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ORIGINAL RESEARCH

Diagnostic Accuracy of Interleukin-27 in Bronchoalveolar Lavage Fluids for Pulmonary Tuberculosis

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Background: The World Health Organization states that China had 0.9 million cases of tuberculosis in 2017, accounting for 9% of cases globally. Despite a decrease in the incidence and mortality of tuberculosis in China over time, development in choosing the appropriate prevention and control of TB is required.

Purpose: The aim of this study was to evaluate the diagnostic significance of interleukin-27 in bronchoalveolar lavage fluids for pulmonary tuberculosis.

Materials and methods: Eventually, 107 bronchoalveolar lavage fluids from patients were included in this study. The concentrations of interleukin-27 and adenosine deaminase were determined in bronchoalveolar lavage fluids using enzyme-linked immunosorbent assay.

Results: It was found that the concentrations of interleukin-27 in bronchoalveolar lavage fluids of sputum-positive pulmonary tuberculosis group were significantly higher than those in sputum-negative pulmonary tuberculosis, lung cancer, and previous pulmonary tuberculosis groups, respectively (all $P<0.001$). Interleukin-27 levels in bronchoalveolar lavage fluids could be used for diagnostic purpose for pulmonary tuberculosis, with the cutoff value of 7.867 pg/mL; interleukin-27 had a sensitivity of 68.8% and specificity of 100% for the differential diagnosis of pulmonary tuberculosis (sputum-negative and sputum-positive PTB) from lung cancer. And with the cutoff value of 6.012 pg/mL, IL-27 had sensitivity and specificity of both 100% for the differential diagnosis of PTB from previous PTB. The risk of pulmonary tuberculosis was positively associated with the concentrations of interleukin-27 and adenosine deaminase in bronchoalveolar lavage fluids.

Conclusion: Interleukin-27 in bronchoalveolar lavage fluids is a sensitive and specific biomarker for the differential diagnosis of pulmonary tuberculosis from lung cancer and previous pulmonary tuberculosis.

Keywords: pulmonary tuberculosis, interleukin-27, adenosine deaminase, bronchoalveolar lavage fluid

Introduction

Tuberculosis (TB) is a global public health problem that is usually caused by *Mycobacterium tuberculosis* (MTB). Globally, it is estimated that 10 million people developed TB in 2017.[1] The World Health Organization states that China had 0.9 million cases of TB in 2017, accounting for 9% of cases globally. Importantly, there are 8.89 million TB cases in China with an incidence rate of 63% until 2017.[1] Despite a decrease in the incidence and mortality of TB in China over time, development in choosing the appropriate prevention and control of TB is required. Acid-fast bacillus (AFB) smears remain the primary means of tuberculosis diagnosis in most

parts of the world where TB is common. However, so far, it is widely believed that AFB smears only detect approximately 50% of the cases of culture-positive TB, and quality control is increasingly difficult.[2,3] Currently, Xpert MTB/RIF technology has been widely used in clinical practice; although it has high specificity and sensitivity for the diagnosis of TB and some extrapulmonary TB, false-positive results still exist.[4–6] The fifth national TB epidemiological survey of China in 2010 showed that the prevalence of active TB and smear-positive TB were 459 per 100,000 and 66 per 100,000, respectively.[7] It can be found that sputum-negative pulmonary TB (PTB) accounts for the significant majority of active TB. Sputum-negative PTB in patients is likely an early stage of PTB, which, if not treated in time, may develop into sputum-positive PTB and eventually lead to death.[8–10]

Sputum-negative PTB is currently defined with TB symptoms in a patient with three sputum smears for AFB and one sputum culture examinations negative in whom PTB is later confirmed by biopsy and other investigations.[11] By now, the accurate diagnosis of sputum-negative PTB has been insufficiently studied, and sputum-negative PTB can also be contagious.[12,13] Hence, early diagnosis and treatment initiation of TB reduce the transmission of the MTB.

