

Monaldi Archives for Chest Disease

eISSN 2532-5264 https://www.monaldi-archives.org/

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Monaldi Arch Chest Dis 2026 [Online ahead of print]

To cite this Article:
Parmar SK, Prem G, Parmar I, et al. **Latent tuberculosis infection in patients with rheumatoid arthritis receiving conventional synthetic disease-modifying antirheumatic drugs: a prospective cross-sectional study from the Himalayas**. *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2026.3953

Submitted: 18-02-2026
Accepted: 1-09-2026

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Latent tuberculosis infection in patients with rheumatoid arthritis receiving conventional synthetic disease-modifying antirheumatic drugs: a prospective cross-sectional study from the Himalayas

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Conflict of interest: the authors declare that they have no competing interests.

Ethics approval and consent to participate: approved by the Institutional Ethics Committee, Indira Gandhi Medical College, Shimla (IGMC/IEC/2023-142; 10 May 2023).

Informed consent: written informed consent was obtained from all participants.

Patient consent for publication: not applicable.

Availability of data and materials: the de-identified dataset is available from the corresponding author on reasonable request, subject to institutional policies.

Declaration of generative artificial intelligence and artificial intelligence-assisted technologies in the writing process: during the course of the work, the authors used ChatGPT 4.0 for the language editing and reference formatting. The authors reviewed and edited all the output and take full responsibility for the content of the publication.

Abstract

Rheumatoid arthritis (RA) and its treatments increase the susceptibility to tuberculosis (TB), mainly in TB-endemic settings. Latent TB infection (LTBI) screening is mandatory before biologic disease-modifying antirheumatic drugs. However, data on LTBI among RA patients receiving conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), particularly from north India, are limited. Tuberculin skin testing (TST) may underestimate LTBI in patients with RA because of impaired cell-mediated immunity and reduced delayed-type hypersensitivity responses.

In this prospective cross-sectional study, to exclude active TB, 146 consecutive adults with RA (ACR/EULAR 2010 criteria) receiving csDMARDs for ≥3 months underwent Mantoux TST (2 tuberculin units purified protein derivative; reading at 48-72 hours) and chest radiography . Using the conventional cut-off (≥10 mm), LTBI prevalence was 8.2% [12/146; 95% confidence interval (CI) 4.3-13.2%]. Using an immunosuppression-adjusted threshold (≥5 mm), prevalence was 23.3% (34/146; 95% CI 17.8-29.4%). The frequency of 0 mm TST induration was high (76.7%; 112/146). According to univariate analysis, LTBI positivity was linked to higher C-reactive protein (CRP), diabetes, fasting glucose ≥100 mg/dL, current smoking, smoking index >200, and urban residency. In multivariable logistic regression (outcome defined as ≥5 mm), smoking index >200 [adjusted odds ratio (OR) 11.47; 95% CI 1.52-86.6; p=0.018] and higher CRP (adjusted OR 1.036 per mg/L; 95% CI 1.006-1.070; p=0.018) remained independently associated with LTBI.

Among RA patients on csDMARDs in a north Indian tertiary center, LTBI prevalence ranged from 8.2% (≥10 mm) to 23.3% (≥5 mm). Application of the ≥5 mm TST threshold substantially increased the estimated LTBI prevalence. The high proportion of participants with 0 mm TST induration suggests reduced TST responsiveness in this population. Further studies incorporating interferon-γ release assays are needed to determine the optimal LTBI screening strategy in patients with rheumatoid arthritis receiving csDMARD therapy.

Key words: latent tuberculosis infection, rheumatoid arthritis, tuberculin skin test, anergy, methotrexate, smoking.

