RESEARCH ARTICLE

Elevated TLR4 as Potential Biomarker for RMT-positive Subclinical Tuberculosis

Riski Irawan Putra Priyono[1,2,*], Kusworini Handono[3], Susanthy Djajalaksana[4,5], Dewi Santosaningsih[5,6]

[1]Doctoral Program in Medical Sciences, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia
[2]Faculty of Medicine, Universitas Islam Al-Azhar, Jl. Unizar No. 20 Turida, Mataram 83237, Indonesia
[3]Department of Clinical Pathology, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia
[4]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia
[5]Dr Saiful Anwar Hospital, Jl. Jaksa Agung Suprapto No.2, Malang 65112, Indonesia
[6]Department of Clinical Microbiology, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia

*Corresponding author. Email: riskyrisa2019@student.ub.ac.id

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Abstract

BACKGROUND: Subclinical tuberculosis (TB) is often underdiagnosed due to the limited sensitivity of sputum-based diagnostics. Host-response biomarkers, particularly pattern recognition receptors (PRRs), offer a potential alternative. Toll-like receptor 4 (TLR4), scavenger receptor class B type 1 (SR-B1), and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) are involved in the early recognition of *Mycobacterium tuberculosis* and may reflect initial immune activation under conditions of low bacillary burden and absent clinical symptoms. However, their diagnostic value in subclinical TB remains unclear. Therefore, this study was conducted to investigate their potential as biomarkers for subclinical TB.

METHODS: Eighty-eight asymptomatic adults with a radiographic suspicion of pulmonary TB were classified into rapid molecular test (RMT)-positive and RMT-negative groups based on GeneXpert MTB/Rifampicin (RIF) results, which served as the reference standard. Blood samples were collected from the subjects, and their serum levels of TLR4, SR-B1, and DC-SIGN were measured using enzyme-linked immunosorbent assay (ELISA).

RESULTS: TLR4 levels were significantly higher in the RMT-positive group (p=0.011), whereas SR-B1 and DC-SIGN showed no significant differences. TLR4 was the only biomarker with strong correlation with subclinical TB status (r=0.861, p<0.001). Based on logistic regression results, TLR4 was identified as the superior predictor with an area under the curve (AUC) of 0.937, 91.3% sensitivity, and 89.8% accuracy. Combining SR-B1 and DC-SIGN with TLR4 did not materially improve diagnostic performance over the TLR4-only model.

CONCLUSION: TLR4 is a promising biomarker associated with RMT-positive status among individuals with suspected subclinical TB, with strong diagnostic performance. Patients with both RMT-positive results and elevated TLR4 levels may require closer monitoring for potential progression to active disease.

KEYWORDS: subclinical tuberculosis, PRRs, TLR4, SR-B1, DC-SIGN, tuberculosis biomarker

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Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and remains one of the leading global public health problems. The World Health Organization (WHO) estimates that approximately 25% of the world's population has been infected, with more than 1.23 million deaths occurring annually. Indonesia ranks second among the countries with the highest TB burden

worldwide, after India, contributing approximately 10% of total global cases.(1–3) These conditions underscore that TB continues to pose a serious threat to public health and human resource productivity.

The major challenge in TB control lies in the subclinical phase of the disease, which represents an intermediate state within the TB spectrum between latent infection and active symptomatic disease. Subclinical TB refers to individuals with evidence of *M. tuberculosis* infection in the absence of overt clinical symptoms, often accompanied by radiographic abnormalities. This phase is particularly concerning because affected individuals may contribute to ongoing transmission while remaining undetected by conventional symptom-based screening approaches.(4–6) In addition, subclinical TB is frequently characterized by low bacillary burden (paucibacillary disease), which reduces the sensitivity of sputum-based diagnostic methods. This difficulty is further pronounced in high-risk groups such as children and individuals with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), who are often unable to produce sputum for examination, as well as those with metabolic disorders such as diabetes, which can alter clinical presentation and complicate traditional diagnostic pathways.(7,8)

Several biomarkers have been investigated to improve the detection of subclinical TB, particularly those reflecting early host immune responses. These include pro-inflammatory cytokines (*e.g.*, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-6), acute-phase proteins such as C-reactive protein (CRP), and transcriptional signatures associated with incipient TB. More recently, attention has shifted toward pattern recognition receptors (PRRs), which are involved in the early recognition of *M. tuberculosis* and the initiation of innate immune responses. Among these, toll-like receptor 4 (TLR4), scavenger receptor class B type 1 (SR-B1), and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) have been implicated in host–pathogen interactions, including mycobacterial recognition, internalization, and modulation of immune signaling pathways. The selection of TLR4, SR-B1, and DC-SIGN in this study is based on their potential relevance in the early phase of TB infection, particularly under conditions of low bacillary load where conventional diagnostic methods are less sensitive. These receptors may reflect subtle immunological changes that occur prior to symptom onset, thereby offering potential utility as biomarkers for subclinical TB. Furthermore, their roles in macrophage and dendritic cell function suggest that they may provide insights into host immune status in populations at high risk for subclinical disease. An immunological biomarker–based approach using non-sputum, serum-based biomarkers offer a promising and potentially useful complementary strategy. PRRs are components of the innate immune system that play a crucial role in pathogen recognition, including *M. tuberculosis*.(9,10)

