

# Soluble CD14 and Th 17-Related Interleukins in Bronchoalveolar Lavage Fluid are Potential Triage Biomarkers for Pulmonary Tuberculosis

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## Research article

**Keywords:** Tuberculosis, bronchoalveolar lavage fluid, biomarker, soluble CD14, IL-17, IL-22

**Posted Date:** January 27th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-153808/v1>

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# Abstract

**Background:** Soluble CD14 (sCD14) and T helper 17-related interleukins (IL-17 and IL-22) in bronchoalveolar lavage fluid (BALF) are preliminarily reported to be increased in pulmonary tuberculosis (PTB). Their significances to the bacteriological confirmation and activity differentiation of PTB, however, are not completely clarified.

**Methods:** A observational study was conducted in 154 consecutive adult patients who were primarily diagnosed with PTB based on radiographic abnormalities. Bacteriological confirmation was made using sputum/BALF smearing, TB-DNA, Xpert test and *Mycobacterium tuberculosis* (Mtb) culture. BALF and serum sCD14, IL-17 and IL-22 were measured using ELISA assays. Their associations with clinical/bacteriological characteristics were analyzed.

**Results:** Finally, 103, 16, 7 and 28 patients were diagnosed with active, inactive, gray zone and non-PTB, respectively. Among active PTB patients, 82 (79.6%) cases, 33 (32.0%) based on sputum and additional 49 (47.6%) depended on BALF were bacteriologically confirmed. BALF levels of sCD14, IL-17 and IL-22 were similar between patients with PTB and non-PTB, but significantly higher in active PTB and bacteriologically-confirmed patients. The BALF levels of these biomarkers to some extent predicted bacteriological confirmation failure (AUC of IL-22 = 0.866), nonresponders to empiric antibacterial therapy, Xpert test (AUC of IL-22 = 0.760) and Mtb cultures. When the sensitivities were set at  $\geq 90\%$  (WHO's minimal requirement for a triage test), the specificities of bacteriological confirmation failure prediction and empiric antibacterial therapy nonresponder prediction in interferon- $\gamma$  release assay-positive patients were about 67%, very close to the WHO's minimal requirement ( $\geq 70\%$ ) for a triage test. In addition, serum levels of sCD14, IL-17 and IL-22 were relatively lower and had no predictive values.

**Conclusions:** BALF levels of sCD14, IL-17 and IL-22, superior to their serum levels, are potential triage biomarkers and may be used to replace the expensive Xpert test in particular occasions or refer proper patients to conduct lung biopsy and expensive molecular tests in the first week or to start nondelayed anti-TB therapy bypassing empiric antibacterial therapy and time-consuming Mtb culture.

## Background

Tuberculosis (TB) is one of the top 10 causes of death worldwide. It caused an estimated 1.5 million deaths in 2018 [1]. When a person is infected by *Mycobacterium tuberculosis* (Mtb), the greatest possibility is to suffer from pulmonary TB (PTB) [2]. All successful infections of Mtb occur by airborne transmission of droplet nuclei containing viable bacteria produced by a sputum-positive individual with active PTB [3]. These imply that to control PTB is very helpful to reduce the global TB incidence and mortality rates.

The diagnosis of PTB generally depends on clinical manifestations, thoracic imaging (e.g. chest computed tomography, CT) and evidences in immunology and etiology. Interferon- $\gamma$  release assay (IGRA), one of the immunological tests, is currently considered as the modern standard for diagnosis of Mtb

infection [2, 4]. However, IGRA is difficult to differentiate active TB from latent TB infection. As for active TB diagnosis, it is difficult to obtain bacteriological evidences. The principal techniques for detection of the Mtb in sputum and tissue samples are culture (the gold standard), microscopy (acid-fast smear) and nucleic acid amplification tests (NAAT). Unfortunately, the sensitivity of common sputum microscopy is about 15%-30% though it is significantly raised by the assistance of sputum induction and sputum NAAT (Xpert MTB/RIF) and culture [5–7]. Compared with sputum, bronchoalveolar lavage fluid (BALF) collected by fiberoptic bronchoscopy has much higher sensitivity for Mtb detection [8, 9]. Nonetheless, more than half of PTB in developing countries and about 20% in developed countries lack bacteriological confirmation [1, 10]. In addition, there are some other limitations of those principal techniques, the time delay of Mtb culture and the high cost of Xpert tests [11, 12]. Therefore, more accurate, rapid, and cost-effective triage tests based on biomarkers are needed to improve case detection, especially to indicate progression from latent infection to clinical disease [13, 14]. In clinical practice, there are still a few IGRA-negative, Mtb culture-positive TB patients who need novel diagnostic biomarkers. In fact, the activity differentiation and bacteriological conformation of a lot of CT-suspected patients are real challenges.

New diagnostic innovations of rapid biomarker-based, non-sputum tests are critical for detecting active TB, ruling out disease or referring patients to the more expensive and accurate molecular testing for confirmation [11, 13–16]. However, few biomarkers progress to a further developmental stage and none has so far led to a diagnostic test that meets the optimum performance requirements of the target product profiles [15, 16]. Therefore, it is imperative to find new biomarkers or to evaluate the significance of classical biomarkers in samples other than serum. Theoretically, BALF has much closer associated with the lesion site of PTB than serum, sputum or urine, which is supported by the fact that BALF has much higher sensitivity for Mtb detection [8, 9]. It is well known that Mtb exists and replicates in alveolar macrophages and membrane-associated cluster of differentiation 14 (CD14) is expressed on the membrane of activated macrophages. Both membrane-associated CD14 and soluble CD14 (sCD14) can facilitate the binding of lipopolysaccharide (LPS)/LPS binding protein to the receptor complex that consists of toll-like receptor-4 and MD-2 [17]. Such binding then activates macrophages to release cytokines, such as interleukin (IL)-6, IL-8 and IL-1 $\beta$  [18], and secondarily activates T helper 17 cells to release IL-17 and IL-22 [19–21]. A small size (n = 12) study has shown that sCD14 level rises in BALF from patients with PTB [22, 23]. IL-17 and IL-22 both decrease in serum, but increase in BALF in patients with TB [19]. However, the significance of these factors as triage tests or diagnostic biomarkers for detecting active PTB has not completely been clarified.

