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Diagnostic Accuracy of Pleural Fluid Interferon-Gamma for Tuberculous Pleural Effusion: A Prospective Cross-Sectional Study

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ABSTRACT: Tuberculous pleural effusion (TPE) remains difficult to diagnose because pleural disease is often paucibacillary, and conventional microbiological tests have limited sensitivity. This prospective cross-sectional diagnostic accuracy study evaluated pleural fluid interferon-gamma (IFN-γ) as a host-response biomarker for TPE. Consecutive adults undergoing clinically indicated thoracentesis at referral hospitals in Makassar, Indonesia, were enrolled, and residual pleural fluid was analyzed using a quantitative sandwich ELISA. Eighty-eight participants were included: 28 with TPE, 30 with parapneumonic pleural effusion (PPE), and 30 with malignant pleural effusion (MPE). Median IFN-γ concentrations were significantly higher in TPE than in non-TPE [803.53 (566.75–1058.06) vs 19.14 (5.50–80.75) pg/mL; $p<0.001$] and differed significantly across TPE, PPE, and MPE ($p<0.001$), with higher levels in TPE than in either comparator. Receiver operating characteristic analysis yielded an area under the curve of 0.992 (95% CI, 0.979–1.000). At the study-derived threshold of ≥346.52 pg/mL, sensitivity was 100.0%, specificity 96.7%, positive predictive value 93.3%, negative predictive value 100.0%, and overall accuracy 97.7%. Pleural fluid IFN-γ showed excellent discrimination for TPE and may be useful as an adjunctive diagnostic biomarker; however, the proposed threshold requires external validation before routine clinical use.

1. INTRODUCTION

Tuberculosis (TB) remains a major global health problem, causing an estimated 10.7 million incident cases and 1.23 million deaths in 2024. Indonesia contributes substantially to this burden, emphasizing the need for timely diagnosis of both pulmonary and extrapulmonary TB [1]. Pleural tuberculosis is the second most common form of extrapulmonary TB after tuberculous lymphadenitis and remains an important cause of exudative pleural effusion in high-burden settings [2,3]. Its clinical manifestations, including fever, cough, pleuritic chest pain, dyspnea, weight loss, and night sweats, are nonspecific and overlap with parapneumonic and malignant pleural effusions, despite these conditions requiring distinct management.

Diagnosing tuberculous pleural effusion (TPE) is challenging because pleural TB is typically paucibacillary. Pleural-fluid acid-fast bacillus smear microscopy has very low sensitivity, while mycobacterial culture provides a variable yield and may require several weeks [2-4]. Molecular assays offer rapid microbiological confirmation but remain insufficiently sensitive in pleural fluid. A comparative meta-analysis reported pooled sensitivity and specificity of 47% and 98%, respectively, for Xpert MTB/RIF Ultra against composite reference standards; thus, a positive result strongly supports TPE, but a negative result does not reliably exclude it [5]. Pleural biopsy may improve diagnostic yield through histopathological and microbiological assessment, but its invasiveness and dependence on procedural expertise limit routine use, particularly in resource-constrained settings [2,4]. Consequently, diagnosis often requires integration of clinical, radiological, biochemical, microbiological, molecular, and histopathological evidence.

Pleural-fluid interferon-gamma (IFN-γ) is a biologically plausible biomarker for TPE. Mycobacterial antigens in the pleural space induce a T-helper 1–dominant immune response, recruiting sensitized T lymphocytes and increasing local IFN-γ production [3,6]. A meta-analysis of 67 studies involving 7,153 participants reported pooled sensitivity and specificity of 93% and 96%, respectively, for unstimulated pleural-fluid IFN-γ [6]. A paired meta-analysis also found higher summary accuracy for IFN-γ than for adenosine deaminase, with sensitivity and specificity of 91% and 96% versus 88% and 91%, respectively [7].