Measuring these receptors in their soluble form in serum provides a direct window into the host's systemic immune activation before clinical symptoms manifest. Among the PRRs that have been extensively studied, we selected three specific PRRs based on their distinct roles in TB pathogenesis. TLR4 has been proposed as a key receptor involved in the immune response to TB through recognition of mycobacterial antigens. TLR4 activation is also associated with various mechanisms employed by *M. tuberculosis* to evade the innate immune system.(11) SR-B1 is known to play a role in the transcytosis of *M. tuberculosis* through microfold cells in the respiratory tract. This receptor recognizes early secreted antigenic target 6 kDa (ESAT-6) secreted by *M. tuberculosis* and facilitates pathogen entry into the host.(12) DC-SIGN is a C-type lectin receptor expressed on dendritic cells that plays a role in the recognition of *M. tuberculosis* and modulation of host immune responses. In addition to serving as a bridge between innate and adaptive immunity, DC-SIGN has also been identified as a potential target in TB vaccine development.(13)

These biomarkers have considerable potential as biological indicators for the detection of subclinical TB. However, PRRs, including TLR4, SR-B1, and DC-SIGN, are not entirely specific to *M. tuberculosis*, as they may also be activated by other pathogens. Moreover, although several host immune biomarkers have been investigated in active and latent TB, their roles in subclinical TB remain poorly understood. In particular, there is a lack of studies that specifically evaluate the expression and diagnostic performance of these PRRs in individuals with subclinical TB, especially in the context of low bacillary burden and absent clinical symptoms. Given these limitations, there is increasing interest in developing non-sputum-based biomarkers that may assist in identifying subclinical TB, particularly in asymptomatic individuals with radiological suspicion. In this context, host immune biomarkers are not intended to replace microbiological confirmation methods such as GeneXpert MTB/RIF, but rather to serve as triage or adjunctive (add-on) diagnostic tools to improve case detection in settings where conventional methods are insufficient. Based on this rationale, the present study

addressed this research gap by specifically investigating the roles of TLR4, SR-B1, and DC-SIGN in subclinical TB, a population that remains underexplored in previous studies. Furthermore, this study evaluated the combined diagnostic performance of these biomarkers through an exploratory derivation analysis, including sensitivity, specificity, and predictive values, both individually and in combination, to assess their potential utility as a non-sputum-based screening approach.

Methods

Study Design and Subjects

This analytic observational study employed a cross-sectional design at Mataram City Regional Public Hospital (RSUD Kota Mataram), Indonesia, from January to December 2025. The study population comprised patients with suspected subclinical pulmonary TB based on chest radiography findings. Study subjects were selected using a consecutive sampling method. Inclusion criteria included patients aged >18 years, absence of respiratory symptoms, radiological features suggestive of pulmonary TB, complete medical records, and provision of written informed consent to participate. The minimum sample size was determined using G*Power with logistic regression analysis and increased by 10%, resulting in a required sample of 82 subjects.

Subject Screening and Radiological Assessment

All subjects without respiratory symptoms underwent chest radiography (posteroanterior view) as an initial screening procedure. Radiological findings suggestive of pulmonary TB included infiltrates, nodular opacities, cavitary lesions, and fibrotic changes, particularly in the upper lung zones (Supplementary 1). The radiographs were independently interpreted by two certified radiologists to ensure diagnostic reliability, and any discrepancies were resolved through consensus discussion.

Microbiological Examination

Respiratory specimens were obtained either through sputum induction or bronchoscopy with bronchial lavage, following standard clinical protocols. The GeneXpert MTB/RIF assay (Rapid Molecular Test/RMT) was used as the reference standard to detect *M. tuberculosis* DNA. Subjects were then classified into two groups based on this gold standard: RMT-positive (subclinical TB) and RMT-negative. However, it is acknowledged that a negative RMT result does not exclude subclinical TB, particularly in paucibacillary cases, and may introduce potential misclassification.

Comorbidity Assessment

Diabetes status was determined by measuring glycated hemoglobin (HbA1c) using the Ion-Exchange High-Performance Liquid Chromatography (IE-HPLC) method on an Arkray Adams A1c HA-8180T analyzer (Arkray Inc., Kyoto, Japan). Following aseptic venipuncture, blood samples were collected in EDTA tubes and analyzed for HbA1c levels. In accordance with American Diabetes Association (ADA) guidelines, diabetes was defined as an HbA1c level of ≥6.5%. Supplementary 2 showed representative results of HPLC chromatogram of the Hb1Ac measurement.

HIV screening was performed using the Chemiluminescent Immunoassay (CLIA) method to detect HIV p24 antigen and antibodies against HIV-1/HIV-2 via a two-site sandwich assay on a Mindray CL-1000i system (Mindray Bio-Medical Electronics Co., Shenzhen, China). Serum was separated from whole blood through centrifugation at 3000 rpm for 15 minutes. Results were interpreted based on the Cut-off Index (COI), where a COI ≥ 1.00 was considered positive for HIV infection. All procedures were conducted with rigorous calibration and quality control protocols.

Measurement of Pattern Recognition Receptors (PRRs)

Peripheral venous blood samples were collected under aseptic conditions. All blood samples were processed within 2 hours after venipuncture to preserve sample integrity. Serum was separated by centrifugation at 3000 rpm for 10 minutes and aliquoted into sterile microtubes.

