Mycobacterium tuberculosis specific antigen-stimulated CD27-CD38+IFN-γ+CD4+ T cells for active tuberculosis diagnosis

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Abstract

Background: Traditional bacteriological tests are time-consuming and of low sensitivity for the diagnosis of active tuberculosis (ATB). The purpose of this study is to investigate the accuracy of $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ for active tuberculosis (ATB) diagnosis.

Methods: This prospective diagnostic accuracy study was conducted at Shanghai Pulmonary Hospital between January 2019 and December 2021. Patients with ATB, non-tuberculosis mycobacterium infection (NTM), latent tuberculosis infection (LTBI), other respiratory diseases (OD), and healthy individuals (HC) were enrolled. The accuracy of $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ and other phenotypic markers for ATB diagnosis was assessed.

Results: A total of 376 patients (237 ATB, 38 LTBI, 8 NTM, 50 OD, and 43 HC) were enrolled. The ratios of $CD4^{+}IFN\text{-}\gamma^{+}CD27^{-}$ and $CD4^{+}IFN\text{-}\gamma^{+}CD27^{-}CD38^{+}$ profiles in $CD4^{+}IFN^{-}\gamma^{+}$ cells and the ratios of $CD4^{+}IFN\text{-}\gamma^{+}CD38^{+}$, $CD4^{+}IFN\text{-}\gamma^{+}CD27^{-}$, and $CD4^{+}IFN\text{-}\gamma^{+}CD38^{+}CD27^{-}$ profiles in $CD4^{+}$ cells in the ATB group were significantly higher than in the other groups. The area under the curve (AUC) of $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ for the diagnosis of ATB was the highest, with a value of 0.890. With the optimal cutoff value of $1.34\text{´}10^{-4}$, the sensitivity and specificity of $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ for ATB diagnosis was 0.869 and 0.849, respectively.

Conclusion: $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ might be an effective biomarker for active tuberculosis diagnosis.

Introduction

Tuberculosis (TB) is the ninth leading cause of death in the world, ahead of HIV/AIDS [1]. Despite great advances in therapy, the world still faces a huge burden of TB. There are about 2 billion people infected with *Mycobacterium tuberculosis* (MTB) worldwide [1, 2], and China is one of the 30 countries with the highest burden of TB and latent TB infection (LTBI) identified by the World Health Organization (WHO) [3]. It was recently estimated that China had the highest LTBI burden in the world, with approximately 350 million persons living with the infection [4, 5].

The rapid and accurate diagnosis of MTB infection is a critical problem in preventing and controlling TB and determining treatment efficacy. Conventional diagnostic methods are time-consuming (e.g., TB culture) or have low specificity (e.g., the tuberculin skin test [TST]) [6]. In addition, some methods cannot distinguish active TB (ATB) from LTBI (e.g., interferon-γ release assays [IGRAs]) [7]. It is challenging to differentiate ATB from LTBI and other respiratory diseases, and misdiagnosis often occurs [8, 9]. Extra-pulmonary TB (EPTB) accounts for a large proportion of TB [10, 11]. Since the results of etiology and pathology are difficult to obtain for tuberculous serous effusion (such as tuberculous pleuritis, tuberculous peritonitis, tuberculous pericarditis, tuberculous meningitis, etc.), the means of diagnosis and differential diagnosis are limited.

The cellular immune response mediated by T lymphocytes plays a key role in TB infection and pathogenesis [12, 13]. The surface molecules of the T cells are the molecular basis for the mutual recognition and interaction between T cells and other cells and molecules [14, 15]. CD27 is a costimulatory receptor expressed on the surface of $CD4^{+}$ T cells [16]. Early-differentiated $CD27^{+}$ memory CD4 T cells are thought to recirculate primarily in secondary lymphoid organs, while late-differentiated $CD27^{-}$memory T cells exhibit additional effector functions [17] and preferentially migrate to peripheral sites of inflammation, such as the lungs during ATB [18]. CD38 is a transmembrane glycoprotein expressed on many types of immune cells. It can catalyze the degradation of nicotinamide adenine dinucleotide (NAD) and its precursors that are necessary for bacterial growth [19, 20]. A study showed that CD38 inhibits the metabolism of pathogens by degrading NAD and its precursors in activated immune cells, thus limiting the development or progression of infection [21].

Recent studies showed that compared with LTBI, $CD27^{-}$ and $CD38^{+}$ are mainly expressed in ATB and could be used as markers to distinguish the two diseases [22–24], but there are few studies on the combined $CD27^{-}$ and $CD38^{+}$ profile. Therefore, this study aimed to detect the co-expression of CD27 and CD38 molecules in $CD4^{+}$ and $CD4^{+}IFN^{-}\gamma^{+}$ T cells and investigate the accuracy of $CD27^{-}CD38^{+}IFN\text{-}\gamma^{+}CD4^{+}/CD4^{+}$ and other MTB-specific phenotypic markers for ATB diagnosis.

