International Journal of Research in Medical Sciences
Chowdhery MKU et al. Int J Res Med Sci. 2026 Feb;14(2):525-530
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20260237

Original Research Article

Prevalence and diagnostic implications of anti-citrullinated peptide antibody in smear-positive pulmonary tuberculosis patients

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Received: 08 November 2025
Accepted: 19 January 2026

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ABSTRACT

Background: Generation of autoantibodies have been described in several infectious diseases including tuberculosis. Rheumatoid arthritis-specific autoantibodies: anti-citrullinated protein antibody presents in pulmonary tuberculosis, patient without evidence of cross-reactivity in tuberculosis bacilli antigens. To evaluate prevalence of ACPA positivity and its association with sputum smear acid-fast bacilli (AFB) grading in pulmonary tuberculosis patients with positive smear.

Methods: This descriptive, observational study was carried out during June 2020 to July 2021 at the Department of Rheumatology, Bangladesh Medical University and National Institute of Diseases of Chest and Hospital Dhaka. One hundred adult, smear-positive pulmonary tuberculosis (TB) patients were recruited by consecutive sampling. Levels of serum ACPA were detected by ELISA. Sociodemographic, clinical and laboratory measures were described with descriptive statistics and compared by chi-square tests.

Results: Positivity of ACPAs was found in 28.0% of patients, with 12.0% showing high levels of ACPA (>60 IU/ml). The median value in the serum ACPA positive patients was 66.0 IU/ml (IQR: 48.5–88.0). Positive-ACPA was also slightly more common in patients with sputum smear grade of 2+ to 3+ compared to that of the lower smearing grades, but without statistical significance (p=0.821).

Conclusions: A significant proportion of smear-positive pulmonary tuberculosis cases showed positivity to ACPA. These results indicated that ACPAs should be interpreted with caution in tuberculosis-endemic regions to prevent the misdiagnosis of inflammatory arthritis.

Keywords: Anti-citrullinated peptide antibody, Autoantibodies, Diagnostic implications, Pulmonary tuberculosis, Smear-positive tuberculosis

INTRODUCTION

According to the World Health Organization, tuberculosis is among the leading causes of death from a single infectious agent, with South-East Asia accounting for a substantial proportion of global cases.[1] Bangladesh is classified as a high tuberculosis burden country, where pulmonary tuberculosis continues to contribute significantly to morbidity, mortality and economic loss.[2] Smear-positive pulmonary tuberculosis patients represent the most infectious group and play a central role in disease transmission within the community.

Tuberculosis is characterized not only by localized pulmonary pathology but also by profound systemic immune activation. Chronic infection with *Mycobacterium tuberculosis* leads to sustained antigenic stimulation, which may disrupt immune tolerance and promote autoantibody production.[3]

Autoimmune phenomena have been increasingly reported in patients with active tuberculosis, including the presence of rheumatoid factor, antinuclear antibodies and other disease-associated autoantibodies.[4] These immunological alterations may complicate clinical interpretation, particularly when serological tests are used to support the diagnosis of autoimmune rheumatic diseases. Anti-citrullinated peptide antibody is a highly specific serological marker for rheumatoid arthritis and is widely used in early diagnosis, prognostication and disease classification.[5] ACPA targets citrullinated proteins generated through post-translational modification, a process closely linked to chronic inflammation. Although ACPA demonstrates high specificity for rheumatoid arthritis in most clinical settings, several studies have reported ACPA positivity in non-rheumatic conditions, including chronic infections such as tuberculosis.[6,7]

The detection of ACPA in tuberculosis patients raises important diagnostic concerns, especially in regions where both tuberculosis and inflammatory arthritis are prevalent. False-positive ACPA results may lead to misdiagnosis, unnecessary investigations or inappropriate initiation of immunosuppressive therapy, which could worsen underlying infection.[3] This issue is particularly relevant in resource-limited settings, where access to advanced imaging and specialist care may be restricted. Previous studies have reported variable prevalence of ACPA positivity among tuberculosis patients, ranging from low to moderately high proportions, depending on study population, assay type and disease severity.[7,8]

However, data from South Asian populations remain limited and few studies have explored the relationship between ACPA positivity and markers of disease burden, such as sputum smear acid-fast bacilli grading. Sputum smear grading reflects bacillary load and disease severity and may influence the degree of immune activation and autoantibody production.[9] Understanding the prevalence and clinical significance of ACPA positivity in smear-positive pulmonary tuberculosis patients is essential to improve diagnostic accuracy and guide appropriate clinical decision-making. In tuberculosis-endemic countries like Bangladesh, distinguishing infection-related autoantibody production from true autoimmune disease is particularly important for both rheumatologists and physicians involved in tuberculosis care.