Recent prospective studies support its diagnostic potential. The multicenter IRISA-TB study reported sensitivity of 81.8% and specificity of 96.6% at a 20.5-pg/mL threshold and greater sensitivity than pleural-fluid Xpert MTB/RIF Ultra against the study reference standard [8]. Huang et al. (2024) likewise found substantially higher pleural-fluid IFN-γ concentrations in TPE than in malignant pleural effusion [9]. However, estimates and thresholds vary across assay platforms, calibration systems, specimen-processing methods, populations, comparator diagnoses, and reference standards. Substantial heterogeneity and risk of bias in the available evidence further limit direct transfer of a single threshold across settings [6].

This prospective cross-sectional study therefore compared pleural-fluid IFN-γ concentrations among tuberculous, parapneumonic, and malignant pleural effusions and evaluated its diagnostic accuracy for distinguishing TPE from non-TPE in an Indonesian clinical population. We also derived an optimal study-specific threshold and estimated its sensitivity, specificity, positive predictive value, and negative predictive value.

2. MATERIALS AND METHODS

2.1 Study Design and Participants

This prospective cross-sectional diagnostic accuracy study was conducted at Dr. Wahidin Sudirohusodo Hospital and affiliated hospitals in Makassar, Indonesia, from April to June 2026. The study evaluated pleural fluid interferon-gamma (IFN-γ) for distinguishing tuberculous pleural effusion (TPE) from non-tuberculous pleural effusion (non-TPE). Parapneumonic pleural effusion (PPE) and malignant pleural effusion (MPE) constituted the non-TPE comparator group.

Consecutive adults aged ≥18 years with pleural effusion undergoing clinically indicated thoracentesis were screened for eligibility. Participants were included when sufficient clinical information was available to establish a final diagnosis of TPE, PPE, or MPE. Patients were excluded if they had received antituberculosis treatment for ≥14 days before pleural fluid sampling, prednisone equivalent ≥20 mg/day for ≥14 consecutive days within the preceding 30 days, potent biological or immunosuppressive therapy within 3 months, or cytotoxic chemotherapy within 14 days. Patients with mixed etiologies or insufficient information for etiological classification were also excluded. Each participant contributed one pleural fluid specimen.

2.2 Sample-Size Determination

Sample size was calculated based on the expected diagnostic sensitivity of pleural fluid IFN-γ. A previous meta-analysis reported a pooled sensitivity of 0.93 for unstimulated pleural fluid IFN-γ [6]. Assuming an anticipated sensitivity of 0.90, an absolute precision of 12%, and a two-sided 95% confidence level, at least 24 evaluable TPE cases were required. After allowing 15% for incomplete data or unusable specimens, the target sample was increased to 29 TPE cases, with at least 29 non-TPE participants. All consecutively eligible participants recruited during the study period were included in the analysis.

2.3 Pleural Fluid Collection and IFN-γ Measurement

Thoracentesis was performed as part of routine clinical care. After routine diagnostic requirements had been completed, approximately 1–2 mL of residual pleural fluid was retained for the study. The specimen was centrifuged according to the laboratory protocol, and the resulting supernatant was stored frozen until analysis.

Pleural fluid IFN-γ concentrations were measured at the Hasanuddin University Medical Research Center using a Human IFN-γ ELISA Kit (Elabscience, catalogue no. E-EL-H0108; Houston, TX, USA) according to the manufacturer's instructions. The quantitative sandwich enzyme-linked immunosorbent assay was read at 450 ± 2 nm, and IFN-γ concentrations were calculated from the standard curve and expressed in pg/mL. The analytical sensitivity of the assay was 9.38 pg/mL, with intra-assay and inter-assay coefficients of variation <10%. Samples with concentrations exceeding the highest calibrator were diluted and reassayed, and final concentrations were corrected according to the dilution factor. Laboratory personnel performing the IFN-γ assay were blinded to participants' clinical information and reference-standard diagnoses.

