients with Tuberculosis.** Patients with pulmonary tuberculosis were those who besides clinical and radiological presentation of pulmonary tuberculosis had positive acid fast bacilli under direct microscopic observation with Ziehl-Neelsen staining of the sputum and became culture positive for *M. tuberculosis*.

**Control subjects.** 32 healthy age and sex matched individuals were tested for the presence of ANCA. None of them had a history of any underlying autoimmune diseases or chronic infectious ones. All the control subjects were health care workers of Alzahra hospital. Patients under 18 years and pregnant women who were culture negative for TB were also excluded. ANCA were detected in patients' sera by using a commercially available Indirect Immunofluorescent (IIF) assay on a substrate of ethanol-fixed human neutrophils (Binding Site, UK). Sera to be tested were diluted 1:20. When a positive result was obtained, further dilutions were made until fluorescence was no longer detectable. Results are expressed as the maximal dilution observed. The specificities of the antibodies were further identified by anti-Myeloperoxidase (anti-MPO) and anti

proteinase 3 (anti-PR3) ELISA, (Genesis UK). A value of <3.1 IU considered as negative, 3.1-4 IU as doubtful and >4 IU as positive.

**Statistical analysis.** To measure the association between ANCA status and tuberculosis, statistical significance was tested by Fisher exact test, t-test, Chi square and the calculation of the 95% confidence intervals. A p value less than 0.05 were considered statistically significant.

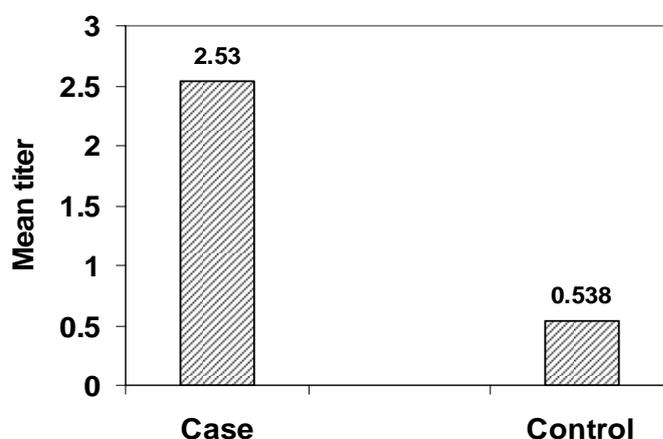
## RESULTS

The demographic characteristics of the cases and controls are summarized in Table 1. The cases and controls ranged from 30-50 years of age with a mean of 36.

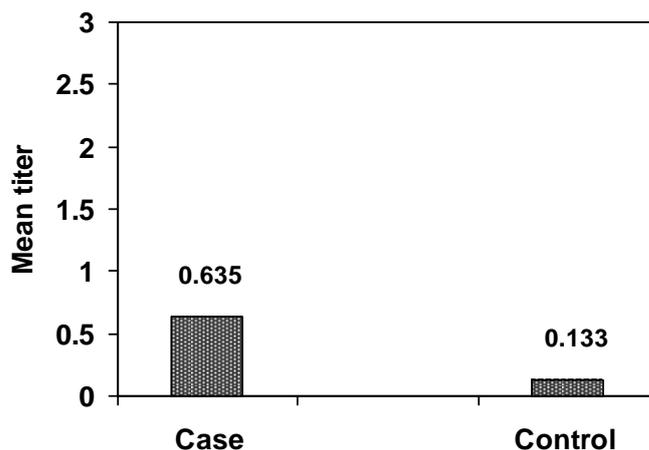
**Table 1. Demographic Characteristics of Cases and Controls.**

	Mean Age	Male Number (%)	Female Number (%)
Case (n=32)	35.44 ± 4.82	17(53.1)	15(46.9)
Control (n=32)	36.98 ± 3.41	17(53.1)	15(46.9)
Total	36.08 ± 3.44	34(53.1)	30(46.9)

Perinuclear patterns (P-ANCA) were detected in 25% of the cases and 6.25% of the controls, while cytoplasmic patterns (C-ANCA) were seen in 3.1% of both the cases and the controls. ANCA specificities revealed 75% anti-myeloperoxidase and 12.5% anti-proteinase 3 in the cases and the control had 3.12% anti-MPO and none had anti-PR3. The overall incidence of C-ANCA was 1.5% in TB patients while P-ANCA positivity was 25%. The control group showed 6.25% positivity for P-ANCA. In our study, the positive C-ANCA did not significantly correlate with tuberculosis ( $p < 0.018$ ). Positive Anti-MPO significantly correlated with TB ( $p > 0.01$ ) (Figure 1). But Anti-PR3 positivity do not correlated with tuberculosis ( $p = 0.851$ ) (Figure 2). Mean Anti-PR3 or the mean Anti-MPO titers were significantly different between the cases and the controls.



