Original Article

Diagnostic accuracy of interleukin 32γ for tuberculous pleural effusion

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Background: Accurate diagnosis of tuberculous pleural effusion (TPE) with biomarkers remains difficult. Interleukin 32gamma (IL-32γ) is a recently discovered proinflammatory cytokines which plays a vital role in the immune response to TPE. This study aimed to assess the diagnostic accuracy of IL-32γ for TPE, especially in different ages of patients.

Methods: Patients with a confirmed diagnosis of pleural effusion were systematically recruited from Beijing Chao-Yang Hospital between June 2019 and May 2022. The concentration of IL-32γ and interferon-gamma (IFN-γ) were evaluated in the pleural effusions with different etiology from 188 patients using enzyme-linked immunosorbent assay method. Adenosine deaminase (ADA) was determined by peroxidase method.

Results: At a threshold value of 181.56 ng/L, IL-32γ demonstrated an area under the curve (AUC) of 0.812 [95% confidence interval (CI): 0.748–0.865], with sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), and negative predictive value (NPV) of 70.2%, 86.0%, 5.0, 0.4, 92.0%, and 55.7%, respectively. In elderly patients (aged >65 years), IL-32γ demonstrated an AUC of 0.984 (95% CI: 0.891–1.000). Notably, the diagnostic accuracy of IL-32γ was significantly higher than that of ADA (P=0.03) and IFN-γ (P=0.02). Similarly, the AUCs for IL-32γ combined with ADA (0.981, 95% CI: 0.886–1.000) and IL-32γ combined with IFN-γ (1.000, 95% CI: 0.920–1.000) were significantly higher than those of ADA (P=0.04) or IFN-γ (P=0.01) alone in elderly patients.

Conclusions: IL-32γ can be used as a valuable biomarker for identifying patients with TPE, especially in elderly patients aged >65 years.

Keywords: Interleukin 32 gamma (IL-32γ); tuberculous pleural effusion (TPE); diagnostic accuracy; age

Submitted Oct 06, 2024. Accepted for publication Feb 18, 2025. Published online Apr 16, 2025.
doi: 10.21037/jtd-24-1676
View this article at: https://dx.doi.org/10.21037/jtd-24-1676

Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). In 2022, approximately 7.5 million new cases of tuberculosis (TB) were reported globally, resulting in an estimated 1.3 million deaths (1). Tuberculous pleural effusion (TPE) is one of the most common forms of extrapulmonary tuberculosis (2). It is definitively diagnosed

through mycobacterial culture of pleural fluid or pleural specimens, although their positive rates are as low as 40% and 65%, respectively (3,4). Another reliable diagnostic method for TPE is the identification of granulomas on pleural biopsy samples (2,5). However, the pleural biopsy is an invasive test with certain surgical risks and complications. Besides, such sophisticated technology may not be available in low resource settings. Therefore, several biochemical assays have been developed to investigate the intrapleural immune response in TPE patients (6). Measuring the concentrations of adenosine deaminase (ADA) and interferon-gamma (IFN-γ) has proven useful for establishing a presumptive diagnosis of TPE compared with non-TPE. However, these biomarkers have limitations in diagnosing TPE; ADA levels may be falsely low in elderly patients and falsely elevated in bacterial pleural co-infections (7). There is considerable variability in the measurement of IFN-γ, including differences in the thresholds for detecting TPE (ranging from 0.3–10 IU/mL and 1.5–300 pg/mL) and in the units used to express its levels in pleural fluid (IU/mL versus pg/mL) (6). Furthermore, the diagnostic accuracy of a single biomarker often decreases in areas of extremely high or low disease prevalence. Therefore, combining different detection methods for pleural effusion can improve the diagnosis of TPE (6,8). Consequently, there is a need for novel and efficient biomarkers for better diagnosis of TPE.

Interleukin 32 (IL-32) is a proinflammatory cytokine that plays a crucial role in the immune response to *Mtb*. Its expression can be induced by *Mtb* in peripheral blood mononuclear cells (PBMCs) and purified monocyte populations (9,10). Studies have shown that IL-32 expression is significantly increased in the pulmonary tissue of patients with active tuberculosis, and it enhances the host's immunity to *Mtb* (11-13). These findings establish IL-32 as a functional marker for the disease and a potential indicator of the body's ability to protect itself against active tuberculosis (14). Among its isoforms, IL-32γ is the most active and the only one that can be secreted by cells due to the signal peptide in its exon (13,15), making it the predominant subtype in the pleural fluid. Our previous study demonstrated that IL-32γ facilitates macrophage proliferation and apoptosis, serving as a potential diagnostic marker for TPE (16). However, the limitations of that study included a small sample size and inadequate investigation of disease variability and patient's age. Given that age may influence immune regulation (17,18), this study, aims to investigate the effect of age as an independent covariate on the diagnostic accuracy of IL-32γ, ADA, and IFN-γ in TPE compared with non-TPE. We present this article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1676/rc).