The serum samples were stored at −80°C until analysis. To minimize protein degradation, repeated freeze–thaw cycles were avoided, and each sample underwent a maximum of one freeze–thaw cycle prior to biomarker measurement. Serum levels of PRRs-related biomarkers (TLR4, SR-B1, and DC-SIGN) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturers' instructions. The kits used were: TLR4 (Cat. No. E-EL-H5820; Elabscience, Wuhan, China) with detection range of 6.5–400 pg/mL and sensitivity of 3.75 pg/mL, SR-B1 (Cat. No. EH1791; FineTest, Boulder, CO, USA) with detection range of 0.313–20 ng/mL and sensitivity of 0.188 ng/mL, and DC-SIGN (Cat. No. KIT102000; SinoBiological, Beijing, China) with detection range of 78.13–5000 pg/mL and sensitivity of 5.6 pg/mL. The analytical performance of the

assays, including detection range and limit of detection, was defined according to the manufacturers' specifications. The intra-assay and inter-assay coefficients of variation were within acceptable limits (<10%), indicating good assay precision. All samples were analyzed in duplicate, and the final concentration for each biomarker was calculated as the mean of the two measurements. If the coefficient of variation between duplicate measurements exceeded 15%, the assay was repeated. Laboratory personnel performing the ELISA assays were blinded to the RMT (GeneXpert) status of the subjects to minimize measurement bias.

Statistical Analysis

Statistical analysis was performed using univariate methods to describe the distribution of variables. Categorical characteristics were compared using chi-square or Fisher's exact tests. Data normality was assessed via the Shapiro–Wilk test, which indicated non-normal distributions (p<0.001). Consequently, differences in PRRs concentrations and their associations with subclinical TB were evaluated using the Mann–Whitney U test and Spearman's correlation, respectively. Diagnostic potential was examined through multivariable logistic regression. Prior to modeling, multicollinearity was ruled out using Variance Inflation Factor (VIF) values. Model stability and generalizability were validated using a 10-fold cross-validation procedure. Discriminatory performance was determined via Receiver Operating Characteristic (ROC) curve analysis to calculate the Area Under the Curve (AUC), sensitivity, specificity, and accuracy at optimal cut-off points.

Results

Baseline Characteristics of Study Participants

A total of 101 potentially eligible participants were initially identified. Of these, 13 individuals were excluded due to not meeting the inclusion criteria (n=9), incomplete data (n=3), and death prior to analysis (n=1). Consequently, 88 participants with radiological findings suggestive of subclinical pulmonary TB were included in the final analysis. All included participants underwent PRRs biomarker testing and were subsequently evaluated using RMT as the reference standard. Based on the RMT results, 46 participants were classified as RMT-positive, while 42 participants were classified as RMT-negative (Figure 1).

The majority of the 88 study subjects were classified as having subclinical TB, had a history of contact with TB patients, were aged ≥40 years, were male, had normal nutritional status, and had no comorbidities. A history of contact was the only variable significantly associated with the occurrence of subclinical TB (p<0.001) (Table 1).

Association of PRRs with Subclinical TB

TLR4 concentrations in the RMT-positive group were significantly higher than those in the RMT-negative group (p=0.011). SR-B1 and DC-SIGN concentrations were slightly lower in the RMT-positive group than in the RMT-negative group, but these differences were not statistically significant (Table 2).

Correlation analysis further showed that TLR4 had a strong and statistically significant association with subclinical TB status (Spearman's r=0.861; p<0.001), whereas SR-B1 and DC-SIGN demonstrated weak and non-significant correlations (r=0.047, p=0.662; and r=0.125, p=0.242, respectively). Overall, these results indicate that TLR4 may have greater potential as a predictor variable in diagnostic modelling for subclinical TB than SR-B1 and DC-SIGN (Table 2).

Diagnostic Potential of PRRs Biomarkers

Before proceeding to further statistical analyses, a normality test was performed on the PRRs biomarker data to evaluate whether the variables followed a normal distribution. This step was essential to determine the most appropriate analytical approach, particularly whether parametric or non-parametric methods should be applied in subsequent

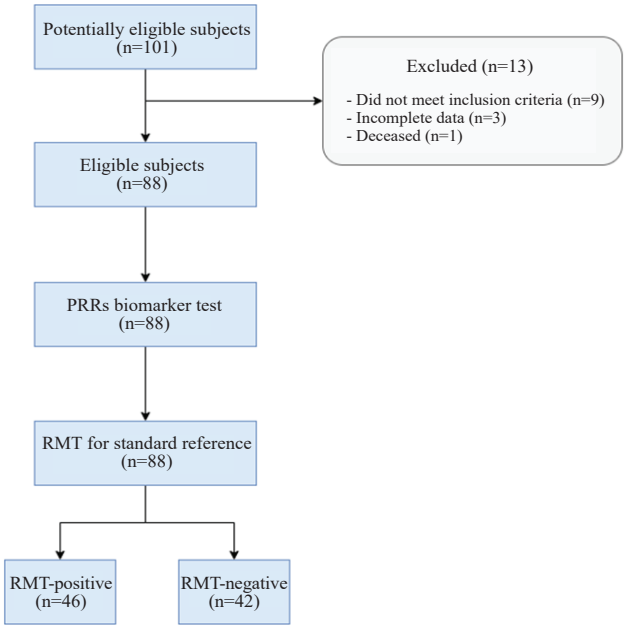

Figure 1. STARD-aligned flow diagram of participant selection and analysis.

