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ORIGINAL RESEARCH

Interleukin −39 Expression in Pleural Effusion and Its Diagnostic Value for Tuberculous Pleurisy

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Objective: This study aimed to assess Interleukin-39 (IL-39) levels in various types of pleural effusion (PE), explore IL-39's diagnostic value in tuberculous pleurisy, analyze its correlation with other PE and tuberculosis indicators, and confirm the involvement of IL-39 in tuberculosis infection and the resulting inflammatory response.

Methods: This study enrolled 113 patients with PE caused by different etiologies: 20 with transudative effusion, 39 with malignant pleural effusion (MPE), 15 with uncomplicated parapneumonic effusion (UPPE), and 39 with tuberculous pleural effusion (TPE). Enzyme-linked immunosorbent assay (ELISA) was used to measure IL-39, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) levels in the pleural fluid (PF) of each group. Adenosine deaminase (ADA) activity was determined using the colorimetric method.

Results: IL-39 concentration was notably higher in the TPE compared to others. The IL-39 demonstrated an AUC of 0.944, with a cut-off value of 39.8 pg/mL, sensitivity of 94.9%, and specificity of 79.7% in distinguishing between the TPE and non-TPE. In discriminating between the TPE and MPE, the AUC for IL-39 was 0.941, with a cut-off value of 39.3 pg/mL, sensitivity of 94.9%, and specificity of 79.5%. For differentiating the TPE and UPPE, IL-39 yielded an AUC of 0.885, with a cut-off value of 235.0 pg/mL, sensitivity of 66.7%, and specificity of 100.0%. Moreover, based on these findings, multivariable diagnostic model and the rapid combination of IL-39 with other tuberculosis biomarkers (such as IFN-γ, TNF-α, and ADA) significantly enhanced the diagnostic and differential diagnostic performance for TPE. Additionally, IL-39, IFN-γ, TNF-α, and ADA levels in PF were positively correlated with each other.

Conclusion: IL-39 demonstrated good diagnostic and differential diagnostic value for TPE. Furthermore, the multivariate diagnostic model, as well as the joint detection of IL-39 with other tuberculosis biomarkers, can further increased the sensitivity or specificity. Additionally, IL-39 exhibited positive correlations with other tuberculosis biomarkers, suggesting its potential involvement in tuberculosis infection and the inflammatory response it may induce.

Keywords: Interleukin-39, pleural effusion, tuberculous pleurisy, diagnosis

Introduction

The World Health Organization's global tuberculosis report for 2023, disclosed that the global count of new tuberculosis cases had surged to 10.6 million in 2022, with 1.3 million deaths attributed to tuberculosis globally. Tuberculosis had then ranked second among the leading causes of infectious disease-related deaths worldwide, second only to COVID-19.[1]

Tuberculous pleural effusion (TPE) stood as the second most prevalent form of extrapulmonary tuberculosis, following lymph node tuberculosis.[2] Swift diagnosis and timely treatment of TPE were vital, given the elevated presence of fibrin proteins and their breakdown products in pleural fluid (PF). Delayed treatment could result in severe

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complications like tuberculous empyema and thickening of the pleura due to fibrosis.[3] Excessive deposition of extracellular matrix after inflammation could also result in lung collapse and chronic respiratory failure,[4] thereby affecting the patient's pulmonary ventilation function and long-term quality of life. Although TPE was clinically common, different etiologies of pleural effusion (PE) could share similarities in presentation and examinations, especially exudative PEs. Therefore, differential diagnosis of TPE presented challenges in clinical practice. Moreover, the diagnosis of TPE relies on the microbiological culture of pleural fluid or tissue, or histopathological biopsy of pleural tissue. However, these methods have certain drawbacks, such as low sensitivity, long cultivation time, and the risk of invasiveness.

Interleukins play a pivotal role as a class of critical cytokines in pleural infections. Interleukin-39 (IL-39), a novel addition to the IL-12 family, was first identified by Wang et al in 2016.[5] Abundant research indicated that IL-39, as a novel pro-inflammatory factor, played a significant role in various autoimmune and inflammatory diseases.[6–9] Recent research established that tuberculosis patients, especially those with active tuberculosis, exhibited notably lower serum IL-39 levels compared to healthy individuals.[10] This raised the possibility of IL-39's involvement in the pathogenesis of TPE, and it could further hold diagnostic significance.

In this study, we assessed the diagnostic performance of IL-39 in discriminating tuberculous pleurisy from PEs caused by other etiologies by comparing its expression levels with tuberculosis biomarkers including adenosine deaminase (ADA), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α). Our findings demonstrated that IL-39 exhibits excellent diagnostic sensitivity for TPE, while offering the advantages of non-invasive, and rapid turnaround time. Additionally, we examined the correlation between IL-39 cytokine levels and common laboratory indicators of PE, as well as other tuberculosis biomarkers, to confirm the involvement of IL-39 in tuberculosis infection and the resulting inflammatory response.

Materials and Methods

Subjects

This study retrospectively collected data from 168 patients with PE who were treated in the Department of Respiratory and Critical Care Medicine at the First Affiliated Hospital of Ningbo University from July 2020 to October 2023. Through strict screening, patients who had received anti-tuberculosis or anti-tumor therapy, or had used corticosteroids or immunosuppressive agents in the past month were excluded. Patients with recent chest trauma within the past 3 months or who had undergone invasive pleural cavity examination and/or treatment were also excluded. Additionally, patients with PE of unknown origin or caused by multiple reasons were excluded, as well as those with compromised immune function or autoimmune diseases, and patients with complicated parapneumonic effusion (CPPE) or empyema. In total, 113 patients were enrolled as subjects in this study. The study protocol received approval from the Medical Ethics Committee of the First Affiliated Hospital of Ningbo University (Approval No. 2024 Research No. 014RS). Informed written consent was obtained from all subjects before collecting PE samples. Figure 1 shows the flowchart of the study.

Transudate was classified according to the Light's criteria.[11] The presence of malignant tumor cells detected in PE or pleural tissue cells could lead to a diagnosis of malignant pleural effusion (MPE).[12] The PE was classified as parapneumonic if it correlated with pulmonary infiltrates showing responsiveness to antibiotic therapy, fever, purulent sputum, or if a microorganism was detected in the PF. Within the parapneumonic effusions, uncomplicated parapneumonic effusion (UPPE) was diagnosed when any of the following criteria are not met: (1) loculated PE; (2) PF lactate dehydrogenase (LDH) > 1000 U/L; PF glucose < 2.2 mmol/L; (3) PF pH < 7.20; (4) positive microbial culture results from PE.[13] The diagnosis of TPE is based on histological examination of pleural biopsy suggesting typical caseating granulomas of tuberculosis infection, with exclusion of other granulomatous diseases of the pleura; or positive culture for *Mycobacterium tuberculosis (Mtb)* in sputum specimens, PE, pleural biopsy, bronchoalveolar lavage fluid specimens.[14]

Sample Collection and Processing

Before treatment initiation, all participants underwent thoracentesis with ultrasound or X-ray guidance to obtain PF samples. Subsequently, PF was immediately subjected to routine analysis, biochemical assays, cytological examination of