In this study, based on a cohort of CT-suspected PTB patients, BALF levels of sCD14, IL-17 and IL-22 were found to be associated with active PTB and to predict the results of bacteriological conformation, empiric antibacterial therapy, Xpert test and Mtb culture, and therefore may be used as triage biomarkers in the future.

## Methods

### Study Design and Subjects

A total of 154 adult patients with typical radiological changes of secondary PTB in CT were consecutively recruited for this study from the fifth Affiliated Hospital of Sun Yat-sen University during May 2017 and October 2018. Patients with recurrent TB and contraindications for fiberoptic bronchoscopy including respiratory failure, severe cardiovascular disease, massive hemoptysis, asthma, liver failure or kidney failure were excluded. The flow diagram of patients' diagnostic process is presented in Fig. 1. All patients were hospitalized, carried out fiberoptic bronchoscopy, diagnosed and started anti-TB therapy abiding by guidelines for treatment of tuberculosis (WHO). The associations of baseline BALF levels of sCD14, IL-17 and IL-22 with the final diagnoses and bacteriological confirmation manners were investigated.

## Data collections

The epidemiological, demographic and clinical data, regular laboratory examination results and treatment regimens of all patients were extracted from the electronic medical records. Regular laboratory examinations included IGRA (TB-specific interferon- $\gamma$  Elispot detection, Diao Biotech, Guangzhou, China), sputum smearing and assays of fluorescent quantitative PCR (TB-DNA) and GeneXpert MTB/RIF (Yimei Scientific, Tianjing, China) to detect TB-DNA in sputum and BALF. Mtb culture was mainly used BALF.

## Sample Preparation

BALF was obtained as reported [24]. A total of 70 mL normal saline was instilled and aspirated from the lung segments involved. Serum samples were regularly collected from each individual. BALF and serum samples were centrifuged at 4000 $\times$ g for 10 minutes to obtain clean supernatants and store them at -80°C until use.

## ELISAs

The levels of sCD14, IL-17 and IL-22 in serum and clean BALF were measured using an enzyme linked immunosorbent assay kit (Zheye Biotech, Guangzhou, China) according to the manufacturer's protocol. The detection limit of these assays was 1.0 ng/mL. Each sample was tested in duplicate.

## Statistical analysis.

Continuous variables are expressed as mean  $\pm$  standard deviation (SD). Categorical variables were expressed as numbers. The statistical significance of various differences was analyzed using the Chi-square, Fisher's exact, or Mann-Whitney U tests as appropriate. The correlations were assessed by Spearman's rank correlation test. In all cases,  $P < 0.05$  was considered to be statistically significant. The data were analyzed using SPSS software (Ver.22, SPSS Inc., Chicago, IL, USA).

## Results

### *Diagnostic process*

The flow diagram of patients' diagnostic process is presented in Fig. 1. Week 1, 70 patients were diagnosed with smear/NAAT-positive outcome either based on sputum or BALF and 67 out of them

immediately started anti-TB therapy. Sixty-one among the rest patients, except for 16 inactive PTB patients and 7 patients with positive culture of common bacteria, were undergone about two weeks of empiric antibacterial therapy (azithromycin + cefixime). Week 2–3, 27 out of 36 nonresponders to empiric antibacterial therapy subsequently started anti-TB therapy. Week 8, final diagnosis of all patients was made. Three nonresponders were confirmed to be infected by non-tuberculosis mycobacterium and 2 of them were excluded from the cohort of anti-TB. Five additional patients started anti-TB therapy due to the positive result of Mtb culture. The overall anti-TB rate was 94.2% (97/103). The anti-TB delay rates caused by empiric antibacterial therapy (Week 2–3) and Mtb culture (Week 8) were 33.0% (34/103) and 4.9% (5/103), respectively. Seven cases were grouped into gray zone (gz-PTB) because they were IGRA-positive, but well responded to antibiotic treatment or had a positive result of common bacterial culture. Among active PTB patients, 82 (79.6%) cases, 33 (32.0%) based on sputum and additional 49 (47.6%) depended on BALF, were microbiologically confirmed. Among those patients undergone anti-TB therapy without microbiologic diagnosis, 15 out of 34 cases were confirmed by Mtb culture. All patients were divided into different groups based on the final diagnosis (Table 1).

Table 1  
Demographics and clinical characteristics of subjects

Characteristics	Active PTB			Inactive PTB (n =16)	gz-PTB <sup>†</sup> (n =7)	Non-PTB (n =28)
	Sputum-based (n =33)	BALF-dependent (n =49)	Clinical (n =21)			
Gender (M/F)	24/9	31/18	16/5	13/3	4/3	13/15
Age (Years old)	43.4±14.8	47.2±15.3	40.8±16.5	50.2±12.6	58.5±12.2	53.0±15.7
TB contact	2	3	0	0	0	0
Smoking	7	14	4	6	1	4
T2DM	5	12	1	2	1	1
GHbA1C (%)	6.78±0.43	6.62±0.38	5.84±0.33	5.95±0.33	5.82±0.24	5.61±0.17
WBC (10 <sup>9</sup> /L)	7.08±1.96	6.43±2.68	5.98±1.63	6.34±2.00	6.77±3.34	6.82±3.22
Neutrophil (%)	68.3±10.5	62.3±11.6	63.2±9.32	63.0±11.6	60.1±17.2	64.1±11.1
ESR (mm/h)	43.3±5.67	40.3±5.22	17.4±4.64	27.6±7.48	32.0±9.46	33.2±5.27
CD4+T (10 <sup>6</sup> /L)	507±32.0*	554±44.0	541±56.0	613±61.0	720±175	736±103
Cavitary lesion	24	13	9	0	1	3
IGRA (+)	32/32 <sup>‡</sup>	40/48	18	15/15	7	0
Sputum (+)						
Smearing	25	0/48	0/19	0	0	0/26
TB-DNA	21/24	0/33	0/15	0/11	0/5	0/19
Xpert test	12/12	0/6	0/3	0/2	0/3	0/6
BALF (+)						
Smearing	21	8	0	0	0	0/27
TB-DNA	27	9/46	0	0	0	0/27
Xpert test	30/32	34/47	0/20	0/14	0	0/21

PTB, pulmonary tuberculosis; BALF, bronchoalveolar lavage fluid; T2DM, type 2 diabetes; GHbA1C, glycated hemoglobin A1c; WBC, white blood cell; ESR, erythrocyte sedimentation rate; IGRA, interferon- $\gamma$  release assay; Xpert, GeneXpert MTB/RIF; *Mtb*, *Mycobacterium tuberculosis*; <sup>†</sup>gz-PTB, gray zone-PTB, IGRA-positive, but well responded to antibiotic treatment or had a positive result of common bacterial culture; <sup>‡</sup>Actual cases involved; \**P* < 0.05 when compared with inactive PTB patients.