Interleukin-27 (IL-27), a member of the interleukin-12 (IL-12) cytokine family, is a heterodimeric cytokine composed of two distinct genes, Epstein-Barr virus-induced gene 3 and IL-27p28, and is mainly produced by the activated antigen-presenting cells.[14,15] Several previous studies have reported that IL-27 was involved in the pathogenesis of tuberculous pleural effusion (TPE) and was higher in TPE compared to non-TPEs, and showed a significant diagnostic value of IL-27 for differentiating TPE from non-TPEs.[16–19] A study in 2012 by Cao found that the concentrations of IL-27 in the sputum and plasma of patients with PTB were higher than that in healthy individuals; moreover, IL-27 in patients with PTB was positively associated with the load of MTB in the sputum.[20] One study by Khair demonstrated that bronchial epithelial cells are capable of expressing and releasing pro-inflammatory mediators that play a key role in airway inflammation.[21] Adenosine deaminase (ADA) is an enzyme involved in purine metabolism, and its primary function is the development and maintenance of the immune system.[22] Several studies have shown that ADA had a high sensitivity and specificity for the differential diagnosis of TPE from non-TPEs.[16–19,23,24]

Considering the facts that IL-27 and ADA are involved and increased in TPE, IL-27 is increased in the sputum of PTB, and its concentrations are positively associated with the load of MTB. Moreover, bronchial epithelial cells play a key role in airway inflammation. More importantly, bronchoalveolar lavage fluid (BALF) originates from the lung segment and subsegment, which is closer to the lesion. BALF can be used for the detection and analysis of cytokines, immune cells, and antibodies, which can directly reflect the condition of the lesion.[25,26] Therefore, we conducted this study to determine whether IL-27 increased in BALFs of PTB and evaluate the diagnostic accuracy of IL-27 in BALFs for PTB.

Materials and Methods

Subjects

This study was approved by the Ethics Committee of Qinghai University Affiliated Hospital, and all patients provided informed consent for inclusion in the study. A total of 248 BALFs from patients with active PTB, previous PTB, and lung cancer were collected from November 2017 to November 2018. Moreover, 141 patients who did not meet the inclusion criteria or whose diagnosis was not clear or anti-HIV antibody was positive were excluded. Eventually, 107 patients were included in this study, and the concentrations of IL-27 and ADA were determined in BALFs using enzyme-linked immunosorbent assay (ELISA).

Lung cancer was confirmed by histopathology. The diagnostic criteria for sputum-negative PTB, sputum-positive PTB, and previous PTB are listed in Table 1.

Sample Collection and Processing

All subjects provided informed consent before bronchoscopy. After the induction of local anesthesia with 2% xylocaine, the transnasal approach was usually selected using an Olympus fiberoptic bronchoscope, and xylocaine was induced through the biopsy channel as necessary. After careful examination of the entire bronchial trees, bronchoalveolar lavage (BAL) was performed in the most affected lobe in the case of local lesions via imaging studies and in the right middle lobe when the disease was diffuse.[27,28] Additionally, 20 mL of normal saline at 37°C was instilled and aspirated into a trap. Instillations of 20-mL normal saline up to a total of 100 mL were repeated, and a collection rate of 40% of the retrieved fluid was considered a qualified lavage.

The BALF was normally performed to detect AFB stain, MTB culture, histopathology, and TB PCR. Other diagnostic examinations of BALF were performed by means of clinical suspicion.

Table 1 The Diagnostic Criteria

Sputum-negative PTB	1) Typical pulmonary tuberculous symptoms and chest X-ray findings 2) Effective antituberculosis treatment 3) Excluding other non-tuberculous lung diseases 4) Strong positive manifestation of PPD (5IU), positive serum antituberculosis antibody 5) Positive tubercle bacillus PCR examination in the sputum 6) Tuberculosis lesions confirmed by extrapulmonary histopathology 7) Positive AFB smears in BALFs 8) Tuberculosis lesions confirmed by bronchial or pulmonary histopathology Three terms of 1 to 6 or any of 7 to 8 can diagnose sputum-negative PTB.[11]
Sputum-positive PTB	1) Positive AFB smears performed twice 2) Positive AFB smears and sputum culture performed once 3) Imaging that supports TB and positive sputum culture or AFB smears Any of 1 to 3 can diagnose sputum-positive PTB.[40]
Previous PTB	1) No tuberculous symptoms such as cough, fever, night sweats 2) Presence of discrete linear or reticular fibrotic scars, or dense nodules with distinct margins, with or without calcification on chest CT 3) Interferon gamma release assay and PPD were all negative. Meeting the above criteria can be diagnosed as previous PTB.[41,42]