2.4 Reference Standard and Diagnostic Classification

The reference standard was the final etiological diagnosis established using available clinical, radiological, microbiological, molecular, cytological, histopathological, and treatment-response information. Pleural fluid IFN-γ results were not used to establish the reference-standard diagnosis. TPE was defined as an exudative pleural effusion with microbiological or molecular evidence of *Mycobacterium tuberculosis*, pleural cytological or histopathological findings compatible with tuberculosis, or a lymphocyte-predominant exudative effusion accompanied by compatible clinical and radiological findings and improvement following guideline-concordant antituberculosis treatment. PPE was defined by clinical and radiological evidence of pneumonia or another non-tuberculous pulmonary infection supported by pleural fluid microbiological findings or clinician adjudication. MPE was defined by the identification of malignant cells in pleural fluid cytology or malignant findings on pleural tissue histopathology.

2.5 Statistical Analysis

Continuous variables were summarized as median with interquartile range (IQR) when non-normally distributed, whereas categorical variables were presented as frequencies and percentages. Normality of continuous variables was assessed using the Shapiro–Wilk test. Age was compared across TPE, PPE, and MPE groups using the Kruskal–Wallis test. Sex and smoking history were compared using Pearson's chi-square test, whereas smoking intensity was evaluated using the Fisher–Freeman–Halton exact test according to cell frequencies.

Pleural fluid IFN-γ concentrations were compared between the TPE and non-TPE groups using the Mann–Whitney U test. Differences among the TPE, PPE, and MPE groups were assessed using the Kruskal–Wallis test. When the overall test was statistically significant, pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni adjustment for multiple comparisons. The diagnostic performance of pleural fluid IFN-γ for distinguishing TPE from non-TPE was evaluated using receiver operating characteristic (ROC) analysis. The area under the ROC curve (AUC) was reported with its 95% confidence interval (CI). The optimal IFN-γ threshold was identified by maximizing the Youden index. At the study-derived threshold, sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy were calculated with exact binomial 95% CIs. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 30 (IBM Corp., Armonk, NY, USA).

3. RESULTS AND DISCUSSION

3.1 Results

A total of 88 participants were analyzed: 28 with tuberculous pleural effusion (TPE; 31.8%), 30 with parapneumonic pleural effusion (PPE; 34.1%), and 30 with malignant pleural effusion (MPE; 34.1%). Age, sex, smoking history, and smoking intensity did not differ significantly among the groups (all P>0.05; Table 1). Among participants with TPE, supporting diagnostic findings included positive sputum molecular testing in 23 of 28 patients (82.1%), pleural cytology compatible with tuberculosis in 15 (53.6%), positive pleural-fluid acid-fast bacilli microscopy in 15 (53.6%), and positive pleural-fluid molecular testing in 2 (7.1%). These findings were not mutually exclusive. All PPE cases had positive pleural-fluid bacterial cultures. MPE was confirmed by malignant pleural-fluid cytology in 28 participants (93.3%) and pleural histopathology in 2 (6.7%).

Table 1. Baseline characteristics according to pleural effusion etiology

Characteristic	TPE (n=28)	PPE (n=30)	MPE (n=30)	P value
Age, years	56.0 (29.5–66.0)	49.5 (30.5–57.5)	56.0 (48.3–61.3)	0.176[a]
Sex				0.241[b]
Male	17 (60.7)	17 (56.7)	12 (40.0)	
Female	11 (39.3)	13 (43.3)	18 (60.0)	
Smoking history				0.961[b]
Never smoker	16 (57.1)	17 (56.7)	18 (60.0)	

Ever smoker	12 (42.9)	13 (43.3)	12 (40.0)	
Smoking intensity				0.516[c]
Never smoker	16 (57.1)	17 (56.7)	18 (60.0)	
Mild	3 (10.7)	6 (20.0)	2 (6.7)	
Moderate	5 (17.9)	3 (10.0)	2 (6.7)	
Severe	4 (14.3)	4 (13.3)	8 (26.7)	

Note: Data are presented as median (Q1–Q3) or n (%). [a]Kruskal–Wallis test. [b]Chi-square test. [c]Fisher–Freeman–Halton exact test. MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion; TPE, tuberculous pleural effusion.