Mtb culture (+)	25	34/47	0	0	0	0/27
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PTB, pulmonary tuberculosis; BALF, bronchoalveolar lavage fluid; T2DM, type 2 diabetes; GHbA1C, glycated hemoglobin A1c; WBC, white blood cell; ESR, erythrocyte sedimentation rate; IGRA, interferon- $\gamma$  release assay; Xpert, GeneXpert MTB/RIF; Mtb, *Mycobacterium tuberculosis*; <sup>†</sup>gz-PTB, gray zone-PTB, IGRA-positive, but well responded to antibiotic treatment or had a positive result of common bacterial culture; <sup>‡</sup>Actual cases involved; \* $P < 0.05$  when compared with inactive PTB patients.

## ***BALF levels of sCD14, IL-17 and IL-22 is similar between PTB and non-PTB***

BALF levels of sCD14, IL-17 and IL-22 have been reported to be increased in PTB and non-PTB patients [18, 23, 25–27], but there is a lack of direct comparison. Here, all of them in BALF were found to be comparable between PTB and non-PTB patients (Fig. 2A). Though BALF level of IL-17 was significantly higher in gz-PTB, the significance is limited due to its small sample size.

## ***BALF levels of sCD14, IL-17 and IL-22 are associated with active PTB***

Compared with patients with inactive PTB, patients with active PTB had significantly higher levels of sCD14 and IL-22, but comparable level of IL-17 (Fig. 2B). Since active PTB patients (Table S1), especially those patients diagnosed based on sputum (Table 1), were slightly younger than inactive PTB patients, the correlation analyses between age and BALF levels of sCD14, IL-17 and IL-22 were conducted (Fig. S1). The un-correlations suggest that the age was not the contributor of the differences in BALF levels of sCD14 and IL-22 between patients with active and inactive PTB.

### *BALF levels of sCD14, IL-17 and IL-22 are associated with bacteriological confirmation manners in active PTB patients*

The final diagnosis of active PTB was made in sputum-based, BALF-dependent and clinical manners (Table 1). Among these three manners, sputum-based and BALF-dependent manners had significantly higher BALF levels of sCD14, IL-17 and IL-22 than clinical manner (Fig. 3A). The IL-17 level of sputum-based manner was also significantly higher than that of BALF-dependent manner. Though there were slight differences in age among these groups (Table 1), those differences were not the contributor of the associations between bacteriological confirmation manners and BALF levels of sCD14, IL-17 and IL-22 since the age was not correlated with the BALF levels of those factors (Fig. S1). In BALF-dependent group, only one patient neither Xpert test nor Mtb culture was positive. The rest patients were divided into Xpert test-, Mtb culture- and Xpert test/Mtb culture-positive groups. BALF levels of sCD14, IL-17 and IL-22 in

Mtb culture-positive group were relatively lower, but not significant than those in Xpert test- or Xpert test/Mtb culture-positive groups (Fig. 3B).

## ***BALF levels of sCD14, IL-17 and IL-22 predict bacteriological confirmation failure***

The associations with PTB bacteriological confirmation manners suggest that BALF levels of sCD14, IL-17 and IL-22 can predict bacteriological confirmation failure. Indeed, BALF levels of sCD14, IL-17 and IL-22 were significantly higher in bacteriologically-confirmed patients, and all of them were significant predictors of bacteriological confirmation (Fig. 4A). When the sensitivities of bacteriological confirmation failure prediction were set at test thresholds of the WHO minimal requirement for triage tests ( $\geq 90\%$ ) [16], the specificities of BALF levels of sCD14, IL-17 and IL-22 were 54.9%, 67.1% and 62.2%, respectively, very close to the WHO requirement ( $\geq 70\%$ ) [16], especially for those of IL-17 and IL-22 (Table 2).

Table 2

Sensitivities and specificities of BALF levels of sCD14, IL-17 and IL-22 in predictions of bacteriological confirmation, antibiotic treatment, Xpert test and Mtb culture in PTB patients

Items	Sensitivity <sup>†</sup> (%)	Specificity (%)	Cut-off value ( $\mu\text{g}$ or ng/mL)	IGRA (+) patients	
				Sensitivity (%)	Specificity (%)
Bacteriological confirmation failure					
sCD14	90.4	54.9	$\leq 3.05$	-	$\neq$
IL-17	90.4	67.1	$\leq 1.23$	-	-
IL-22	90.4	62.2	$\leq 2.04$	-	-
Empiric anti-bacterial nonresponder <sup>§</sup>					
sCD14	91.2	33.3	$\leq 3.72$	100.0	66.7
IL-17	91.2	37.0	$\leq 2.17$	92.0	66.7
IL-22	91.2	14.8	$\leq 5.80$	96.0	66.7
Xpert test (+)					
sCD14	90.3	37.3	$\geq 1.47$	-	-
IL-17	90.3	31.4	$\geq 0.72$	-	-
IL-22	90.3	59.2	$\geq 1.52$	-	-
Mtb culture (+)					
sCD14	91.5	27.5	$\geq 1.46$	-	-
IL-17	91.5	8.6	$\geq 0.64$	-	-
IL-22	91.5	36.2	$\geq 1.42$	-	-
IGRA, interferon- $\gamma$ release assay; Xpert, GeneXpert MTB/RIF; Mtb, <i>Mycobacterium tuberculosis</i> ; <sup>†</sup> minimal requirement of WHO ( $\geq 90\%$ ); <sup>‡</sup> IGRA is unsuitable for bacteriological confirmation in PTB; <sup>§</sup> nonresponders to empiric anti-bacteria (potential PTB patients).					