Abbreviations: TB, tuberculosis; PTB, pulmonary tuberculosis; PPD, purified protein derivative; AFB, acid-fast bacillus; PCR, polymerase chain reaction; BALF, bronchoalveolar lavage fluid; CT, computed tomography.

A 10-mL lavage fluid was filtered using a double-layer sterile gauze and placed in a silicone plastic bottle. Subsequently, specimens were immediately immersed in ice and were centrifuged at 1800 rpm for 10 mins at 4°C. The cell-free supernatants of BALF were immediately frozen at −80°C after centrifugation to determine the concentrations of IL-27 and ADA.

Measurement of Interleukin-27 (IL-27) and Adenosine Deaminase (ADA)

The concentrations of IL-27 and ADA in the supernatants of BALFs were measured using ELISA kit according to the manufacturer's instructions (Kenuodi Biotech Co., Ltd., China). The minimum detectable concentrations of IL-27 and ADA were 1.0 pg/mL and 0.1 U/L, respectively.

Statistical Analysis

Data for continuous variables were expressed as mean ± standard deviation, and those of categorical variables were presented as percentages. Groups were compared using the chi-squared test for categorical variables and one-way analysis of variance for continuous variables. Receiver operating characteristic (ROC) curves were used to evaluate the capacity of IL-27 and ADA in BALFs for differentiating PTB from non-PTB and sputum-negative PTB from sputum-positive PTB. Youden's index was calculated to determine the best threshold of IL-27 and ADA. Smooth curve fitting was used to investigate the association between PTB with IL-27 and ADA using a generalized additive model. A two-piecewise linear regression model was applied to examine the threshold effect. A log likelihood ratio test was also conducted to compare the one-line linear regression model with a two-piecewise linear model. All data were analyzed using EmpowerStats(R) (www.empowerstats.com, X&Y solutions, Inc., Boston, MA, USA) and R (http://www.R-project.org). *P*-values <0.05 were considered significant.

Results

Characteristics of the Subjects

A total of 248 BALFs from patients were collected from November 2017 to November 2018. Additionally, 141 patients who did not meet the inclusion criteria or whose diagnosis was not clear or anti-HIV antibody was positive were excluded. Eventually, 25 patients with sputum-negative pulmonary tuberculosis, 23 sputum-positive pulmonary tuberculosis, 35 lung cancer, and 24 previous pulmonary tuberculosis were included with no significant difference in gender and age (all $P>0.05$).

Concentrations of IL-27 and ADA in Bronchoalveolar Lavage Fluids

The concentrations of IL-27 in BALFs of the sputum-positive PTB group were significantly higher than those in

Table 2 The Concentrations of IL-27 and ADA in BALFs of Different Groups (Mean±SD)

Groups	Previous PTB (n =24)	Sputum-Positive PTB (n =23)	Sputum-Negative PTB (n =25)	Lung cancer (n =35)	F value	*P*-value
IL-27 (pg/mL)	5.3±0.4	12.8±3.6*	7.6±0.9*▲	6.5±0.9*▲■	80.386	<0.001
ADA (U/L)	0.9±0.0	1.1±0.5*	1.1±0.2*	1.0±0.1*	6.411	<0.001

Notes: *Compared with previous PTB (*P*<0.001); ▲Compared with sputum-positive PTB (*P*<0.001); ■Compared with sputum-negative PTB (*P*<0.001).

the sputum-negative PTB, lung cancer, and previous PTB groups, respectively (all *P*<0.001). The concentrations of ADA in BALFs were significantly lower in the previous PTB group than those in the sputum-negative PTB, sputum-negative PTB, and lung cancer groups, respectively (all *P*<0.001), and there was no significant difference between the sputum-positive PTB, sputum-negative PTB, and lung cancer groups (all *P*>0.05) (Table 2 and Figure 1).