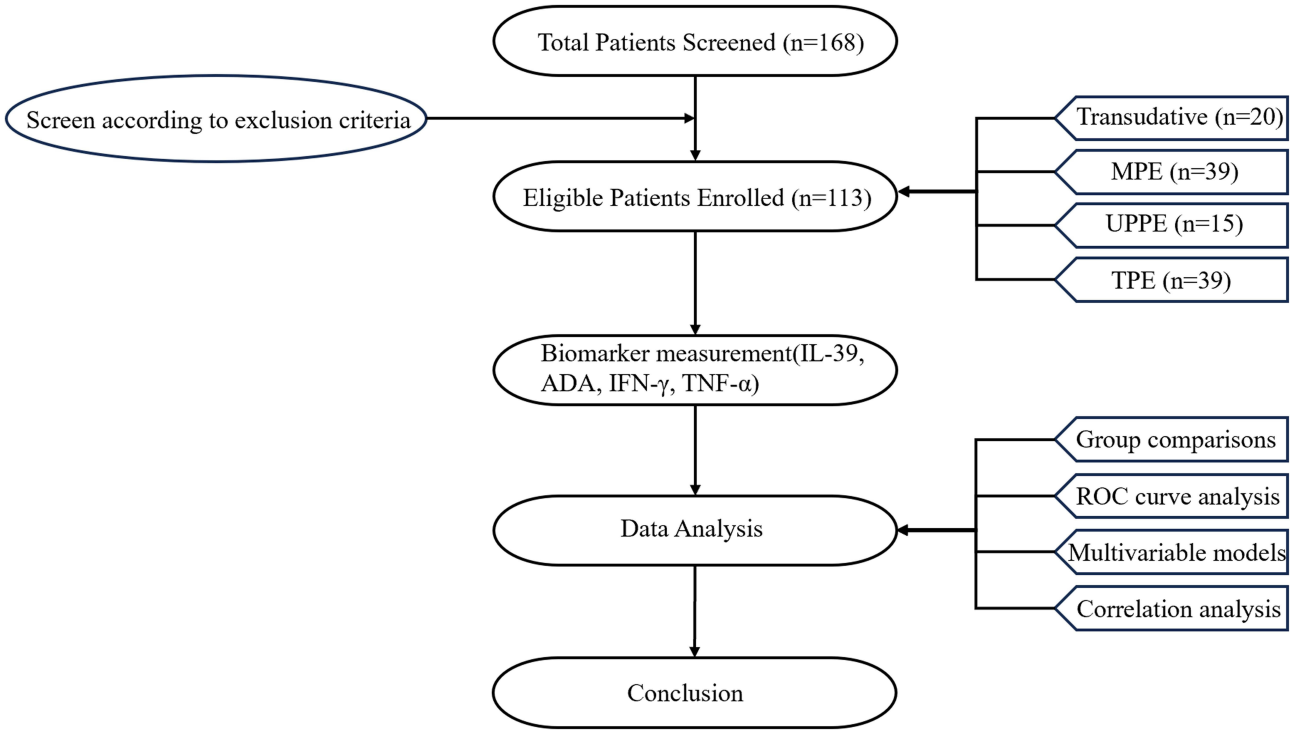

Figure 1 Flowchart of the study.

PF sediment cells, and microbial culture of PE. The remaining PE was centrifuged at 1000g for 15 minutes, then the supernatant was aspirated and stored at −80°C.

Measurement of IFN-γ, TNF-α, ADA and IL-39 Levels in PE

The concentrations of IFN-γ, TNF-α, and IL-39 in PE supernatant were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Jianglai Biotechnology Co., Ltd). The assay detection ranges were 1.56–100.0 pg/mL for IFN-γ and TNF-α, and 2.5–80.0 pg/mL for IL-39. Samples exceeding these limits were diluted or concentrated as needed to ensure accurate quantification within the assay range. ADA activity was determined using the colorimetric method (Shanghai Bohu Biological technology Co., Ltd).

Statistical Analysis

Continuous data were expressed as median (interquartile range), and the comparison between two sample groups was conducted using the Mann–Whitney *U*-test. The correlation between two variables was analyzed using Spearman's rank correlation test. Categorical data were depicted as frequencies and evaluated utilizing either the chi-square test or Fisher's exact test. Additionally, this study utilized binary logistic regression to construct multivariate diagnostic models. Receiver operating characteristic (ROC) curve analysis was conducted to assess the effectiveness of individual biomarkers, the multivariate diagnostic models, and combined diagnosis in distinguishing the etiology of PE. The statistical analysis was carried out with SPSS version 25.0 and GraphPad Prism version 9.0. $P < 0.05$ was considered statistically significant.

Results

Demographic Characteristics and Laboratory Test results of Study Participants

Following stringent criteria for diagnosis and exclusion, a total of 113 subjects were included in the study. Of these, 20 cases were in the transudate group, 39 in the MPE group, 15 in the UPPE group, and 39 in the TPE group. In this study, the transudate group, MPE group, and UPPE group were combined into the non-TPE group, totaling 74 cases. Demographic characteristics

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Table 1 The Demographic Data and Laboratory Test Results of the Study Participants (n = 113)

Variable	Transudate (n=20)	MPE (n=39)	UPPE (n=15)	TPE (n=39)
Age (years)	76.0 (64.5, 84.0)	68.0 (63.0, 77.0)	71.0 (63.5, 81.5)	47.0 (26.5, 67.5) **
Gender (male/female)	13/7	29/10	15/0$^{\#}$	31/8
WBC (cells/μL)	565.0 (355.0, 1050.0) *	900.0 (600.0, 1910.0) *	3100.0 (1700.0, 6688.5) $^{\#\#\#\#}$	2180.0 (1395.0, 3450.0) $^{\#\#\#\#}$
Neutrophils (%)	5.0 (3.5, 8.0) ***	15.0 (8.5, 24.5) $^{\#\#\#}$	61.0 (50.0, 72.5) ****	15.0 (9.0, 25.5) $^{\#\#\#\#}$
Lymphocytes (%)	49.5 (40.0, 56.5)	44.0 (29.5, 67.0)	26.0 (12.5, 33.5) *	72.0 (65.0, 81.0) ****
Total protein (g/l)	21.1 (16.2, 24.6) ****	44.6 (38.7, 49.6) $^{\#\#\#\#}$	43.3 (30.1, 49.3) $^{\#\#\#\#}$	49.0 (46.2, 53.7) *
LDH (U/l)	106.5 (89.0, 121.0) ***	345.0 (192.5, 533.0) $^{\#\#\#\#}$	195.0 (159.0, 306.5) $^{\#\#\#}$	591.0 (384.0, 787.0) **
Glucose (mmol/l)	7.6 (6.7, 9.4)	6.4 (5.6, 7.9) $^{\#}$	7.0 (6.0, 8.2)	5.0 (3.6, 6.0) **
ADA (U/l)	5.0 (3.5, 5.5) ***	9.0 (6.0, 12.0) $^{\#\#\#}$	10.0 (8.0, 13.0) $^{\#\#\#\#}$	43.0 (36.0, 51.5) ****
IFN-γ (pg/mL)	11.1 (8.5, 15.7) ***	62.0 (20.2, 113.4) $^{\#\#\#}$	54.0 (38.7, 83.5) $^{\#\#\#\#}$	252.2 (106.1, 378.4) ****
TNF-α (pg/mL)	80.5 (50.2, 114.5) *	122.7 (71.2, 190.0) *	201.4 (93.4, 325.8) *	1882.6 (1417.0, 2033.8) ****
IL-39 (pg/mL)	20.7 (20.5, 20.9) ****	32.1 (28.0, 37.3) $^{\#\#\#\#}$	39.8 (28.6, 101.2) $^{\#\#\#\#}$	313.3 (113.9, 412.5) ****

Notes: Data are displayed as median (interquartile range); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, compared with each of the other three groups; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.001$, $^{\#\#\#\#}$ $P < 0.0001$, compared with transudative group; The comparisons were determined by the Mann–Whitney *U*-test or the chi-square test.

Abbreviations: WBC, white blood cell count; NEU, neutrophils; LYM, lymphocytes; ADA, adenosine deaminase; LDH, lactate dehydrogenase; Glu, glucose; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; IL-39, interleukin-39; MPE, malignant pleural effusion; TPE, tuberculous pleural effusion; UPPE, uncomplicated parapneumonic pleural effusion.

and laboratory test results of study participants are detailed in Table 1. The results revealed that the median age (interquartile range) of patients in the TPE group was 47.0 (26.5, 67.5) years, with statistical differences observed compared to other groups (all $P < 0.01$). As expected, patients in the TPE group exhibited predominantly lymphocytic characteristics in PF cytology, with a significantly higher percentage of lymphocytes compared to PEs caused by other reasons (all $P < 0.0001$). Additionally, patients in the TPE group exhibited higher levels of total protein and LDH in PF compared to other groups, with statistically significant differences ($P < 0.05$ and $P < 0.01$, respectively), along with significantly lower glucose content in PF (all $P < 0.01$).