Highlight box

Key findings

- Interleukin 32 gamma (IL-32γ) can be used as a valuable biomarker for identifying patients with tuberculous pleural effusion (TPE), especially in elderly patients aged >65 years. At a threshold value of 181.56 ng/L, IL-32γ demonstrated an area under the curve (AUC) of 0.812 [95% confidence interval (CI): 0.748–0.865]. In elderly patients (aged >65 years), IL-32γ demonstrated an AUC of 0.984 (95% CI: 0.891–1.000).

What is known and what is new?

- IL-32 is a proinflammatory cytokine that plays a crucial role in the immune response to *Mycobacterium tuberculosis* (*Mtb*). Our previous study demonstrated that IL-32γ facilitates macrophage proliferation and apoptosis, serving as a potential diagnostic marker for TPE.
- This study incorporated a larger sample size and further investigated the disease variability and patient's age.

What is the implication, and what should change now?

- Age should be considered in the diagnosis of TPE or other etiologies of pleural effusion. Further research is required to investigate the specific mechanism by which age modulates the expression of IL-32γ, adenosine deaminase (ADA), and interferon-gamma (IFN-γ).

Methods

Study populations

The prospective study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Beijing Chaoyang Hospital, Capital Medical University (No. 2024-ke-15) and informed consent was obtained from all individual participants. Patients with a confirmed diagnosis of pleural effusion were systematically recruited from Beijing Chao-Yang Hospital between June 2019 and May 2022. Only patients who met the predetermined inclusion criteria were included in the study, while those who did not were excluded.

Diagnostic criteria and sample collection

TPE was diagnosed if any of the following criteria were met: positive *Mtb* culture from pleural effusion or pleural

biopsy specimen; the presence of epithelioid cell granuloma and/or caseating granulomas in the pleural biopsy specimen, with no evidence of other granulomatous diseases; exudative effusion accompanied by elevated ADA levels and either moderately or strongly positive skin tests for pure protein derivatives of tuberculin (PPD); positive IFN-γ release test; or a positive *Mtb* antibody. Malignant pleural effusion (MPE) was diagnosed by the presence of malignant cells in pleural effusion or pleural biopsy specimens. Parapneumonic pleural effusion (PPE) was diagnosed as pleural effusion associated with bacterial pneumonia, bronchiectasis, or lung abscess. Transudative pleural effusion was classified based on Light's criteria and attributed to other underlying conditions. The exclusion criteria included patients who had received antituberculosis or antitumor therapy prior to admission, those with immunosuppressed conditions (such as diabetes, hepatitis B/C infection, chronic glomerulonephritis, or immunosuppressive therapy), and patients who had undergone any invasive intrathoracic procedures or experienced chest trauma within three months.

Pleural effusion samples were collected through diagnostic thoracentesis or medical thoracoscopy. The samples were centrifuged at 400 g for 6 min, and the supernatant was separated and stored at –80 ℃ for further analysis.

Measurement of IL-32γ, ADA, and IFN-γ

The concentrations of IL-32γ and IFN-γ in the pleural effusion samples were measured using ELISA kits (IL-32γ, DY3040-05, R&D systems, USA; IFN-γ, KHC402, Invitrogen, USA) following the manufacturer's instructions. ADA levels were determined using an automatic biochemical analyzer (TBA40FR, Toshiba, Japan) with a peroxidase method kit (B1036, XinChuangYuan Biotech, China) following the manufacturer's instructions.

Statistical analysis

Categorical variables were presented as frequencies, while continuous variables were presented as medians with 25th to 75th percentiles. Significant differences in categorical variables were determined using the Chi-squared (χ^2) test. The Mann-Whitney *U* test and Kruskal-Wallis test were used to evaluate differences between two or multiple groups of continuous variables, respectively. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic value of variables, with the areas under the curves (AUC) calculated and compared using the z-test. All of the statistical analyses were performed using MedCalc 22.0 (MedCalc Software, Belgium) and GraphPad Prism 8.3 (GraphPad Software, USA), with P<0.05 considered to be statistically significant.

Results

Clinical and demographic parameters of patients with pleural effusions

A total of 188 patients with pleural effusions were enrolled in this study. The clinicopathological characteristics of patients with TPE and non-TPE are summarized in *Table 1*. Non-TPE cases were further classified as MPE, PPE, and transudative pleural effusion. The results showed that patients with TPE were significantly younger than those with non-TPE (P<0.001) across all of the etiological subgroups (P<0.01). However, there was no significant difference in gender distribution between the two groups (*Table 1*). The concentrations of total protein, glucose, and lactate dehydrogenase, as well as white blood cell (WBC) counts, were significantly higher in TPE than those in non-TPE (*Table 1*). There was no significant difference in chlorine concentration or the percentages of pleural fluid mononuclear cells (PFMCs) and polymorphonuclear leukocytes (PMNs) in WBC between the two groups (*Table 1*).