Introduction

Tuberculosis remains a major public health problem in India and globally.The World Health Organization (WHO) continues to identify India as among the highest-burden countries with Tuberculosis [1]. Immune sensitization to Mycobacterium tuberculosis antigens without radiographic or clinical signs of active disease is known as latent TB infection (LTBI) [2]. Cell-mediated immunity is a key component of inflammatory rheumatic diseases. The treatments of rheumatoid arthritis and its impairment significantly increases the risk of reactivation of TB [3,4]. Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with chronic inflammation, immune dysregulation, and excess infection risk [5,6]. Immunomodulatory therapy is often necessary for the management of RA in the modern era. Tumor necrosis factor (TNF)-targeted biologics have been shown to produce the strongest TB signal, which is in line with TNF's crucial function in maintaining granulomas [3,7]. In India, csDMARDs are commonly used for a long time, and even though biologics are the most known risk, patients may become eligible for biologics after years of csDMARD exposure, comorbidity, and advanced age.

LTBI screening in RA is challenging. The tuberculin skin test (TST) is inexpensive and accessible but can be falsely negative in immunosuppressed states because of diminished delayed-type hypersensitivity (anergy) [8,9]. In RA patients, high rates of 0 mm induration have been reported and have been associated with methotrexate dose and steroid exposure [8]. Interferon-gamma release assays (IGRA) can mitigate some TST limitations, but cost and availability restrict routine use in many Indian settings [10].

Prospective data describing LTBI prevalence and clinical correlates among csDMARD-treated RA patients from North India are limited. This study aimed to estimate LTBI prevalence by TST using two clinically relevant induration thresholds (≥10 mm and ≥5 mm) and to examine factors associated with LTBI in an RA population receiving csDMARDs.

Although previous Indian studies have evaluated LTBI screening among patients with rheumatoid arthritis, most have focused on patients being evaluated before initiation of biologic therapy or have included mixed treatment populations receiving different immunosuppressive regimens. Data specifically evaluating patients receiving only conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) from North India remain limited. In addition, few studies have compared LTBI prevalence using both conventional (≥10 mm) and

immunosuppression-adjusted (≥5 mm) TST thresholds in this population. The present study was undertaken to address these knowledge gaps.

Primary objective: to estimate the point prevalence of LTBI among RA patients receiving csDMARDs using TST ≥10 mm.

Secondary objectives: (1) To estimate an immunosuppression-adjusted LTBI prevalence using TST ≥5 mm. (2) To describe the distribution of TST induration and quantify the proportion with 0 mm TST induration and (3) To explore demographic and clinical factors associated with LTBI in this population.

Materials and Methods

Study design, setting, and ethics

This was a prospective hospital based cross sectional observational study conducted in the Departments of Pulmonary Medicine and Rheumatology at Indira Gandhi Medical College (IGMC), Shimla, Himachal Pradesh, India, between May 2023 and May 2024. Consecutive eligible patients were prospectively recruited during the study period. It was Reported by following the STROBE guidance for observational studies [11]. Institutional Ethics Committee approval was obtained for the study (IGMC/IEC/2023-142; 10 May 2023). Written informed consent was taken from all participants.

Participants

Participants were consecutive adults (≥18 years) with RA who met the ACR/EULAR 2010 classification criteria and had been taking csDMARDs for at least three months. Patients with active tuberculosis, a history of anti-tubercular therapy, a current systemic corticosteroid use equivalent to prednisone ≥5 mg/day, pregnancy, or the use or planned initiation of biologic or targeted synthetic DMARDs were excluded.

A participant flow diagram has been added showing the enrollment and analysis of all included participants. The number of patients screened before eligibility assessment was not prospectively recorded.

Sample size

The required sample size was estimated using the formula for estimating a single population proportion, $n = Z^2P(1 - P)/d^2$, assuming an expected LTBI prevalence of 20.4% based on the Indian study by Agarwal et al., a 95% confidence level, and an absolute precision of 6.5%, yielding a minimum sample size of 146 participants. Consecutive eligible patients attending the Rheumatology outpatient clinic during the 12 month study period were recruited until the target sample size was achieved. The planned sample size was also considered feasible based on the expected outpatient volume and the ability to complete standardized TST administration and reading at 48 to 72 hours for all enrolled participants. The analysis was primarily intended for prevalence estimation, with exploratory analyses of factors associated with LTBI.