***BALF levels of sCD14, IL-17 and IL-22 predict nonresponders to empiric antibacterial therapy in active PTB patients***

At the end of Week 1, 61 smear/NAAT-negative patients needed empiric antibacterial therapy. On Week 2–3, there were a total of 36 nonresponders. Since 2 of them were diagnosed with infection of nontuberculous mycobacterium at last (Fig. 1), the exact anti-TB delay rate on Week 2–3 was 33.0% (34/103). The nonresponders in active PTB patients had significantly lower BALF levels of IL-17 and IL-22 than responders, and the BALF levels of IL-17 and IL-22 were significant predictors of empiric antibacterial therapy (Fig. 4B), suggesting that BALF levels of IL-17 and IL-22 may help avoiding anti-TB delay by predicting nonresponders to empiric antibacterial therapy. When matching the WHO minimal requirement for sensitivity of a triage test ( $\geq 90\%$ ) [16], nonresponders-predicting specificities of the BALF levels of sCD14, IL-17 and IL-22 were 33.3%, 37.0% and 14.8%, which were far below the WHO requirement for specificity ( $\geq 70\%$ ) (Table 2). However, together with IGRA that is usually used to differentiate TB from non-TB, the sensitivities of BALF levels of sCD14, IL-17 and IL-22 all were superior to WHO requirement and the specificities (66.7%) were very close to the WHO requirement (Table 2).

## ***BALF levels of sCD14, IL-17 and IL-22 predict results of Xpert test and Mtb culture***

In this study, Xpert test and Mtb culture were the major bacteriological confirmation measures, especially in patients who etiologically diagnosed dependent on BALF (Table 1). Therefore, the associations of bacteriological confirmation manners suggest that BALF levels of sCD14, IL-17 and IL-22 may have predictive values for the results of Xpert test and Mtb culture. Among 119 cases of active and inactive PTB patients, Xpert test was conducted in 113 cases with 62 positive and 51 negative results, respectively. The BALF level of IL-22 rather than sCD14 and IL-17 was significantly higher in Xpert-positive patients, but all of them were significant predictors of Xpert test (Fig. 4C). When matching the WHO minimal requirement for sensitivity of a triage test ( $\geq 90\%$ ) [13], Xpert test-predicting specificities of BALF levels of sCD14, IL-17 and IL-22 were 37.3%, 31.4% and 59.2%, respectively (Table 2). Those specificities were far below the WHO requirement for specificity ( $\geq 70\%$ ) [16]. Mtb culture was conducted in 117 cases with 59 positive and 58 negative results. The BALF levels of sCD14, IL-17 and IL-22 in Mtb culture-positive patients were all insignificantly higher, but sCD14 and IL-22 were significant predictors of Mtb culture (Fig. 4D). Similarly, Mtb culture-predicting specificities were very low if matching the WHO minimal requirement for sensitivity (Table 2).

## ***Significance of serum levels of sCD14, IL-17 and IL-22 is limited in PTB patients***

Compared with BALF, serum is more convenient to be obtained in clinical practice. A total of 76 out of 119 PTB patients had serum data of sCD14, IL-17 and IL-22. The average serum levels of sCD14 and IL-22 but IL-17 were significantly lower than average BALF levels in active PTB patients and to some extent among different diagnostic manners (Table S2). The serum levels of sCD14, IL-17 and IL-22 were comparable in patients with active and inactive PTB, and were not associated with bacteriological

conformation manners (Fig. S2). Concordantly, serum levels of sCD14, IL-17 and IL-22 were unable to predict the results of Xpert test and Mtb culture (Fig. S3), and the bacteriological confirmation failure prediction performance was far below the minimal requirement of WHO (Table S3).

## Discussion

Bacteriological confirmation based on sputum and BALF using acid fast bacilli smear, Xpert test and Mtb culture can be realized in about 80% of PTB patients [6, 10]. In this study, 79.6% (82/103) of active PTB, including 32.0% (33/103) based on sputum and 47.6% (49/103) depended on BALF further, were bacteriologically confirmed, suggesting that our cohort of patients is a typical example of the current status and suitable for biomarker study. The BALF levels of sCD14, IL-17 and IL-22 were similar between patients with PTB and non-PTB, but significantly higher in patients with active PTB and patients who were successfully etiologically diagnosed. The BALF levels of sCD14, IL-17 and IL-22 to some extent predicted bacteriological confirmation failure, nonresponders to empiric antibacterial therapy and the results of Xpert test and Mtb cultures. The serum levels of these factors, however, were relatively lower and had no predictive values. When the sensitivity matches the WHO requirement for a triage test ( $\geq 90\%$ ), the bacteriological confirmation failure prediction specificities of IL-17 and IL-22, and empiric antibacterial therapy-nonresponder prediction specificities in IGRA-positive patients were very close to the minimal WHO requirement ( $\geq 70\%$ ). Therefore, the BALF levels of sCD14, IL-17 and IL-22, superior to their serum levels, can be used as triage tests to refer patients to conduct lung biopsy and other expensive molecular tests in the first week and as alternative measures to replace the expensive Xpert test and time-consuming Mtb culture in particular occasions, implying that the BALF levels of sCD14, IL-17 and IL-22 may help avoiding anti-TB delays to some extent in the future.

sCD14, IL-17 and IL-22 in BALF were successfully detected as reported [18, 23, 25–27]. Concordantly, their levels were significantly higher in patients with active PTB [18, 23, 26]. However, their BALF levels were not significantly different between PTB and non-PTB patients, suggesting that the BALF levels of these factors cannot be directly used to differentiate PTB from non-PTB. Indeed, BALF levels of sCD14, IL-17 and IL-22 all rise in other lung infectious diseases [25, 27, 28], concordant to sCD14 as a general receptor of LPS and IL-17 and IL-22 as general mediators of T helper 17 cells. Though IGRA can help us to differentiate PTB from non-PTB, it still a pity for these biomarkers could not be diagnostic markers of TB since there were a few IGRA-negative patients with positive results of Xpert test or Mtb culture as reported [29, 30]. However, the levels of sCD14, IL-17 and IL-22 reflect the activity of inflammation. Therefore, the high BALF levels were naturally associated with active PTB. Moreover, the BALF levels of sCD14, IL-17 and IL-22 were found to be associated with bacteriological confirmation manners and the low levels predicted the final bacteriological confirmation failure with performance close to the WHO's minimal requirement for a triage test. Since biomarkers meet such requirement are rarely seen [16], sCD14, IL-17 and IL-22 in BALF may develop into triage biomarkers for PTB and improve bacteriological confirmation by referring patients with extremely low BALF levels of those biomarkers to conduct lung biopsy or other expensive examinations as early as possible. The nature of the triage effect of these biomarkers in BALF

is because they well represent the connection efficiency between TB focus and bronchia. It is well known that this connection is very important to the spread of Mtb by determining its number in sputum [3, 31].