Pleural fluid interferon-gamma (IFN-γ) concentrations were substantially higher in TPE than in non-TPE [median, 803.53 pg/mL (Q1–Q3, 566.75–1058.06) versus 19.14 pg/mL (Q1–Q3, 5.50–80.75); P<0.001; Table 2]. Concentrations also differed significantly across TPE, PPE, and MPE (P<0.001), with median values of 803.53, 30.02, and 13.15 pg/mL, respectively. Bonferroni-adjusted pairwise analyses showed significantly higher IFN-γ concentrations in TPE than in PPE and MPE (both adjusted P<0.003), whereas PPE and MPE did not differ significantly (adjusted P=0.501).

Table 2. Pleural fluid IFN-γ concentrations according to diagnostic group

Analysis and group	IFN-γ, pg/mL	P value
Primary comparison		
TPE (n=28)	803.53 (566.75–1058.06)	<0.001[a]
Non-TPE (n=60)	19.14 (5.50–80.75)	
Etiology-specific comparison		
TPE (n=28)	803.53 (566.75–1058.06)	<0.001[b]
PPE (n=30)	30.02 (5.74–168.33)	
MPE (n=30)	13.15 (4.84–50.40)	
Pairwise comparison		**Adjusted P value**
TPE versus PPE	–	<0.003[c]
TPE versus MPE	–	<0.003[c]
PPE versus MPE	–	0.501[c]

Note: IFN-γ concentrations are presented as median (Q1–Q3). [a]Mann–Whitney U test. [b]Kruskal–Wallis test. [c]Pairwise Mann–Whitney U tests with Bonferroni adjustment. IFN-γ, interferon-gamma; MPE, malignant pleural effusion; non-TPE, non-tuberculous pleural effusion; PPE, parapneumonic pleural effusion; TPE, tuberculous pleural effusion.

Pleural fluid IFN-γ showed an area under the receiver operating characteristic curve of 0.992 (95% confidence interval [CI], 0.979–1.000; P<0.001) for distinguishing TPE from non-TPE (Figure 1).

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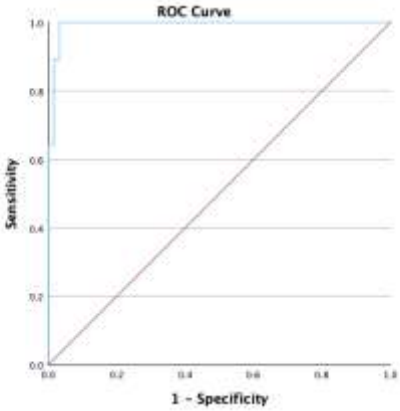

Figure 1. Receiver operating characteristic curve of pleural fluid IFN-γ for distinguishing TPE from non-TPE. The area under the receiver operating characteristic curve was 0.992 (95% CI, 0.979–1.000; P<0.001). TPE was designated as the positive condition, whereas PPE and MPE constituted the non-TPE comparator group. The optimal study-derived threshold was ≥346.52 pg/mL.

The study-derived threshold of ≥346.52 pg/mL yielded 28 true-positive, 58 true-negative, 2 false-positive, and no false-negative results. Sensitivity was 100.0% (95% CI, 87.7–100.0), specificity 96.7% (95% CI, 88.5–99.6), positive predictive value 93.3% (95% CI, 77.9–99.2), negative predictive value 100.0% (95% CI, 93.8–100.0), and overall accuracy 97.7% (95% CI, 92.0–99.7; Table 3).

Table 3. Diagnostic accuracy of pleural fluid IFN-γ for identifying tuberculous pleural effusion

Parameter	Estimate
Study-derived threshold	≥346.52 pg/mL
AUC (95% CI)	0.992 (0.979–1.000)
Youden index	0.967
True positive	28
False negative	0
True negative	58
False positive	2
Sensitivity, % (95% CI)	100.0 (87.7–100.0)
Specificity, % (95% CI)	96.7 (88.5–99.6)
Positive predictive value, % (95% CI)	93.3 (77.9–99.2)
Negative predictive value, % (95% CI)	100.0 (93.8–100.0)
Overall accuracy, % (95% CI)	97.7 (92.0–99.7)

Note: TPE was designated as the positive condition, whereas PPE and MPE comprised the non-TPE comparator group. The threshold was selected by maximizing the Youden index. AUC, area under the receiver operating characteristic curve; CI, confidence interval; IFN-γ, interferon-gamma; MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion; TPE, tuberculous pleural effusion.