Data collection

Demographics, residence (urban/rural), TB exposure history, comorbidities (diabetes, hypertension) smoking status, and smoking index (cigarettes per day × years; heavy smoking defined as >200) were recorded using a well-structured pro forma. The RA factors recorded were the length of the disease, the csDMARD class (methotrexate, sulfasalazine, leflunomide), and the duration of methotrexate treatment. Fasting blood glucose and CRP were determined during the same visit or the latest result obtained within one month, if appropriate.

Tuberculin skin test and chest radiography

Mantoux TST was performed using 0.1 mL containing 2 TU purified protein derivative (PPD) injected intradermally into the volar forearm. Induration was measured transversely at 48–72 hours [9]. Erythema was not measured for the analysis. For analysis, TST was categorized as 0 mm, 1–9 mm, or ≥10 mm. The LTBI prevalence was measured using (a) ≥10 mm as the conventional threshold and (b) ≥5 mm as an immunosuppression-adjusted threshold used in many rheumatology screening contexts. The ≥10 mm threshold was used as the conventional criterion for TST positivity in the general adult population. The ≥5 mm threshold was selected because several international recommendations for LTBI screening in immunocompromised individuals, including patients with rheumatoid arthritis being evaluated before biologic therapy, consider an induration of ≥5 mm as positive. Since patients with rheumatoid arthritis receiving

csDMARDs may have impaired cell mediated immunity and reduced TST responsiveness, both thresholds were evaluated to estimate the potential effect of diagnostic cut-off selection on LTBI prevalence. All participants underwent chest radiography (PA view) to exclude active pulmonary TB.

Statistical analysis

Data were analysed in IBM SPSS v25.0. Categorical variables were summarized as n (%) and continuous variables as mean ± SD or median (IQR) as appropriate. Univariate comparisons used chi-square/Fisher's exact tests for categorical variables and independent-samples t tests for continuous variables. A binary logistic regression model (forward stepwise likelihood ratio) was fitted to identify variables independently associated with LTBI when LTBI was defined as TST ≥5 mm. Candidate predictors included age, sex, residence, smoking variables, comorbidities, fasting glucose, CRP, and methotrexate exposure duration. Results are presented as adjusted odds ratios (aOR) with 95% confidence intervals (CI). Statistical significance was set at $p<0.05$.
Variables demonstrating clinical relevance or a significant association in univariable analysis were considered for inclusion in the multivariable logistic regression model. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test and Nagelkerke R^2. Given the limited number of LTBI-positive events, the multivariable analysis was considered exploratory. Potential multicollinearity was minimized by avoiding inclusion of clinically overlapping variables in the same model.

Results

Participant characteristics

The study included 146 participants; 122 (83.6%) were female. Mean age was 53.1 ± 12.6 years. Most participants were from rural areas (117/146; 80.1%). Methotrexate was the most used csDMARD (127/146; 87.0%) with median weekly dose 15 mg and mean exposure duration 3.62 ± 4.67 years. Diabetes mellitus was present in 13 (8.9%) and hypertension in 13 (8.9%). Current smoking was reported by 9 (6.2%) and ex-smoking by 12 (8.2%).

TST distribution and LTBI prevalence

TST induration was 0 mm in 112/146 (76.7%), 1–9 mm in 22/146 (15.1%), and ≥10 mm in 12/146 (8.2%). Using the ≥10 mm cut-off, LTBI prevalence was 8.2% (12/146; 95% CI 4.3–13.2%). When an immunosuppression-adjusted threshold of ≥5 mm was applied, LTBI prevalence increased to 23.3% (34/146; 95% CI 17.8–29.4%).