PTB in some patients is initially suspected by means of computed tomography. Bacteriological confirmation based on sputum and BALF using acid fast bacilli smear and Xpert test were realized in most patients. However, 39.6% of patients in this study needed an empiric antibacterial therapy to make differential diagnosis, which theoretically led anti-TB delay. The lower BALF levels of IL-17 and IL-22 weakly predicted nonresponders to empiric antibacterial therapy, but in IGRA-positive patients, the predicting performance close to the WHO's minimal requirement for a triage test. Therefore BALF levels of sCD14, IL-17 and IL-22 may be used as triage biomarkers to not only refer patients with low levels to conduct lung biopsy, but also refer patients with moderate BALF levels to start anti-TB therapy immediately. Delays in treatment initiation may lead to worse clinical outcomes and increased transmission [32, 33]. Empiric antibacterial therapy was the major cause of anti-TB delay in this study. The nature of the triage effect of moderate levels of these biomarkers on empiric antibacterial therapy is perhaps because PTB (smear/NAAT-positive patients) rather than non-PTB patients with higher levels of those biomarkers were selected out by acid fast bacilli smear and Xpert test based on sputum and BALF.

Xpert and Mtb culture are key measures to increase the microbiologic diagnosis rate in smearing-negative PTB [6–9, 34–36]. However, Xpert test is relatively expensive and cannot be broadly used in developing countries and Mtb culture not only needs sophisticated research-type laboratories, but also is time-consuming [11, 12, 37]. In this study, the BALF levels of sCD14, IL-17 and IL-22 to some extent predicted the results of Xpert and Mtb culture, especially the level of IL-22 for Xpert test having a performance worthy of more further studies, suggesting that the BALF levels of IL-22 can be used to replace the expensive Xpert test in particular occasions (e.g. in developing countries) and to direct early anti-TB treatment before the result of Mtb culture comes out. The predictions of Xpert test and Mtb culture are the basics for BALF levels of sCD14, IL-17 and IL-22 to predict bacteriological confirmation failure. Compared with Mtb culture, Xpert test was much better predicted. The possible explanation is that the mentioned factors are involved in protective mechanisms against Mtb infection and Xpert detects both live and dead Mtb, but Mtb culture only detected live bacteria.

Compared with BALF levels, serum levels of sCD14, IL-17 and IL-22 were significantly lower and of no significance in differentiating activity of PTB and predicting Xpert and Mtb culture, which is in concordance with the increases in BALF and decreases in serum of IL-17 and IL-22 [18, 26]. In BALF, IL-22 had the best and sCD14 had the moderate comprehensive performance to differentiate the activity of PTB and predict bacteriological confirmation failure, empiric antibacterial therapy, Xpert test and Mtb culture, while IL-17 was only good at predicting bacteriological confirmation failure and empiric antibacterial therapy nonresponders. The possible explanation is that these factors have different protecting efficacy. Namely, IL-22 is the lowest one, which is in concordance with its antagonism in production with IFN- $\gamma$  [18, 26]. Though the bacteriological confirmation failure prediction specificities and empiric antibacterial therapy-nonresponder prediction specificities in IGRA-positive patients were very close to the minimal WHO requirement, the general performances of these biomarkers in BALF were

moderate. Therefore, our results suggest that non-protective biomarkers in BALF may be even worthy of more attentions in future. In addition, the significance of IL-17 and IL-22 in BALF to predictions of bacteriological confirmation failure and empiric antibacterial therapy nonresponders is worthy of prospective, random and multi-center studies.

## Conclusions

BALF largely increased the bacteriological confirmation rate of PTB. The BALF levels of sCD14, IL-17 and IL-22 were efficient triage biomarkers possessing comprehensive performance to differentiate the activity of PTB and predict bacteriological confirmation failure, empiric antibacterial therapy, Xpert test and Mtb culture. Therefore, those biomarkers in BALF may be used as triage indicators to refer patients with low levels to conduct lung biopsy or other expensive tests and smear/NAAT-negative patients with moderate to start anti-TB as early as possible, and to replace the expensive Xpert in developing countries sometimes and to direct early anti-TB treatment before the results Mtb culture come out. However, the predicting values of these factors are limited. The significance of IL-17 and IL-22 in BALF to predictions of bacteriological confirmation failure and empiric antibacterial therapy nonresponders is worthy of prospective, random and multi-center studies in the future.

## Abbreviations

BALF, bronchoalveolar lavage fluid; CD, cluster of differentiation; CT, computed tomography; gz, gray zone; IGRA, Interferon- $\gamma$  release assay; IL, interleukin; LPS, lipopolysaccharide; Mtb, *Mycobacterium tuberculosis*; NAAT, nucleic acid amplification tests; PTB, pulmonary tuberculosis;

SD, standard deviation; TB, tuberculosis.

## Declarations

### Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the fifth Affiliated Hospital of Sun Yat-sen University. All patients had signed the informed consent.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interest

The authors declare that they have no competing interest.

## Funding

This work was supported by grants from “Five and Five” projects of Sun Yat-Sen University and the Scientific and Technological Bureau of Guangzhou, Guangdong Province (No. 201508020059) , China.

## Authors' contributions

XMP is the guarantor of the article. XMP and ZSH brought the concept; JL, WXD and QDL collected the data and performed the tests; JL, WXD and XMP made the statistical analysis and wrote the paper; All co-authors approved the final version of the paper.