Table 1. Characteristics of research subjects regarding subclinical TB.

Variable	Categories	RMT-positive (n)	RMT-negative (n)	*p*-value
Age	Adult (18–40 years)	15	10	0.361
	Older (>40 years)	31	32	
Sex	Male	31	27	0.759
	Female	15	15	
Nutritional status	Undernutrition (BMI <18.5 kg/m^2)	20	17	0.776
	Normal (BMI 18.5-24.9 kg/m^2)	26	25	
Contact history	With contact	44	11	0.000*
	No contact	2	31	
HIV	HIV positive	9	5	0.326
	HIV negative	37	37	
Diabetes Mellitus	DM	15	12	0.682
	Non-DM	31	30	

Categorical variables were compared using the chi-square test or Fisher's exact test. *Significant if $p<0.05$.

analyses. In the context of assessing the diagnostic potential of PRRs biomarkers, the distributional characteristics of the data may influence both the choice of statistical tests and the interpretation of relationships among variables.

The results of the Shapiro–Wilk normality test indicated that all PRRs biomarkers, namely TLR4, SR-B1, and DC-SIGN, were not normally distributed, with *p*-values of less than 0.001 for all variables. The relatively low Shapiro–Wilk statistics, 0.3767 for TLR4, 0.2866 for SR-B1, and 0.1942 for DC-SIGN, further support the presence of substantial deviation from normality. These findings suggest that the distributions of the PRRs biomarker variables differ significantly from a normal distribution and should therefore be interpreted using statistical methods appropriate for non-normally distributed data. To further assess the suitability of the variables for multivariable analysis, a multicollinearity test was subsequently conducted.

Multicollinearity testing was performed across all candidate logistic regression models to evaluate the degree of correlation among predictors prior to model estimation. For the single-predictor models, VIF values were not applicable because multicollinearity cannot be assessed when only one independent variable is included. In the two-predictor and three-predictor models, the VIF values ranged from 10.002 to 10.034 for TLR4, SR-B1, and DC-SIGN. Based on the assessment criterion applied in this study, these values were interpreted as indicating no problematic multicollinearity, suggesting that the included predictors could be entered simultaneously into the multivariable logistic regression models without substantial distortion of coefficient estimates. Following the assessment of distributional characteristics and collinearity among predictors, logistic regression analysis was conducted to examine the diagnostic contribution of each PRRs biomarker, both individually and in combination.

As shown in Table 3, TLR4 emerged as the only biomarker consistently associated with RMT status across all fitted logistic regression models. In the univariable model, TLR4 was a significant predictor, with a coefficient of 0.1818, a *p*-value of less than 0.001, and an odds ratio of 1.1994 (95% CI: 1.1149 to 1.2904), indicating that each one-unit increase in TLR4 was associated with an approximately

Table 2. Relationship of PRRs with subclinical TB.

Biomarker	RMT-positive (Mean±SD)	RMT-negative (Mean±SD)	Mann-Whitney *p*-value	Spearman r value	Spearman *p*-value
TLR4	113.94±10.51	89.53±11.53	0.011*	0.861	<0.001*
SR-B1	20.82±5.93	20.90±5.25	0.628	0.047	0.662
DC-SIGN	4.30±1.28	4.38±1.48	0.223	0.125	0.242

Mann-whitney analysis was performed to analyze mean difference between RMT-positive and RMT-negative. Meanwhile, Spearman's correlation test was performed to examine the association between PRR levels and subclinical TB status. *Significant if $p<0.05$.

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Table 3. Logistic regression analysis results.

Model	Term	Coefficient	*p*-value	Odds Ratio	95% CI	Events	Non-events	Exact Model Equation
TLR4	Intercept	-18.462	<0.001*	—	—	46	42	logit(P(TCM=1)) = -18.4620 + 0.1818(TLR4)
	TLR4	0.1818	<0.001*	11.994	1.1149–1.2904			
SR-B1	Intercept	0.1459	0.860	—	—	46	42	logit(P(TCM=1)) = 0.1459 – 0.0026(SR-B1)
	SR-B1	-0.0026	0.945	0.9974	0.9250–1.0754			
DC-SIGN	Intercept	0.2827	0.692	—	—	46	42	logit(P(TCM=1)) = 0.2827 – 0.0442(DC-SIGN)
	DC-SIGN	-0.0442	0.778	0.9567	0.7034–1.3013			
TLR4 + SR-B1	Intercept	-190.722	<0.001*	—	—	46	42	logit(P(TCM=1)) = -19.0722 + 0.1836(TLR4) + 0.0213(SR-B1)
	TLR4	0.1836	<0.001*	12.015	1.1152–1.2944			
	SR-B1	0.0213	0.730	10.215	0.9051–1.1529			
TLR4 + DC-SIGN	Intercept	-180.098	<0.001*	—	—	46	42	logit(P(TCM=1)) = -18.0098 + 0.1842(TLR4) - 0.1595(DC-SIGN)
	TLR4	0.1842	<0.001*	12.022	1.1163–1.2948			
	DC-SIGN	-0.1595	0.526	0.8526	0.5208–1.3959			
SR-B1 + DC-SIGN	Intercept	0.3534	0.749	—	—	46	42	logit(P(TCM=1)) = 0.3534 - 0.0032(SR-B1) – 0.0449(DC-SIGN)
	SR-B1	-0.0032	0.933	0.9968	0.9243–1.0750			
	DC-SIGN	-0.0449	0.775	0.9561	0.7026–1.3009			
TLR4 + SR-B1 + DC-SIGN	Intercept	-184.786	<0.001*	—	—	46	42	logit(P(TCM=1)) = -18.4786 + 0.1852(TLR4) + 0.0156(SR-B1) – 0.1500(DC-SIGN)
	TLR4	0.1852	<0.001*	12.035	1.1164–1.2974			
	SR-B1	0.0156	0.800	10.157	0.9001–1.1462			
	DC-SIGN	-0.15	0.555	0.8607	0.5229–1.4166			