METHODS

This study was conducted in Department of Rheumatology of BMU and NIDCH, Dhaka, Bangladesh from June, 2020 to July, 2021. A total of 100 sputum smear positive adult pulmonary tuberculosis patients of both genders were enrolled to this study by consecutive sampling method after taking informed written consent. Ethical clearance for the study was taken from the Institutional Review Board (IRB) of BMU as well as, Ethical Clearance Committee of BCPS. The clinical history and sociodemographic parameters such as, age, sex, monthly income, occupation, educational level, marital status, past history of contact with pulmonary tuberculosis patient, history of taking anti-TB drug and sputum smear AFB gradings were collected from enrolled cases.

Inclusion criteria

Adult patients aged 18 years and above. Patients of either sex, Patients diagnosed with sputum smear–positive pulmonary tuberculosis based on acid-fast bacilli microscopy. Patients enrolled before or within seven days of initiation of anti-tuberculosis therapy. Patients willing to participate in the study and providing written informed consent

Exclusion criteria

Patients with a previously diagnosed rheumatoid arthritis or other autoimmune rheumatic diseases. Patients receiving immunosuppressive or disease-modifying anti-rheumatic drugs. Patients with known chronic inflammatory or connective tissue disorders. Patients with extra-pulmonary tuberculosis only. Patients with severe concurrent medical illnesses that could affect immune status. Patients unwilling or unable to provide informed written consent.

Study procedure

At first patients briefed about the objectives of the study risks and benefits for participating in the study and confidentiality. Written informed consent obtained accordingly. Medical history and sociodemographic data were collected by face-to- face interview and recorded in a semi-structured data sheet. 10 ml venous blood collected from antecuevital vein and serum prepared for estimation of auto antibodies. Serum sample was collected from patient before or within 7days after starting anti TB treatment.

Blood collection procedure

Blood was collected before or within seven days of anti-TB treatment. With all aseptic precautions: Using a pressure calf, a suitable vein in the arm was located. The skin over the vein cleansed thoroughly, center to periphery (up to 5 cm) using spirit (ethanol-ether). After drying up, washed with povidone iodine or tincture iodine. 10 ml of blood was drawn with sterile 5cc disposable syringe and taken a sterile test tube. Using a lead pencil, each bottle was labeled with patient's name, ID no. and collection time and date; then sent for laboratory test. Those sample collected in NIDCH, was preserved at -20°C prior to laboratory test at BMU later on.

Ethical considerations

Ethical clearance for the study was taken from the Institutional Review Board (IRB) of both BSMMU and NIDCH as well as Ethical Clearance Committee of BCPS. The study population were thoroughly appraised about the nature, purpose and implication of the study as well as entire spectrum of benefits and risks of the study. Subjects were assured that about their confidentiality, anonymity and free do to refuge to participant and to withdraw them from the study at any time. The withdrawal from the study would not deprive the patients from their deserved medical services. Informed written consent from all the study subjects were taken free of duress and without exploiting any weakness of the subjects.

Assay procedure of anti-citrullinated peptide antibody by ELISA method

All reagents must be brought to room temperature (20-26°c) prior to beginning the assay. Place the required number of microwells/strips in the holder. Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor. Add 100 µl of the prediluted CCP 3.1 IgG/IgA ELISA Low Positive, the CCP 3.1 IgG/IgA ELISA High Positive, Calibrators B through E if desired, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.

Wash step

Thoroughly aspirate the contents of each well. Add 200-300 µl of the diluted HRP Wash buffer to all wells, then aspirate. Maintain the same sequence for the aspiration as was used for the sample addition. Add 100 µl of the HRP CCP 3.1 IgG/IgA conjugate to each well. Add 100 µl of TMB Chromogen to each well and incubate in the dark for 30 minutes at room temperature. Add 100 µl of HRP stop solution to each well. Read the absorbance (OD) of each well at 450 nm within one hour of stopping the reaction. If biochromatic measurements are desired, 620 nm can be used as a reference wavelength.

Data analysis

After cleaning and check, qualitative data was expressed in number and percentage. The quantitative data was presented in means and standard deviations. To determine the association, X^2 test was used for categorical variables where appropriate. A p value <0.05 was considered statistically significant. All analysis was performed using the SPSS software version 26.00.