3.2 Discussion

Pleural fluid interferon-gamma (IFN-γ) concentrations were markedly higher in tuberculous pleural effusion (TPE) than in non-tuberculous effusions, both collectively and when compared separately with parapneumonic pleural effusion (PPE) and malignant pleural effusion (MPE). IFN-γ showed excellent discrimination, with an area under the curve of 0.992. At the study-derived threshold of ≥346.52 pg/mL, sensitivity was 100.0%, specificity was 96.7%, and overall accuracy was 97.7%. These findings indicate strong separation between TPE and the two principal alternative diagnoses represented in this cohort. However, the apparently perfect sensitivity should be interpreted cautiously because it was based on only 28 TPE cases and the threshold was derived and evaluated within the same dataset.

The marked elevation of IFN-γ in TPE is biologically plausible. Mycobacterial antigens entering the pleural space induce a delayed T-helper 1–dominant immune response, characterized by recruitment of sensitized lymphocytes and compartmentalized IFN-γ production [3,4]. IFN-γ activates macrophages and supports cellular mechanisms that restrict intracellular mycobacterial proliferation. This localized immune response may partly explain why pleural tuberculosis is often paucibacillary despite intense inflammation [3,10]. Nevertheless, *Mycobacterium tuberculosis* can persist through modulation of macrophage function and other host immune pathways [11]. Thus, high pleural IFN-γ concentrations may coexist with a low microbiological yield.

Our findings are consistent with the broader diagnostic literature. A meta-analysis of 67 studies involving 7,153 participants reported pooled sensitivity and specificity of 93% and 96%, respectively, for unstimulated pleural fluid IFN-γ [6]. A paired comparative meta-analysis also found higher summary accuracy for IFN-γ than for adenosine deaminase (ADA), with pooled sensitivity and specificity of 91% and 96% for IFN-γ compared with 88% and 91% for ADA [7]. Because both biomarkers were evaluated in the same participants, this paired evidence reduces bias arising from indirect comparisons. However, diagnostic accuracy alone does not establish superiority in availability, cost, turnaround time, or clinical utility.

Prospective studies have reported findings in the same direction, although performance estimates vary. A two-centre Chinese study demonstrated high diagnostic accuracy for pleural IFN-γ in separate training and validation cohorts [12]. Nguyen et al. also observed higher IFN-γ concentrations in TPE than in non-TPE, although the reported area under the curve was more modest at 0.84 [13]. Huang et al. reported an area under the curve of 0.999 in a small cohort comparing TPE with MPE [9]. Conversely, the multicentre IRISA-TB study from South Africa and India reported sensitivity of 81.8% and specificity of 96.6% at a cut-point of 20.5 pg/mL [8]. A meta-analysis of T-helper 1 and T-helper 2 cytokines similarly found substantially higher pleural IFN-γ concentrations in TPE than in MPE, supporting a predominantly T-helper 1–associated response in pleural tuberculosis [14].

The sensitivity and area under the curve observed in our study were at the upper end of published estimates. This may reflect differences in patient spectrum, the proportion of microbiologically or histologically supported diagnoses, disease prevalence, comparator composition, and the reference standard. The clear separation observed here may partly result from restricting the non-TPE group to PPE and MPE and from the relatively small number of TPE cases. Comparable performance should not be assumed in populations containing a wider range of lymphocytic, autoimmune, or other inflammatory pleural diseases.