19.9% increase in the odds of RMT. This association remained stable after adjustment for SR-B1 and DC-SIGN in the multivariable models, with odds ratios ranging from 1.2015 to 1.2035 and all *p*-values remaining below 0.001. In contrast, SR-B1 and DC-SIGN were not significantly associated with RMT, either as single predictors or when included in combination models, as reflected by *p*-values greater than 0.05 and confidence intervals that included 1. These findings indicate that, among the evaluated PRRs biomarkers, TLR4 demonstrated the strongest and most consistent diagnostic potential, whereas SR-B1 and DC-SIGN did not provide meaningful independent predictive value in the current dataset.

To further evaluate the robustness and generalizability of the logistic regression models, a 10-fold cross-validation procedure was performed for each biomarker model and biomarker combination. Model performance was assessed using the area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). This validation step was intended to determine whether the observed diagnostic performance remained consistent across different data partitions and to identify the most stable predictive model.

The TLR4-only model demonstrated the most consistently strong predictive performance. It achieved a mean AUC of approximately 0.93 (ranging from 0.75 to 1.00) and a mean accuracy of approximately 0.90. In sharp contrast, the SR-B1 and DC-SIGN models, when used alone, showed poor and unstable performance, with mean AUCs of 0.29 and 0.47, respectively (Table 4). These results indicate that TLR4 is the only single biomarker with substantial diagnostic utility for classifying RMT status.

Models combining multiple biomarkers also achieved high performance, provided they included TLR4. While the full model (TLR4 + SR-B1 + DC-SIGN) produced the highest mean AUC of approximately 0.93, this improvement over the TLR4-only model was marginal and did not result in higher mean accuracy. Furthermore, the poor performance of the SR-B1 + DC-SIGN combination (Mean AUC ~0.25) confirms that the absence of TLR4 substantially reduces model discrimination. Overall, these findings suggest that the addition of SR-B1 and DC-SIGN provides little meaningful improvement over the use of TLR4 alone.

To further assess the discriminatory performance of each biomarker model, receiver operating characteristic (ROC) curve analysis was performed for all single- and multi-biomarker combinations. The ROC curves presented in Figures 2 and 3 provide a visual comparison of the diagnostic ability of each model, while Table 5 summarizes the corresponding AUC values, 95% confidence intervals, optimal cut-off points, and classification metrics. This analysis was conducted to identify the most accurate and clinically relevant PRRs-based model for subclinical TB diagnosis.

As illustrated in Figures 2 and Figure 3, models that included TLR4 consistently demonstrated superior discrimination, with ROC curves located near the upper-left corner of the plot, indicating strong sensitivity-specificity trade-offs. In contrast, the ROC curves for SR-B1, DC-SIGN, and the SR-B1 + DC-SIGN combination remained close to the diagonal reference line, suggesting limited discriminatory capacity. Visually, the TLR4-only model and the models combining TLR4 with SR-B1 and/or DC-SIGN showed highly similar ROC profiles, indicating that the addition of the other biomarkers did not materially improve overall discrimination beyond that achieved by TLR4 alone.

As shown in Table 5, the TLR4 model achieved the highest standalone diagnostic performance, with an AUC of 0.9369 (95% CI: 0.8879 to 0.9858), an optimal cut-off of 0.5119, an accuracy of 0.8977, a sensitivity of 0.913,

Table 4. Cross validation analysis of all parameters.

Biomarker Model	Mean AUC	Mean Accuracy	Performance Description
TLR4	0.93	0.90	Strong & Robust: The most reliable single predictor
SR-B1	0.29	0.38	Poor: Unstable and low diagnostic utility
DC-SIGN	0.47	0.50	Limited: Low discriminatory ability
TLR4, SR-B1	0.92	0.85	High: Similar to TLR4 but with lower accuracy
TLR4, DC-SIGN	0.93	0.88	High: Comparable to the TLR4-only model
SR-B1, DC-SIGN	0.25	0.42	Poor: Performance drops significantly without TLR4
TLR4, SR-B1, DC-SIGN	0.93	0.84	Marginal: Highest AUC but no accuracy gain over TLR4