Concentrations of IL-32γ, ADA, and IFN-γ in pleural effusions

Regardless of age, the concentrations of IL-32γ, ADA, and IFN-γ were significantly higher in TPE groups than those in non-TPE groups (P<0.001) (*Figure 1A-1C*, *Table 2*). In the different etiological subgroups, these biomarkers were also significantly higher in TPE groups than those in MPE, PPE, or transudative pleural effusion subgroups (P<0.01, *Table 2*). Based on previous research and our preliminary studies (19,20), 65 years was set as the benchmark for two subgroups: the young group (≤65 years of age) and the elderly group (>65 years of age). In the young group, IL-32γ, ADA, and IFN-γ levels were significantly higher in TPE groups than those in non-TPE groups (P<0.001) (*Table 2*). Among the different etiological subgroups, these biomarkers were significantly higher in TPE groups than those in MPE subgroup (*Table 2*). However, no significant differences were observed between TPE and transudative pleural effusion for IL-32γ or ADA. In the elderly group, IL-32γ and ADA levels were

Table 1 Demographic, cytological and biochemical characteristics in pleural effusions

Variable	TPE (n=131)	Non-TPE				P value			
		Total (n=57)	MPE (n=45)	PPE (n=6)	Transudative (n=6)	TPE *vs.* non-TPE	TPE *vs.* MPE	TPE *vs.* PPE	TPE *vs.* transudative
Age, years	30.0 (24.0, 42.0)	69.0 (60.5, 75.5)	67.0 (57.5, 74.5)	73.5 (72.0, 76.8)	74.0 (57.3, 80.8)	<0.001	<0.001	<0.001	0.002
Sex, n (%)									
Male	79 (60.3)	34 (59.6)	24 (53.3)	4 (67.7)	6 (100.0)	0.93	0.41	0.76	0.05
Female	52 (39.7)	23 (40.4)	21 (46.7)	2 (33.3)	0 (–)	0.93	0.41	0.76	0.05
Pleural effusion									
Protein, g/L	52.3 (48.3, 55.2)	43.3 (38.9, 49.0)	46.6 (41.5, 49.5)	34.8 (29.4, 37.6)	33.1 (27.7, 44.2)	<0.001	<0.001	<0.001	0.003
Glucose, mmol/L	4.7 (3.9, 5.5)	5.4 (3.8, 6.8)	5.3 (3.6, 6.7)	7.1 (4.5, 8.5)	6.0 (4.4, 7.0)	0.02	0.76	0.02	0.07
Chloride, mmol/L	106.2 (103.2, 108.2)	106.7 (103.1, 108.8)	106.1 (103.1, 108.8)	106.2 (100.2, 108.7)	108.4 (106.2, 112.8)	0.35	0.58	0.84	0.058
LDH, U/L	520.0 (332.0, 701.0)	312.0 (181.5, 441.5)	349.0 (232.5, 468.0)	258.5 (131.8, 561.8)	107.0 (68.5, 156.3)	<0.001	0.001	0.04	<0.001
WBC, $\times10^9$/L	3.6 (2.1, 5.7)	1.1 (0.6, 1.7)	1.2 (0.7, 1.6)	0.6 (0.1, 2.0)	0.7 (0.5, 2.4)	<0.001	<0.001	<0.001	0.002
PFMC, %	86.0 (76.6, 93.4)	89.0 (75.0, 93.0)	89.0 (75.0, 92.5)	76.5 (48.2, 89.0)	95.0 (92.3, 98.1)	0.98	0.93	0.12	0.005
PMN, %	13.4 (6.1, 22.9)	11.0 (7.0, 25.1)	11.0 (7.5, 25.1)	23.5 (11.0, 51.8)	5.0 (1.9, 7.8)	0.98	0.78	0.09	0.008

Data are presented as median (25^{th}–75^{th} centile) unless otherwise stated. Transudative represents the transudative pleural effusion. LDH, lactate dehydrogenase; MPE, malignant pleural effusion; PFMC, pleural fluid mononuclear cell; PPE, parapneumonic pleural effusion; PMN, polymorphonuclear leukocyte; TPE, tuberculous pleural effusion; WBC, white blood cell.

significantly higher in TPE groups than those in non-TPE groups (IL-32γ, P<0.001; ADA, P=0.001), but no significant difference was found in IFN-γ levels between the two groups (*Table 2*). In the different etiological subgroups, IL-32γ levels were significantly higher in TPE than those in MPE, PPE, or transudative pleural effusion subgroups, ADA levels were significantly higher in TPE than those in MPE or transudative pleural effusion subgroups, but no significant difference was found in IFN-γ levels between TPE and the other etiological subgroups (*Table 2*).

Diagnostic values of IL-32γ, ADA, and IFN-γ in pleural effusions

The diagnostic capacity of IL-32γ, ADA, and IFN-γ for TPE was determined using ROC curve analysis (*Table 3*). Generally, IL-32γ demonstrated an AUC of 0.812 [95% confidence interval (CI): 0.748–0.865, P<0.001] with a threshold level of 181.56 ng/L (*Figure 1D*, *Table 3*). As shown in *Table 3*, the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), and negative predictive value (NPV) of IL-32γ were 70.2%, 86.0%, 5.0, 0.4, 92.0%, and 55.7%, respectively. The diagnostic accuracy parameters of ADA and IFN-γ are presented in *Figure 1E*,*1F* and *Table 3*. ADA and IFN-γ demonstrated AUCs of 0.946 (95% CI: 0.904–0.974, P<0.001) and 0.964 (95% CI: 0.926–0.985, P<0.001), respectively.