The study-derived threshold of ≥346.52 pg/mL also differed substantially from thresholds reported elsewhere. Such variation is expected because measured concentrations depend on assay platform, antibody pairs, calibration systems, analytical range, dilution procedures, specimen processing, storage conditions, and reporting units. Patient selection, comparator diagnoses, reference standards, and the statistical method used to select the operating point also affect the resulting threshold [6,15]. The IRISA-TB threshold of 20.5 pg/mL, for example, was developed for a specific rapid immunosuspension assay and cannot be transferred directly to an enzyme-linked immunosorbent assay platform [8]. Our threshold should therefore be considered assay- and cohort-specific until independently reproduced using the same laboratory procedure.

Unstimulated pleural fluid IFN-γ should also be distinguished from antigen-stimulated interferon-gamma release assays. Unstimulated testing measures cytokine already present in pleural fluid, whereas assays such as T-SPOT.TB measure IFN-γ released after lymphocytes are exposed to *M. tuberculosis*-specific antigens. These methods differ in biological target, specimen preparation, analytical procedure, numerical scale, and diagnostic performance and should not be interpreted interchangeably [8].

The etiology-specific analysis showed higher IFN-γ concentrations in TPE than in either PPE or MPE, whereas PPE and MPE did not differ significantly. This pattern does not imply that non-tuberculous effusions lack cytokine-mediated inflammation. Pleural infection is biologically heterogeneous and varies with pathogen, disease stage, host response, and previous antimicrobial exposure [16]. MPE also contains a complex immunoregulatory microenvironment involving T-cell subsets, macrophages, stromal cells, immune checkpoints, and soluble mediators [17,18]. Rather, our results suggest that IFN-γ is more useful for identifying a tuberculosis-associated pleural response than for distinguishing among non-tuberculous etiologies. The two false-positive results further demonstrate that an elevated concentration is not pathognomonic for TPE.

Clinically, pleural fluid IFN-γ may be useful as an adjunct when TPE remains likely despite negative or inconclusive microbiological testing. A markedly elevated value may increase diagnostic confidence when interpreted with pre-test probability and other pleural findings. Nevertheless, this study does not establish IFN-γ as a universal rule-in or rule-out test. Xpert MTB/RIF Ultra provides rapid and highly specific microbiological evidence but has limited sensitivity in pleural fluid [5]. Pleural biopsy can provide histopathological and microbiological confirmation when fluid-based tests are nondiagnostic, although it is invasive and depends on appropriate expertise and facilities [2,19].

IFN-γ should therefore complement rather than replace microbiological, molecular, cytological, and histopathological evaluation. As a host-response biomarker, it does not demonstrate viable organisms, identify drug resistance, provide an isolate for susceptibility testing, or establish the alternative etiology of an effusion. Interpretation should remain integrated with clinical probability, imaging, pleural fluid biochemistry and cellular composition, microbiological findings, cytology, and pleural histology. Predictive values are also prevalence-dependent and cannot be transferred directly to primary care, low-burden settings, or populations with a different disease spectrum.

This study has several strengths. Participants were recruited prospectively and consecutively, pleural fluid was obtained during clinically indicated thoracentesis, laboratory personnel were blinded to the reference-standard diagnosis, and the comparator group included both parapneumonic and malignant effusions. IFN-γ was also evaluated as both a continuous biomarker and a threshold-based diagnostic test.

Several limitations require consideration. The study was conducted in referral hospitals within one geographical area and included only 28 TPE cases, limiting precision and generalizability. The composite reference standard incorporated microbiological, molecular, cytological, histopathological, and treatment-response criteria. Although such standards are often necessary when no single sufficiently sensitive reference test exists, variation in their components can introduce diagnostic misclassification [20]. Some cases classified partly by treatment response lacked microbiological or histological confirmation. The threshold was neither internally validated through resampling nor evaluated in an independent cohort. IFN-γ was not compared directly with ADA, preventing conclusions regarding comparative or incremental accuracy. The effects of human immunodeficiency virus infection, immunosuppression, previous antituberculosis therapy, and prior antimicrobial exposure could not be fully evaluated. Finally, the non-TPE group was limited to PPE and MPE, leaving performance against other inflammatory and lymphocytic effusions uncertain.