Methods

Study design and population

This prospective diagnostic study was conducted in Shanghai Pulmonary Hospital, School of Medicine of Tongji University, between January 2019 and December 2021. The inclusion criteria were 1) aged 18-70 years, 2) healthy volunteers (HC) or patients with ATB, LTBI, non-tuberculosis mycobacterium infection (NTM), or other respiratory diseases (OD). The exclusion criteria were 1) primary or secondary immunodeficiency, including HIV, long-term steroid use, or co-existing autoimmune diseases, 2) diabetes or viral hepatitis, 3) history of anti-tuberculosis treatments. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Shanghai Pulmonary Hospital. All participants signed the informed consent forms.

ATB (including active pulmonary TB (PTB) and active EPTB) was diagnosed according to i) no previous history of TB, ii) positive sputum, bronchoalveolar lavage fluid (BALF), or bacteriological tests, including positive acid-fast staining, positive MTB culture (Bectec960), or positive

Gene-XPERT test, and iii) no other lung diseases [25-27]. LTBI was defined as household contacts of newly diagnosed TB patients with positive IGRA, with no evidence of clinically manifest active TB[28]. OD was defined as patients with any other respiratory diseases except TB, and with negative IGRA results. Healthy volunteers were those had negative IGRA results and no history of TB exposure. Sputum smear-positive patients with negative sputum MTB culture results were diagnosed as non-tuberculous mycobacteria (NTM).

Data collection

Demographic information including gender and age of all participants was recorded. For each participant, 0.5 ml whole blood was collected in heparin anticoagulant tubes. Acid fast and Gene-XPERT were used for ATB screening. The results of MTB culture and IGRA were recorded.

ESAT6/CFP10 stimulation and flow cytometry

Within 4 h of blood collection, the whole blood was added into sample tubes containing 10μg/mL ESAT6/CFP10 polypeptide antigen (Beckman Coulter, Brea, CA, USA). Brefeldin A (10μg/mL, Biolegend, San Diego, CA, USA) was added as a Golgi transport inhibitor, and the whole blood was incubated at 37°C with 5% CO_2 for 16 h. After culture, 2.5 mL of erythrocyte lysis solution and fixative solution (3% diethylene glycol, 2% formaldehyde, and 0.75% methanol) were added and incubated at room temperature for 10 min. The cells were centrifuged at 600 ×*g* for 5 min. The supernatant was discarded, and 375 μL Perfix-NC membrane breaking solution (Beckman Coulter) was added and mixed. IFN-γ-FITC was added. Then, 5μL of CD4-PE, CD3-ECD, CD27-PE-CY5.5, and CD38-PE-CY7 were mixed and incubated for 45 min in the dark. Phosphate-buffered saline (PBS, 3 mL) was added. The cells were centrifuged at 600 ×*g* for 5 min, and the supernatant was discarded. After adding 300μL PBS, a Beckman Coulter DxFLEX flow cytometer was used for rapid collection (60μL/min). The collection stop condition was set to 100,000 $CD4^+$ cells or 300 s of whole blood. FlowJo V10 (Treestar, Ashland, OR) was used for data analysis. The ratio of $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD27^-/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$, $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+$, $CD4^+IFN\text{-}\gamma^+CD27^-/CD4^+$ was measured.

Statistical analysis

Statistical analyses were performed using SPSS 18 (SPSS, Armonk, NY, USA) and Graph Pad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). Continuous variables are described as means±standard deviation (SD), and categorical variables as n (%). The Kruskal-Wallis test was used for comparison of independent samples between multiple groups, Dunn's test was used for pairwise comparison between multiple groups, and the Mann-Whitney U-test was used for comparison of independent samples between two groups. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic performance of each biomarker. The areas under the curves (AUCs) were compared by the DeLong test. The optimal cutoff value of variables was identified according to the maximum Youden index. Two-sided P-values <0.05 were considered statistically significant.

Results

A total of 376 participants, including 216 males and 160 females with an average age of 41.2±16.9 years were enrolled (Table 1). There were 237 cases of ATB (177 cases of PTB and 60 cases of EPTB), 38 cases of LTBI, 8 cases of NTM, 50 cases with OD, and 43 HC (Fig. 1).