At enrollment, all study participants were deemed clinically stable. Additionally, there was no evidence among study participants regarding any constitutional symptoms that might indicate active TB, as all the chest X-rays were read as normal, thereby minimizing the likelihood of active pulmonary tuberculosis at enrollment. Figure 1 presents the distribution of TST induration categories.

Factors associated with LTBI

In univariate analyses (LTBI defined as ≥10 mm), LTBI positivity was more frequent among current smokers, individuals with smoking index >200, participants with diabetes, those with fasting glucose ≥100 mg/dL, those living in urban areas, and those with higher CRP. Mean CRP was higher among LTBI-positive participants than LTBI-negative participants (22.10 ± 16.99 mg/L vs 12.63 ± 12.19 mg/L).

In multivariable logistic regression using the ≥5 mm threshold (34 events), heavy smoking (smoking index >200) and higher CRP remained independently associated with LTBI (Tables 1 and 2).

Discussion

This prospective cross-sectional research showed a relatively low prevalence of LTBI based on the application of the TST threshold of ≥10 mm (8.2%), but there was an increase in the estimated LTBI prevalence when a lower immunosuppression-adjusted threshold of ≥5 mm was used (23.3%). One interesting thing about the study findings is that about 76.7% of the participants did not demonstrate any TST results. The high proportion of participants with 0 mm TST induration suggests reduced TST responsiveness in this population. However, true TST anergy cannot be confirmed because neither IGRA nor complementary immunological assessment nor a non immunosuppressed control group was included [8].

Interpretation of prevalence estimates

A prevalence of 8.2% using ≥10 mm is lower than several RA populations screened before biologic therapy, where TST-based LTBI prevalence commonly ranges around 10–30% depending on population risk and the degree of immunosuppression [8,12,13]. In North India, Agarwal et al. observed that only 20.4% of RA patients were TST positive. Even lower positivity was seen in those who had used steroid treatment recently [13]. The lower prevalence in the current study likely reflects (i) exclusion of steroid users; (ii) differences in background TB exposure and/or health-seeking patterns in Himachal Pradesh; and (iii) imperfect sensitivity of TST in RA.

Application of the ≥5 mm threshold increased the estimated prevalence of LTBI from 8.2% to 23.3%, demonstrating the influence of the diagnostic threshold on prevalence estimates in immunosuppressed individuals. However, because IGRA or another reference standard was not performed, it cannot be concluded that the higher estimate more accurately reflects the true prevalence of LTBI [2,10]. The prior Bacillus Calmette-Guérin (BCG) vaccination must also be taken into account during the analysis of these results. Childhood BCG vaccination usually causes a decline in TST positivity over time, yet positive TSTs may still be recorded when a lower threshold is used. As BCG vaccination details were not available for the current study, the possible influence of vaccination on the prevalence of LTBI could not be established.

Interpretation and study limitations

The prospective design, standardized TST administration, and systematic exclusion of clinically apparent pulmonary tuberculosis strengthen the internal validity of the present study. Nevertheless, the absence of IGRA remains an important limitation because studies from India have demonstrated improved LTBI detection when TST and IGRA are used together. However, the cost, limited accessibility, and occurrence of indeterminate IGRA results continue to restrict their routine use in many resource-constrained settings [10].

Smoking and inflammation as associated factors

Heavy smokers were defined as individuals with a smoking index greater than 200. Heavy smoking has been independently associated with LTBI positivity with an adjusted odds ratio of 11.47. However, due to the large confidence intervals, one can deduce that there is a small

number of heavy smokers included in the pilot study. In addition, only 34 people fulfilled the≥ 5 mm criterion of LTBI, which did not allow defining many predictors in multivariate model. Therefore, these regression findings should be interpreted as exploratory and hypothesis generating rather than confirmatory. From a mechanistic perspective, smoking acts as a TB risk factor via its impact on airway defenses and immune responses. Smoking is also a known risk factor for developing respiratory infections [14]. However, care should be taken in interpreting this result due to its precision and the extent to which residual confounding may play a role in this assessment. Nevertheless, these findings suggest that smoking may represent an important factor for evaluation in future studies investigating LTBI risk among patients with rheumatoid arthritis.