In the young group, IL-32γ demonstrated an AUC of 0.789 (95% CI: 0.713–0.853, P<0.001) at a threshold of 175.95 ng/L. The AUCs of ADA (0.991; 95% CI: 0.959–1.000) and IFN-γ (0.992; 95% CI: 0.961–1.000) were significantly higher than that of IL-32γ.

Notably, in the elderly group, IL-32γ demonstrated an AUC of 0.984 (95% CI: 0.891–1.000), which was significantly higher than ADA (0.817; 95% CI: 0.672–0.918, P=0.03) and IFN-γ (0.578; 95% CI: 0.420–0.725, P=0.02), at a threshold of 257.44 ng/L (*Figure 1G-1I*, *Table 3*). In this group, the sensitivity, specificity, PLR, NLR, PPV, and NPV of IL-32γ were 88.9%, 100.0%, –, 0.1, 100.0%, and 97.2%, respectively (*Table 3*).

Diagnostic values of combinations of IL-32γ and ADA, IL-32γ and IFN-γ, and ADA and IFN-γ in pleural effusions

In all of the age groups, the AUCs for IL-32γ combined

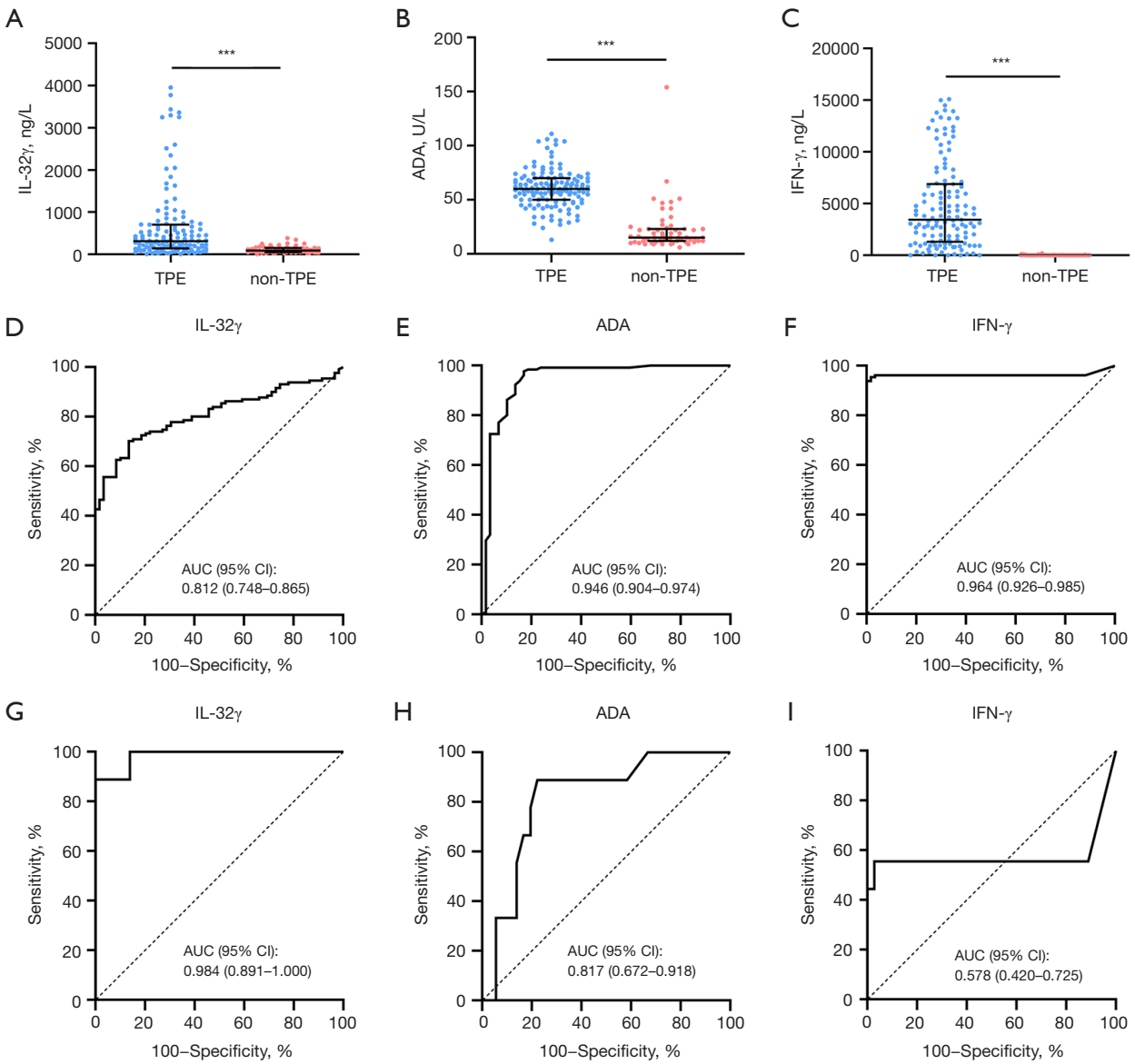

Figure 1 Concentrations and diagnostic capacity of IL-32γ, ADA, and IFN-γ for TPE overall or by age. (A-C) Concentrations of IL-32γ, ADA, and IFN-γ in PE from TPE and non-TPE patients, respectively. (D-F) ROC curves show the diagnostic capacity of the IL-32γ, ADA, and IFN-γ in pleural effusion. (G-I) ROC curves show the diagnostic capacity of the IL-32γ, ADA, and IFN-γ in TPE of patients aged >65 years. ***, P<0.001. ADA, adenosine deaminase; AUC, area under the curve; CI, confidence interval; IL-32γ, interleukin 32γ; IFN-γ, interferon-γ; PE, pleural effusion; ROC, receiver operating characteristic; TPE, tuberculous pleural effusion.

with ADA (0.970, 95% CI: 0.934–0.989) and IL-32γ combined with IFN-γ (0.999, 95% CI: 0.979–1.000) were significantly higher than those of IL-32γ (P<0.001), ADA (P=0.02), or IFN-γ (P=0.02) alone (*Figure 2A,2B*, *Tables 4,5*). Similarly, in the elderly group, the AUCs for IL-32γ combined with ADA (0.981, 95% CI: 0.886–1.000) and IL-32γ combined with IFN-γ (1.000, 95% CI: 0.920–1.000) were significantly higher than those of ADA (P=0.04) or IFN-γ (P=0.01) alone (*Figure 2C,2D*, *Tables 4,5*). However, in the young group, no significant differences were observed in the AUCs for IL-32γ combined with ADA or IL-32γ combined with IFN-γ compared with ADA or IFN-γ alone (*Table 5*).