Diagnostic Value of IL-27

The diagnostic accuracy of IL-27 in BALFs was assessed using ROC curve analyses. The area under the ROC curve (AUC) was 0.885 (95% confidence interval [CI], 0.817–0.953), with the cutoff value of 7.867 pg/mL, and IL-27 had a sensitivity of 68.8%, specificity of 100%, and negative likelihood ratio (NLR) of 0.313 for the differential diagnosis of PTB (sputum-negative and sputum-positive PTB) from lung cancer (Table 3 and Figure 2). With the cutoff value of 6.012 pg/mL, IL-27 had sensitivity and specificity of both 100% for the differential diagnosis of PTB from previous PTB (Table 4 and Figure 3). In the differential diagnosis of sputum-positive PTB and sputum-negative PTB, the AUC was 0.988 (95% CI, 0.968–1.000), with the cutoff value of 8.222 pg/mL, and IL-27 had a sensitivity of 88%, specificity of 100%, and NLR of 0.12 (Table 5 and Figure 4).

Diagnostic Value of ADA

The concentrations of ADA in BALFs had an AUC of 0.641 (95% CI, 0.521–0.761) for the differential diagnosis of PTB from lung cancer (Figure 2). With the cutoff value of 1.087 U/L, the sensitivity, specificity, positive likelihood ration (PLR), and NLR were 62.5%, 60%, 1.563, and 0.625, respectively