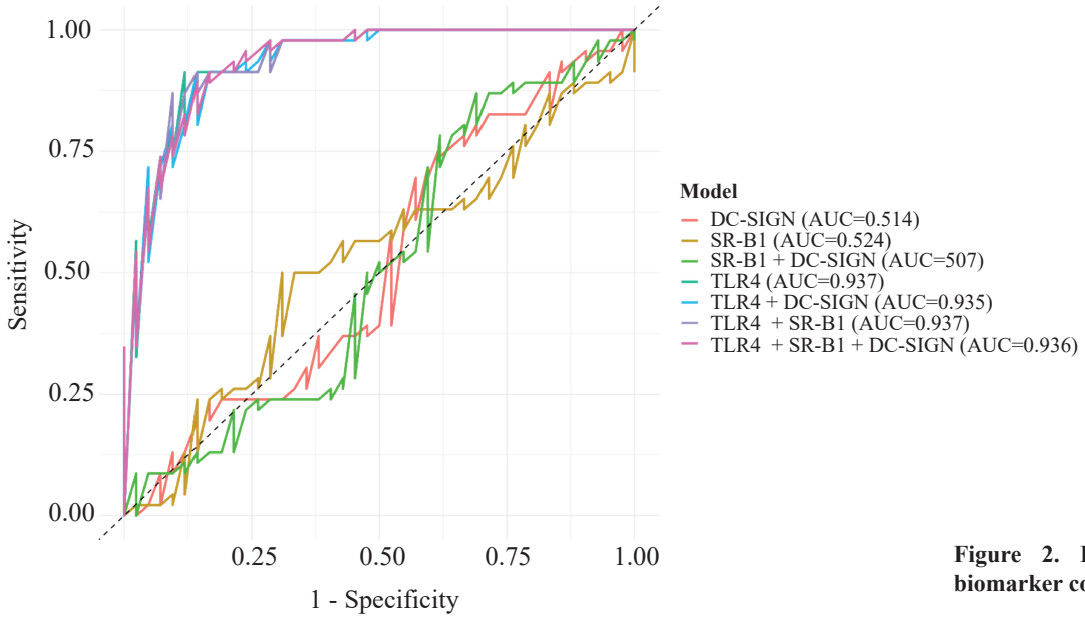

Figure 2. ROC curves for all biomarker combinations.

and a specificity of 0.881. The combined TLR4 + SR-B1 model yielded an identical AUC of 0.9369, but with slightly lower accuracy (0.8864) and sensitivity (0.8696), although specificity was marginally higher (0.9048). Similarly, the TLR4 + DC-SIGN model and the full TLR4 + SR-B1 + DC-SIGN model also showed excellent performance, with AUC values of 0.9348 and 0.9363, respectively, but neither model demonstrated a clear improvement over TLR4 alone. These findings suggest that adding SR-B1 and DC-SIGN to TLR4 provided little additional diagnostic benefit.

By contrast, SR-B1 alone, DC-SIGN alone, and the SR-B1 + DC-SIGN combination exhibited poor discriminatory performance, with AUC values of 0.5241, 0.5145, and 0.5072, respectively. Although DC-SIGN showed relatively high sensitivity (0.7609) and the SR-B1 + DC-SIGN model showed even higher sensitivity (0.8696), their low specificity values of 0.3810 and 0.3095 indicate poor ability to correctly classify non-subclinical TB cases. Overall, these results demonstrate that TLR4 was the dominant biomarker for subclinical TB discrimination and that it offered the best balance between sensitivity and specificity. Taken together, the ROC analysis supports TLR4 as the most promising PRRs biomarker and the most parsimonious model for the diagnosis of subclinical TB in the present study.

Discussion

Demographic factors, including age, sex, and nutritional status, were not significantly associated with subclinical TB in this study. This is consistent with longitudinal data from South Africa.(14) Although aging is biologically linked to immunosenescence (15,16), and male predominance is often associated with external risks like smoking (16,17), our findings align with global meta-regressions and studies in South Korea indicating that these factors may be context-dependent and do not always reflect the subclinical spectrum (4,14-23). Furthermore, the prevalence of normal nutritional status among our subjects supports the concept that subclinical TB often involves a low bacillary burden that has not yet triggered systemic inflammation or weight loss.(18,19,22)

In contrast to demographic traits, a history of contact was significantly associated with subclinical TB. The majority of subjects with positive RMT results had documented exposure, reinforcing epidemiological evidence that household contacts face a high risk of latent infection. (24-26) Specifically, studies in Semarang have shown that prolonged contact duration significantly increases the risk of TB progression (26), highlighting the importance of targeted screening in high-exposure groups regardless of clinical symptoms.