The Concentrations of IL-39, ADA, IFN-γ, and TNF-α in PE

Patients in the TPE group showed notably higher IL-39 levels in PE compared to other groups (all $P < 0.0001$), with no significant differences in IL-39 concentrations between the MPE and UPPE groups ($P > 0.05$) (Table 1 and Figure 2A). Further comparison of IL-39 concentrations in PE between the TPE and non-TPE groups revealed significantly higher levels in the TPE group ($P < 0.0001$) (Figure 2B).

As recognized tuberculosis biomarkers, consistent with expectations, patients in the TPE group exhibited significantly higher levels of ADA, IFN-γ, and TNF-α compared to all other groups ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively) (Table 1 and Figure 2C, E, G). The concentrations of ADA, IFN-γ, and TNF-α in the TPE group were markedly higher compared to the non-TPE group ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively) (Figure 2D, F, H).

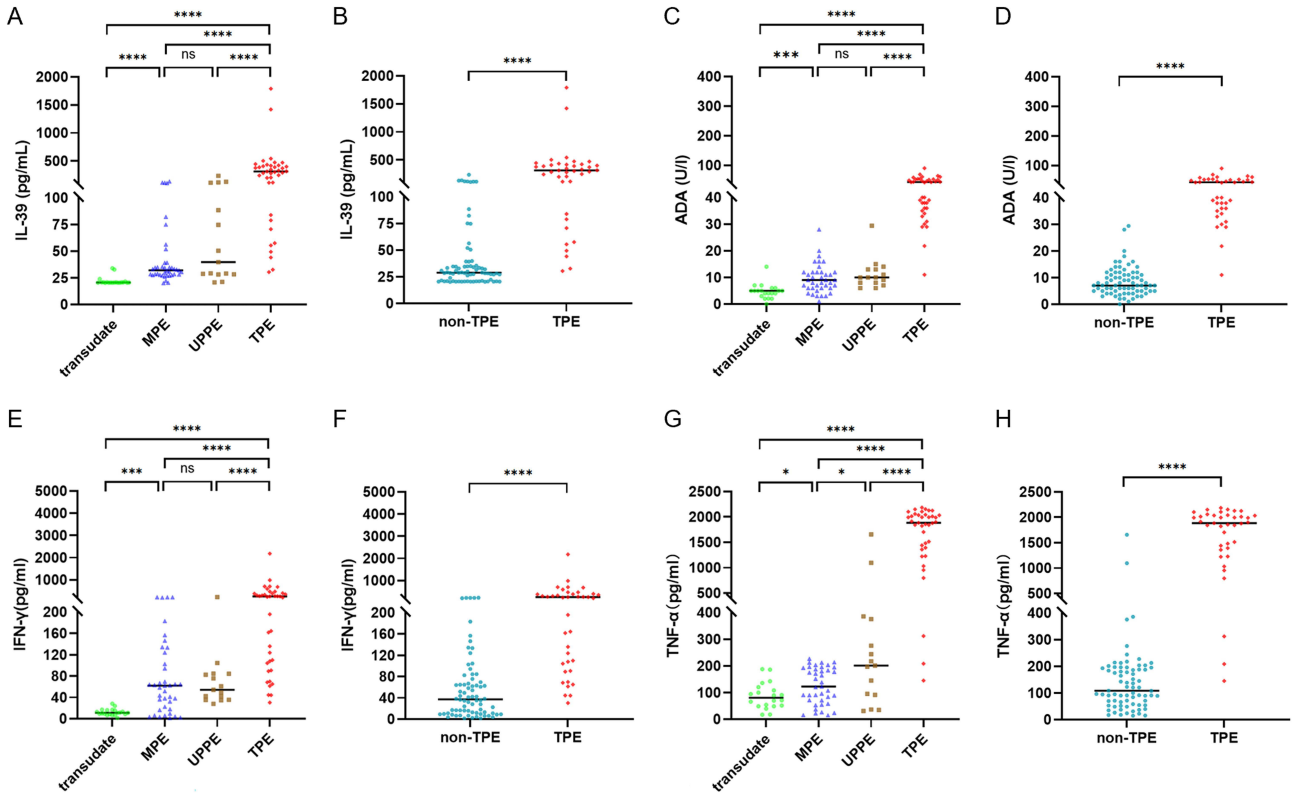

Figure 2 The level of PF parameters. (**A**) IL-39 levels across transudate, MPE, UPPE, and TPE. (**B**) Differences in IL-39 levels between TPE and non-TPE. (**C**) ADA levels across transudate, MPE, UPPE, TPE. (**D**) Differences in ADA levels between TPE and non-TPE. (**E**) IFN-γ levels across transudate, MPE, UPPE, TPE. (**F**) Differences in IFN-γ levels between TPE and non-TPE. (**G**) TNF-α levels across transudate, MPE, UPPE, TPE. (**H**) Differences in TNF-α levels between TPE and non-TPE. Horizontal bars denote the median. ns, not significant; *, $P < 0.05$; ***, $P < 0.001$; ****, $P < 0.0001$.

The Discriminative Diagnostic Value of IL-39, ADA, IFN-γ, TNF-α, Multivariate Model and Combined Testing for PE Etiologies

The Discriminative Diagnostic Value of IL-39, ADA, IFN-γ, TNF-α, Model 1 and Combined Testing for TPE and Non-TPE

ROC curve analysis demonstrated that IL-39 showed high diagnostic performance for distinguishing between TPE and non-TPE, with an AUC of 0.944 at a cut-off value of 39.8 pg/mL. Similarly, ADA, IFN-γ, and TNF-α also exhibited strong diagnostic values, with AUCs of 0.992, 0.900, and 0.980, respectively (Table 2 and Figure 3A).

We employed binary logistic regression to construct a diagnostic model for differentiating between TPE and non-TPE (Table 3), referred to as Model 1. IL-39, lymphocyte percentage, and glucose were included in the final multivariable model. Model 1 is described as follows: Model 1 = [0.024 ×IL-39 (pg/mL)] + [0.070 ×lymphocyte (%)] - [0.318 ×glucose (mmol/l)] - 4.981.

Table 2 Diagnostic Accuracy of Pleural Parameters for Distinguishing TPE From Non-TPE (n = 113)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39	> 39.8 pg/mL	0.944 (0.884–0.979)	< 0.0001	94.9	79.7	4.7	0.1	71.2	96.7
ADA	> 20.0 U/l	0.992 (0.953–1.000)	< 0.0001	97.4	97.3	36.1	0.0	95.0	98.6
IFN-γ	> 84.6 pg/mL	0.900 (0.829–0.948)	< 0.0001	82.1	81.1	4.3	0.2	69.6	89.6
TNF-α	> 385.9 pg/mL	0.980 (0.934–0.997)	< 0.0001	92.3	97.3	34.2	0.1	94.7	96.0
Model 1	> −1.007	0.970 (0.919–0.993)	< 0.0001	92.3	90.5	9.8	0.1	83.7	95.7

Notes: The Model 1 included IL-39, lymphocytes and glucose.
Abbreviations: AUC, area under the curve; PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

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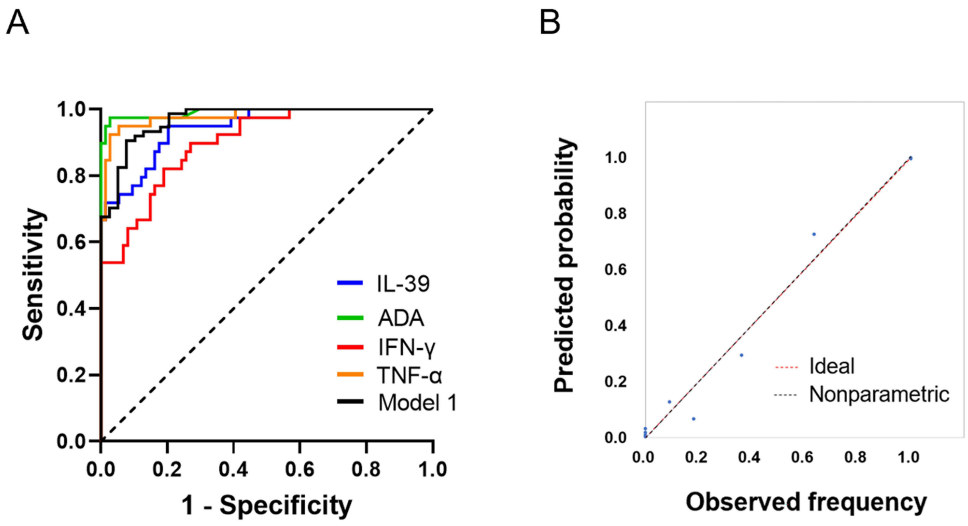

Figure 3 Diagnostic performance of multivariate model. (**A**) ROC curve comparison of Model 1 with IL-39, ADA, IFN-γ and TNF-α for distinguishing TPE from non-TPE. (**B**) Calibration plot of the multivariable Model 1.