RESULTS

Table 1 show that mean age of the participants was 42.81±16.30 years, ranging from 18 to 84 years. Of the total participants, 55 (55%) were male, with a male-to-female ratio of 1.2:1. The mean body mass index (BMI) was 19.10±2.09 kg/m² and 67 (67%) participants were underweight. The average monthly family income was 17,310±9,865 Bangladeshi Taka. Regarding occupation, 36 (36%) participants were service holders, while 32 (32%) were housewives. In terms of educational status, 33 (33%) participants were illiterate, whereas 18 (18%) had completed higher secondary education or above. A history of contact with a pulmonary tuberculosis patient was reported by 25 (25%) participants and 21 (21%) had previously received anti-tuberculosis treatment. The detailed demographic characteristics of the study participants are presented. ACPA was defected in 28% of study subjects. Among them twelve were only ACPA positive shown in Table 2. Among autoantibody-positive participants, the mean serum anti-CCP (ACPA) level was 68.70±25.40 IU/ml, with values ranging from 28 to 122 IU/ml. Estimation of median and interquartile range of serum ACPA level among autoantibody positive study subjects are shown in Table 3.

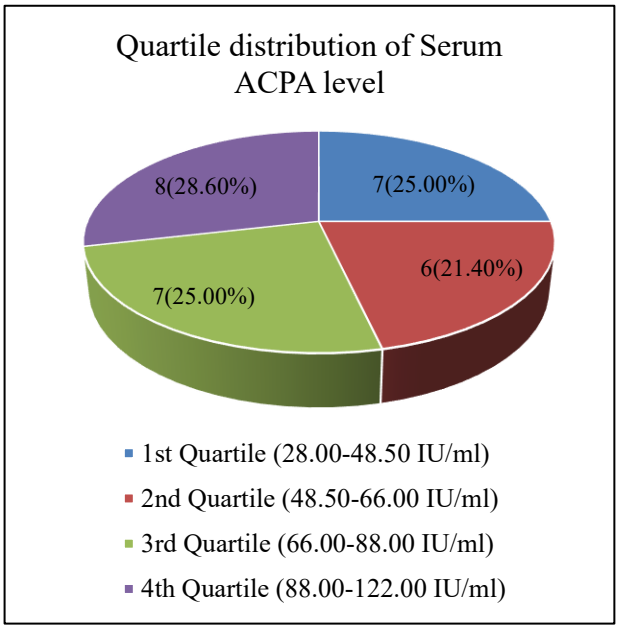

Figure 1: The quartile distribution of serum ACPA level among ACPA positive study subjects.

Shown in figure first quartile comprised patients with serum ACPA levels ranging from 28.00 to 48.50 IU/ml and included 7 patients (25.0%). The second quartile, representing ACPA levels between 48.50 and 66.00 IU/ml, included 6 patients (21.4%). The third quartile (66.00–88.00 IU/ml) also consisted of 7 patients (25.0%). The fourth quartile included patients with the highest ACPA concentrations, ranging from 88.00 to 122.00 IU/ml and comprised 8 patients (28.6%). Patients were considered ACPA positive when the serum ACPA level exceeded 20 IU/ml. Among patients with lower bacillary load (scanty to 1+ AFB grading, n=66), ACPA positivity was observed in 18 patients (27.30%). In contrast, a higher proportion of RF positivity was noted among patients with higher bacillary load (2+ to 3+ AFB grading, n=34), where 10 patients (29.40%) were ACPA positive. Although ACPA positivity appeared to be more frequent in patients with higher sputum smear grading, the association did not reach statistical significance (p=0.821). This finding suggests a trend toward increased ACPA positivity with increasing mycobacterial burden, but without a statistically significant correlation in the present study are shown in Table 4.

Table 1: Demographic characteristics of the study subjects.

Characteristics	Mean±SD	No. of patients (N)
Age (in years)	42.80±16.30	—
Sex		
Male	—	55
Female	—	45
Body weight		
Underweight	—	67
Normal	—	31
Overweight and above	—	2
BMI[b]	19.11±2.09	—
Marital status		
Married	—	86
Unmarried	—	14
Monthly family income (Tk.)	17,310±9,865	—
Occupational status		
Service	—	36
Housewife	—	32
Others[c]	—	32
Educational status		
Illiterate	—	33
Primary	—	26
Secondary	—	23
High secondary and above	—	18
Residence		
Rural	—	63
Urban	—	37
Past history of contact with smear-positive pulmonary tuberculosis patient	—	25
History of taking anti-TB	—	21

Table 2: Presence of ACPA either in single or in combination among study subjects.

Autoantibody/combination of autoantibodies	No. of patients (N)
Only ACPA positive	12
Total ACPA positive	28

Table 3: Estimation of median and interquartile range of serum ACPA level among autoantibody positive study subjects.