In univariate analysis, higher levels of CRP were significantly associated with LTBI positivity. However, since CRP is non-specific inflammatory marker, the correlation should not be interpreted as causative relationship. High levels of CRP could indicate other types of systemic inflammation rather than latent tuberculosis infection [5,6]. Further studies incorporating IGRA and longitudinal follow-up are required to clarify the biological basis of this association.

Diabetes and metabolic risk

Diabetes mellitus and Impaired fasting glucose were found to be significantly correlated with LTBI in univariate analysis, but not in multivariate analysis. The lack of significance might be due to insufficient power, the presence of collinearity between these factors, and some other confounders, but not necessarily due to the lack of association. DM is a known risk factor for TB infection and progression to TB [15], and further larger prospective studies should be done to determine the association between LTBI and DM in RA patients.

There were many factors affecting TST reactivity which were not assessed in the current study, such as previous TB exposure, RA disease activity, cumulative immunosuppressant therapy, nutrition, and cell mediated immunity.. These variables may have influenced TST responses and should be incorporated into future studies together with IGRA testing.

Potential clinical implications

The study population is broadly representative of patients receiving csDMARD therapy at tertiary rheumatology centers in India. Although these findings are exploratory, they may help

inform the design of future prospective studies evaluating LTBI screening strategies in tuberculosis-endemic settings [3,7].

Although these findings should not be used to modify current screening recommendations, they suggest that further evaluation of lower TST thresholds in immunosuppressed patients with rheumatoid arthritis may be worthwhile, particularly in tuberculosis endemic settings. Future prospective studies incorporating both TST and IGRA are required before any changes to LTBI screening strategies can be recommended.

Strengths and limitations

Strengths of this study are: prospective study enrollment, uniform TST method, reading window [9], active TB disease exclusion by symptom screen and chest radiograph and multivariable modeling. The major limitations are: no IGRA testing, single-center study, possible selection bias of tertiary care referral, and statistical imprecision (large confidence intervals) due to the small number of LTBI outcomes, especially for the ≥10 mm cutoff. The multivariable model accounted for a small proportion of outcome variation (Nagelkerke R^2 = 0.17), and results should be interpreted as associations rather than as causal predictors.

Information regarding cumulative corticosteroid exposure before study enrollment was not available and therefore could not be evaluated. Active tuberculosis was excluded using symptom screening and chest radiography. However, extrapulmonary tuberculosis could not be completely excluded because routine microbiological or advanced imaging investigations were not performed.

The relatively small number of LTBI positive events may have resulted in model instability and wide confidence intervals, particularly for smoking index. Disease activity indices such as DAS28 were not available for analysis. Consequently, the influence of rheumatoid arthritis disease activity on TST responsiveness could not be evaluated.

Conclusions

In this prospective cross sectional study of patients with rheumatoid arthritis receiving csDMARDs in North India, LTBI prevalence was 8.2% using the conventional TST threshold (≥10 mm) and 23.3% using an immunosuppression adjusted threshold (≥5 mm). The high proportion of participants with 0 mm TST induration suggests reduced TST responsiveness in this

population. In the absence of IGRA, the optimal TST threshold cannot be determined. These findings support further evaluation of lower TST thresholds and combined TST and IGRA based screening strategies in larger prospective studies, particularly before initiation of biologic therapy.