ROC curve analysis was conducted to evaluate the discriminative diagnostic value of Model 1 for distinguishing between TPE and non-TPE (Figure 3A). The findings indicated that Model 1 demonstrated superior diagnostic performance compared to individual IL-39. With a cut-off value set at −1.007, the AUC was 0.970 (95% *CI*, 0.919–0.993; $P < 0.0001$) (Table 2). Furthermore, the tolerance for all independent variables exceeded 0.1, and the variance inflation factors were all below 2, suggesting the absence of multicollinearity among the predictor variables. With a *P* value of 0.865 from the Hosmer-Lemeshow test, the good fit of the model was further confirmed ($P > 0.05$). The calibration plot also demonstrated a high level of agreement between the actual and predicted probabilities, with an intercept of −0.0022 and a slope of 1.0067, confirming the acceptable calibration of the model (Figure 3B).

We further explored the diagnostic efficacy of combining IL-39 with other tuberculosis biomarkers (ADA, IFN-γ, TNF-α) to differentiate between TPE and non-TPE (Table 4). We found that combining two, three, or four biomarkers for diagnosis, with the requirement that all markers must be positive to diagnose TPE as positive, effectively improved the specificity of the diagnosis. Except for the IL-39+IFN-γ combination, all other combinations increased the specificity for distinguishing between TPE and non-TPE, maintaining high levels ranging from 98.6% to 100.0%. On the other hand, combining two, three, or four biomarkers for diagnosis, with any one or more positive results considered as a positive diagnosis for TPE, significantly improved the sensitivity of the diagnosis. Among these combinations, except for the IL-39 or IFN-γ combination, all other combinations exhibited 100% sensitivity for distinguishing between TPE and non-TPE, albeit with a relatively lower specificity ranging from 62.2% to 78.4%. Notably, combinations such as IL-39 or ADA and IL-39 or TNF-α showed the highest specificity.

Table 3 Univariate and Multivariate Analyses of Risk Factors for Distinguishing TPE From Non-TPE

Variable	Univariate		Multivariate	
	OR (95% *CI*)	***P* value**	**OR (95% *CI*)**	***P* value**
Total protein (g/l)	1.100(1.050–1.152)	< 0.0001	1.031(0.949–1.120)	0.472
LDH (U/l)	1.003(1.001–1.004)	< 0.001	0.999(0.996–1.002)	0.384
Glucose (mmol/l)	0.701(0.576–0.854)	< 0.001	0.697(0.490–0.990)	0.044
Lymphocytes (%)	1.069(1.041–1.097)	< 0.0001	1.072(1.030–1.115)	0.001
IL-39 (pg/mL)	1.022(1.012–1.032)	< 0.0001	1.023(1.009–1.037)	0.001

Table 4 The Value of IL-39 Combined With Tuberculosis Biomarkers in Discriminating TPE From Non-TPE (n = 113)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39+ADA	-	0.955 (0.898–0.985)	< 0.0001	92.3	98.6	68.3	0.1	97.3	96.1
IL-39+IFN-γ	-	0.877 (0.802–0.931)	< 0.0001	79.5	95.9	19.6	0.2	91.2	89.9
IL-39+TNF-α	-	0.929 (0.865–0.969)	< 0.0001	87.2	98.6	64.5	0.1	97.1	93.6
IL39+ADA+IFN-γ	-	0.897 (0.826–0.947)	< 0.0001	79.5	100.0	-	0.2	100.0	90.2
IL39+ADA+TNF-α	-	0.936 (0.874–0.973)	< 0.0001	87.2	100.0	-	0.1	100.0	93.7
IL-39+IFN-γ+TNF-α	-	0.897 (0.826–0.947)	< 0.0001	79.5	100.0	-	0.2	100.0	90.2
IL-39+IFN-γ+TNF-α +ADA	-	0.897 (0.826–0.947)	< 0.0001	79.5	100.0	-	0.2	100.0	90.2
IL-39 or ADA	-	0.892 (0.820–0.942)	< 0.0001	100.0	78.4	4.6	0.0	70.9	100.0
IL-39 or IFN-γ	-	0.812 (0.727–0.879)	< 0.0001	97.4	64.9	2.8	0.0	59.4	98.0
IL-39 or TNF-α	-	0.892 (0.820–0.942)	< 0.0001	100.0	78.4	4.6	0.0	70.9	100.0
IL-39 or ADA or IFN-γ	-	0.818 (0.734–0.884)	< 0.0001	100.0	63.5	2.7	0.0	59.1	100.0
IL-39 or ADA or TNF-α	-	0.885 (0.812–0.937)	< 0.0001	100.0	77.0	4.4	0.0	69.6	100.0
IL-39 or IFN-γ or TNF-α	-	0.818 (0.734–0.884)	< 0.0001	100.0	63.5	2.7	0.0	59.1	100.0
IL-39 or IFN-γ or TNF-α or ADA	-	0.811 (0.726–0.878)	< 0.0001	100.0	62.2	2.6	0.0	58.2	100.0

Notes: IL-39 > 39.8 pg/mL; ADA > 20.0 U/l; IFN-γ > 84.6 pg/mL; TNF-α > 385.9 pg/mL; "+" indicates that all biomarkers must be positive for a positive diagnosis; "or" indicates that a positive result for either one of the biomarkers is sufficient for a positive diagnosis.

The Discriminative Diagnostic Value of IL-39, ADA, IFN-γ, TNF-α and Combined Testing for TPE and MPE

ROC curve analysis demonstrated that IL-39 exhibited high diagnostic value for distinguishing between TPE and MPE, with an AUC of 0.941 at a cut-off value of 39.3 pg/mL. Similarly, ADA, IFN-γ, and TNF-α showed strong diagnostic performance with AUCs of 0.990, 0.855, and 0.986, respectively (Table 5 and Figure 4).

We further investigated the diagnostic efficacy of combining IL-39 with other tuberculosis biomarkers for distinguishing between TPE and MPE (Table 6). We found that combining two, three, or four biomarkers for diagnosis, with the requirement that all markers be positive to diagnose TPE, effectively enhanced the specificity for discriminating between TPE and MPE. Among these seven combinations, except for the IL-39 + IFN-γ combination, all others demonstrated 100% specificity, while maintaining high sensitivity levels ranging from 84.6% to 92.3%, with the IL-39 + ADA combination showing the highest sensitivity. On the other hand, combining two, three, or four biomarkers for diagnosis, with any positive result of one or more biomarkers considered as a positive diagnosis of TPE, although it increased the sensitivity of distinguishing TPE and MPE, resulting in 100% sensitivity for all combinations, specificity decreased, ranging from 51.3% to 79.5%. Among these combinations, the IL-39 or TNF-α combination exhibited the highest specificity.

