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A combination of iron metabolism indexes and tuberculosis-specific antigen/phytohemagglutinin ratio for distinguishing active tuberculosis from latent tuberculosis infection

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ABSTRACT

Background: Discriminating active tuberculosis (ATB) from latent tuberculosis infection (LTBI) remains challenging. This study aimed to investigate a diagnostic model based on a combination of iron metabolism and the TB-specific antigen/phytohemagglutinin ratio (TBAg/PHA ratio) in T-SPOT.TB assay for differentiation between ATB and LTBI.

Methods: A total of 345 participants with ATB (n = 191) and LTBI (n = 154) were recruited based on positive T-SPOT.TB results at Tongji hospital between January 2017 and January 2020. Iron metabolism analysis was performed simultaneously. A diagnostic model for distinguishing ATB from LTBI was established according to multivariate logistic regression.

Results: The TBAg/PHA ratio showed 64.00% sensitivity and 90.10% specificity in distinguishing ATB from LTBI when a threshold of 0.22 was used. All iron metabolism biomarkers in the ATB group were significantly different from those in the LTBI group. Specifically, serum ferritin and soluble transferrin receptor in ATB were significantly higher than LTBI. On the contrary, serum iron, transferrin, total iron binding capacity, and unsaturated iron binding capacity in ATB were significantly lower than LTBI. The combination of iron metabolism indicators accurately predicted 60.00% of ATB cases and 91.09% of LTBI subjects, respectively. Moreover, the combination of iron metabolism indexes and TBAg/PHA ratio resulted in a sensitivity of 88.80% and specificity of 90.10%. Furthermore, the performance of models established in the Qiaokou cohort was confirmed in the Caidian cohort.

Conclusions: The data suggest that the combination of iron metabolism indexes and TBAg/PHA ratio could serve as a biomarker to distinguish ATB from LTBI in T-SPOT-positive individuals.

Introduction

Tuberculosis (TB) remains the leading cause of an infectious disease death worldwide (Furin et al., 2019). There were approximately 10.0 million new cases and 1.5 million deaths from TB in 2018 (World Health Organization, 2019). Most individuals infected with *Mycobacterium tuberculosis* (MTB) remain asymptomatic, despite a continued immune response, which is a condition termed latent tuberculosis infection (LTBI). Of individuals with LTBI, 5–10% will progress to active TB (ATB) during their lifetime (Hoppe et al., 2016, Sudre et al., 1992). The differential diagnosis between ATB and LTBI is the key point to control or end TB, especially in countries with a high burden of TB such as China (Gao et al., 2017, World Health Organization, 2015a).

There are still no gold standards for diagnosing MTB infection. The sensitivities of current approaches for ATB diagnosis – including smear microscopy, mycobacterial culture and molecular detection by Xpert MTB/RIF – are unsatisfactory under low bacterial loads and the scope of their application is not included for diagnosing LTBI (World Health Organization, 2015b). The

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tuberculin skin test (TST) is the only available tool for detecting LTBI. Interferon-gamma release assays (IGRAs), which depend on the detection of interferon-gamma (IFN-γ) in response to MTB-specific antigens, have been introduced as an alternative to TST for the diagnosis of MTB infection. However, both TST and IGRAs are intrinsically unable to discriminate between ATB and LTBI (Cohen et al., 2019, Wallis et al., 2013). Recently, Wang et al. proposed a new calculation-TB-specific antigen/phytohemagglutinin ratio (TBAg/PHA ratio) for the T-SPOT.TB (T-SPOT) (Oxford Immunotec Ltd., Oxford, UK) assay and found that it had potential value on differentiating ATB from LTBI (Wang et al., 2016). Other studies have also confirmed the moderate value of this indicator (Bosco et al., 2017, Zhou et al., 2017). On the other hand, iron metabolism in TB is receiving more and more attention (Boelaert et al., 2007, McDermid et al., 2013). A hallmark of TB-associated anemia is the 'iron delivery problem' (Camaschella, 2015, Minchella et al., 2015b). Several studies have shown that biomarkers of iron metabolism could facilitate clinical diagnosis in MTB infection (Chegou et al., 2016, Dai et al., 2019, Minchella et al., 2015a). It was therefore wondered whether it would be possible to combine these two methods to achieve a better distinction between ATB and LTBI.