Table 1
Characteristics of the participants

	ATB (n = 237)	LTBI (n = 38)	NTM (n = 8)	OD (n = 50)	HC (n = 43)	P
Male (n, %)	155 (65.4%)	15 (39.5%)	5 (62.5%)	25 (50.0%)	16 (37.2%)	P < 0.001
Age (year)	38.0±16.4	44.6±13.9	48.8±11.0	56.3±16.5	36.5±12.8	P < 0.001
EPTB (n, %)	60 (25.3%)	/	/	/	/	
TB culture						
N/A (n, %)	0	0	5 (62.5%)	3 (6.0%)	0	
Negative (n, %)	48(20.3%)	38 (100%)	3 (37.5%)	47 (94.0%)	43 (100%)	
Positive (n, %)	189(79.7%)	0	0	0	0	
IGRA						
N/A (n, %)	21 (8.9%)	0	1 (12.5%)	3 (6.0%)	0	
Indeterminate (n, %)	1 (0.4%)	0	0	0	0	
Negative (n, %)	10 (4.2%)	0	4 (50.0%)	47 (94.0%)	43 (100%)	
Positive (n, %)	205 (86.5%)	38 (100%)	3 (37.5%)	0	0	

ATB: active tuberculosis; EPTB: extrapulmonary tuberculosis; LTBI: latent tuberculosis infection; NTM: non-tuberculous mycobacterium pulmonary disease; HC: healthy control; OD: other respiratory diseases; N/A: not available.

The CD27 or CD38 expression in ESAT-6/CFP-10 peptides stimulated $CD4^+IFN\text{-}\gamma^+$ T cells was compared with the unstimulated cells (Fig. 2). The rates of $CD27^-$, $CD38^+$, and $CD27^-CD38^+$ were significantly higher in the ATB group than in the non-ATB groups (all $P < 0.001$) (Fig. 3A-C). This also indicates that CD27, $CD38^+$, and $CD27^-CD38^+$ are mainly found in the $CD4^+IFN\text{-}\gamma^+$ T cells of ATB patients. The ratio of $CD4^+IFN\text{-}\gamma^+CD27^-$ and $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ profiles in $CD4^+IFN\text{-}\gamma^+$ cells stimulated by ESAT 6/CFP10 in the ATB group were higher than that in LTBI ($P = 0.02$ and $P < 0.001$, respectively), NTM ($P = 0.001$ and $P < 0.001$, respectively), OD (both $P < 0.001$), and HC groups (both $P < 0.001$) (Fig. 3D, 3F). The ratio of $CD4^+IFN\text{-}\gamma^+CD38^+$ profile in $CD4^+IFN\text{-}\gamma^+$ cells of the ATB group was higher than the LTBI ($P = 0.01$) and OD groups (all $P < 0.001$), but no difference was found between the ATB and the NTM or HC group (Fig. 3E). The rates of $CD4^+IFN\text{-}\gamma^+CD38^+$, $CD4^+IFN\text{-}\gamma^+CD27^-$, and $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ profiles in $CD4^+$ T cell subsets in the ATB group were significantly higher than in the other groups (all $P < 0.001$) (Fig. 3G-I).

The diagnostic values (including sensitivity, specificity, negative predictive value, positive predictive value, positive likelihood ratio, negative likelihood ratio, and accuracy) of $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD27^-/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+IFN\text{-}\gamma^+$, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$, $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+$, $CD4^+IFN\text{-}\gamma^+CD27^-/CD4^+$ for the diagnosis and differential diagnosis of TB were evaluated (Table 2). $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+IFN\text{-}\gamma^+$ had the highest sensitivity (0.899) and $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ had the highest specificity (0.849). ROC curves for ATB diagnosis were performed and the AUCs of the six markers were greater than 0.7 except $CD4^+IFN\text{-}\gamma^+CD38^+/CD4^+$ $IFN\text{-}\gamma^+$ (Fig. 4A). The AUC of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$, which was 0.890, was the highest compared with the other markers (Delong test, $P < 0.05$), indicating that the $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ had the highest value in the diagnosis and differential diagnosis of ATB. At the cutoff value, the sensitivity and specificity of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ were both above 80% (Fig. 4B). At the cutoff value of $1.34{\cdot}10^{-4}$, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ exhibited good differential diagnosis ability for ATB, as most ATB cases had $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ values over $1.34{\cdot}10^{-4}$ and most non-ATB cases had values below $1.34{\cdot}10^{-4}$ (Fig. 4C). The sensitivity, specificity, and accuracy of $CD4^+IFN\gamma^+CD38^+CD27^-/CD4^+$ were 0.869, 0.849, and 0.862, respectively. The positive predictive value was 0.907, and the negative predictive value was 0.792.