The Discriminative Diagnostic Value of IL-39, ADA, IFN-γ, TNF-α and Combined Testing for TPE and UPPE

ROC curve analysis showed that IL-39 had a high diagnostic value for distinguishing between TPE and UPPE, with an AUC of 0.885 (95% *CI*, 0.769–0.956; $P < 0.0001$), at a cut-off value of 235.0 pg/mL. Comparatively, ADA had an AUC

Table 5 Diagnostic Accuracy of Pleural Parameters for Distinguishing TPE From MPE (n = 78)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39	> 39.3 pg/mL	0.941 (0.863–0.982)	< 0.0001	94.9	79.5	4.6	0.1	82.2	93.9
ADA	> 20.0 U/l	0.990 (0.936–1.000)	< 0.0001	97.4	97.4	38.0	0.0	97.4	97.4
IFN-γ	> 64.8 pg/mL	0.855 (0.757–0.925)	< 0.0001	89.7	64.1	2.5	0.2	71.4	86.2
TNF-α	> 226.9 pg/mL	0.986 (0.928–0.999)	< 0.0001	94.9	100.0	-	0.1	100.0	95.1

Abbreviations: AUC, area under the curve; PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

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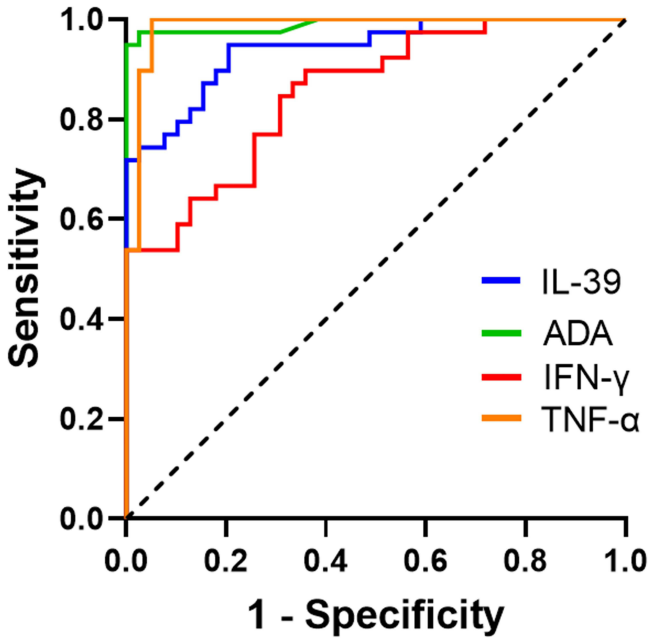

Figure 4 ROC curve comparison of IL-39, ADA, IFN-γ and TNF-α for distinguishing TPE from MPE.

of 0.985 (95% *CI*, 0.908–1.000; $P < 0.0001$) at a cut-off value of 15.0 U/L. IFN-γ had an AUC of 0.880 (95% *CI*, 0.763–0.953; $P < 0.0001$) at a cut-off value of 84.6 pg/mL. TNF-α had an AUC of 0.942 (95% *CI*, 0.843–0.987; $P < 0.0001$) at a cut-off value of 385.9 pg/mL (Table 7 and Figure 5).

We further explored the diagnostic efficacy of combining IL-39 with other tuberculosis biomarkers for distinguishing between TPE and UPPE (Table 8). We found that when combining two, three, or four biomarkers for diagnosis, and requiring all markers to be positive to make a positive diagnosis of TPE, although it significantly increased the specificity of distinguishing between TPE and UPPE, with all combinations achieving 100% specificity, the sensitivity was relatively low, ranging from 56.4% to 66.7%. Among them, the combination of IL-39 + ADA had the highest sensitivity, but compared to IL-39 alone, it did not significantly improve the diagnostic performance for differentiating TPE and UPPE. On the other hand, when considering any one or more positive results of biomarkers as a positive diagnosis of TPE, these seven combinations had sensitivities ranging from 92.3% to 97.4% in distinguishing TPE and UPPE. However, compared to ADA alone in PF, these combinations did not significantly improve the diagnostic performance for differentiating TPE and UPPE.

Correlation Analysis of IL-39 Concentration Levels with Other Common Parameters in the TPE Group

To explore the relationship between the novel tuberculosis biomarker IL-39 concentration and other common parameters, we constructed a correlation heatmap (Figure 6A). Based on this heatmap, we conducted Spearman correlation analysis on the portion with a *P*-value less than 0.05. The results showed that in the TPE group, IL-39 concentration was positively correlated with total protein ($r = 0.3496$, $P = 0.0291$, Figure 6B), positively correlated with LDH ($r = 0.6197$, $P < 0.0001$, Figure 6C), and negatively correlated with glucose ($r = -0.3561$, $P = 0.0261$, Figure 6D).

Correlation Analysis Between IL-39 and Tuberculosis Biomarkers

Given that IL-39 is a relatively novel cytokine, understanding its relationship with well-characterized biomarkers helps to clarify its role in the pathophysiology of tuberculous pleurisy and assess its potential as a complementary diagnostic marker. We further investigated the relationship between IL-39 levels and tuberculosis biomarkers (IFN-γ, TNF-α, and ADA). The results revealed a positive correlation between IL-39 levels and IFN-γ ($r = 0.6694$, $P < 0.0001$, Figure 7A), IL-39 levels and TNF-α ($r = 0.7567$, $P < 0.0001$, Figure 7B), IL-39 levels and ADA ($r = 0.7974$, $P < 0.0001$, Figure 7C).

Table 6 The Value of IL-39 Combined With Tuberculosis Biomarkers in Discriminating TPE From MPE (n = 78)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39+ADA	-	0.962 (0.892–0.992)	< 0.0001	92.3	100.0	-	0.1	100.0	92.9
IL-39+IFN-γ	-	0.872 (0.777–0.937)	< 0.0001	84.6	89.7	8.3	0.2	89.2	85.4
IL-39+TNF-α	-	0.949 (0.874–0.986)	< 0.0001	89.7	100.0	-	0.1	100.0	90.7
IL39+ADA+IFN-γ	-	0.923 (0.840–0.971)	< 0.0001	84.6	100.0	-	0.2	100.0	86.7
IL39+ADA+TNF-α	-	0.949 (0.874–0.986)	< 0.0001	89.7	100.0	-	0.1	100.0	90.7
IL-39+IFN-γ+TNF-α	-	0.923 (0.840–0.971)	< 0.0001	84.6	100.0	-	0.2	100.0	86.7
IL-39+IFN-γ+TNF-α +ADA	-	0.923 (0.840–0.971)	< 0.0001	84.6	100.0	-	0.2	100.0	86.7
IL-39 or ADA	-	0.885 (0.792–0.946)	< 0.0001	100.0	76.9	4.3	0.0	81.3	100.0
IL-39 or IFN-γ	-	0.769 (0.660–0.857)	< 0.0001	100.0	53.8	2.2	0.0	68.4	100.0
IL-39 or TNF-α	-	0.897 (0.808–0.955)	< 0.0001	100.0	79.5	4.9	0.0	83.0	100.0
IL-39 or ADA or IFN-γ	-	0.756 (0.646–0.847)	< 0.0001	100.0	51.3	2.1	0.0	67.2	100.0
IL-39 or ADA or TNF-α	-	0.885 (0.792–0.946)	< 0.0001	100.0	76.9	4.3	0.0	81.3	100.0
IL-39 or IFN-γ or TNF-α	-	0.769 (0.660–0.857)	< 0.0001	100.0	53.8	2.2	0.0	68.4	100.0
IL-39 or IFN-γ or TNF-α or ADA	-	0.756 (0.646–0.847)	< 0.0001	100.0	51.3	2.1	0.0	67.2	100.0

Notes: IL-39 > 39.3 pg/mL; ADA > 20.0 U/l; IFN-γ > 64.8 pg/mL; TNF-α > 226.9 pg/mL.

Table 7 Diagnostic Accuracy of Pleural Parameters for Distinguishing TPE From UPPE (n = 54)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39	> 235.0 pg/mL	0.885 (0.769–0.956)	< 0.0001	66.7	100.0	-	0.3	100.0	53.6
ADA	> 15.0 U/l	0.985 (0.908–1.000)	< 0.0001	97.4	93.3	14.6	0.0	97.4	93.3
IFN-γ	> 84.6 pg/mL	0.880 (0.763–0.953)	< 0.0001	82.1	86.7	6.2	0.2	94.1	65.0
TNF-α	> 385.9 pg/mL	0.942 (0.843–0.987)	< 0.0001	92.3	86.7	6.9	0.1	94.7	81.3

Abbreviations: AUC, area under the curve; PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

Additionally, a positive correlation was observed between IFN-γ levels and TNF-α (r = 0.7706, $P < 0.0001$, Figure 7D), IFN-γ levels and ADA (r = 0.7410, $P < 0.0001$, Figure 7E), and TNF-α levels and ADA (r = 0.7871, $P < 0.0001$, Figure 7F).

Discussion

PE, as a common complication in clinical practice, has diverse etiologies involving numerous diseases. Despite the availability of many markers for diagnosing the etiology of PE, the quest for novel biomarkers remains a challenging and crucial research focus in this field.