## Acknowledgements

We thank the rest of clerks in Clinical laboratory, the Fifth Affiliated Hospital, Sun Yat-Sen University, Zhuhai, China, for technical assistance.

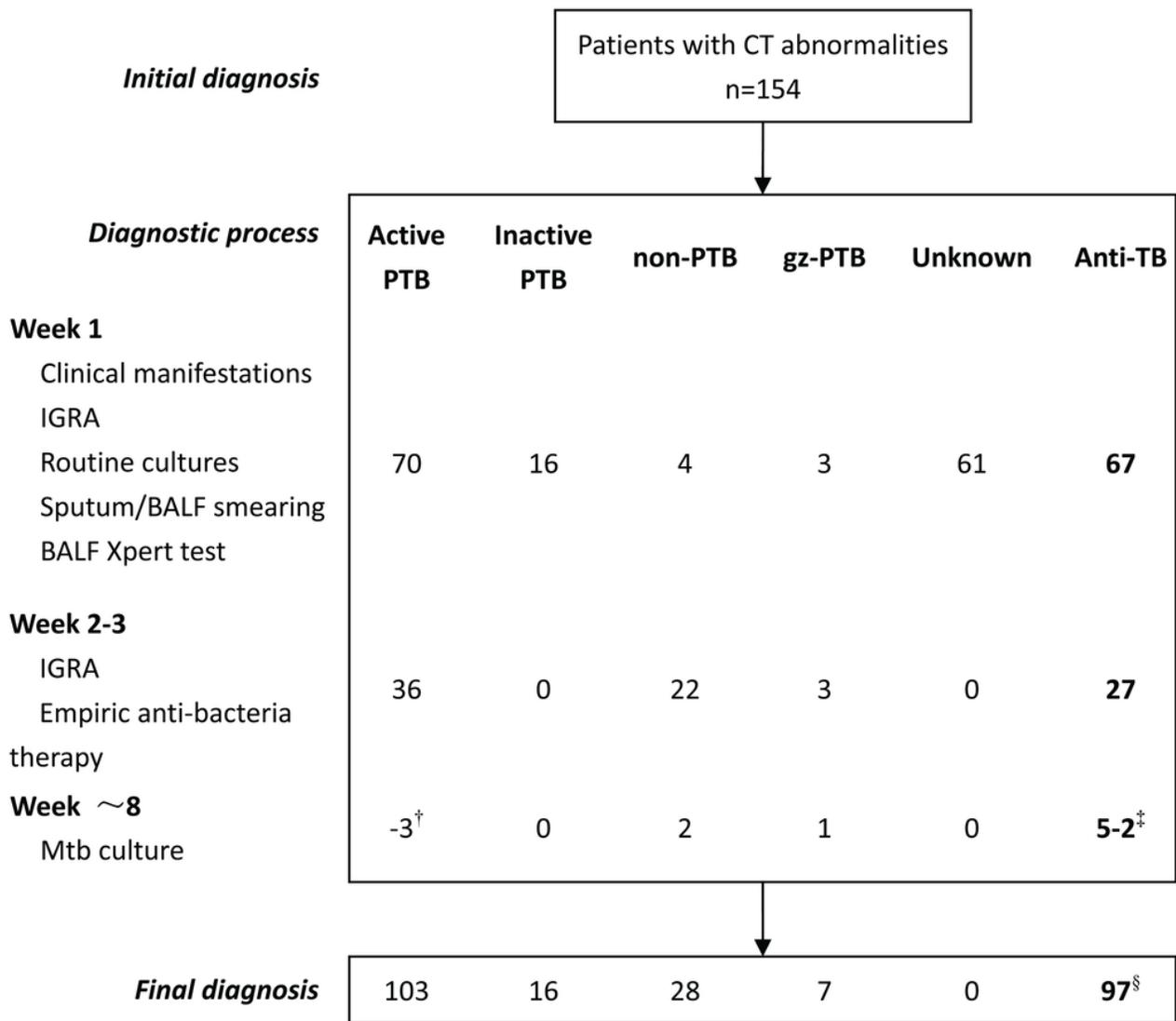
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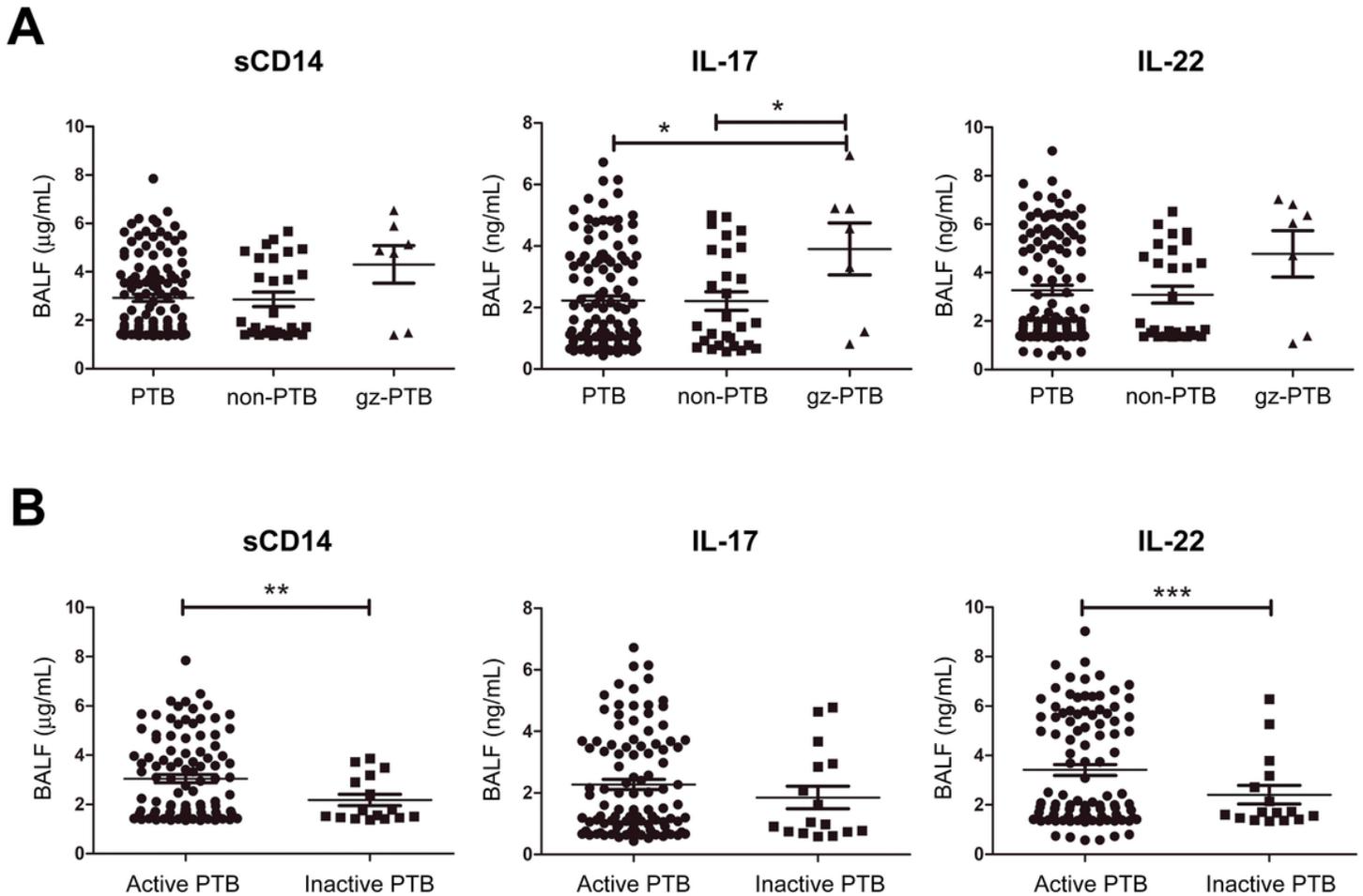
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## Figures