The present study investigated the TBAg/PHA ratio in T-SPOT assay and iron metabolism indexes – including serum ferritin (SF), serum iron (SI), transferrin (TF), total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and soluble transferrin receptor (STFR) – in LTBI individuals and those with ATB. It aimed to establish a discriminatory model based on the combination of iron metabolism indexes and the TBAg/PHA ratio, which can be applied to differentiate ATB from LTBI.

Materials and methods

Study design

This study was carried out between January 2017 and January 2020 at Tongji Hospital (Qiaokou cohort, the largest hospital in central China) and Sino-French New City Hospital (Caidian cohort, a branch hospital of Tongji Hospital). All participants were recruited based on positive T-SPOT results. Iron metabolism analysis was simultaneously performed in all subjects. The diagnosis of ATB was based on the following criteria: (1) clinical characteristics and symptoms, including fever, cough and productive sputum; and (2) a positive Xpert MTB/RIF result and/or a positive culture for MTB. LTBI was defined as a subject with positive T-SPOT test results and meeting the following criteria: (1) absence of symptomatic, microbiological or radiological evidence of ATB and (2) no history of TB. Participants with the following conditions were excluded: 1) age <17 years and 2) undergoing anti-TB treatment for >2 weeks. The study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent was obtained from all participants.

Iron metabolism analysis

Serum samples were collected from patients and iron metabolism indicators were measured using ROCHE COBAS 8000 (Mannheim, Germany) according to the manufacturer's instructions. The indexes obtained were as follows: serum ferritin (SF), serum iron (SI), transferrin (TF), total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and soluble transferrin receptor (STFR).

T-SPOT assay

Samples of heparinized peripheral blood were collected and analyzed using T-SPOT assay (Oxford Immunotec, Oxford, UK) according to the manufacturer's instructions. Briefly, the isolated peripheral blood mononuclear cells (PBMCs) (2.5×10^5) were added to 96-well plates precoated with anti-IFN-γ antibody. Four wells were used for each participant: medium well, PHA well, early secreted antigenic target 6 (ESAT-6), and culture filtrate protein 10 (CFP-10) wells. Plates were incubated for 16–20 h at 37 C° with 5% CO_2 and developed using an anti-IFN-γ antibody conjugate and substrate to detect the presence of secreted IFN-γ. Spot-forming cells (SFC) were counted with an automated ELISPOT reader (CTL Analyzers, Cleveland, OH, USA). The test result was positive if the ESAT-6 minus negative control and/or CFP-10 minus negative control ≥6 spots. The test result was negative if both ESAT-6 minus negative control and CFP-10 minus negative control ≤5 spots. Results were considered undetermined if the spot amounts in the PHA well were <20 or if spot amounts in the medium well were >10.

Table 1
Demographic, clinical and laboratory characteristics of study participants.

Variables	Qiaokou (training cohort)			Caidian (validation cohort)			P^b
	ATB (n = 125)	LTBI (n = 101)	P^a	ATB (n = 66)	LTBI (n = 53)	P^a	
Sex, male, %	79 (63.20%)	61 (60.40%)	0.681	45 (68.18%)	33 (62.26%)	0.562	0.558
Age, years	51.94 ± 13.94	52.01 ± 14.42	0.909	51.74 ± 13.81	50.38 ± 12.95	0.371	0.591
Presence of BCG scar	58 (46.40%)	40 (39.60%)	0.345	32 (48.48%)	22 (41.51%)	0.465	0.733
TB history	31 (24.80%)	0 (0%)	< 0.001	14 (21.21%)	0 (0%)	< 0.001	0.737
Underlying condition or illness							
HIV infection	1 (0.80%)	0 (0%)	1	1 (1.52%)	0 (0%)	1	1
Diabetes mellitus	10 (8.80%)	5 (4.95%)	0.428	6 (9.09%)	3 (5.66%)	0.729	0.825
End-stage renal disease	7 (5.60%)	4 (3.96%)	0.758	3 (4.55%)	1 (1.89%)	0.628	0.591
Liver cirrhosis	4 (3.20%)	2 (1.98%)	0.694	1 (1.52%)	3 (5.66%)	0.322	0.742
Hematological malignancy	5 (4.00%)	5 (4.95%)	0.755	2 (3.03%)	1 (1.89%)	1	0.554
Solid tumor	12 (9.60%)	10 (9.9%)	1	5 (7.58%)	5 (9.43%)	0.75	0.846
Organ transplantation	9 (7.20%)	6 (5.94%)	0.792	4 (6.06%)	2 (3.77%)	0.691	0.642
Immunosuppressive condition[c]	13 (10.40%)	9 (8.91%)	0.823	7 (10.61%)	4 (7.55%)	0.753	1
Positive mycobacterial culture	105 (84.00%)	NA	NA	57 (86.36%)	NA	NA	NA
Positive Xpert MTB/RIF	92 (73.60%)	NA	NA	50 (75.76%)	NA	NA	NA

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; BCG, Bacille Calmette-Guérin; TB, tuberculosis; NA, not applicable; HIV, human immunodeficiency virus.