The cytokine IL-39, as a novel member of the IL-12 family, was first discovered by Wang et al in 2016.[5] In addition to IL-39, the IL-12 family also includes IL-12, IL-23, IL-27, and IL-35. All members of the IL-12 cytokine family are composed of two subunits: an α cytokine subunit and a β cytokine subunit.[15] This family is closely associated with the occurrence and development of inflammation, with chain pairing rearrangement (disruption) being a notable feature. Aberrant expression of this cytokine is closely associated with various pathological processes such as inflammation, autoimmune diseases, cancer, and endocrine disorders.[8,16–18] However, there is limited research investigating the presence and expression levels of the IL-39 cytokine in PE. This study innovatively assessed the expression levels of IL-39 in PE caused by different etiologies and thoroughly evaluated its efficacy in distinguishing between different etiologies of PE.

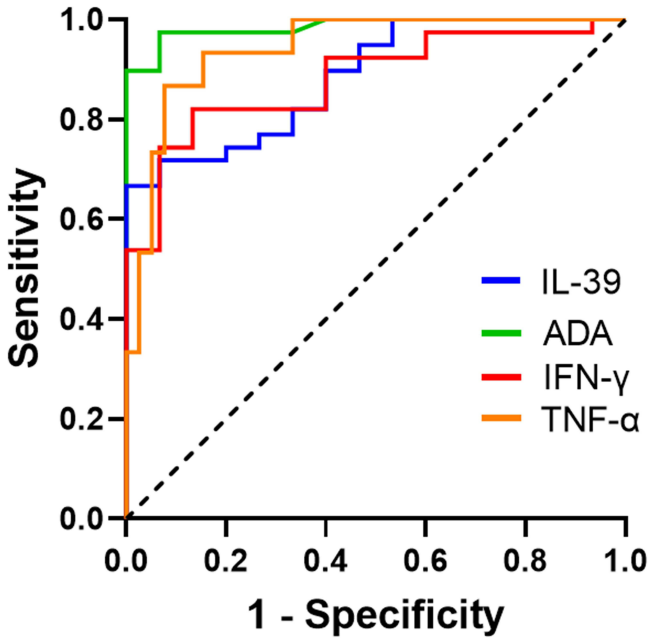

Figure 5 ROC curve comparison of IL-39, ADA, IFN-γ and TNF-α for distinguishing TPE from UPPE.

Recent studies have shown a significant decrease in serum IL-39 levels in tuberculosis patients compared to healthy individuals, particularly in active cases.[10] In this study, our findings demonstrate that IL-39 is expressed at varying levels in PEs caused by various etiologies, with differences observed in IL-39 expression levels among different types of PEs. Specifically, the expression of IL-39 in the PE of the TPE group was significantly elevated compared to the non-TPE group. IL-39 exhibited high diagnostic efficacy in distinguishing between TPE and non-TPE, with an AUC value of 0.944 (95% *CI*, 0.884–0.979). Based on the principle of maximizing the sum of sensitivity and specificity, the IL-39 cut-off value was established at 39.8 pg/mL. At this cut-off value, the sensitivity and specificity of IL-39 for distinguishing between TPE and non-TPE were 94.9% and 79.7%, respectively. Considering the AUC value, sensitivity, and specificity, IL-39 can be considered a valuable new biomarker for distinguishing between TPE and non-TPE. As ADA, IFN-γ, and TNF-α are recognized tuberculosis biomarkers,[19–21] we further compared the performance of IL-39 in distinguishing between TPE and non-TPE with these biomarkers. Our findings revealed that ADA and TNF-α had better AUC values in distinguishing between TPE and non-TPE, while IFN-γ showed slightly lower efficacy. However, heightened ADA levels might also manifest in a range of other conditions, including empyema, rheumatoid arthritis, and diverse infectious diseases (such as brucellosis, Q fever, histoplasmosis, and coccidioidomycosis).[22] Similarly, elevated levels of TNF-α can be seen in empyema, and studies have reported a sensitivity of 79% and specificity of 82% for TNF-α in diagnosing TPE, indicating its limited diagnostic accuracy.[23] Elevated levels of IFN-γ may also be observed in empyema and hematological malignancies.[24,25] Therefore, the use of a single biomarker in clinical differentiation between TPE and non-TPE may sometimes be limited, necessitating consideration of multiple factors. Consequently, we attempted to combine multiple tuberculosis biomarkers to distinguish between TPE and non-TPE. Our results demonstrate that these combined tests can further improve the sensitivity or specificity of the diagnosis. Thus, rapid combination testing of these tuberculosis biomarkers can effectively differentiate between TPE and non-TPE, particularly in initial screening, aiding in more effective management of the diagnostic strategy for tuberculous pleurisy. To further improve the diagnostic performance of TPE and non-TPE, we also developed a multivariate diagnostic Model 1 composed of IL-39, lymphocyte percentage and glucose. Validation showed that this model exhibited good discriminatory diagnostic performance for TPE and non-TPE.

Wang et al revealed the significant role of IL-39 in inducing neutrophil differentiation and/or proliferation, further discovering its ability to activate neutrophils, prompting the secretion of B cell activating factor (BAFF), thereby enhancing IL-39 expression in B cells, thus forming a positive feedback loop.[6] Notably, studies have found

Table 8 The Value of IL-39 Combined With Tuberculosis Biomarkers in Discriminating TPE From UPPE (n = 54)

Variable	Cut-off Value	AUC (95% *CI*)	*P* value	Sensitivity (%)	Specificity (%)	PLR	NLR	PPV (%)	NPV (%)
IL-39+ADA	-	0.833 (0.707–0.921)	< 0.0001	66.7	100.0	-	0.3	100.0	53.6
IL-39+IFN-γ	-	0.782 (0.649–0.883)	< 0.0001	56.4	100.0	-	0.4	100.0	46.9
IL-39+TNF-α	-	0.821 (0.692–0.912)	< 0.0001	64.1	100.0	-	0.4	100.0	51.7
IL39+ADA+IFN-γ	-	0.782 (0.649–0.883)	< 0.0001	56.4	100.0	-	0.4	100.0	46.9
IL39+ADA+TNF-α	-	0.821 (0.692–0.912)	< 0.0001	64.1	100.0	-	0.4	100.0	51.7
IL-39+IFN-γ+TNF-α	-	0.782 (0.649–0.883)	< 0.0001	56.4	100.0	-	0.4	100.0	46.9
IL-39+IFN-γ+TNF-α +ADA	-	0.782 (0.649–0.883)	< 0.0001	56.4	100.0	-	0.4	100.0	46.9
IL-39 or ADA	-	0.954 (0.859–0.992)	< 0.0001	97.4	93.3	14.6	0.0	97.4	93.3
IL-39 or IFN-γ	-	0.895 (0.781–0.962)	< 0.0001	92.3	86.7	6.9	0.1	94.7	81.3
IL-39 or TNF-α	-	0.908 (0.797–0.969)	< 0.0001	94.9	86.7	7.1	0.1	94.9	86.7
IL-39 or ADA or IFN-γ	-	0.887 (0.772–0.957)	< 0.0001	97.4	80.0	4.9	0.0	92.7	92.3
IL-39 or ADA or TNF-α	-	0.887 (0.772–0.957)	< 0.0001	97.4	80.0	4.9	0.0	92.7	92.3
IL-39 or IFN-γ or TNF-α	-	0.841 (0.716–0.926)	< 0.0001	94.9	73.3	3.6	0.1	90.2	84.6
IL-39 or IFN-γ or TNF-α or ADA	-	0.821 (0.692–0.912)	< 0.0001	97.4	66.7	2.9	0.0	88.4	90.9

Notes: IL-39 > 235.0 pg/mL; ADA > 15.0 U/l; IFN-γ > 84.6 pg/mL; TNF-α > 385.9 pg/mL.

a significant elevation in BAFF levels in the PE of TPE patients.[26] BAFF plays a crucial role in regulating T cell survival and activation, and it also promotes Th1 responses, thus playing a pivotal role in the pathogenesis of TPE.[27,28] Additionally, research has identified a self-sustaining inflammatory loop between BAFF and IFN-γ.[29] On the other hand, studies have reported that IL-39 can induce the production of IFN-γ in T cells in human lungs.[8] Moreover, when using IFN-γ inhibitors to suppress B cell activation, IL-39 expression significantly decreases.[5] Given the significant roles of IFN-γ and BAFF in the pathogenesis of TPE, as well as the complex relationship among IL-39, BAFF, and IFN-γ, we cannot help but speculate whether IL-39 also participates in the pathogenesis of TPE.