Table 2 Concentrations of IL-32γ, ADA, and IFN-γ in pleural fluid according to age

Variable	TPE (n=131)	Non-TPE				P value			
		Total (n=57)	MPE (n=45)	PPE (n=6)	Transudative (n=6)	TPE *vs.* non-TPE	TPE *vs.* MPE	TPE *vs.* PPE	TPE *vs.* transudative
IL-32γ, ng/L									
All ages	314.05 (145.64, 705.45)	92.81 (49.22, 157.35)	112.01 (53.55, 160.61)	72.90 (45.10, 117.93)	54.49 (33.17, 119.32)	<0.001	<0.001	0.003	0.003
≤65 years	287.70 (143.94, 663.87)	87.73 (51.55, 165.44)	99.89 (49.10, 167.08)	–	66.55 (–)	<0.001	<0.001	–	0.29
>65 years	582.65 (396.33, 2,184.92)	92.81 (46.87, 157.29)	129.03 (55.79, 157.38)	72.90 (45.10, 117.93)	42.43 (32.73, 163.98)	<0.001	<0.001	<0.001	0.003
ADA, U/L									
All ages	60.00 (50.00, 70.00)	15.00 (11.50, 23.00)	15.00 (12.00, 23.00)	15.50 (10.50, 34.00)	11.00 (9.75, 21.75)	<0.001	<0.001	0.002	0.001
≤65 years	61.50 (51.50, 70.25)	15.00 (11.75, 19.75)	15.00 (11.50, 19.00)	–	42.00 (–)	<0.001	<0.001	–	0.20
>65 years	40.00 (28.00, 58.50)	15.00 (11.00, 24.00)	20.50 (12.25, 30.00)	15.50 (10.50, 34.00)	10.00 (9.50, 13.50)	0.001	0.003	0.07	0.001
IFN-γ, ng/L									
All ages	3,444.30 (1,314.84, 6,894.71)	3.31 (1.49, 10.55)	3.04 (1.23, 10.55)	8.97 (3.09, 31.65)	3.81 (2.19, 4.62)	<0.001	<0.001	<0.001	<0.001
≤65 years	3,759.68 (1,821.51, 7,138.64)	3.18 (1.10, 8.15)	3.31 (1.08, 8.57)	–	2.03 (–)	<0.001	<0.001	–	0.03
>65 years	173.26 (0.00, 1,653.15)	3.48 (1.57, 12.18)	2.51 (1.32, 14.56)	8.97 (3.09, 31.65)	4.41 (2.72, 4.67)	0.26	0.25	0.56	0.59

Data are presented as median (25^{th}–75^{th} centile). TPE, tuberculous pleural effusion. Transudative represents the transudative pleural effusion. ADA, adenosine deaminase; IFN-γ, interferon-γ; IL-32γ, interleukin 32γ; MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion.