[a] Comparisons were performed between ATB and LTBI groups using the Chi-squared test or Mann–Whitney U test.

[b] Comparisons were performed between the Qiaokou and Caidian cohorts using the Chi-squared test or Mann–Whitney U test.

[c] Patients who underwent organ transplantation, chemotherapy or took immunosuppressants within 2 months. Data are presented as means ± SD or numbers (percentages).

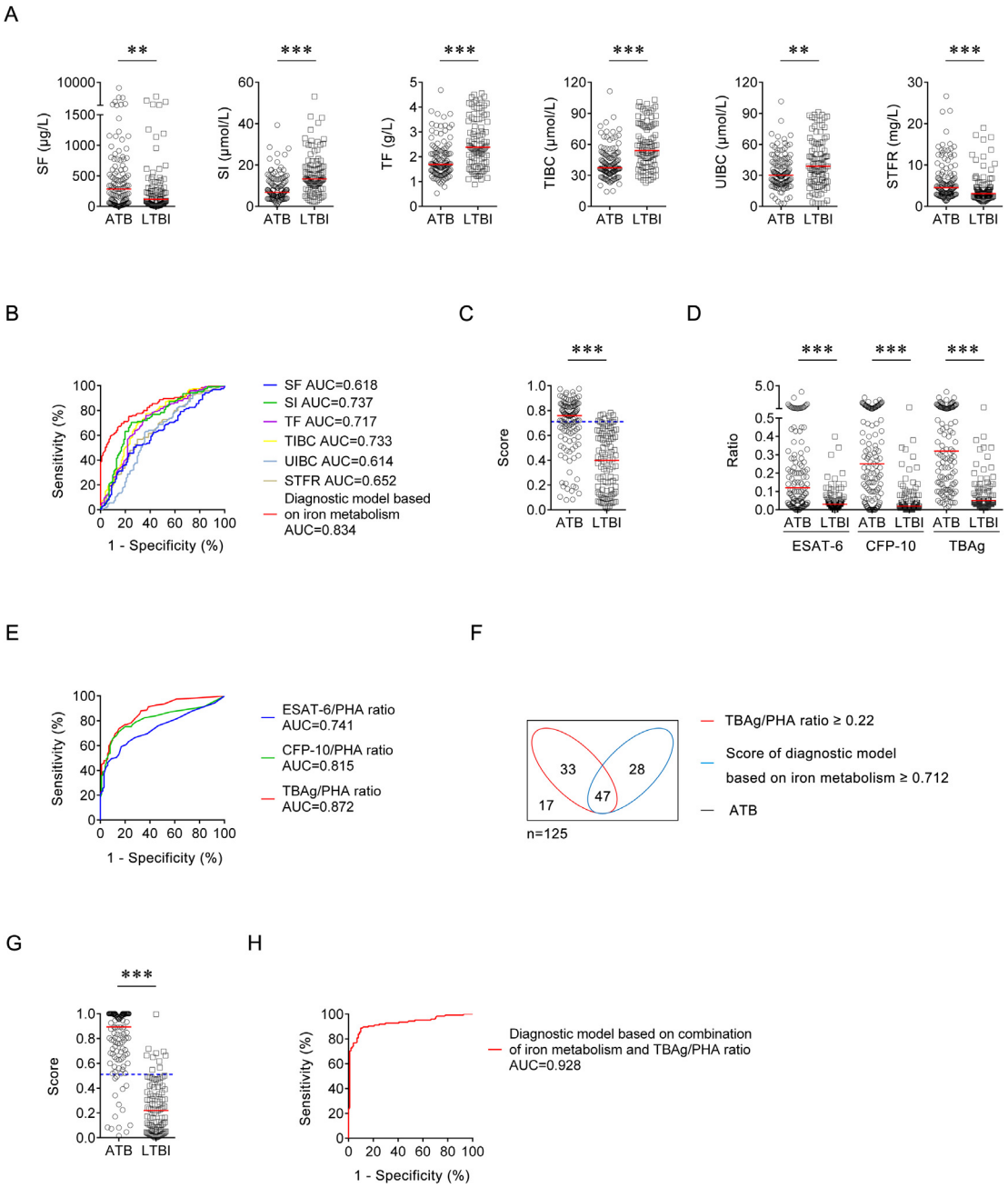

Figure 1. Establishment of a diagnostic model based on a combination of iron metabolism and the TBAg/PHA ratio in the Qiaokou cohort.
(A) Scatter plots showing the levels of SF, SI, TF, TIBC, UIBC, and STFR in ATB patients (n = 125) and LTBI individuals (n = 101).
Horizontal lines indicate the median.
$^{**}p < 0.01$, $^{***}p < 0.001$ (Mann–Whitney U test).
(B) ROC analysis showing the performance of SF, SI, TF, TIBC, UIBC, STFR, and the diagnostic model based on iron metabolism in distinguishing ATB from LTBI.
(C) Scatter plots showing the score of the diagnostic model based on iron metabolism in ATB patients (n = 125) and LTBI individuals (n = 101).
Horizontal lines indicate the median.
$^{***}p < 0.001$ (Mann–Whitney U test).
Blue dotted lines indicate the cut-off value in distinguishing these two groups.
(D) Scatter plots showing ESAT-6/PHA ratio, CFP-10/PHA ratio, and TBAg/PHA ratio in ATB patients (n = 125) and LTBI individuals (n = 101).
Horizontal lines indicate the median.
$^{***}p < 0.001$ (Mann–Whitney U test).
(E) ROC analysis showing the performance of ESAT-6/PHA ratio, CFP-10/PHA ratio, and TBAg/PHA ratio in distinguishing ATB from LTBI.
(F) Venn diagrams showing the overlap of TBAg/PHA ratio and the diagnostic model based on iron metabolism in ATB patients (n = 125).
(G) Scatter plots showing the score of the diagnostic model based on the combination of iron metabolism and TBAg/PHA ratio in ATB patients (n = 125) and LTBI individuals (n = 101).
Horizontal lines indicate the median.
$^{***}p < 0.001$ (Mann–Whitney U test).
Blue dotted lines indicate the cut-off value in distinguishing these two groups.
(H) ROC analysis showing the performance of the diagnostic model based on the combination of iron metabolism and the TBAg/PHA ratio in distinguishing ATB from LTBI.
Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; SF, serum ferritin; SI, serum iron; TF, transferrin; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; STFR, soluble transferrin receptor; ESAT-6, early secreted antigenic target 6; CFP-10, culture filtrate protein 10; TBAg, tuberculosis-specific antigens; PHA, phytohemagglutinin; ROC, receiver operating characteristic; AUC, area under the curve.

To ensure the reliability of T-SPOT assay, the following points need to be considered: (1) PBMCs were isolated within 4 h of blood collection; (2) each new batch of T-SPOT reagent must be validated before use; (3) T-SPOT assay was performed strictly according to the manufacturer's protocol; and (4) the automated ELISPOT reader was calibrated with a reference plate every month.

This study calculated the ratios of (1) ESAT-6 SFC to PHA SFC and (2) CFP-10 SFC to PHA SFC. The larger of these two values was defined as the TBAg/PHA ratio of one participant.

Statistical analysis

Differences between the ATB and LTBI groups were compared using the Mann–Whitney U test or Chi-square test. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic ability of various methods to distinguish ATB from LTBI. Area under the curve (AUC), sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and accuracy, together with their 95% confidence intervals (CI), were calculated. To build the diagnostic model for differentiating ATB from LTBI, all variables with statistical significance were taken as candidates for further multivariable logistic regression analyses; and then the regression equation (diagnostic model) was obtained and a score for each individual was calculated. Statistical analysis and graphing were performed using SPSS 25.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 6 (GraphPad Software, CA, USA). All statistical tests were two-sided. $P<0.05$ was considered statistically significant.

Results

Cohort characteristics

A total of 226 participants were recruited and grouped into either ATB (n = 125) or LTBI (n = 101) in the Qiaokou cohort, based on pre-defined criteria. Another 119 subjects, including 66 ATB and 53 LTBI, were enrolled in the Caidian cohort. The clinical and demographic characteristics of the recruited participants are presented in Table 1. There was no significant between-group difference in age and gender. About 63% of subjects were male, and the mean age was around 51 years.