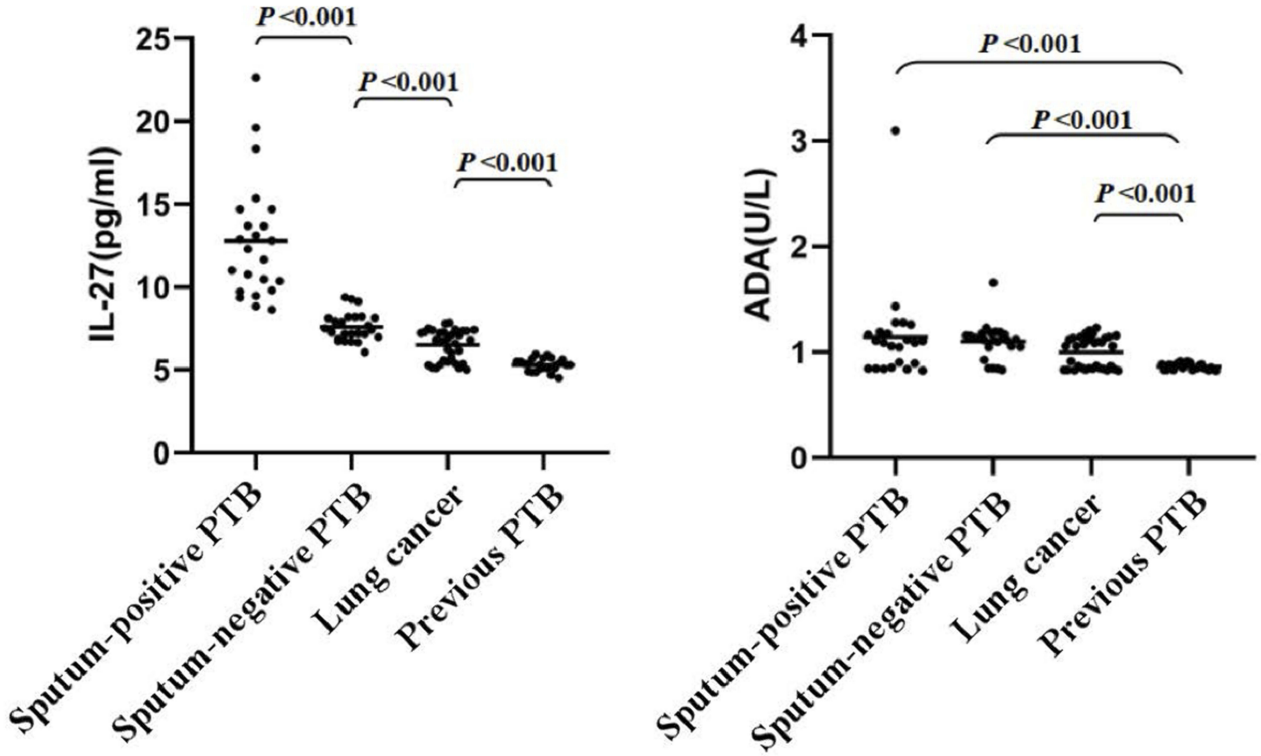

Figure 1 The concentrations of IL-27 and ADA in BALFs of different groups.

Table 3 Diagnostic Performance of IL-27 and ADA in Differentiating PTB from Lung Cancer

Variables	AUC (95% CI)	Cut-Off	Specificity	Sensitivity	PLR	NLR
IL-27	0.885 (0.817–0.953)	7.867	1.000	0.688	–	0.313
ADA	0.641 (0.521–0.761)	1.087	0.600	0.625	1.563	0.625
IL-27+ADA	0.883 (0.814–0.952)	–	1.000	0.688	–	0.313

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

(Table 3). With the cutoff value of 0.922 U/L, ADA had a sensitivity of 75% and a specificity of 100% for the differential diagnosis of PTB from previous PTB (Table 4 and Figure 3). However, with the cutoff value of 1.109 U/L, the AUC was 0.557 (95% CI, 0.384–0.730) and IL-27 had a sensitivity of 64%, specificity of 60.9%, PLR of 1.636, and NLR of 0.591 for the differential diagnosis of sputum-positive PTB from sputum-negative PTB (Table 5 and Figure 4).

Diagnostic Value with the Combinations of IL-27 and ADA

Combinations of IL-27 and ADA had a sensitivity of 68.8%, specificity of 100%, and NLR of 0.313 for the differential diagnosis of PTB from lung cancer, and the AUC was 0.883 (95% CI, 0.814–0.952) (Table 3 and Figure 2), and IL-27 had sensitivity and specificity of both 100% for the differential diagnosis of PTB from previous PTB (Table 4 and Figure 3). However, there was no significant difference in the diagnostic performance between IL-27 and IL-27+ADA in differentiating PTB from lung cancer and previous PTB (all P >0.05) (Tables S1 and S2). We also merged lung cancer and previous PTB groups as non-PTB group for ROC analysis and there was no significant difference in the diagnostic performance between IL-27 and IL-27+ADA in differentiating PTB from non-PTB (Tables S3 and S4, Figure S1).

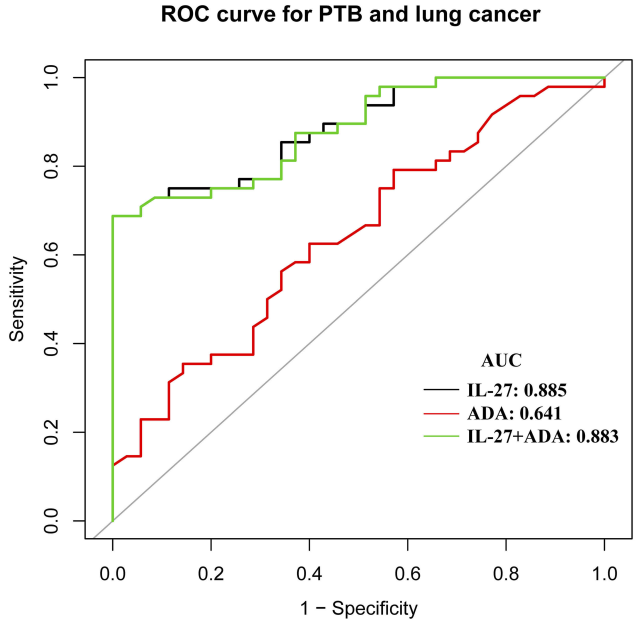

Figure 2 ROC curve for differential diagnosing PTB from lung cancer.
Notes: PTB including sputum-negative PTB and sputum-positive PTB.