Serum autoantibody	Number of patients	Median (IU/ml)	IQR (IU/ml)
Serum ACPA	28	66.00	48.50-88.00

Table 4: Association of sputum smear for AFB gradings with serum autoantibody positivity among enrolled subjects.

Auto-antibodies status	Scanty to 1+AFB gradings (n=66)	2+ to 3+ AFB gradings (n=34)	P value
Serum ACPA	18 (27.30)	10 (29.40)	0.821

DISCUSSION

The findings demonstrate that a considerable proportion of patients with active pulmonary tuberculosis exhibit ACPA positivity, highlighting important diagnostic and clinical implications in tuberculosis-endemic regions. A total of 100 sputum smear positive adult pulmonary tuberculosis patients of both genders were enrolled to this study by consecutive sampling method after taking informed written consent. Ethical clearance for the study was taken from the IRB of BMU as well as, Ethical Clearance Committee of BCPS. The clinical history and sociodemographic parameters such as, age, sex, monthly income, occupation, educational level, marital status, past history of contact with pulmonary tuberculosis patient, history of taking anti-TB drug and sputum smear AFB gradings were collected from enrolled cases.

In current study, 28% of study subjects were ACPA positive. Elkayam et al, found 32 % of active pulmonary tuberculosis patients were ACPA positive, which was nearly consistent with present study.[10] Contrary to this finding, lower frequency (6.7%) of ACPA positivity was reported by Mori et al, in pulmonary tuberculosis patients.[11] In this study, mean serum ACPA level was 68.70±25.40 IU/ml (28-122 IU/ml), among ACPA positive study subjects. Contrary to this findings, lower mean serum ACPA value (15.40±72.40 IU/ml) was reported by Mori et al.[11] This difference in the prevalence and level of serum ACPA in tuberculosis patients between two studies were not clear. One possible explanation may be a difference in genetic and racial backgrounds of these two patient populations.

For example, a strong association of ACPA production with HLA-DRB1 alleles carrying a shared epitope has been reported and an association of ACPA levels with polymorphisms in the peptidyl arginine deaminase 4 (PAD4) has been reported in Japanese and Korean population, but no such study has not yet done in Bangladesh.[12,13] Further studies on genetic backgrounds of the investigated populations will be required to clarify the significance of ACPA in conditions other than RA. Another possible explanation for the discrepancy in ACPA prevalence and mean were due to the different assay kits used to detect serum ACPA. In our study, we used a commercially available anti-CCP3 assay kit from Inova Diagnostics, while Mori et al, used an anti-CCP2 assay kit from Axis Shield Diagnostics (cut off value for ACPA positivity were >20 IU/ml and >5 IU/ml respectively).[11] One study has reported some discrepancies in sensitivity between manufacturers.[14] For that reason, standardization of anti-CCP kits and of the definition of a positive results will be needed for each ethnic group. Tuberculosis causes chronic inflammation and excessive tissue destruction, which ultimately leads to cellular apoptosis and protein citrullination in lung.[15] Thus, the citrullinated proteins in inflammatory tissue may play an important role to produce ACPA as a result of autoimmune response. Therefore, ACPA may be produced in tuberculosis even without arthritis. Kakumanu et al showed, among ACPA positive pulmonary tuberculosis patients in most cases, the ACPA reactivity were citrulline independent.[16] For that reason, a positive ACPA test in such patient carefully interpreted with care.

According to American College of Rheumatology (ACR), serum ACPA level more than three times the upper limit of normal (level >60 IU/ml) was considered high serum ACPA level. In present study, among ACPA positive study subjects, 57% of total observations of serum ACPA value were within high serum ACPA level. Though 46.40% of ACPA positive study participants complaints of arthralgia, none of them fulfil the features of inflammatory arthritis. These are the subjects, who may be a candidate for longitudinal follow up regarding to determine the outcome of ACPA positivity.[17,18] The findings of this study are particularly relevant for rheumatologists and physicians involved in tuberculosis care. In resource-limited settings, reliance on serological markers without adequate clinical correlation may contribute to diagnostic errors.

Several authors have suggested repeating autoantibody testing after completion of anti-tuberculosis therapy, as declining antibody levels may help differentiate infection-related autoimmunity from true autoimmune disease.[8] The single-center design and relatively small sample size may limit generalizability. Longitudinal follow-up was not conducted to assess changes in ACPA levels following anti-tuberculosis treatment. Additionally, assays to differentiate citrullinated from non-citrullinated peptide reactivity were not performed, which may have provided further insight into antibody specificity.