Table 3 Diagnostic performance of pleural IL-32γ, ADA and IFN-γ in differentiating between patients with TPE and non-TPE according to age

Variable	Cut-off value	AUC (95%CI)	Sensitivity (95% CI)	Specificity (95% CI)	PLR	NLR	PPV (%)	NPV (%)
IL-32γ, ng/L								
All ages	181.56	0.812 (0.748–0.865)	70.2 (61.6–77.9)	86.0 (74.2–93.7)	5.0 (2.6–9.6)	0.4 (0.3–0.5)	92.0 (85.7–95.7)	55.7 (48.6–62.5)
≤65 years	175.95	0.789 (0.713–0.853)	68.9 (59.8–76.9)	86.4 (65.1–97.1)	5.1 (1.8–14.6)	0.4 (0.3–0.5)	96.6 (90.7–98.8)	33.3 (26.8–40.6)
>65 years	257.44	0.984 (0.891–1.000)	88.9 (51.8–99.7)	100.0 (90.0–100.0)	–	0.1 (0–0.7)	100.0 (–)	97.2 (84.7–99.6)
ADA, U/L								
All ages	27	0.946 (0.904–0.974)	97.7 (93.5–99.5)	84.2 (72.1–92.5)	6.2 (3.4–11.2)	0 (0–0.1)	93.4 (88.6–96.3)	94.1 (83.9–98.0)
≤65 years	26	0.991 (0.959–1.000)	99.2 (95.5–100.0)	90.1 (70.8–98.9)	10.9 (2.9–40.9)	0 (0–0.1)	98.4 (94.2–99.6)	95.2 (73.9–99.3)
>65 years	24	0.817* (0.672–0.918)	88.9 (51.8–99.7)	77.1 (59.9–89.6)	3.9 (2.0–7.5)	0.1 (0–0.9)	50.0 (34.3–65.7)	96.4 (80.8–99.4)
IFN-γ, ng/L								
All ages	191.32	0.964 (0.926–0.985)	93.9 (88.3–97.3)	100.0 (93.7–100.0)	–	0 (0–0.1)	100.0 (–)	87.7 (78.5–93.3)
≤65 years	87.18	0.992 (0.961–1.000)	99.2 (95.5–100.0)	100.0 (84.6–100.0)	–	0 (0–0.1)	100.0 (–)	95.7 (75.8–99.4)
>65 years	119.37	0.578† (0.420–0.725)	55.6 (21.2–86.3)	97.1 (85.1–99.9)	19.4 (2.6–146.3)	0.5 (0.2–1.0)	83.3 (39.9–97.4)	89.5 (80.3–94.6)

*, P=0.03; †, P=0.02, compared with AUC of IL-32γ using the z statistic, respectively. ADA, adenosine deaminase; AUC, area under the curve; CI, confidence interval; IFN-γ, interferon-γ; IL-32γ, interleukin 32γ; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