Results of iron metabolism analysis and T-SPOT in ATB and LTBI

All indicators associated with iron metabolism significantly differed between individuals with ATB and LTBI in the Qiaokou cohort. Specifically, SF and STFR in ATB were significantly higher than LTBI. In contrast, SI, TF, TIBC, and UIBC in ATB were significantly lower than LTBI (Figure 1A). The best AUC was obtained with SI (0.737), while the AUC of TF was 0.717 (Figure 1B). These findings indicated that it was possible to apply iron metabolism indicators to distinguish ATB from LTBI. To establish a diagnostic model based on indicators in iron metabolism for distinguishing ATB from LTBI, all variables with statistical significance were used for multivariable logistic regression analysis. A diagnostic model was built as the following:

$P = 1/[1 + e^{-(2.951 - 0.08 \times SI - 1.138 \times TF + 0.135 \times STFR)}]$ (P, predictive value; e, natural logarithm). ROC analysis showed that the AUC of the diagnostic model was 0.834 (95% CI, 0.783–0.885) (Figure 1B and C). When the cut-off value was set at 0.712, the sensitivity and specificity were 60.00% and 91.09%, respectively (Table 2).

For T-SPOT assay, ESAT-6/PHA ratio, CFP-10/PHA ratio and TBAg/PHA ratio in ATB were significantly higher than LTBI (Figure 1D). When using the TBAg/PHA ratio as an indicator, the sensitivity and specificity in discriminating ATB cases and LTBI individuals were 64.00% and 90.10%, respectively, with a threshold of 0.22 (Table 2; Figure 1E).

Diagnostic model based on the combination of iron metabolism indicators and TBAg/PHA ratio for discriminating ATB from LTBI

Although iron metabolism indexes and the TBAg/PHA ratio showed potential value with moderate performance in discriminating between ATB and LTBI, both of their sensitivities were relatively low. However, the overlap between the TBAg/PHA ratio and iron metabolism showed that a combination of these two tools could improve the diagnostic value (Figure 1F). Thus, a new diagnostic model was obtained by logistic regression analysis:

$P = 1/[1 + e^{-(1.268 + 8.824 \times \text{TBAg/PHA ratio} - 0.073 \times SI - 1.281 \times TF + 0.187 \times STFR)}]$ (P, predictive value; e, natural logarithm).

ROC analysis showed that the AUC of this model to differentiate ATB from LTBI was 0.928 (95% CI, 0.893–0.964), with a sensitivity of 88.80% and specificity of 90.10% when using 0.512 as the threshold (Table 2; Figure 1G and H).

Validation of the diagnostic model in the Caidian cohort

Another blinded validation study was performed in an independent population in the Caidian cohort. There was a significant difference in all indicators of iron metabolism between individuals with ATB and LTBI in this cohort (Figure 2A). Similar performance was observed in the Caidian cohort. Validation of the diagnostic model based on iron metabolism produced an AUC of 0.867 (95% CI, 0.801–0.932) with 63.64% sensitivity and 90.57% specificity (Table 3; Figure 2B and C). If using 0.22, which was obtained from the training cohort, as the cut-off value of the TBAg/PHA ratio, the sensitivity and specificity were 65.15% and 90.57% in differentiating ATB from LTBI, respectively (Table 3; Figure 2D and E). The diagnostic model based on a combination of iron

Table 2
The performance of various methods for distinguishing between ATB and LTBI in the Qiaokou cohort.

Variables	Cut-off value	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)	Accuracy
TBAg/PHA ratio	0.22	0.872 (0.828–0.917)	64.00% (54.88–72.25%)	90.10% (82.13–94.89%)	88.89% (80.08–94.25%)	66.91% (58.26–74.60%)	6.46 (3.54–11.81)	0.40 (0.32–0.51)	75.66%
Diagnostic model based on iron metabolism indexes	0.712	0.834 (0.783–0.885)	60.00% (50.84–68.54%)	91.09% (83.32–95.59%)	89.29% (80.16–94.68%)	64.79% (56.28–72.49%)	6.73 (3.55–12.77)	0.44 (0.35–0.55)	73.89%
Diagnostic model based on combination of iron metabolism indexes and TBAg/PHA ratio	0.512	0.928 (0.893–0.964)	88.80% (81.61–93.52%)	90.10% (82.13–94.89%)	91.74% (84.95–95.75%)	86.67% (78.31–92.26%)	8.97 (4.96–16.21)	0.12 (0.08–0.20)	89.38%

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; TBAg, tuberculosis-specific antigens; PHA, phytohemagglutinin; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; CI, confidence interval.