List of abbreviations

ACR - American College of Rheumatology

anti-CCP - anti–cyclic citrullinated peptide

bDMARD - biologic disease-modifying antirheumatic drug

CI - confidence interval

CRP - C-reactive protein

csDMARD - conventional synthetic disease-modifying antirheumatic drug

IGRA - interferon-gamma release assay

LTBI - latent tuberculosis infection

MTX - methotrexate

OR - odds ratio

PPD - purified protein derivative

RA - rheumatoid arthritis

TB - tuberculosis

TNF - tumor necrosis factor

TST - tuberculin skin test

WHO - World Health Organization

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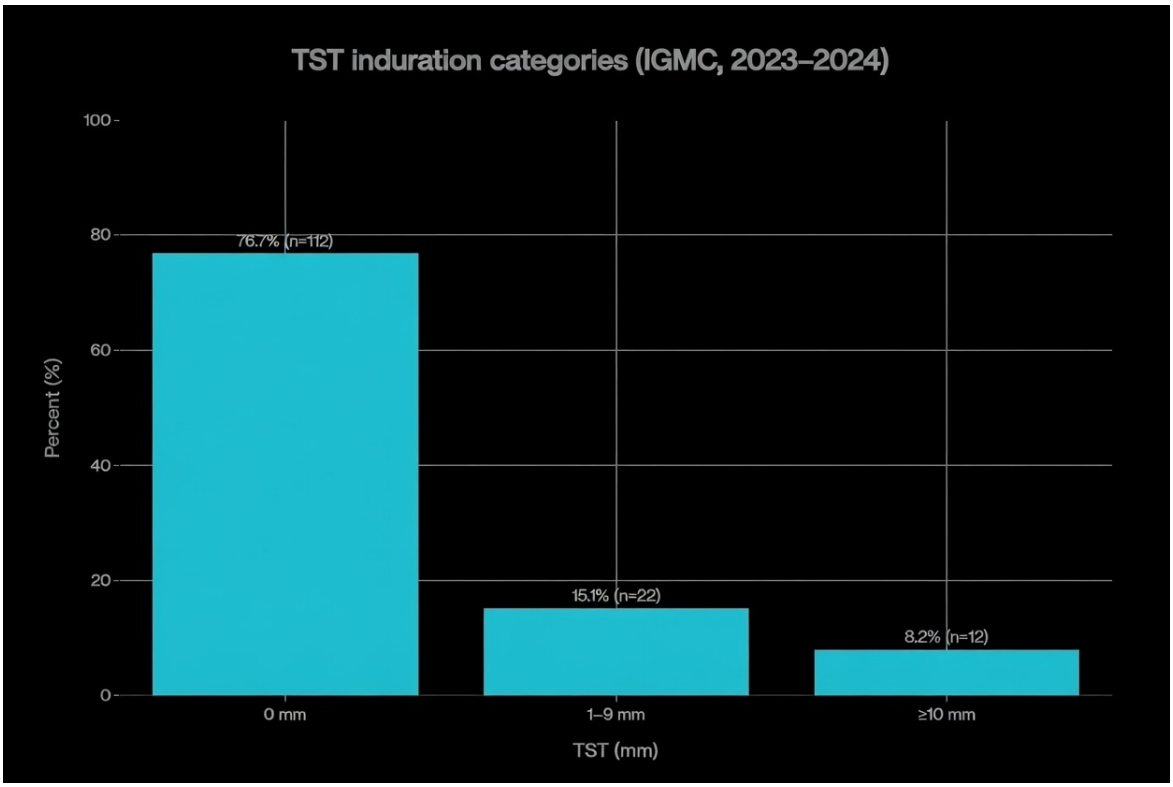


Figure 1. Distribution of TST induration categories.

Table 1. TST categories and LTBI prevalence (n=146)

TST category	n (%)
0 mm	112 (76.7)
1–9 mm	22 (15.1)
≥10 mm	12 (8.2)
LTBI (≥10 mm)	12/146 (8.2)
LTBI (≥5 mm)	34/146 (23.3)

Table 2. Multivariable logistic regression for LTBI (TST ≥5 mm).

Predictor	aOR	95% CI	p-value
Smoking index >200	11.47	1.52–86.6	0.018
CRP (per 1 mg/L increase)	1.036	1.006–1.070	0.018

Model diagnostics: Nagelkerke R^2 0.17; Hosmer–Lemeshow p=0.32.