**Figure 1.** Comparison of the Mean Anti-MPO titer in cases and controls.



**Figure 2.** Comparison of Mean Anti-PR3 Titer in Cases and Controls.

## DISCUSSION

Tuberculosis (TB) is still one of the major causes of morbidity and mortality worldwide. Iranian data shows a prevalence of 27/100000 case/yr with a mortality rate of 2.6/100000 case/yr for TB in 2006 (17). ANCA, especially anti-proteinase 3 (PR3) and Anti-myeloperoxidase (MPO) have been used as diagnostic markers of primary systemic vasculitis. Antineutrophil cytoplasmic autoantibodies (ANCA's) directed to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) are strongly associated with the ANCA-associated vasculitides: Wegener's granulomatosis, microscopic polyangiitis, and its renal limited form, idiopathic necrotizing crescentic glomerulonephritis, and Churg-Strauss syndrome (18). In recent studies the positivity of ANCA'S subtypes in non-vasculitis diseases and infections has been shown (3,4,15, 16). The development of autoantibodies in TB patients could have a multifactorial etiology. The presence of T lymphocytes reactive to heat shock proteins (Hsp), and the important aim of immune response against certain intracellular auto antigens such as MPO from PMN, also the mechanism of molecular mimicry, could explain the association of ANCA and TB in patients with severe alterations of their immune response (19). Distinction between TB and WG can be difficult at disease onset, thereby conferring a potentially elementary differential diagnosis role for ANCA testing (20).

In our study, there were no correlation between C-ANCA and TB; however P-ANCA positivity significantly correlated with TB. Furthermore, it is observed that P-ANCA positivity together with anti-MPO specificity is commonly noticed in TB cases.

Pradhan et al., also have reported the presence of ANCA in 30% of TB patients. They detected P-ANCA in 52.4% and C-ANCA in 38.1% of patients with pulmonary TB and showed that 47.6% had anti-MPO and 28.6% had anti-PR3 (15). Teixeira et al., detected ANCA in 10% of the patients with TB including 4% C-ANCA and 6% P-ANCA, but only one IIF-negative specimen was anti-PR3 positive (16). In Contrast, Flores-Suarez et al. using IIF showed that ANCA's were present in 44.4% of the tuberculosis patients (16 C-ANCA, 4 P-ANCA), while and in 40% of patients showed anti-PR3 and anti-MPO (21).

Due to clinical similarities in presentation of Tuberculosis and Wegener's granulomatosis and the lack of specificity of ANCA for vasculitides disorders and also the presence of a high frequency of TB in our country, we suggest that:

- 1) Tuberculosis must be actively sought and excluded in ANCA positive patients especially if they are living in countries with a high prevalence of TB.
- 2) Treatment decisions should be focused on the clinical presentation of the patient (pre-test probability) and histological findings and not on the result of ANCA testing alone.
- 3) The diagnosis of WG should not be based on lung biopsy specimen until the special stains and cultures for the infection are obtained, because granulomatous infections of the lung (e.g mycobacteria or fungi) may also cause vasculitis and necrosis.
- 4) The use of tests to clinical situations with a rather high pretest probability for ANCA associated vasculitis should be restricted.
- 5) The application of ANCA testing as the only clinical diagnostic hallmark for vasculitides disorders to be regarded as controversial and has to be used properly in clinical settings.