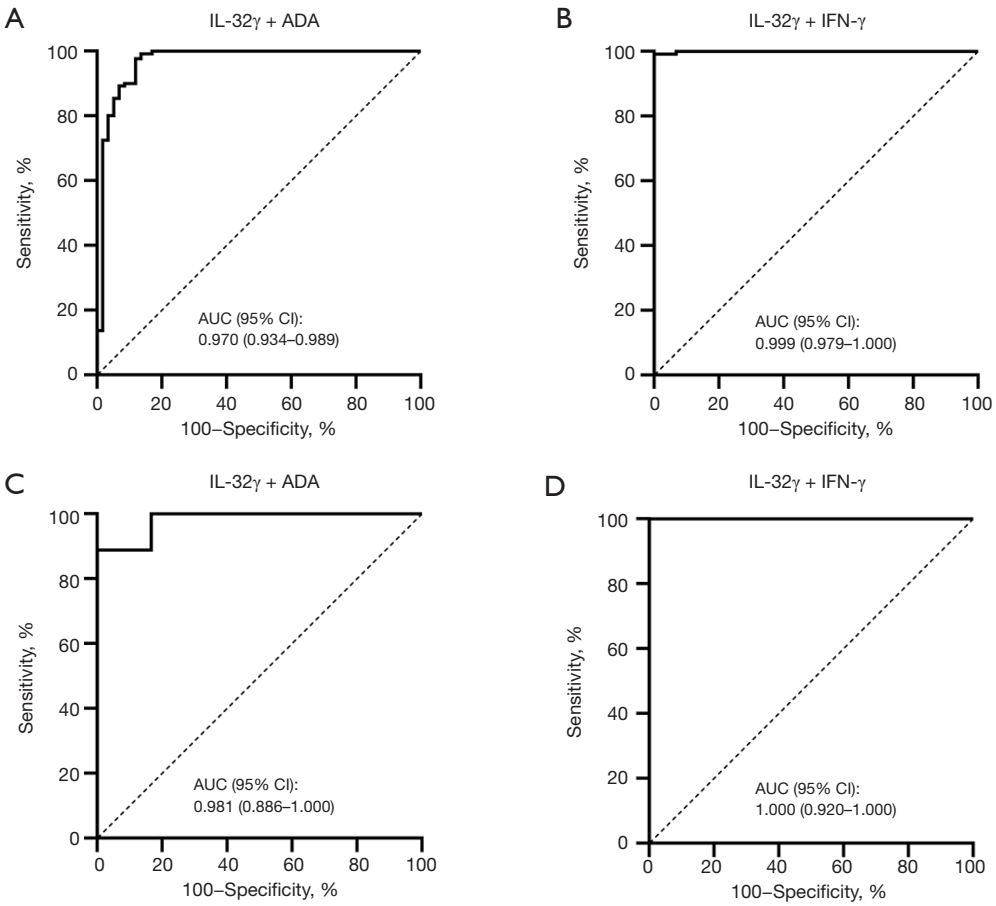

Figure 2 Diagnostic capacity of IL-32γ combined with ADA or IFN-γ for TPE overall or by age. (A,B) ROC curves show the diagnostic capacity of IL-32γ combined with ADA or IFN-γ for TPE in all of the age groups, respectively. (C,D) ROC curves show the diagnostic capacity of IL-32γ combined with ADA or IFN-γ for TPE in the elderly group, respectively. ADA, adenosine deaminase; AUC, area under the curve; CI, confidence interval; IL-32γ, interleukin 32γ; IFN-γ, interferon-γ; ROC, receiver operating characteristic; TPE, tuberculous pleural effusion.

Discussion

IL-32γ is a recently discovered proinflammatory cytokine that plays a crucial role in the immune response to intracellular pathogens such as *Mtb* (11,12). In this study, the concentration and diagnostic accuracy of IL-32γ were investigated. The results of this study showed that IL-32γ levels were significantly increased in TPE compared with non-TPE; establishing IL-32γ as a useful diagnostic biomarker for TPE, with an AUC of 0.812. Further age-based subgroup analysis revealed that IL-32γ had better diagnostic efficacy in distinguishing TPE from non-TPE in the elderly group (aged >65 years), with an AUC of 0.984, exceeding the diagnostic efficacy of both ADA and IFN-γ. Moreover, combining multiple factors for diagnosis enhanced the diagnostic efficacy between TPE and non-TPE. In all ages, especially in the elderly group (aged >65 years), the diagnostic efficacy of IL-32γ combined with ADA or with IFN-γ was significantly higher than that of ADA or IFN-γ alone. These findings revealed that IL-32γ serve as a potential diagnostic biomarker for distinguishing TPE from non-TPE, especially in elderly patients.

Some studies have shown that IL-32γ levels are significantly higher in TPE than that in non-TPE (16,21). Our previous study suggested that IL-32γ in pleural effusion serves as a potential diagnostic marker for distinguishing TPE from non-TPE (16). However, the limitations of that study included a small sample size and inadequate investigation of disease variability or patient's age. Therefore, in this study, we collected additional samples to

Table 4 Diagnostic performance of IL-32γ + ADA, IL-32γ + IFN-γ, and ADA + IFN-γ in differentiating between patients with TPE and non-TPE according to age

Variable	Cut-off value	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PLR	NLR	PPV (%)	NPV (%)
IL-32γ + ADA								
All ages	0.476	0.970 (0.934–0.989)	97.7 (93.5–99.5)	89.5 (78.5–96.0)	9.3 (4.4–19.8)	0 (0–0.1)	95.5 (90.9–97.8)	94.4 (84.7–98.1)
≤65 years	0.728	0.999 (0.972–1.000)	98.4 (94.2–99.8)	100.0 (84.6–100.0)	–	0 (0–0.1)	100.0 (–)	91.7 (73.6–97.8)
>65 years	0.281	0.981 (0.886–1.000)	88.9 (51.8–99.7)	100.0 (90.0–100.0)	–	0.1 (0–0.7)	100.0 (–)	97.2 (84.7–99.6)
IL-32γ + IFN-γ								
All ages	0.425	0.999 (0.979–1.000)	99.2 (95.8–100.0)	100.0 (93.7–100.0)	–	0 (0–0.1)	100.0 (–)	98.3 (89.0–99.8)
≤65 years	0	1.000 (0.975–1.000)	100.0 (97.0–100.0)	100.0 (84.6–100.0)	–	0 (–)	100.0 (–)	100.0 (–)
>65 years	0	1.000 (0.920–1.000)	100.0 (66.4–100.0)	100.0 (90.0–100.0)	–	0 (–)	100.0 (–)	100.0 (–)
ADA + IFN-γ								
All ages	0.666	0.985 (0.955–0.997)	94.7 (89.3–97.8)	100.0 (93.7–100.0)	–	0.1 (0–0.1)	100.0 (–)	89.1 (79.8–94.4)
≤65 years	0.196	0.998 (0.970–1.000)	99.2 (95.5–100.0)	100.0 (84.6–100.0)	–	0 (0–0.1)	100.0 (–)	95.7 (75.8–99.4)
>65 years	0.117	0.825 (0.681–0.923)	77.8 (40.0–97.2)	80.0 (63.1–91.6)	3.9 (1.8–8.2)	0.3 (0.1–1.0)	50.0 (32.1–67.9)	93.3 (80.3–98.0)

ADA, adenosine deaminase; AUC, area under the curve; CI, confidence interval; IFN-γ, interferon-γ; IL-32γ, interleukin 32γ; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value.