Table 2
Diagnostic performance of different phenotypic markers

	Cutoff	Se	Sp	PPV	NPV	FPR	FNR	$^{+}$LR	$^{-}$LR	Accuracy	Youden
$CD4^+IFN\gamma^+CD27^-CD38^+/CD4^+$	$1.34\cdot10^{-4}$	0.869	0.849	0.907	0.792	0.151	0.130	5.753	0.154	0.862	0.718
$CD4^+IFN\gamma^+CD27^-/CD4^+$	$2.11\cdot10^{-4}$	0.781	0.849	0.898	0.694	0.151	0.219	5.167	0.258	0.806	0.630
$CD4^+IFN\gamma^+CD38^+/CD4^+$	$2.56\cdot10^{-4}$	0.776	0.791	0.864	0.675	0.209	0.224	3.721	0.283	0.782	0.568
$CD4^+IFN\gamma^+CD27^-CD38^+/CD4^+IFN\gamma^+$	$1.91\cdot10^{-1}$	0.793	0.705	0.821	0.667	0.295	0.207	2.689	0.293	0.761	0.498
$CD4^+IFN\gamma^+CD27^-/CD4^+IFN\gamma^+$	$3.21\cdot10^{-1}$	0.624	0.777	0.827	0.548	0.223	0.376	2.800	0.483	0.681	0.401
$CD4^+IFN\gamma^+CD38^+/CD4^+IFN\gamma^+$	$1.80\cdot10^{-1}$	0.899	0.417	0.724	0.707	0.583	0.101	1.542	0.243	0.721	0.316

Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; FPR: false positive rate; FNR: false-negative rate; $^{+}$LR: positive likelihood ratio; $^{-}$LR: negative likelihood ratio.

In addition, we investigated the difference in the rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ between TB culture-negative ($culture^-$) and TB culture-positive ($culture^+$) ATB patients. Among the 237 ATB cases, 189 cases were TB $culture^+$, and 48 were TB $culture^-$. The rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ in $CD4^+$ T cells stimulated by ESAT 6/CFP10 showed no significant difference between TB $culture^+$ and TB $culture^-$ ($P > 0.05$). Compared with the other groups, the rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ in the TB $culture^-$ group were higher than in the LTBI ($P < 0.001$), NTM ($P = 0.003$), OD ($P < 0.001$), and HC groups ($P < 0.001$) (Fig. 5A). The rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ on $CD4^+$ cells of PTB and EPTB were also compared (Fig. 5B). There were 177 cases of PTB and 60 cases of EPTB among the 237 ATB patients. The rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ in $CD4^+$ cells showed no significant difference between PTB and EPTB ($P > 0.05$). The rates of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ in EPTB were higher than in the LTBI, NTM, OD, and HC groups (all $P < 0.001$). Therefore, $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ could also be used as a marker the diagnosis and differential diagnosis of ATB patients with TB $culture^-$ and EPTB.

Discussion

The diagnostic performance of $CD27^-CD38^+IFN\text{-}\gamma^+CD4^+/CD4^+$ and other MTB-specific phenotypic markers for ATB diagnosis was evaluated in this study. The AUC of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ for the diagnosis of ATB was the highest (0.890), and the sensitivity and specificity was 0.869 and 0.849 with the optimal cutoff value of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ as $1.34\cdot10^{-4}$. Therefore, $CD27^-CD38^+IFN\text{-}\gamma^+CD4^+/CD4^+$ might be an effective biomarker for ATB diagnosis and differential diagnosis in future clinical application.

In recent years, the research on T cell-related molecular markers has been a hot topic. Xu et al. [24] evaluated CD27 in $CD27^-IFN\text{-}\gamma^+CD4^+$ T cells for differential diagnosis in TB-unexposed healthy people, TB contacts, and smear-negative TB and concluded that the percentage of $CD27^-IFN\text{-}\gamma^+CD4^+$ cells can distinguish smear-negative TB patients from the other two groups (AUC = 0.88, sensitivity 82.1%, specificity 80.0%). The study focused on the differential diagnosis of sputum smear-negative ATB and LTB, but many of the smear-negative patients were culture-positive. There are still some ATB patients with both negative smear and culture, and it is challenging to distinguish ATB from LTBI in these patients. Therefore, it is more meaningful to compare ATB with LTBI in sputum culture-negative, which is also one of the key contents of the present study. Latorre et al. [22] found that the rates of $CD27^-$ and $CCR4^+$ in $IFN\text{-}\gamma^+$ $TNF\text{-}\alpha^+CD4^+$ T cells stimulated by $ESAT^-6/CFP^-10$ or PPD had a high diagnostic value and a high diagnostic accuracy between ATB and LTBI, but the ATB and LTBI participants in the study were enrolled within the first 4 weeks of initiation of anti-TB therapy or prophylactic anti-TB therapy, and it is questionable whether anti-TB therapy interfered with the results. Silveira-mattos et al. [23] focused on CD38, HLADR, and Ki67. The results showed that the rates of $CD38^+$, $HLADR^+$, or $Ki67^+$ in $IFN\text{-}\gamma^+CD4^+$ T cells could differentiate LTBI from ATB. $HLADR^+$ and $Ki67^+$ could identify EPTB and PTB accurately. HIV infection did not affect the ability of these markers to distinguish between ATB and LTBI, EPTB, and PTB. Still, a large proportion of EPTB tends to be combined with PTB, so the comparison is worth considering.