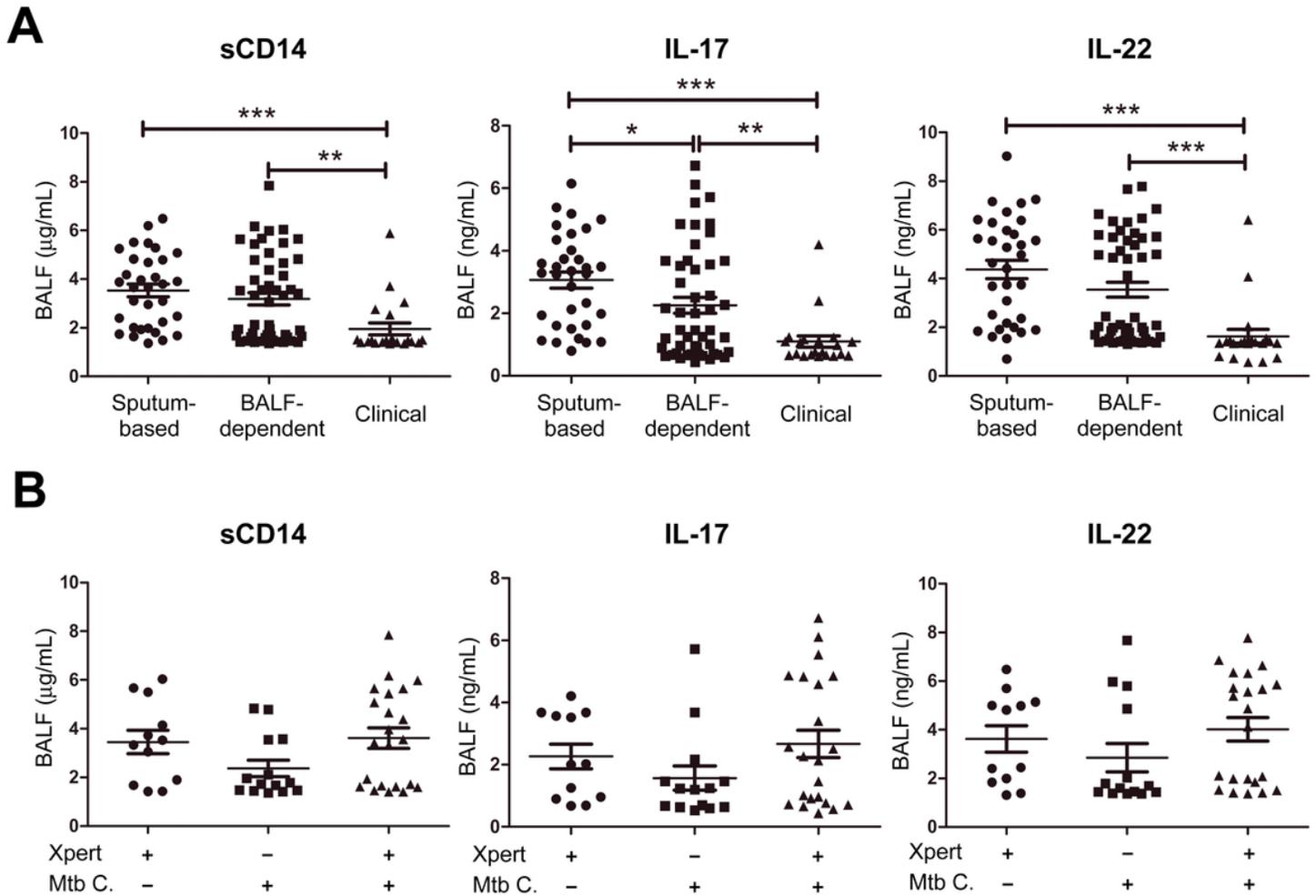
**Figure 1**

Flow diagram of patients' diagnostic process. CT, computed tomography; PTB, pulmonary tuberculosis; gz, gray zone (IGRA-positive, but well responded to empiric antibacterial therapy). † Three patients were excluded from active PTB and ‡ two patients switched to anti-non-tuberculosis mycobacterium therapy due to existence of non-tuberculosis mycobacterium in Mtb culture. § Three smear/NAAT-positive and 3 clinically-diagnosed patients refused to start anti-TB treatment in active PTB patients.