Future studies should validate the proposed threshold in larger multicentre cohorts using the same assay, calibration system, specimen-processing protocol, and a prespecified decision rule. Analyses restricted to microbiologically or histopathologically confirmed TPE would clarify the influence of the composite reference standard. Direct comparisons with ADA, Xpert MTB/RIF Ultra, culture, and pleural biopsy are also needed to determine whether IFN-γ provides clinically useful incremental information. Local economic evaluations remain necessary because cost-effectiveness depends on disease prevalence, assay costs, existing diagnostic pathways, access to pleural biopsy, and the consequences of diagnostic error. Although IRISA-TB appeared cost-effective in a South African decision model, those findings cannot be assumed to apply to other healthcare systems [21].

4. CONCLUSION

Pleural fluid IFN-γ showed excellent diagnostic discrimination for tuberculous pleural effusion and distinguished TPE from parapneumonic and malignant pleural effusions in this cohort. At the study-derived threshold of ≥346.52 pg/mL, diagnostic performance was high; however, this cut-off remains assay- and population-specific and requires independent validation. Pleural fluid IFN-γ is therefore best positioned as a complementary biomarker within a multimodal diagnostic approach rather than as a stand-alone replacement for microbiological, molecular, or histopathological confirmation.

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AUTHOR CONTRIBUTIONS

All authors approved the submitted manuscript and agreed to be accountable for their respective contributions.

Fadli: conceptualization, methodology, investigation, data curation, formal analysis, visualization, project administration, and preparation of the original manuscript draft.

Irawaty Djaharuddin: conceptualization, methodology, supervision, validation, interpretation of findings, and critical review and editing of the manuscript.

Erwin Arief: methodology, validation, supervision, interpretation of findings, and critical review and editing of the manuscript.

Nurjannah Lihawa: validation, clinical interpretation, and critical review and editing of the manuscript.

Harun Iskandar: validation, interpretation of findings, and critical review and editing of the manuscript.

Harry Akza Putrawan: validation, clinical interpretation, and critical review and editing of the manuscript.

All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for the integrity and accuracy of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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ETHICS STATEMENT

The study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (Approval No. 525/UN4.6.4.5.31/PP36/2026; Protocol No. UH26020287; April 16, 2026). Written informed consent was obtained from all participants before enrollment and before the research use of residual pleural fluid and clinical data. Thoracentesis was performed solely as part of routine clinical care and was not undertaken specifically for research purposes. Participant specimens and clinical data were coded to maintain confidentiality.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional restrictions.

REFERENCES

[1] World Health Organization, 2025. *Global Tuberculosis Report 2025*. World Health Organization, Geneva. https://www.who.int/publications/i/item/9789240116924

[2] Lo Cascio, C.M., Kaul, V., Dhooria, S., Agrawal, A., Chaddha, U., 2021. Diagnosis of tuberculous pleural effusions: A review. *Respiratory Medicine* 188, 106607.

[3] McNally, E., Ross, C., Gleeson, L.E., 2023. The tuberculous pleural effusion. *Breathe* 19(4), 230143.

[4] Shaw, J.A., Koegelenberg, C.F.N., 2021. Pleural tuberculosis. *Clinical Chest Medicine* 42(4), 649–666.

[5] Aggarwal, A.N., Agarwal, R., Dhooria, S., Prasad, K.T., Sehgal, I.S., Muthu, V., 2022. Xpert MTB/RIF Ultra versus Xpert MTB/RIF for diagnosis of tuberculous pleural effusion: A systematic review and comparative meta-analysis. *PLoS One* 17(7), e0268483.

[6] Aggarwal, A.N., Agarwal, R., Dhooria, S., Prasad, K.T., Sehgal, I.S., Muthu, V., 2021. Unstimulated pleural fluid interferon gamma for diagnosis of tuberculous pleural effusion: A systematic review and meta-analysis. *Journal of Clinical Microbiology* 59(5), e02112-20.