This study focused on the rates of $CD27^-$ and $CD38^+$ and their co-occurrence. The results showed that after peripheral blood was stimulated by ESAT 6/CFP10, the rates of $CD4^+IFN\text{-}\gamma^+CD27^-$ and $CD4^+IFN\text{-}\gamma^+CD27^-$ on $CD4^+$ $IFN\text{-}\gamma^+$ cells of ATB were higher than in the other groups. The rate of $CD4^+IFN\text{-}\gamma^+CD38^+$ in ATB was higher than in LTBI and OD, but there were no differences with NTM and HC groups. The rates of $CD4^+IFN\text{-}\gamma^+CD38^+$, $CD4^+IFN\text{-}\gamma^+CD27^-$, and $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$ subsets in $CD4^+$ cells were higher in ATB than in the other groups. Similar results were observed for

the rates of $CD4^+IFN\text{-}\gamma^+CD38^+$ and $CD4^+IFN^-\gamma^+CD27^-$, consistent with the previous study mentioned above. Nevertheless, there are few studies on $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$, and additional studies are necessary to strengthen the results. ROC curves were performed for the proportions of $CD4^+IFN\text{-}\gamma^+CD38^+$, $CD4^+IFN\text{-}\gamma^+CD27^-$, and $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$ in $CD4^+$ and $CD4^+$ $IFN\text{-}\gamma^+$ as diagnostic indexes. According to their AUC, $CD4^+IFN\text{-}\gamma^+CD27^-/CD4^+IFN\text{-}\gamma^+$ and $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+/CD4^+IFN\text{-}\gamma^+$ have diagnostic value for ATB. The AUC of $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+/CD4^+$ was 0.890, indicating the highest diagnosis value. The reason that the diagnostic value of each biomarker on $CD4^+$ cells is higher than $CD4^+IFN\text{-}\gamma^+$ may be related to the both effect of $CD27^-CD38^+$ and IFN-γ, but the exact mechanisms remain to be explored in future research.

As it is difficult to diagnose culture-negative TB clinically, acid-fast sputum staining tests cannot distinguish TB from NTM. In countries and regions with relatively poor public health resources, the gene-XPERT test is not easily accessible. On the other hand, EPTB is more difficult to diagnose than PTB. The diagnosis is usually made by pathology, excluding other diseases and diagnostic treatment, which is expensive in time and resources. In this study, the results showed no difference of $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$ on $CD4^+$ neither between TB $culture^+$ and TB $culture^-$ nor between PTB and EPTB. Still, the rates were higher in TB $culture^-$ and EPTB than in the LTBI, NTM, OD, and HC groups. Therefore, $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$ cell subsets could be helpful for $culture^-$ TB and EPTB diagnosis.

This study has certain limitations. The study aimed to explore the indicators for ATB diagnosis, and the phenotypic markers in the ATB group were compared with the LTBI, NTM, OD, and HC groups because the differential diagnosis of these groups is also of great clinical value. Unfortunately, the sample size for the NTM group was relatively small, mainly because it was a single-center study with a limited sample size. Therefore, multicenter studies with larger samples should be carried out. In addition, this study did not examine the effects of treatments on $CD4^+IFN\text{-}\gamma^+CD27^-CD38^+$ cell subsets.

In conclusion, $CD27^-CD38^+IFN\text{-}\gamma^+CD4^+/CD4^+$ could be used as an effective biomarker for TB diagnosis and differential diagnosis.

Declarations

Ethics approval and consent to participate

This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Shanghai Pulmonary Hospital. And informed consent was obtained from All participants.

Consent for publication

All authors consent to publication if our article is accepted for publication by the journal.

Availability of data and material

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

Author contributions

Bo Su, Wei Sha and Yong Fang designed the study protocol. Yong Fang, Na Wang and Lin Li recruited subjects and collected peripheral blood samples. Liang Tang and Xiao-Jun Yang performed flow cytometry on peripheral blood samples. Yuan Tang analyzed flow cytometry data. Yong Fang and Na Wang collated data. Yong Fang and Bo Su prepared all the figures and Tables. Yong Fang wrote the main manuscript text. All authors reviewed the manuscript. Bo Su, Wei Sha and Wen-Fei Wu put forward suggestions for revision.

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Competing interests

There were no conflicts of interest.