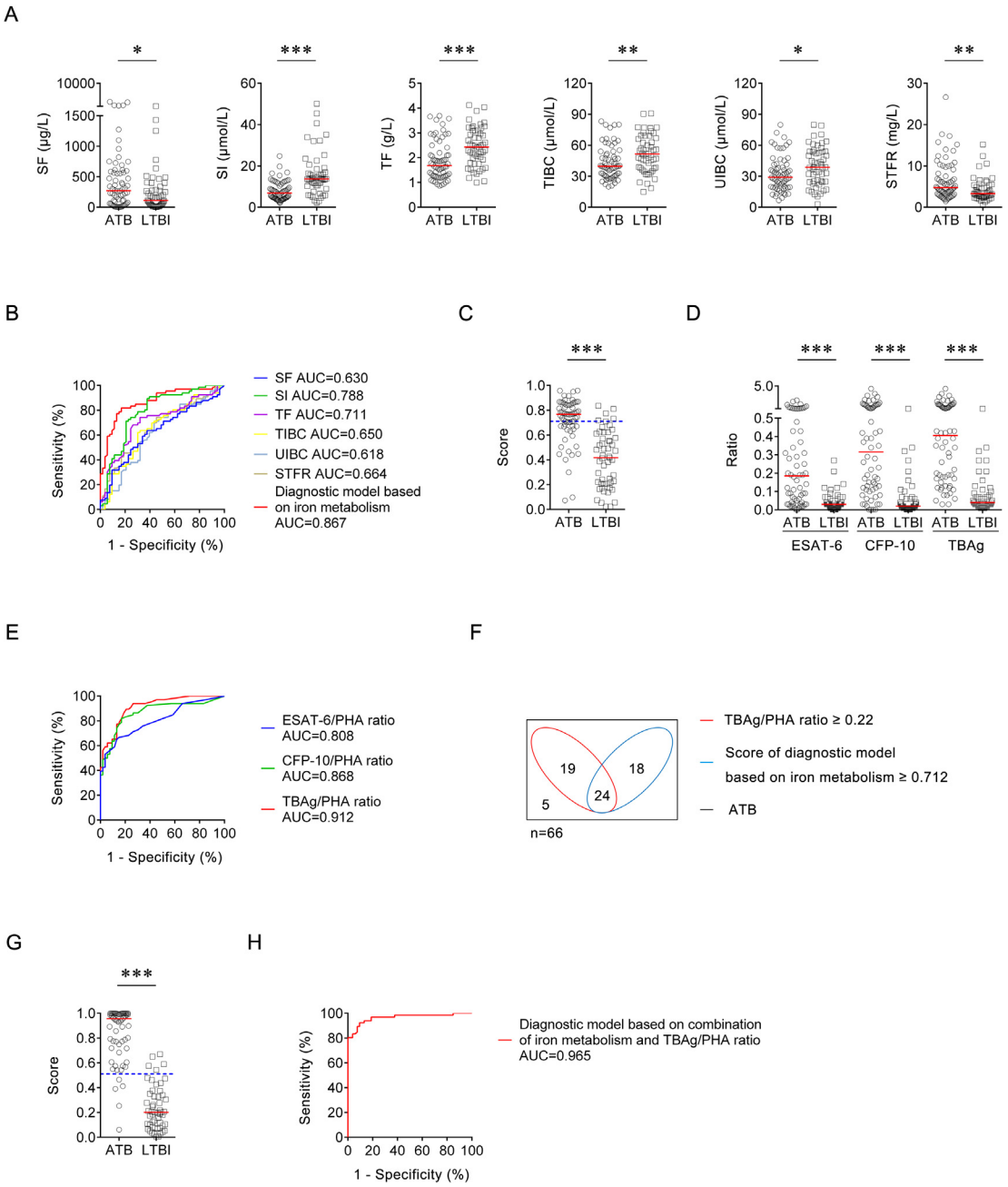

Figure 2. Validation of the diagnostic model based on a combination of iron metabolism and the TBAg/PHA ratio in the Caidian cohort.
(A) Scatter plots showing the levels of SF, SI, TF, TIBC, UIBC and STFR in ATB patients (n = 66) and LTBI individuals (n = 53).
Horizontal lines indicate the median.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Mann–Whitney U test).
(B) ROC analysis showing the performance of SF, SI, TF, TIBC, UIBC, STFR, and the diagnostic model based on iron metabolism in distinguishing ATB from LTBI.
(C) Scatter plots showing the score of the diagnostic model based on iron metabolism in ATB patients (n = 66) and LTBI individuals (n = 53).
Horizontal lines indicate the median.
***$p < 0.001$ (Mann–Whitney U test).
Blue dotted lines indicate the cut-off value in distinguishing these two groups.
(D) Scatter plots showing the ESAT-6/PHA ratio, CFP-10/PHA ratio and TBAg/PHA ratio in ATB patients (n = 66) and LTBI individuals (n = 53).
Horizontal lines indicate the median.
***$p < 0.001$ (Mann–Whitney U test).
(E) ROC analysis showing the performance of the ESAT-6/PHA ratio, CFP-10/PHA ratio and TBAg/PHA ratio in distinguishing ATB from LTBI.
(F) Venn diagrams showing the overlap of the TBAg/PHA ratio and the diagnostic model based on iron metabolism in ATB patients (n = 66).
(G) Scatter plots showing the score of the diagnostic model based on the combination of iron metabolism and the TBAg/PHA ratio in ATB patients (n = 66) and LTBI individuals (n = 53).
Horizontal lines indicate the median.
***$p < 0.001$ (Mann–Whitney U test).
Blue dotted lines indicate the cut-off value in distinguishing these two groups.
(H) ROC analysis showing the performance of the diagnostic model based on the combination of iron metabolism and TBAg/PHA ratio in distinguishing ATB from LTBI.
Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; SF, serum ferritin; SI, serum iron; TF, transferrin; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity; STFR, soluble transferrin receptor; ESAT-6, early secreted antigenic target 6; CFP-10, culture filtrate protein 10; TBAg, tuberculosis-specific antigens; PHA, phytohemagglutinin; ROC, receiver operating characteristic; AUC, area under the curve.

Table 3
The performance of various methods for distinguishing between ATB and LTBI in the Caidian cohort.

Variables	Cut-off value	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)	Accuracy