[7] Aggarwal, A.N., Agarwal, R., Dhooria, S., Prasad, K.T., Sehgal, I.S., Muthu, V., 2021. Comparative accuracy of pleural fluid unstimulated interferon-gamma and adenosine deaminase for diagnosing pleural tuberculosis: A systematic review and meta-analysis. *PLoS One* 16(6), e0253525.

[8] Christopher, D.J., Esmail, A., Scott, A.J., Wilson, L., Randall, P., Thangakunam, B., et al., 2024. Diagnostic performance of unstimulated IFN-γ (IRISA-TB) for pleural tuberculosis: A prospective study in South Africa and India. *Open Forum Infectious Diseases* 11(10), ofae533.

[9] Huang, H., Li, Y., Cao, X., Yang, M., Shen, J., 2024. Diagnostic value of IFN-gamma in tuberculous pleural effusion. *Cytokine* 184, 156773.

[10] Shanmuganathan, G., Orujyan, D., Narinyan, W., Poladian, N., Dhama, S., Parthasarathy, A., et al., 2022. Role of interferons in *Mycobacterium tuberculosis* infection. *Clinics and Practice* 12(5), 788–796.

[11] Chandra, P., Grigsby, S.J., Philips, J.A., 2022. Immune evasion and provocation by *Mycobacterium tuberculosis*. *Nature Reviews Microbiology* 20(12), 750–766.

[12] Luo, Y., Yan, F., Xue, Y., Mao, L., Lin, Q., Tang, G., et al., 2020. Diagnostic utility of pleural fluid T-SPOT and interferon-gamma for tuberculous pleurisy: A two-center prospective cohort study in China. *International Journal of Infectious Diseases* 99, 515–521.

[13] Nguyen, M.H., Dao, Q.M., Bui, T.T.H., Le, V.H.T., 2020. Diagnostic values of different cytokines in identifying tuberculous pleural effusion. *Tropical Biomedicine* 37(2), 372–378.

[14] Zeng, Y., Wang, L., Zhou, H., Qi, Y., 2022. A meta-analysis of Th1 and Th2 cytokine profiles differentiating tuberculous from malignant pleural effusion. *Scientific Reports* 12(1), 2743.

[15] Hassanzad, M., Hajian-Tilaki, K., 2024. Methods of determining optimal cut-point of diagnostic biomarkers with application of clinical data in ROC analysis: An update review. *BMC Medical Research Methodology* 24, 84.

[16] Bedawi, E.O., Ricciardi, S., Hassan, M., Gooseman, M.R., Asciak, R., Castro-Añón, O., et al., 2023. ERS/ESTS statement on the management of pleural infection in adults. *European Respiratory Journal* 61(2), 2201062.

[17] Ge, S., Zhao, Y., Liang, J., He, Z., Li, K., Zhang, G., et al., 2024. Immune modulation in malignant pleural effusion: From microenvironment to therapeutic implications. *Cancer Cell International* 24(1), 105.

[18] Wu, Y.Y., Hsu, Y.L., Huang, Y.C., Su, Y.C., Wu, K.L., Chang, C.Y., et al., 2023. Characterization of the pleural microenvironment niche and cancer transition using single-cell RNA sequencing in EGFR-mutated lung cancer. *Theranostics* 13(13), 4412.

[19] Deschuyteneer, E.P., De Keukeleire, T., 2022. Diagnostic value and safety of thoracoscopic pleural biopsies in pleural exudative effusions of unknown origin, including follow-up. *BMJ Open Respiratory Research* 9(1), e001161.

[20] Patel, R., Tsalik, E.L., Evans, S., Fowler, V.G., Doernberg, S.B., 2023. Clinically adjudicated reference standards for evaluation of infectious diseases diagnostics. *Clinical Infectious Diseases* 76(5), 938.

[21] Pooran, A., Randall, P., Meldau, R., Maine, R., Esmail, A., 2022. Economic evaluation of microbiological and host biomarker-based tests for the diagnosis of pleural tuberculosis in a high burden setting. *Journal of Thoracic Disease* 14(9), 3167–3177.