TPE is a delayed hypersensitivity reaction caused by *Mtb*, initiated when caseous lesions beneath the pleura rupture, releasing *Mtb* antigens into the pleural cavity, triggering the body's immune defense mechanisms.[30,31] The initial inflammatory response to antigens leads to increased capillary permeability, allowing proteins to flow into the pleural space, resulting in a significant rise in PF total protein levels. Additionally, inflammatory substances can block lymphatic pores in the pleura, further reducing the clearance rate of PE.[14] In the early stages of *Mtb*-induced pleural injury, polymorphonuclear leukocytes, especially neutrophils, rapidly infiltrate the pleural cavity. Subsequently, macrophages infiltrate in large numbers, initiating an immune response driven by lymphocytes, accompanied by the release of ADA and the formation of pleural granulomas.[32,33] It is noteworthy that in most cases of TPE, the infiltrating lymphocytes are primarily memory helper Th1 cells, which dominate the immune response.[34] In Th1 cell-mediated immune responses, the abundant release of IFN-γ, TNF-α, and other related cytokines plays a crucial role.[35] TNF-α can synergize with IFN-γ, stimulating the production of reactive nitrogen intermediates, thereby mediating macrophage phagocytosis and further enhancing the clearance of *Mtb*.[36,37] Furthermore, TNF-α stimulates immune cell migration to the site of infection, aiding in granuloma formation, thereby controlling disease progression.[38] In TPE, elevated levels of LDH often reflect the extent of *Mtb* damage to pleural tissue.[39] Meanwhile, studies have indicated a decrease in glucose levels in TPE.[40] Our data demonstrate that in TPE patients, IL-39 concentration levels are positively correlated with total protein ($r = 0.3496$, $P = 0.0291$) and LDH ($r = 0.6197$, $P < 0.0001$), while negatively correlated with glucose ($r = -0.3561$, $P = 0.0261$). This finding increases the possibility of IL-39 involvement in the pathogenesis of TPE.

Correlation analysis in our study demonstrated that IL-39 levels were positively associated with IFN-γ and TNF-α, both of which are key cytokines in the Th1-mediated immune response. Similarly, ADA, a widely recognized

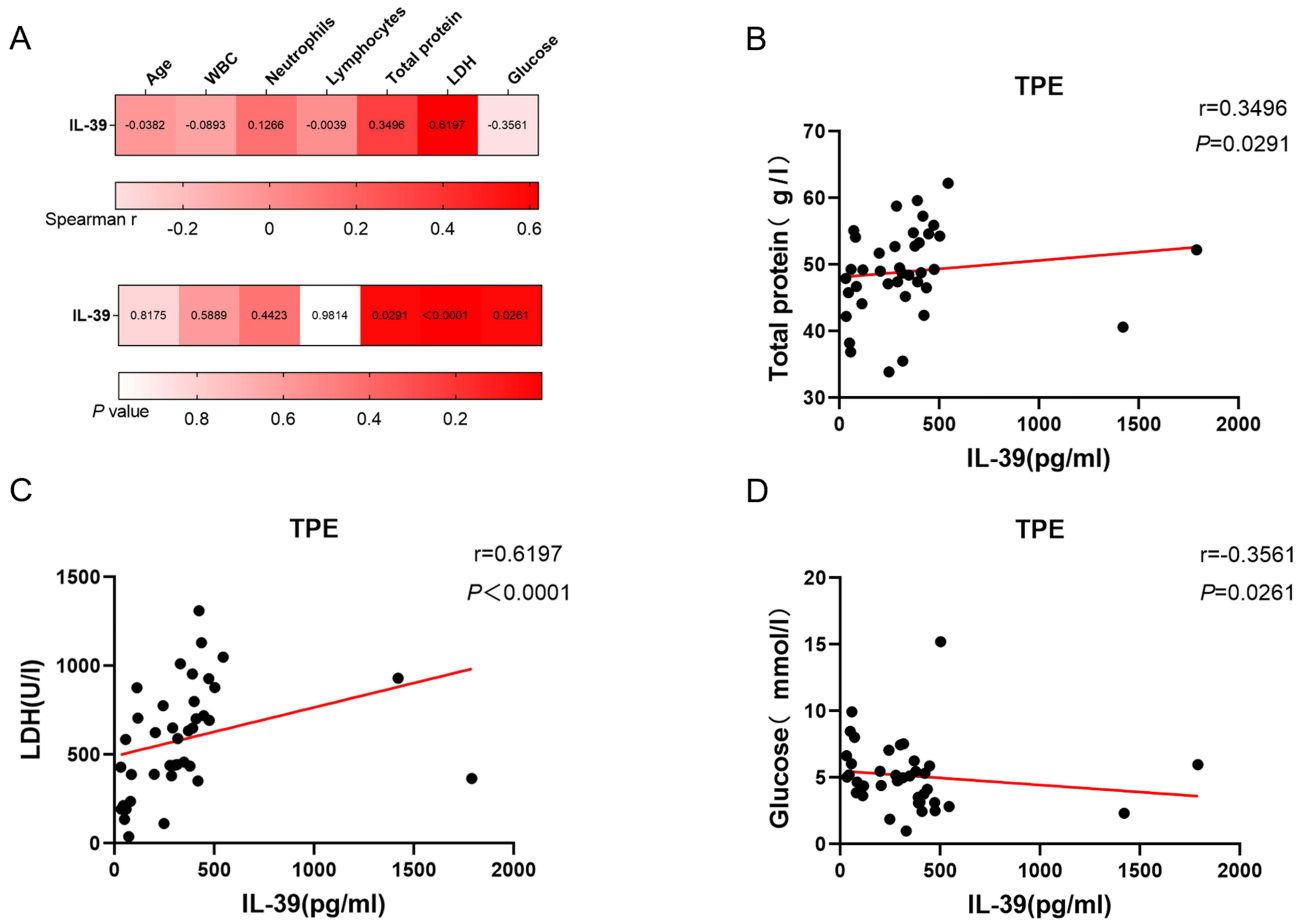

Figure 6 Correlation between IL-39 concentrations and PF related parameters in TPE, assessed via Spearman correlation coefficients. (**A**) Heatmap of the correlation between IL-39 and pleural fluid parameters in TPE. (**B**) Correlation analysis between IL-39 and total protein. (**C**) Correlation analysis between IL-39 and LDH. (**D**) Correlation analysis between IL-39 and glucose.

tuberculosis biomarker, showed significant correlations with IL-39, IFN-γ, and TNF-α, suggesting potential interactions between these markers in TPE pathophysiology.

While these findings indicate that IL-39 may play a role in the immune response to tuberculosis, further studies are required to elucidate its exact function and mechanism. Future research should focus on validating these associations and exploring the potential of IL-39 as a diagnostic or prognostic biomarker for TPE.