Smooth Curve Fitting and Threshold Effect Analysis

Smooth curve fitting showed that the risk of PTB was positively associated with the concentrations of IL-27 and ADA in BALFs, but no significant nonlinear association or threshold effect between them was observed (the log likelihood ratio tests were all >0.05) (Figures 5 and 6).

Discussion

Cellular immunity is one of the main responses for anti-TB.[29] At early stage of MTB infection, macrophages recognize MTB through pattern recognition receptors, resulting in the transcription of IL-27 and other cytokines that stimulate lymphocytes to generate adaptive immune response.[30] IL-27 is produced by antigen-presenting cells in the early stage to exhibit an antimicrobial infection.[14] IL-27 can effectively induce CD4+T cell proliferation and promote the production of interferon gamma (IFN-γ) by CD4+T and inflammatory effects together with IL-12.[14] The anti-inflammatory response of IL-27 is through the promotion of interleukin-10, which acts in an anti-inflammatory manner by inhibiting the inflammatory response.[31]

To date, several studies have demonstrated the association between IL-27 and TPE, and the concentrations of IL-27 in TPE were significantly higher than those in malignant, infectious, and transudative PE, respectively.[16–19] Yang further confirmed the IL-27 produced by pleural CD4+T, CD8+T, monocytes, macrophages, and mesothelial cells by flow cytometry.[16] Pearl and González-Juarrero have shown that the number of macrophages in the airway of TB patients has increased.[32,33] Activated macrophages and dendritic cells are the main sources of IL-27.[34] Therefore, we hypothesized that IL-27 might be involved in the development of TB.

Table 4 Diagnostic Performance of IL-27 and ADA in Differentiating PTB from Previous PTB

Variables	AUC (95% CI)	Cut-Off	Specificity	Sensitivity	PLR	NLR
IL-27	1.000 (1.000–1.000)	6.012	1.000	1.000	–	0.000
ADA	0.851 (0.762–0.939)	0.922	1.000	0.750	–	0.250
IL-27+ADA	1.000 (1.000–1.000)	–	1.000	1.000	–	0.000

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

In this study, our data have shown for the first time that IL-27 could be detected in BALFs, and the levels of IL-27 in BALFs were significantly higher in PTB patients compared with non-PTB; furthermore, IL-27 concentration in sputum-positive PTB was also significantly higher than that in sputum-negative PTB. Cao found that the concentrations of IL-27 in the sputum of patients with PTB were positively associated with the load of MTB in the sputum.[20] We speculated that the load of MTB in sputum-positive PTB was higher than that in sputum-negative PTB, resulting in an increasing production of IL-27 in sputum-positive PTB. In the lung cancer group and previous PTB group, our data showed that the concentration of IL-27 in lung cancer was higher than that in previous PTB probably because the loads of MTB were extremely low or almost none; moreover, due to the dual effects of anti-inflammatory and pro-inflammatory mediators of IL-27, it can promote the production of cytokines, such as IFN-γ, and also play a pivotal role in antiviral and antitumor effects.[14,15,35] IFN-γ can effectively suppress the rapid growth of cancer cells and promote NK cell activity and production of tumor necrosis factor-α, leading to the death of cancer cells.[36] Therefore, this reverse change resulted in higher levels of IL-27 in lung cancer than in previous PTB.