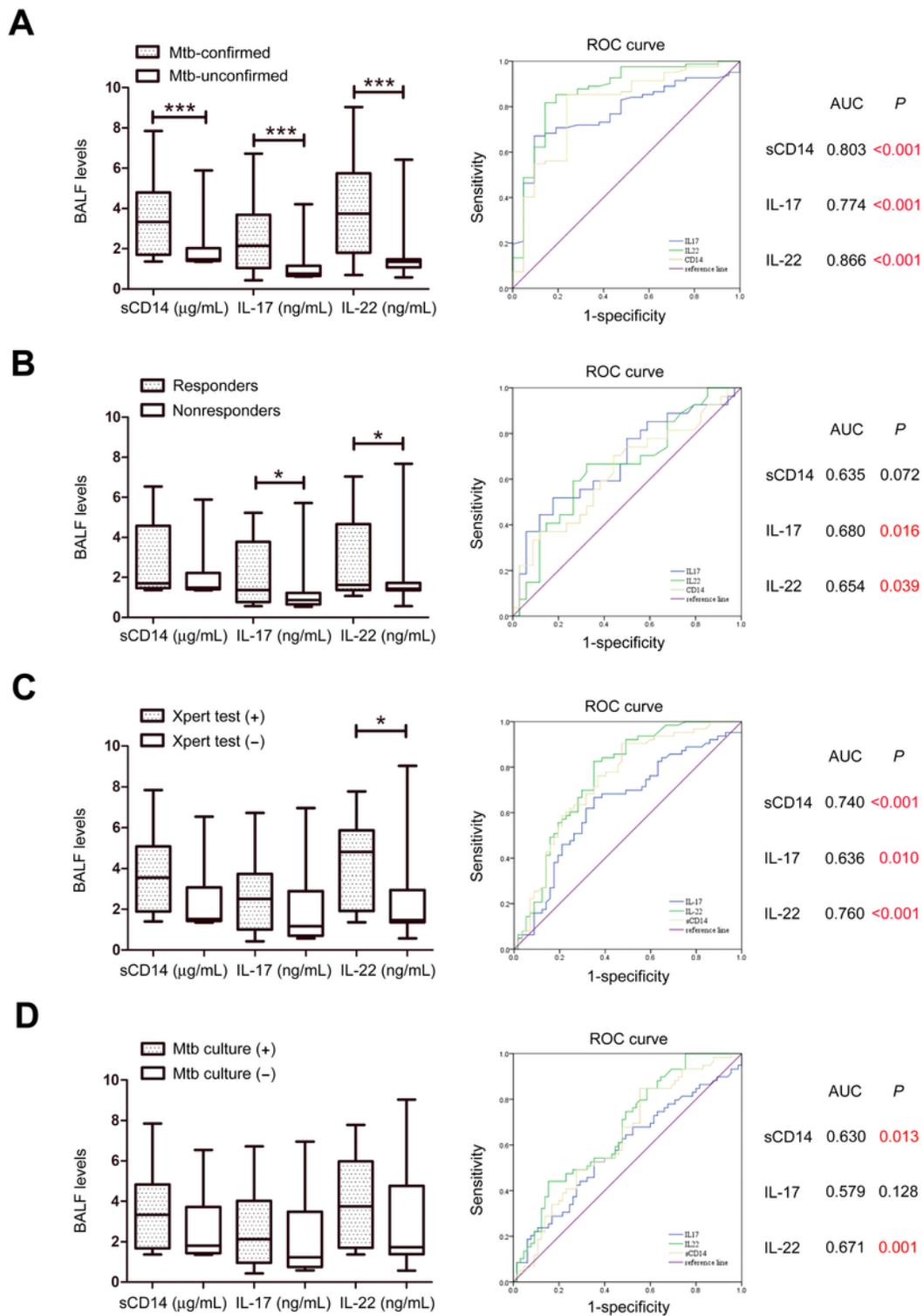
**Figure 2**

BALF levels of sCD14, IL-17 and IL-22 in active or inactive PTB and non-PTB patients. PTB, pulmonary tuberculosis; gz, gray zone (IGRA-positive, but well responded to empiric antibacterial therapy). (A) No significant differences in BALF levels of sCD14, IL-17 and IL-22 were found between PTB and non-PTB patients. BALF level of IL-17 was significantly higher, but had limited significance in gz-PTB due to the small sample size. (B) Patients with active PTB had significantly higher BALF levels of sCD14 and IL-22 but IL-17. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 3**

Relationships between PTB bacteriological confirmation manners and BALF levels of sCD14, IL-17 and IL-22 in active PTB patients. Xpert, GeneXpert MTB/RIF; Mtb C., Mycobacterium tuberculosis culture. (A) Active PTB was diagnosed based on sputum (sputum-based), dependent on BALF (BALF-dependent) and clinically (clinical), respectively. Sputum-based and BALF-dependent patients had higher BALF levels of sCD14, IL-17 and IL-22. As for IL-17, sputum-based patients had the highest level. (B) In BALF-dependent patients, BALF levels of sCD14, IL-17 and IL-22 in Mtb culture-positive group were relatively lower, but not significant than those in Xpert test- or Xpert test/Mtb culture-positive groups.



**Figure 4**

Predict values of BALF levels of sCD14, IL-17 and IL-22 in PTB patients. Xpert, GeneXpert MTB/RIF; Mtb, Mycobacterium tuberculosis; ROC, receiver operating characteristic curve; AUC, area under roc curve. (A) BALF levels of sCD14, IL-17 and IL-22 were significantly higher in bacteriologically-confirmed patients, and all of them were significant predictors of bacteriological confirmation. (B) BALF levels of IL-17 and IL-22 rather than sCD14 were significantly lower in nonresponders to empiric antibacterial therapy, the BALF

levels of IL-17 and IL-22 were significant predictors of empiric antibacterial therapy. (C) BALF level of IL-22 rather than sCD14 and IL-17 was significantly higher in Xpert-positive patients, but all of them were significant predictors of Xpert test. (D) BALF levels of sCD14, IL-17 and IL-22 were all insignificantly higher in Mtb culture-positive patients, but sCD14 and IL-22 were significant predictors of Mtb culture.

## Supplementary Files

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