Regarding comorbidities, HIV and diabetes mellitus did not show a significant association with subclinical TB in this cohort. While global literature emphasizes HIV as a major driver of TB progression through class of differentiation (CD)4 dysfunction (27-30) and diabetes mellitus as a factor increasing risk by 20–60% via macrophage and T-lymphocyte impairment (31,32), the lack of significance here may reflect the early subclinical phase. At this stage, severe immunological impairment may not yet be manifest, or the population size may limit the detection

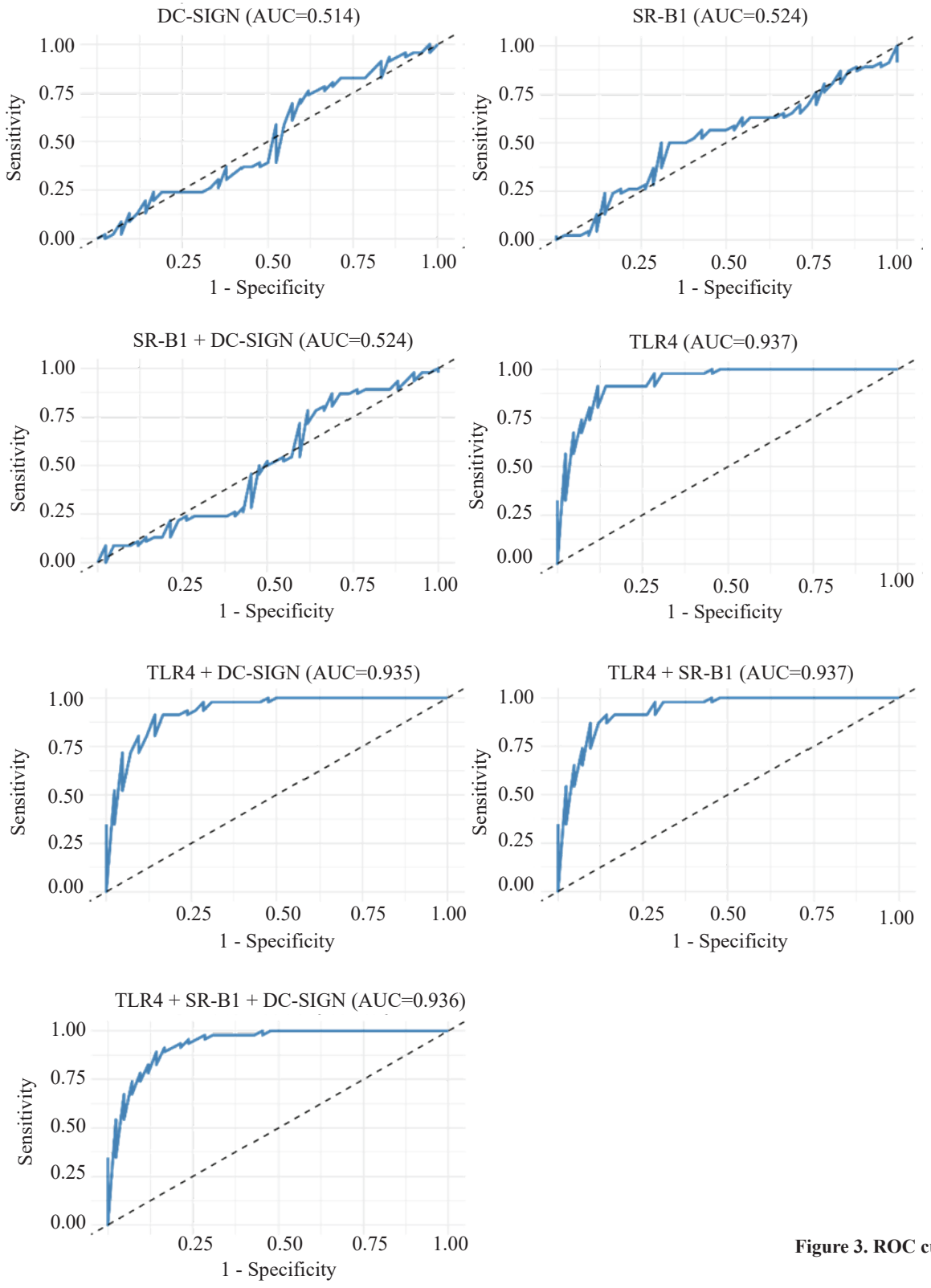

Figure 3. ROC curve per model.

of these well-established associations. Biomarker level analysis in this study suggests the presence of innate immune activation in individuals with subclinical TB. These findings indicate that measurable host immune responses may occur even in the absence of overt clinical symptoms. However, these observations should be interpreted cautiously, as the underlying immunological mechanisms cannot be fully established from this cross-sectional analysis.

Table 5. Comparison of PRRs in subclinical TB diagnosis.

Model	AUC	AUC 95% CI	Cut-off	Accuracy	Sensitivity	Specificity	PPV	NPV
TLR4	0.9369	0.8879–0.9858	0.5119	0.8977	0.9130	0.8810	0.8936	0.9024
TLR4 + SR-B1	0.9369	0.8880–0.9857	0.5458	0.8864	0.8696	0.9048	0.9091	0.8636
TLR4 + SR-B1 + DC-SIGN	0.9363	0.8876–0.9850	0.4829	0.8750	0.8913	0.8571	0.8723	0.8780
TLR4 + DC-SIGN	0.9348	0.8853–0.9843	0.4567	0.8864	0.9130	0.8571	0.8750	0.9000
SR-B1	0.5241	0.4010–0.6472	0.5237	0.5909	0.5000	0.6905	0.6389	0.5577
DC-SIGN	0.5145	0.3903–0.6387	0.5151	0.5795	0.7609	0.3810	0.5738	0.5926
SR-B1 + DC-SIGN	0.5072	0.3817–0.6328	0.5097	0.6023	0.8696	0.3095	0.5797	0.6842