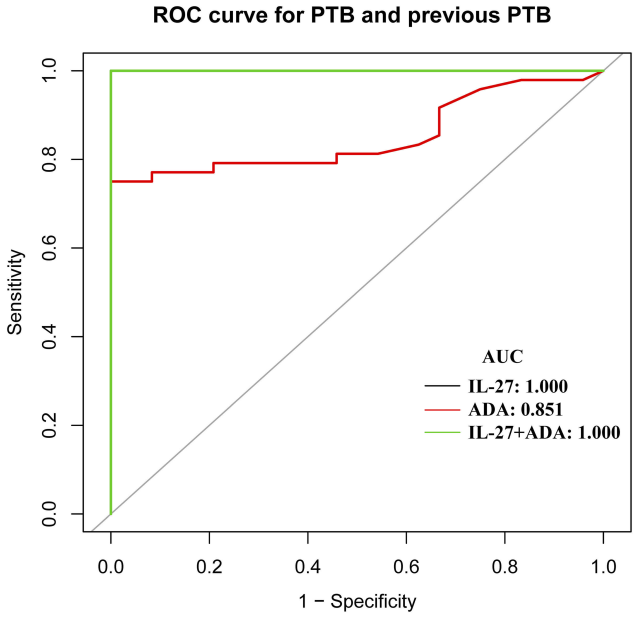

Figure 3 ROC curve for differential diagnosing PTB from previous PTB.
Notes: PTB including sputum-negative PTB and sputum-positive PTB.

ADA has been used as a valuable biomarker to differentiate TPE from non-TPEs.[16–19,23,24] Ainslie showed that CD4+T cells evidently increased in TPE; the number of lymphocytes in BALFs of the affected lungs was higher than those in the healthy side, and lymphocytes in the BALFs of patients with miliary PTB were the highest among other causes.[25] Our data demonstrated that the concentrations of ADA in BALFs were significantly lower in the previous PTB group than those in the sputum-negative PTB, sputum-negative PTB, and lung cancer groups, respectively, and because of insufficient sample size, there was no significant difference between the sputum-positive PTB (1.1±0.5 U/L), sputum-negative PTB (1.1±0.2 U/L), and lung cancer (1.0±0.1 U/L) groups. However, considering the numerical value, the ADA levels of the PTB groups (including sputum-positive PTB and sputum-negative PTB) were slightly higher than that of the lung cancer group, and the previous PTB (0.9±0.0 U/L) was the lowest among the four groups. The concentrations of ADA in BALFs had a sensitivity of 62.5% and specificity of 60% for the differential diagnosis of PTB from lung cancer with the cutoff value of 1.087 U/L, and the AUC was 0.641. Orphanidou and Boonsarngsuk suggested that ADA in BALFs has limited value in differentiating PTB from some pulmonary diseases. Boonsarngsuk et al showed that the AUC was 0.70 with the ADA cutoff value of 3.0 U/L in differentiating PTB.[37,38] Combinations of IL-27 and ADA had a sensitivity of 68.8% and specificity of 100% for the differential diagnosis of PTB from lung cancer, and the AUC was 0.883. However, there was no difference in the diagnostic performance between IL-27 and IL-27+ADA in differentiating PTB from lung cancer (P =0.609) probably because the ADA concentrations were not significantly different among the sputum-positive PTB, sputum-negative PTB, and lung cancer

Table 5 Diagnostic Performance of IL-27 and ADA in Differentiating Sputum-Negative PTB from sputum-Positive PTB

Variables	AUC (95% CI)	Cut-Off	Specificity	Sensitivity	PLR	NLR
IL-27	0.988 (0.968–1.000)	8.222	1.000	0.880	–	0.120
ADA	0.557 (0.384–0.730)	1.109	0.609	0.640	1.636	0.591

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

groups. Our results were consistent with that of the previous studies, but not identical.

To the best of our knowledge, this study was the first one to investigate the diagnostic value of BALFs IL-27 in the differential diagnosis of PTB from lung cancer and previous PTB and sputum-positive PTB from sputum-negative PTB.

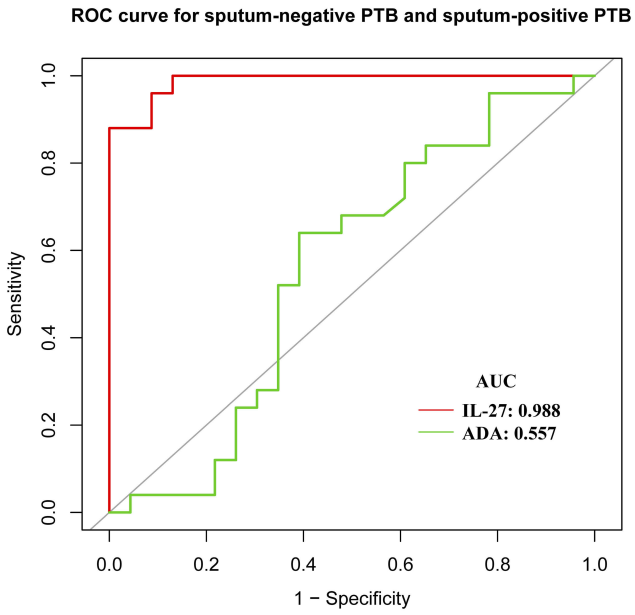

Figure 4 ROC curve for differential diagnosing sputum-negative PTB from sputum-positive PTB.