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## REFERENCES

- 1 van der Woude FJ, Daha MR, van ES LA. The current status of neutrophil cytoplasmic antibodies. *Clin Exp Immunol.* 1989; 78:143-8.
- 2 Venning MC, Quinn A, Broomhead V, Bird AG. Antibodies directed against neutrophils (c-ANCA and p-ANCA) are of distinct diagnostic value in systemic vasculitis. *Q J Med.* 1990; 77:1287-96.
- 3 Schmitt WH, van der Woude FJ. Clinical application of antineutrophil cytoplasmic antibody testing. *Curr Opin Rheumatol.* 2004; 16:9-17.
- 4 Hoffman GS, Specks U. Antineutrophil cytoplasmic antibodies. *Bull Rheum Dis.* 1998; 47:5-8.
- 5 Gallin MY, Jacobi AB, Buttner DW, Schonberger O, Marti T, Eritmann KD. Human autoantibody to defensin: disease association with Hyperreaction onchocerciasis (sowda). *J Exp Med.* 1995; 182:41-7.
- 6 Pudifin DJ, Duursma J, Gathiram V, Jackson TF. Invasive amoebiasis is associated with the development of antineutrophil cytoplasmic antibody. *Clin Exp Immunol.* 1994; 97:48-51.
- 7 Subra JF, Michelet C, Laprote J, Carrere F, Reboul P, Cartier F, et al. The presence of cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA) in the course of subacute bacterial endocarditis with glomerular involvement, coincidence or association? *Clin Nephrol.* 1998; 49:15-8.
- 8 Yahya TM, Benedict S, Shalabi A, Bayoumi R. Antineutrophil cytoplasmic antibody (ANCA) in malaria is directed against cathepsin G. *Clin Exp Immunol.* 1997; 110:41-4.
- 9 Davenport A, Lock RJ, Wallington TB. Clinical relevance of testing for antineutrophil cytoplasmic antibodies (ANCA) with a standard indirect immunofluorescence ANCA test in patients with upper or lower respiratory tract symptoms. *Thorax.* 1994; 49:213-7.
- 10 Ardiles LG, Valderrama G, Moya P, Mezzano SA. Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic autoantibodies (ANCA) in poststreptococcal glomerulonephritis. *Clin Nephrol.* 1997; 47:1-5.
- 11 Stappaerts I, Bogers J, Ebo D, van den Broecke E, Stevens WJ, Van Marck E, et al. c-ANCA positivity in a Belgian patient with pulmonary paracoccidioidomycosis. *Eur Respir J.* 1997; 10:2419-22.
- 12 Galperin C, Shoenfeld Y, Gilburd B, Esterre P, Meroni PL, Del Papa N, et al. Anti-neutrophil cytoplasmic antibodies in patients with chromomycosis. *Clin Exp Rheumatol.* 1996; 14:479-83.
- 13 Constantin A, Marin F, Oksman F, Bouteiller G. Antineutrophil cytoplasmic antibodies in leptospirosis. *J Rheumatol.* 1996; 23:411.
- 14 Medina F, Camargo A, Moreno J, Zonana-Nacach A, Aceves-avila J, Fraga A. Anti-neutrophil cytoplasmic autoantibodies in leprosy. *Br J Rheumatol.* 1998; 37:270-3
- 15 Pradhan VD, Badakere SS, Ghosh K, Pawar AR. Spectrum of anti-neutrophil cytoplasmic in patients with pulmonary tuberculosis overlaps with that of Wegener's granulomatosis. *Indian J Med Sci.* 2004; 58:283-8
- 16 Teixeira L, Mahr A, Jauregui F, Noël LH, Nunes H, Lefort A, et al. low seroprevalence and poor specificity of antineutrophil cytoplasmic antibodies in tuberculosis. *Rheumatology(oxford).* 2005; 44:247-50
- 17 World Health Organization [online] 2010 [cited 2010 Apr 5]. Available from: URL: [http://apps.who.int/globalatlas/predefinedReports/TB/PDF\\_Files/irn.pdf](http://apps.who.int/globalatlas/predefinedReports/TB/PDF_Files/irn.pdf)

#### Rate of ANCA positivity in TB

- 18 Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin G, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. *Kidney Int.* 1998; 53:743-53.
- 19 Habegger de sorrentino A, Motta P, Iliovich E, Sorrentino AP. Anti-neutrophil cytoplasmic antibodies (ANCA) in patients with symptomatic and asymptomatic HIV infection. *Medicina (B Aires).* 1997; 57:294-8
- 20 Gordon C, Luqmani R, Fields P, Howie AJ, Emery P. Two cases of 'Wegner's tuberculosis'. *Br J Rhomatol.* 1993; 32:143-9
- 21 Flores-suárez LF, Cabiedes J, Villa AR, van der Woude FJ, Alcocer-varela J. Prevalence of antineutrophil cytoplasmic autoantibodies in patients with tuberculosis. *Rheumatology(oxford).* 2003; 42:223-9.