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Figures

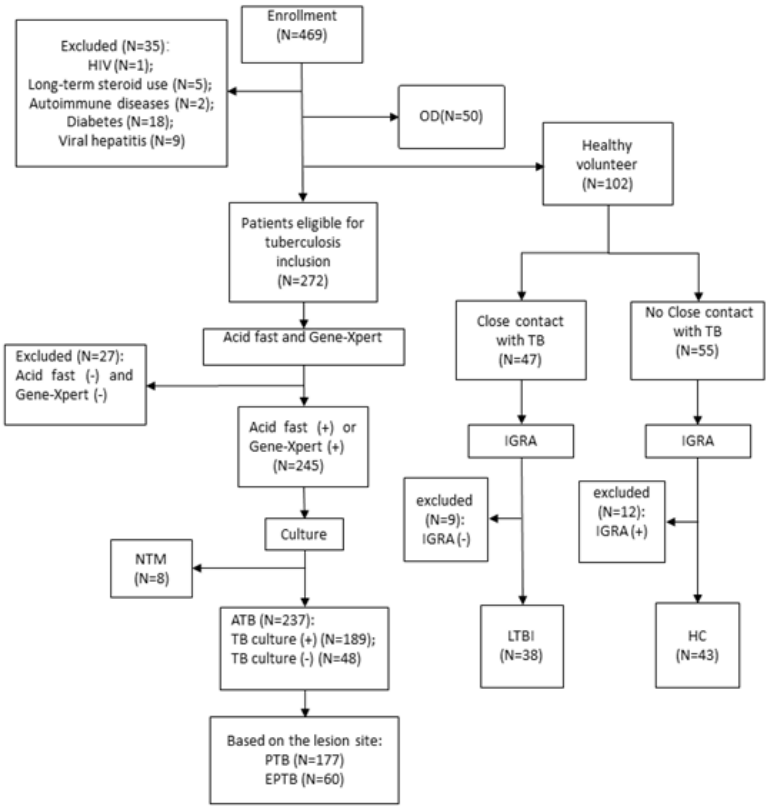

Figure 1

Participant enrollment flowchart. TB: tuberculosis; ATB: active tuberculosis; LTBI: latent tuberculosis infection; NTM: non-tuberculous mycobacterium pulmonary disease; OD: other respiratory diseases; HC: healthy control; PTB: pulmonary tuberculosis; EPTB: extra-pulmonary tuberculosis; IGRA: interferon-γ release assay.

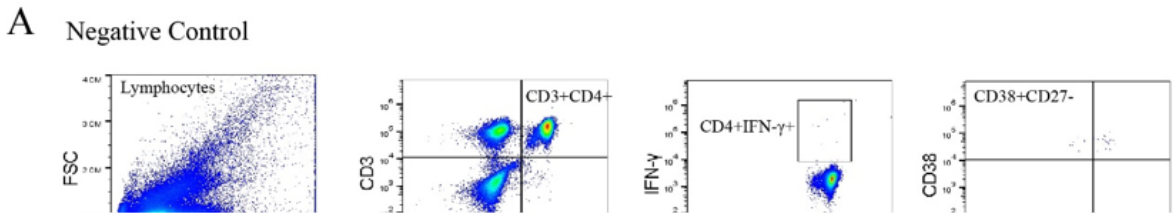

Figure 2

Flow cytometry gating strategy for $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ T cells from the peripheral blood lymphocyte of the participants. (A) Peripheral blood lymphocyte without stimulation by ESAT-6/CFP-10 peptides (negative control) and with 16-h stimulation by ESAT-6/CFP-10 peptides (E6C10). (B) The comparison of CD27 or CD38 expression in ESAT-6/CFP-10 peptides stimulated $CD4^+IFN\text{-}\gamma^+$ T cells to the unstimulated cells.