Table 5 Statistic tests of AUCs in IL-32γ + ADA, IL-32γ + IFN-γ, and ADA + IFN-γ compared with IL-32γ, ADA, or IFN-γ respectively

Variable	P value			Z score		
	IL-32γ	ADA	IFN-γ	IL-32γ	ADA	IFN-γ
IL-32γ + ADA						
All ages	<0.001	0.02	–	4.789	2.364	–
≤65 years	<0.001	0.19	–	4.883	1.307	–
>65 years	0.48	0.04	–	0.707	2.110	–
IL-32γ + IFN-γ						
All ages	<0.001	–	0.02	6.155	–	2.301
≤65 years	<0.001	–	0.32	4.913	–	0.999
>65 years	0.36	–	0.01	0.922	–	2.556
ADA + IFN-γ						
All ages	–	0.08	0.045	–	1.769	2.003
≤65 years	–	0.27	0.32	–	1.107	0.990
>65 years	–	0.90	0.04	–	0.130	2.086

AUCs are compared using the z statistic, respectively. ADA, adenosine deaminase; AUC, area under the curve; IFN-γ, interferon-γ; IL-32γ, interleukin 32γ.

expand the sample size and analyzed the diagnostic efficacy of IL-32γ based on disease etiology and age. Our results showed that the AUC of IL-32γ was lower than that of our previous study (16). These differences may be attributed to variations in the number and characteristics of the patients, as well as the etiology of the pleural effusions analyzed in both studies. Furthermore, we investigated the diagnostic efficacy of IL-32γ in different age subgroups, as well as the

efficacy of IL-32γ combined with ADA or IL-32γ combined with IFN-γ in these age groups. Thus, this study provides novel insight into the diagnostic performance of IL-32γ in TPE.

ADA has consistently shown high diagnostic accuracy for TPE since it was first reported in 1978 (22). However, some studies have established a negative correlation between ADA levels in pleural fluid and age. This suggests that ADA serves as a more accurate diagnostic marker in young patients than in older patients (18,23-26). Also, ADA levels in TPE decreased with increasing age, resulting in reduced diagnostic accuracy in older patients. This age-related decline in immune function likely impacts ADA expression (18,25,26). Similarly, the diagnostic accuracy of IFN-γ was influenced by age (18,26). Our results were consistent with prior studies, demonstrating a decline in the diagnostic efficacy of ADA and IFN-γ in the elderly group (aged >65 years). Conversely, IL-32γ exhibited higher diagnostic efficacy in elderly patients compared with younger patients. Additionally, the diagnostic efficacy of IL-32γ was significantly higher than that of ADA and IFN-γ in the elderly group (aged >65 years).

Research has shown that age-related changes in immunity can alter the fundamental mechanisms of the immune system (17,27). This change can be attributed to the decline in immune function with aging in elderly individuals. This decline in immune response results from a reduction in lymphocyte function, including disruption of cell activation, senescence of immunocytes, and disorder in the maturation processes of these cells (28). These findings suggest that the expression and secretion of IL-32γ, ADA, and IFN-γ might be influenced by age. Notably, in elderly patients (age >65 years), diagnostic performance was greatly enhanced using IL-32γ alone or in combination with ADA or IFN-γ, making these markers especially useful for identifying patients with TPE. However, further research is required to investigate the specific mechanism by which age modulates the expression of IL-32γ, ADA, and IFN-γ.

It should be mentioned that the current guideline recommends ADA as the preferred test for the diagnosis of TPE, because it is less expensive and simpler (29). In contrast, despite the superiority of IFN-γ in diagnosing TPE, the high cost and the lack of an accepted cut-off value for the discrimination of TPE limit its daily clinical use (6). Our data demonstrate that the measurement of IL-32γ obtained from pleural fluid may provide high levels of sensitivity and specificity for TPE, which may be applicable for the development of a rapid and non-invasive diagnostic test in low resource settings.

This study has some limitations. First, the number of older patients with TPE and younger patients with non-TPE we included were small, which might have biased the estimation of test accuracy. Second, the number of non-TPE subjects was limited, especially in the PPE or Transudative group, which might also lead to biased results. Therefore, we will consider increasing the sample size to verify the diagnostic accuracy of IL-32γ in pleural effusions. In addition, further studies are required to confirm the mechanism associated with the IL-32γ in pleural effusions.

Conclusions

In conclusion, this study highlights the importance of IL-32γ as a biomarker for diagnosing patients with TPE. The concentration of IL-32γ was significantly elevated in patients with TPE compared with non-TPE. Furthermore, IL-32γ, either alone or in combination with ADA or IFN-γ, exhibited high diagnostic efficacy in distinguishing TPE and non-TPE, especially in patients over the age of 65 years.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1676/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1676/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1676/prf

Funding: This work was supported by the National Natural Science Foundation of China (82200111), Beijing Scholars Program (048), and Reform and Development Program of Beijing Institute of Respiratory Medicine (Ggyfz202406).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1676/coif). The authors

have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The prospective study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of Beijing Chaoyang Hospital, Capital Medical University (No: 2024-ke-15) and informed consent was obtained from all individual participants.

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Cite this article as: Wang Q, Ma L, Zhai K, Yi FS, Shi HZ, Shao MM. Diagnostic accuracy of interleukin 32γ for tuberculous pleural effusion. J Thorac Dis 2025;17(4):2339-2349. doi: 10.21037/jtd-24-1676