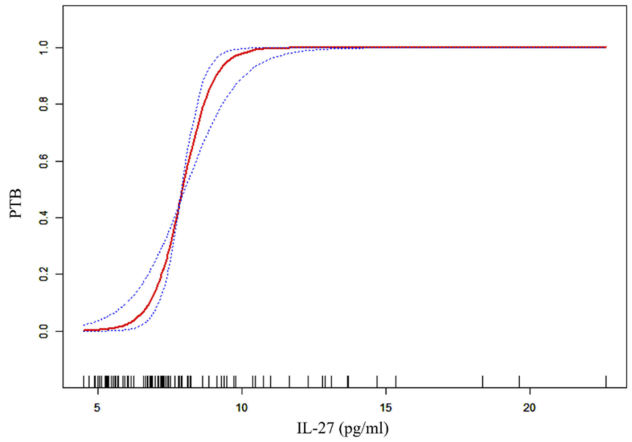

Figure 5 Smooth curve fitting for IL-27 with the risk of PTB.

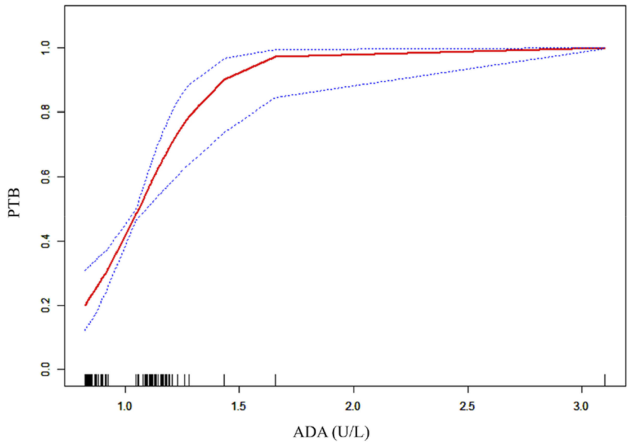

Figure 6 Smooth curve fitting for ADA with the risk of PTB.

Based on our current data, IL-27 has an excellent diagnostic performance in PTB, but this study has some limitations. First, we did not perform BALF differential cell counts. Hence, we were not able to investigate the association between IL-27 and ADA and different cell types in BALFs and thoroughly explain why some BALFs had higher IL-27 and ADA in PTB than other causes, although the source of IL-27 has been elucidated. Second, we did not correct the values for BALFs' urea or total protein concentration. Some investigators believed that the use of local ADA activity was more appropriate, which can be calculated from BALF ADA corrected by serum ADA, BALF urea (or albumin), and serum urea (or albumin).[37] However, Kayacan showed that BALF ADA activity had a higher diagnostic value than ADA in the local area.[39] Similarly, Yang believed that IL-27 is produced by the local area as the levels of IL-27 in serum were significantly lower than that in TPE.[16] Therefore, this requires further research to confirm whether the results are consistent in BALFs. Third, sample size in our study was limited; thus, there may be some bias. The concentration of IL-27 in BALFs is not conclusive for the diagnosis of PTB. Hence, increasing the sample size study is required in the future. Fourth, the ROC analysis in a first study such as this might always be more sensitive and specific than observed in real clinical practice since the cutoff value was calculated by the subjects included. It should therefore be mentioned that

further prospective studies are required to verify the diagnostic value of BALFs' IL-27 in a larger number of patients with PTB and other pulmonary diseases.

Conclusion

In conclusion, our data showed that IL-27 in BALFs is a sensitive and specific biomarker for the differential diagnosis of PTB from lung cancer and previous PTB.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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