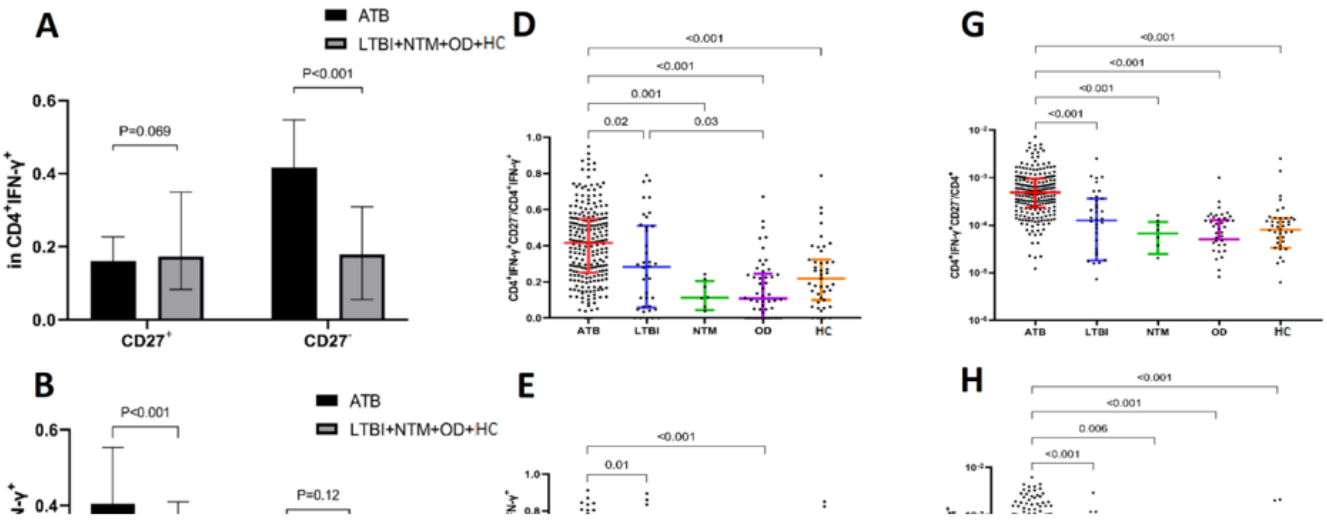

Figure 3

The phenotypic markers on $CD4^+$ IFN-γ^+ T cells and on $CD4^+$ T cells in peripheral blood of different groups. The phenotypic markers (A) $CD4^+IFN\text{-}\gamma^+CD27^+$ and $CD4^+IFN^-\gamma^+CD27^-$, (B) $CD4^+IFN^-\gamma^+CD38^+$ and $CD4^+IFN\text{-}\gamma^+CD38^-$, (C) $CD4^+IFN^-\gamma^+CD38^+CD27^-$ and $CD4^+IFN\text{-}\gamma^+CD27^+CD38^-$, (D)$CD4^+IFN^-\gamma^+CD27^-$, (E) $CD4^+IFN^-\gamma^+CD38^+$, and (F) $CD4^+IFN^-\gamma^+CD38^+CD27^-$ on $CD4^+$ IFN-γ^+ T cell in peripheral blood stimulated with ESAT 6/CFP10 were compared in ATB and other groups. The phenotypic markers (G) $CD4^+IFN^-\gamma^+CD27^-$, (H) $CD4^+IFN^-\gamma^+CD38^+$, and (I) $CD4^+IFN^-\gamma^+CD38^+CD27^-$ on $CD4^+$ T cell in peripheral blood stimulated with ESAT 6/CFP10 in each group was compared. ATB: active tuberculosis; LTBI: latent tuberculosis infection; NTM: non-tuberculous mycobacterium pulmonary disease; OD: other respiratory diseases; HC: healthy control.