TBAg/PHA ratio	0.22	0.912 (0.863–0.962)	65.15% (52.34–76.19%)	90.57% (78.58–96.47%)	89.58% (76.56–96.10%)	67.61% (55.34–77.95%)	6.91 (2.94–16.20)	0.38 (0.28–0.54)	76.47%
Diagnostic model based on iron metabolism indexes	0.712	0.867 (0.801–0.932)	63.64% (50.82–74.86%)	90.57% (78.58–96.47%)	89.36% (76.11–96.02%)	66.67% (54.47–77.07%)	6.75 (2.87–15.84)	0.40 (0.29–0.55)	75.63%
Diagnostic model based on combination of iron metabolism indexes and TBAg/PHA ratio	0.512	0.965 (0.934–0.997)	92.42% (82.50–97.18%)	90.57% (78.58–96.47%)	92.42% (82.50–97.18%)	90.57% (78.58–96.47%)	9.80 (4.24–22.63)	0.08 (0.04–0.20)	91.60%

Abbreviations: ATB, active tuberculosis; LTBI, latent tuberculosis infection; TBAg, tuberculosis-specific antigens; PHA, phytohemagglutinin; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; CI, confidence interval.

metabolism indexes and TBAg/PHA ratio also performed well in the validation cohort: 0.965 (95% CI, 0.934–0.997) AUC, 92.42% sensitivity, and 90.57% specificity (Table 3; Figure 2F–H).

Discussion

TB remains a leading cause of global morbidity and mortality (Collaborators, 2018). Millions of TB cases go undiagnosed each year due to the limitations of current tools, and delays in diagnosis of ATB contribute to its high death toll and facilitate onward transmission of infection (Bayer and Castro, 2017, Dheda et al., 2016). Until now, novel diagnostic biomarkers were still urgently needed to detect MTB infection (Hatherill et al., 2019). The clinical management is complicated by difficulty in discriminating between latent infection and active disease (Blumberg and Ernst, 2016, Churchyard and Swindells, 2019, Petersen et al., 2019). However, existing biomarker research for this target has thus far yielded limited success (Menzies et al., 2018, Walzl et al., 2018). Although more recent studies have demonstrated that transcriptome (Singhania et al., 2018, Turner et al., 2020), metabolome (Weiner et al., 2018), proteome (Chaisson et al., 2019, Yang et al., 2020) and genome (Suliman et al., 2018, Warsinske et al., 2019) correlate with MTB infection, few markers have progressed to a further developmental stage. Any identified biomarkers have considerably varied among studies, limiting their use for clinical diagnosis (MacLean et al., 2019). Thus, the development of a rapid diagnostic test that can distinguish between ATB and LTBI with satisfactory performance is very important for TB control.