Multivariate analysis showed that while TLR4 serves as a highly robust independent predictor, the integration of multiple PRRs biomarkers showed that combining TLR4 with SR-B1 and DC-SIGN yielded high sensitivity but did not materially improve overall accuracy or AUC compared to the TLR4-only model. The observed diagnostic performance should be interpreted with caution, as the model was derived and evaluated within the same dataset and may be subject to overestimation. The evaluation of these markers reflects different aspects of host innate immune responses, ranging from pathogen recognition to downstream immunological signaling. This is consistent with the concept that the immune system operates through interconnected pathogen-recognition pathways rather than isolated mechanisms.(7) However, the integration of PRRs biomarkers in this study should be considered exploratory, and their role in representing subclinical TB pathogenesis remains to be further elucidated. Although a multi-marker approach may provide a broader overview of host responses, its clinical applicability requires validation in larger and independent cohorts. Overall, these suggest that subclinical TB involves complex interactions where TLR4 acts as a dominant indicator that may not require additional markers like SR-B1 or DC-SIGN for effective discrimination.

Previous studies have highlighted the role of host immune responses in tuberculosis pathogenesis and diagnosis. Macrophage migration inhibitory factor (MIF) expression varies according to granuloma organization, suggesting that immune mediator levels may reflect disease activity.(33) Urinary lipoarabinomannan (LAM) detection may improve diagnostic yield in extrapulmonary TB, underscoring the need for alternative biomarkers beyond conventional sputum-based approaches.(34) In addition, immune stimulation enhances IFN-γ and IL-12 production in peripheral blood mononuclear cells exposed to *M. tuberculosis* DNA, indicating measurable host immune activation.(35)

Some studies have also demonstrated the relevance of innate immune signaling pathways in TB. For example, Toll-like receptor–related pathways have been implicated in host–microbial interactions (36), while host biological parameters have been associated with clinical outcomes (37). Furthermore, hypoxia-inducible factor (HIF) has been shown to regulate macrophage antimicrobial responses against *M. tuberculosis*.(38) Taken together, these findings provide a biological rationale for exploring PRRs-related molecules such as TLR4, SR-B1, and DC-SIGN. However, when compared with the broader literature on host-derived blood biomarkers for TB, the diagnostic performance of such biomarkers remains variable across studies.

These findings should therefore be interpreted cautiously in light of several methodological limitations. The relatively small sample size and single-center setting may limit generalizability to populations with different host genetics or *M. tuberculosis* strain diversity. In addition, the use of RMT as the reference standard may have led to misclassification, particularly in paucibacillary cases where bacterial load is low. Consequently, some subjects in the RMT-negative group may have had undetected subclinical TB, which could affect the estimated diagnostic performance of the biomarkers. An important consideration in interpreting these findings is the distinction between RMT-negative individuals and truly healthy populations. Subjects in the RMT-negative group were not asymptomatic healthy controls but individuals with clinical or radiological suspicion of subclinical TB. Therefore, it is plausible that biomarker levels in the RMT-negative group in this study may differ from those observed in truly healthy populations, potentially narrowing the observed differences between groups and contributing to reduced discriminatory performance. In this context, the limited independent predictive value observed for SR-B1 and DC-SIGN may be partly explained by their broader roles in innate immune recognition, which are not specific to TB. Taken together, these findings suggest that

while SR-B1 and DC-SIGN contribute to the biological understanding of host responses to *M. tuberculosis*, their role as independent diagnostic markers appears limited. Their potential utility may be better realized as part of a multi-marker panel reflecting complementary immune pathways rather than as single discriminatory indicators. Future research is warranted to validate these findings in larger, independent, and more diverse populations, ideally using longitudinal designs to assess the predictive value of these biomarkers for progression from subclinical to active TB. Additionally, incorporating healthy control groups and more sensitive or composite reference standards may help to better define the diagnostic performance and biological relevance of these host immune markers.

Conclusion

Among the innate immune biomarkers evaluated, TLR4 demonstrated the most robust and consistent diagnostic potential for distinguishing RMT-positive status among individuals with suspected subclinical TB, suggesting its role as a primary indicator of early host immune activation. While multi-biomarker models incorporating SR-B1 and DC-SIGN achieved high sensitivity, they provided limited additional benefit in overall accuracy compared to the more parsimonious TLR4-only model. These findings highlight the potential utility of TLR4 as a non-sputum-based biomarker associated with RMT-positive cases in this population, while SR-B1 and DC-SIGN appear to have a more complementary role within an exploratory multi-marker framework. Importantly, individuals with both RMT-positive results and elevated TLR4 levels may require closer clinical monitoring due to the potential risk of progression to active TB.

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Authors Contribution

RIPP conceptualized and designed the study, and was responsible for data collection and data analysis. K contributed to data interpretation and provided important intellectual input. DS and SD contributed to data acquisition and supported the analytical process. RIPP drafted the manuscript and prepared the figures. All authors critically revised the manuscript and approved the final version for publication.

Ethical Statement

The study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Islam Al-Azhar Mataram (No: 079/EC-03/FK-06/UNIZAR/VII/2025) and by the Health Research Ethics Committee of Regional General Hospital, Mataram (No: 021/Etik Pen./RSUD/VI/2025). Written informed consent was obtained from all subjects and the study was conducted in accordance with the Declaration of Helsinki principles.

Conflict of Interest

Authors declare that there are no conflict of interest.

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