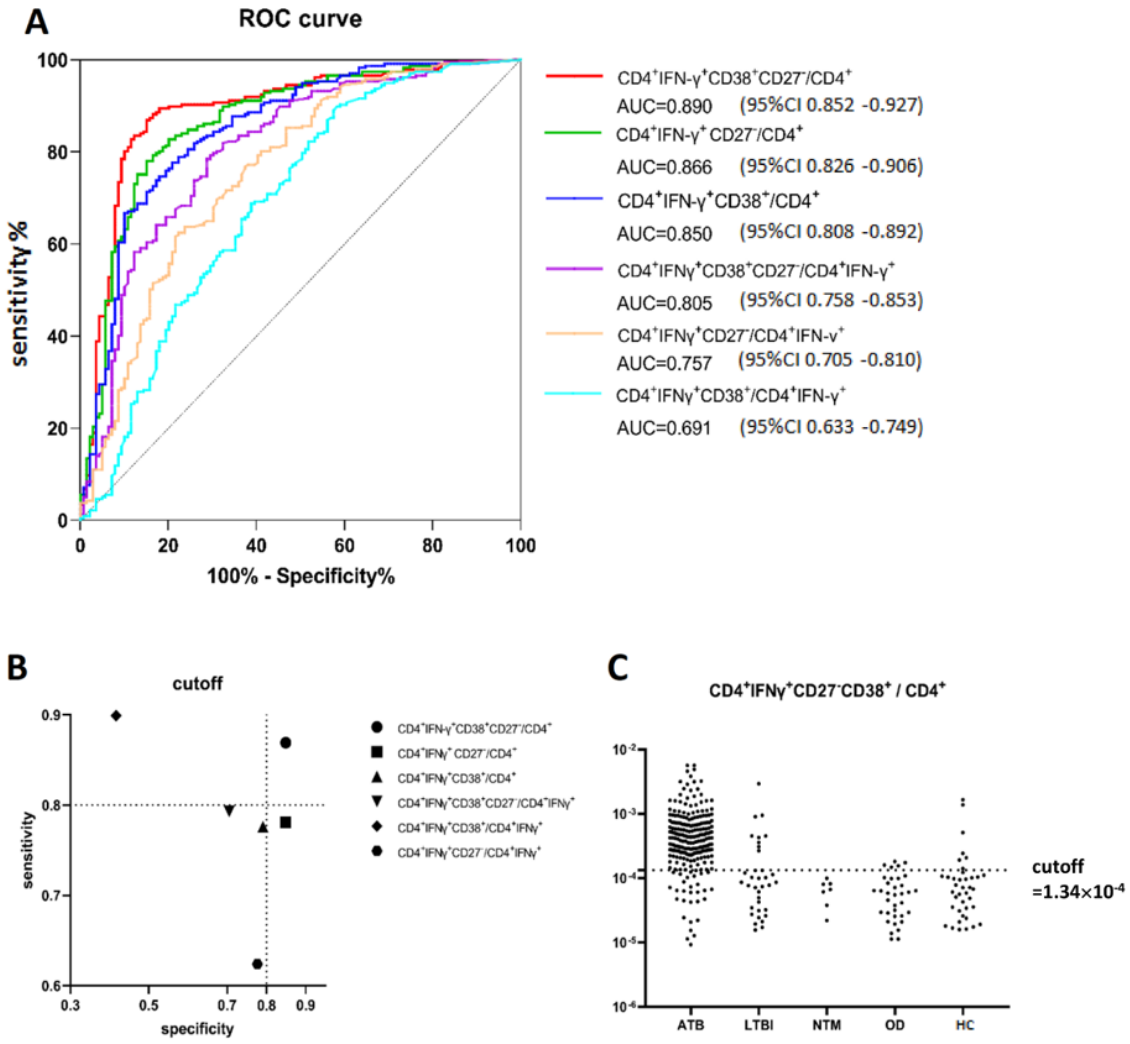

Figure 4

Receiver operating characteristics (ROC) curves and accuracy for the diagnosis of active tuberculosis (ATB). (A) ROC curves of different indicators for the diagnosis of ATB. (B) Sensitivity and specificity corresponding to the cutoff values. The dotted line indicates 80% sensitivity and 80% specificity. (C) The dotted line represents the cutoff value of $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-/CD4^+$ as 1.34×10^{-4}. ATB: active tuberculosis; LTBI: latent tuberculosis infection; NTM: non-tuberculous mycobacterium pulmonary disease; OD: other respiratory diseases; HC: Healthy control.

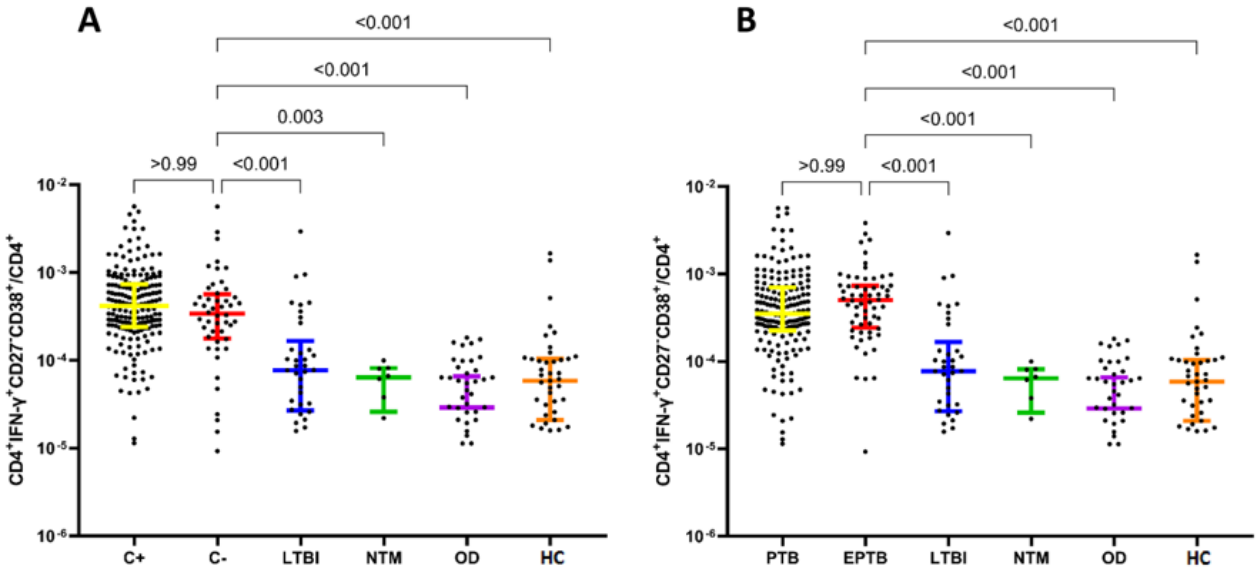

Figure 5

The phenotypic marker $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ on $CD4^+$ cells in different subgroups. (A) The phenotypic marker $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ on $CD4^+$ cells with negative TB culture stimulated with ESAT 6/CFP10. (B) The phenotypic marker $CD4^+IFN\text{-}\gamma^+CD38^+CD27^-$ on $CD4^+$ cells with EPTB stimulated with ESAT 6/CFP10. C^+: TB culture$^+$; C^-: TB culture$^-$; PTB: pulmonary tuberculosis; EPTB: extrapulmonary tuberculosis; LTBI: latent tuberculosis infection; NTM: non-tuberculous mycobacterium pulmonary disease; OD: other respiratory diseases; HC: healthy control.