CONCLUSION

This study found that anti-citrullinated peptide antibody positivity was present in a considerable proportion of smear-positive pulmonary tuberculosis patients, including cases with high antibody titers. These findings suggest that ACPA production may occur as a consequence of infection-related immune activation rather than true autoimmune disease. No significant association was observed between sputum smear acid-fast bacilli grading and ACPA positivity. In tuberculosis-endemic settings, positive ACPA results should be interpreted with caution

to avoid misdiagnosis and inappropriate immunosuppressive therapy. Further longitudinal studies are needed to clarify the clinical relevance of ACPA positivity following tuberculosis treatment.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. World Health Organization. Global tuberculosis report 2023. Geneva: World Health Organization. 2023.
2. Hossain S, Quaiyum MA, Zaman K, Banu S, Husain MA. Tuberculosis control in Bangladesh: progress, challenges and future directions. J Infect Public Health. 2020;13(7):973–81.
3. Elkayam O, Yaron M, Caspi D. Tuberculosis and autoimmune diseases. Autoimmun Rev. 2006;5(3):174–8.
4. Kroot EJ, Hazes JM, Colin EM, Dolhain RJ, van Leeuwen MA, van Rijswijk MH, et al. Rheumatoid factor, anti-citrullinated peptide antibodies and prognosis in rheumatoid arthritis. Ann Rheum Dis. 2000;59(11):884–9.
5. van der Helm-van Mil AH, Huizinga TW, de Vries RR, Toes RE. Emerging patterns of risk factor make-up enable subclassification of rheumatoid arthritis. Arthritis Rheum. 2005;52(6):1728–35.
6. Yamada R, Suzuki A, Chang X, Yamamoto K. Anti-citrullinated protein antibody production is enhanced in chronic infectious diseases. Rheumatology (Oxford). 2007;46(8):1179–83.
7. Kakumanu P, Yamagata H, Sobel ES, Reeves WH, Satoh M. Patients with pulmonary tuberculosis are frequently positive for anti-cyclic citrullinated peptide antibodies, but their sera also react with uncitrullinated peptides. Arthritis Rheum. 2009;60(6):1576–81.
8. Singal R, Goyal S, Sharma S, Singla P. Autoantibody profile in patients with active pulmonary tuberculosis. Int J Rheum Dis. 2013;16(5):546–9.
9. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011;378(9785):57–72.
10. Elkayam O, Gat A, Lidgi M, Segal R, Yaron M, Caspi D. Atypical cutaneous findings in a patient with systemic lupus erythematosus. Lupus. 2003;12(6):413–7.
11. Mori S, Naito H, Ohtani S, Yamanaka T, Sugimoto M. Diagnostic utility of anti-cyclic citrullinated peptide antibodies for rheumatoid arthritis in patients with active lung tuberculosis. Clin Rheumatol. 2009;28(3):277–83.
12. Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D, et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum. 2005;52(11):3433–8.
13. Cha S, Choi CB, Han TU, Kang CP, Kang C, Bae SC. Association of anti-cyclic citrullinated peptide antibody levels with PADI4 haplotypes in early rheumatoid arthritis and with shared epitope alleles in very late rheumatoid arthritis. Arthritis Rheum. 2007;56(5):1454–63.
14. García-Berrocal B, González C, Pérez M, Navajo JA, Moreta I, Dávila C, et al. Anti-cyclic citrullinated peptide autoantibodies in IgM rheumatoid factor-positive patients. Clin Chim Acta. 2005;354(1–2):123–30.
15. Makrygiannakis D, af Klint E, Lundberg IE, Löfberg R, Ulfgren AK, Klareskog L, et al. Citrullination is an inflammation-dependent process. Ann Rheum Dis. 2006;65(9):1219–22.
16. Kakumanu P, Yamagata H, Sobel ES, Reeves WH, Chan EK, Satoh M. Patients with pulmonary tuberculosis are frequently positive for anti-cyclic citrullinated peptide antibodies, but their sera also react with unmodified arginine-containing peptide. Arthritis Rheum. 2008;58(6):1576–81.
17. Lima I, Santiago M. Antibodies against cyclic citrullinated peptides in infectious diseases: a systematic review. Clin Rheumatol. 2010;29(12):1345–51.
18. Hayashi N, Kawamoto T, Mukai M, Morinobu A, Koshiba M, Kondo S, et al. Detection of antinuclear antibodies by enzyme immunoassay using nuclear HEp-2 cell extract and recombinant antigens: comparison with immunofluorescence assay. Clin Chem. 2001;47(9):1649–59.

Cite this article as: Chowdhery MKU, Sultan MI, Islam MN, Zaman MSI, Begum M, Rajan S, et al. Prevalence and diagnostic implications of anti-citrullinated peptide antibody in smear-positive pulmonary tuberculosis patients. Int J Res Med Sci 2026;14:525-30.