Currently, there were few available reports that have elaborated the value of the combination of iron metabolism indexes and other indicators on diagnosing TB. It is believed that the current study is the first comprehensive investigation on iron metabolism indexes and TBAg/PHA ratio for discriminating between ATB and LTBI. For iron metabolism, a three-indicators biosignature was developed, which could efficiently discriminate ATB from LTBI with moderate accuracy. Meanwhile, the TBAg/PHA ratio in T-SPOT assay also presented an equivalent use on this target. Furthermore, a discriminatory four-indicator biosignature based on the combination of iron metabolism and TBAg/PHA ratio that differed among these two conditions was identified. Subsequent validation with an independent cohort finally confirmed the discriminatory value of these models for discriminating between ATB and LTBI.

Another interesting question is why iron metabolism indicators and the TBAg/PHA ratio have complementary effects on TB diagnosis. It was speculated that these two methods exhibit different performance in MTB-infected individuals with different immune status. Previous studies have shown that the TBAg/PHA ratio was obviously decreased in immunocompromised ATB patients (Wang et al., 2018b). Therefore, it is very difficult to distinguish ATB from LTBI in this condition because most times the low TBAg/PHA ratio results are attributed to LTBI. However, indicators in iron metabolism, such as SI, are decreased in immunocompromised patients (MacKenzie et al., 2008). Thus, it is reasonable that combining these two methods could improve the performance of TB diagnosis.

The TBAg/PHA ratio is a specific indicator for TB diagnosis. Apart from distinguishing between ATB and LTBI it can also be used to discriminate TB from other respiratory diseases such as lung cancer, bacterial pneumonia and fungal pneumonia (Wang et al., 2018a, Wang et al., 2020). Meanwhile, iron metabolism indexes – including SI, SF and TF – have also been reported to have potential value in differentiating ATB from other lung illness such as chronic obstructive pulmonary disease and respiratory tract infections (Dai et al., 2019, Jacobs et al., 2016).

The present study had some limitations. First, although it was a two-center study, the sample size was relatively small in each center. Therefore, the accuracy of the models should be evaluated with more participants in further studies. Second, since all participants in this study were recruited based on a positive T-SPOT assay; MTB-infected patients with negative T-SPOT results were not involved in this study. Given that the proportion of ATB patients with negative T-SPOT results may reach 14% (Liao et al., 2009), the inclusion of this part of the population may have led to a biased diagnostic performance. Third, since the subjects enrolled in this study were aged $\geq$17 years, the performance of the diagnostic model in individuals aged <17 years was unclear. Fourth, the effect of anti-TB treatment on various diagnostic indicators was not investigated. However, previous studies have found that the TBAg/PHA ratio would decrease after anti-TB treatment (Wang et al., 2016, Zhou et al., 2017). On the other hand, it was reported that iron metabolism indexes, including SI and TF, would increase after anti-TB treatment, and the levels of SF and STFR would decrease after anti-TB treatment (Dai et al., 2019, Minchella et al., 2015a, Miranda et al., 2017, Sepehri et al., 2018). These findings indicate that dynamic monitoring of the TBAg/PHA ratio and iron metabolism indexes could be used to guide anti-TB treatment in ATB patients. Finally, notwithstanding the relatively great performance of this established model, further validations are needed to assess the application of the model in different settings alongside head-to-head comparisons with other biomarkers.

In summary, this study comprehensively analyzed iron metabolism indexes and the TBAg/PHA ratio associated with different TB infectious statuses and identified a diagnostic model capable of distinguishing ATB from LTBI in individuals with positive T-SPOT results. These findings represent a significant advance in current tests of TB infection.

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Author contributions

YL, FW and ZS designed the research and drafted the manuscript. YL and YX managed statistical analyses. YL, QL, GT and XY collected the samples and clinical data. LM and HS performed the experiment. All authors contributed to manuscript revision, read and approved the final version.

Competing interests

The authors declare there are no competing interests.

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