The cytological characteristics of PE in both TPE and MPE typically consist mainly of lymphocytes, with similar clinical and laboratory presentations. Distinguishing between the two becomes particularly challenging in the absence of clear pathological or etiological evidence. Although thoracoscopy is an effective diagnostic method, its invasiveness limits its widespread clinical application.[41–43] Additionally, tumor biomarkers such as vascular endothelial growth factor, carcinoembryonic antigen, carbohydrate antigen 125, carbohydrate antigen 15–3, carbohydrate antigen 19–9, and cytokeratin fragment 21–1 have been utilized in the diagnosis of MPE, but their accuracy still needs improvement.[44–46] Therefore, the search for a non-invasive, rapid, and effective new biomarker to differentiate between TPE and MPE is urgent and important. In this study, we found that the concentration of pleural IL-39 in TPE patients was significantly higher than in MPE patients, providing us with a new approach for differential diagnosis. Therefore, we further explored the diagnostic performance of pleural IL-39 in distinguishing between TPE and MPE. The results showed that IL-39 exhibited good performance in differentiating TPE and MPE, with an AUC value as high as 0.941 (95% *CI*, 0.863–0.982). Following the principle of maximizing the sum of sensitivity and specificity, we selected a cut-off value of 39.3 pg/mL for IL-39. At this cut-off value, the sensitivity and specificity of IL-39 for distinguishing between TPE and MPE were 94.9% and 79.5%, respectively. Considering the AUC value, sensitivity, and specificity, IL-39 was suggested

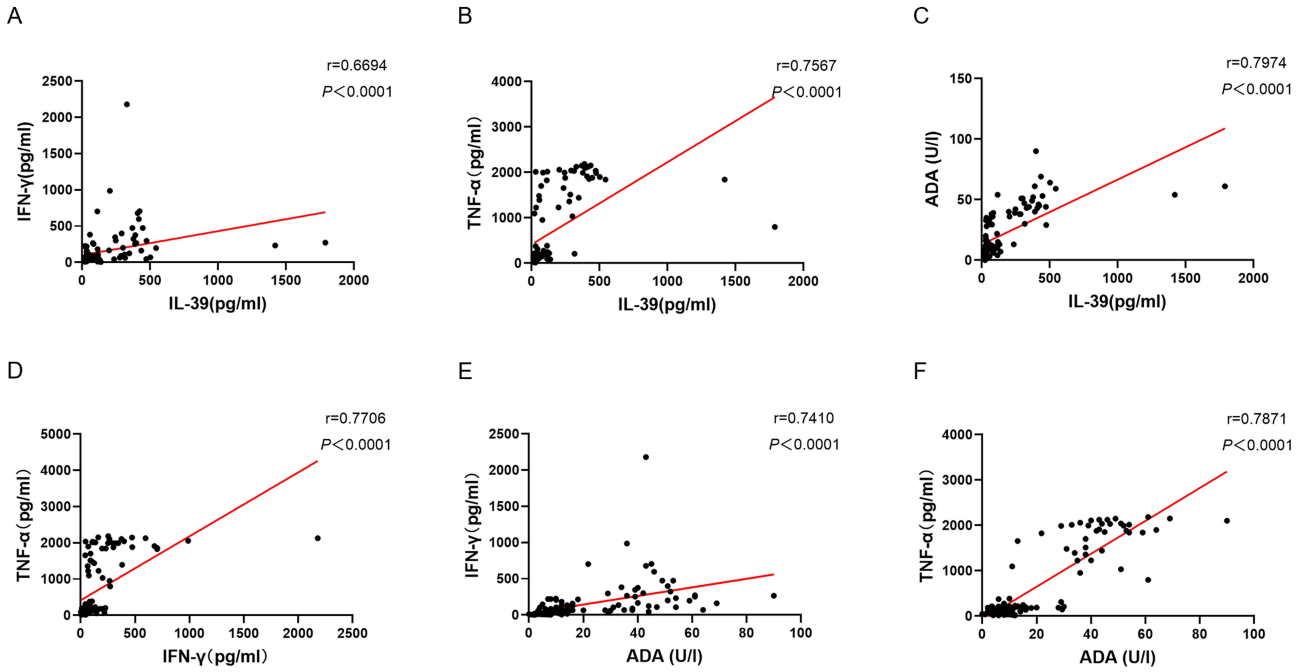

Figure 7 Spearman correlation coefficient analysis of four pleural markers. (**A**) Correlation analysis between IL-39 and IFN-γ. (**B**) Correlation analysis between IL-39 and TNF-α. (**C**) Correlation analysis between IL-39 and ADA. (**D**) Correlation analysis between IFN-γ and TNF-α. (**E**) Correlation analysis between ADA and IFN-γ. (**F**) Correlation analysis between ADA and TNF-α.

to be a valuable biomarker for the differential diagnosis of TPE and MPE. Additionally, we evaluated the diagnostic performance of pleural ADA, pleural IFN-γ, and pleural TNF-α for distinguishing between TPE and MPE. This can greatly reduce the misdiagnosis of MPE as TPE, which is crucial for formulating subsequent treatment plans and prognosis for patients. Moreover, we attempted to combine these biomarkers to differentiate between TPE and MPE. The results showed that combining these biomarkers could effectively distinguish between TPE and MPE.

TPE and UPPE are the primary causes of PE resulting from pleural infection, and in most cases, distinguishing between them is not difficult. This is mainly due to the cytological characteristics of TPE, which typically consist predominantly of lymphocytes, while UPPE is characterized by neutrophil predominance. However, exceptions exist in clinical practice. For instance, TPE may sometimes exhibit cytological characteristics predominantly comprising neutrophils,[47,48] and in other clinical and laboratory aspects, similarities between TPE and UPPE exist, especially in cases where PF Gram stain is negative and bacterial/mycobacterial cultures are negative, making the differentiation between the two particularly challenging. Therefore, the pursuit of novel biomarkers to enhance the accuracy of differential diagnosis is of utmost importance. In this study, we found that the expression level of IL-39 in the TPE group was significantly higher than in the UPPE group, prompting us to explore the potential value of IL-39 in the differential diagnosis of TPE and UPPE. Through further analysis, we found that the AUC of IL-39 for distinguishing between TPE and UPPE was 0.885 (95% *CI*, 0.769–0.956). Following the principle of maximizing the sum of sensitivity and specificity, we selected a cut-off value of 235.0 pg/mL for IL-39. At this cut-off value, the sensitivity and specificity of IL-39 for distinguishing between TPE and UPPE were 66.7% and 100.0%, respectively. It is worth noting that UPPE can usually be significantly relieved with effective antibiotic treatment, whereas once TPE is diagnosed, long-term anti-tuberculosis drug therapy is often required. These anti-tuberculosis drugs may cause a range of adverse reactions, such as hepatic impairment, allergic reactions, and visual impairment.[49] Therefore, the high specificity demonstrated by IL-39 in distinguishing between TPE and UPPE is particularly important. It helps to reduce the risk of misdiagnosing UPPE as TPE, optimizing the utilization of medical resources and avoiding the discomfort and potential harm caused by unnecessary anti-tuberculosis treatment. Similarly, we also evaluated the diagnostic performance of ADA, IFN-γ and TNF-α for distinguishing between TPE and UPPE. We found that ADA (sensitivity 97.4%, specificity 93.3%) performed

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better in distinguishing between TPPE and UPPE than TNF-α (sensitivity 92.3%, specificity 86.7%), and was superior to IFN-γ (sensitivity 82.1%, specificity 86.7%). Although the sensitivity of these three biomarkers in distinguishing between TPE and UPPE was higher than that of IL-39, their specificity was lower than IL-39. When combining these biomarkers to differentiate between TPE and UPPE, we found no significant improvement in the diagnostic performance for distinguishing between the two.

While our study has yielded some meaningful findings, there are still some limitations to consider. Firstly, the number of study subjects included was limited, particularly in the UPPE group, because of the strict adherence to inclusion and exclusion criteria during case selection. Hence, additional confirmation of the diagnostic accuracy of IL-39 cytokine and multivariate models in PEs resulting from various causes is necessary in future large-scale, multicenter cross-sectional studies to verify the precision and dependability of the findings. What's more, given the limited research data on IL-39 in other infectious diseases, further studies are needed to explore this issue in greater depth. Additionally, we did not perform flow cytometry experiments on PEs, nor did we include the detection of other cytokines (such as BAFF). This prevents us from further elucidating the pathogenic mechanism of IL-39 cytokine in PF.

Conclusion

IL-39 was expressed at varying levels across different etiologies of PE. It demonstrated good diagnostic value in differentiating TPE from PEs caused by other etiologies. Additionally, IL-39 exhibited positive correlations with other tuberculosis biomarkers, suggesting its potential involvement in tuberculosis infection and the inflammatory response it may induce.

Data Sharing Statement

Data will be made available on request.

Ethics Approval

This research protocol has been approved by the Medical Ethics Committee of The First Affiliated Hospital of Ningbo University, with the ethics approval number: The First Affiliated Hospital of Ningbo University Ethics 2024 Research No. 014RS. This research is conducted according to the World Medical Association Declaration of Helsinki.

Acknowledgments

Informed consent was obtained from all study participants prior to the commencement of the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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