Construction of a risk model and deep learning network based on patients with active pulmonary tuberculosis and pulmonary inflammation

DECHANG XU[1*], JIANG ZENG[2*], FANGFANG XIE[3*], QIANTING YANG[4],
KAISONG HUANG[5], WEI XIAO[5], HOUWEN ZOU[1] and HUIHUA ZHANG[5]

[1]Internal Medicine Center of Ganzhou Cancer Hospital; [2]Ganzhou Fifth People's Hospital,
The Ninth Affiliated Clinical College, Gannan Medical University; [3]Department of Endocrinology,
Ganzhou People's Hospital, Ganzhou, Jiangxi 341000; [4]Shenzhen Institute of Liver Diseases, Third People's Hospital,
Shenzhen, Guangdong 518112; [5]Guangdong Provincial Key Laboratory of Regional Immunity and Disease, Department of
Pathogen Biology, School of Medicine, Shenzhen University, Shenzhen, Guangdong 518052, P.R. China

Abstract. Most patients with active pulmonary tuberculosis (TB) are difficult to be differentiated from pneumonia (PN), especially those with acid-fast bacillus smear-negative (AFB⁻) and interferon-γ release assay-positive (IGRA⁺) results. Thus, the aim of the present study was to develop a risk model of low-cost and rapid test for the diagnosis of AFB⁻ IGRA⁺ TB from PN. A total of 41 laboratory variables of 204 AFB⁻ IGRA⁺ TB and 156 PN participants were retrospectively analyzed. Candidate variables were identified by t-statistic test and univariate logistic model. The logistic regression analysis was used to construct the multivariate risk model and nomogram with internal and external validation. A total of 13 statistically differential variables were compared between AFB⁻ IGRA⁺ TB and PN by false discovery rate (FDR) and odds ratio (OR). By integrating five variables, including age, uric acid (UA), albumin (ALB), hemoglobin (Hb) and white blood cell counts (WBC), a multivariate risk model with a concordance index (C-index) of 0.7 (95% CI: 0.61, 0.8) was constructed. The nomogram showed that UA and Hb acted as protective factors with an OR <1, while age, WBC and ALB were risk factors for TB occurrence. Internal and external validation revealed that nomogram prediction was consistent with the actual observations. Collectively, it was revealed that an integration of five biomarkers (age, UA, ALB, Hb and WBC) may be used to quickly predict TB in AFB⁻ IGRA⁺ clinical samples from PN.

Introduction

Tuberculosis (TB) remains a severe global public health problem, especially in developing countries (1). How to reduce the TB epidemic is a major issue hindering economic and social development. Detection of *Mycobacterium tuberculosis* (*Mtb*) DNA using Gene Xpert MTB/RIF assay is more sensitive and rapid for diagnosing TB and rifampicin resistance (2). However, due to its costs, environmental limitations, and difficulties in its supply, it is difficult to carry out screening in a low-income rural area (3). Interferon-γ release assay (IGRA) is commonly used in the diagnostic workup of *Mtb* but distinguishes poorly between TB and latent TB infection (LTBI) (4,5).

In addition, the symptoms of patients with TB are very similar to those of bacterial community-acquired pneumonia (CAP). Both TB and CAP are infections of the lower respiratory tract, but they are often considered as separate entities (6). TB is classically a more indolent disease presenting cavitating lung lesions observed in patients with a history of cough for three months or longer accompanied by weight loss, and, often, is not associated with acute respiratory compromise (7). By contrast, CAP is generally associated with a short history of several days, is rapidly progressive, and is more often associated with respiratory compromise (8). The diagnosis of CAP is based on the detection of a new infiltrate on a chest radiograph or other imaging technique in the presence of recently acquired respiratory signs and symptoms (9). However, clinical findings do not reliably predict radiologically confirmed PN, as features of TB may sometimes be quite similar to those of CAP among patients who experience symptoms at the early stage (10). In

Correspondence to: Dr Huihua Zhang, Guangdong Provincial Key Laboratory of Regional Immunity and Disease, Department of Pathogen Biology, School of Medicine, Shenzhen University, 3688 Nanhai Avenue, Nanshan, Shenzhen, Guangdong 518052, P.R. China
E-mail: hh_zhang@szu.edu.cn

Mr. Houwen Zou, Internal Medicine Center of Ganzhou Cancer Hospital, 19 Huayuanqian, Shuidong, Zhanggong, Ganzhou, Jiangxi 341000, P.R. China
E-mail: zhw8956@163.com

*Contributed equally

addition, the etiology cannot be simply differentiated clinically or radiologically and is undefined in ~50% of patients (8). Because most patients with TB have no sputum specimens or are *Mtb* smear/culture-negative, this makes diagnosis more intractable (11).

Host markers including secreted molecules in blood have been reported as novel candidate markers to distinguish TB, such as interferon-γ inducible protein 10 kDa (12), interleukin-2 (IL-2) (13), IL-6 (14), C-reactive protein (CRP) (15) and vascular endothelial growth factor (14). However, the diagnostic performances of reported biomarkers cannot be applied in low-income areas due to technical, instrumentation or cost limitations. To address this problem, the present study retrospectively analyzed the differences in routinely monitored laboratory variables in blood tests between AFB⁻ IGRA⁺ TB and PN, and built a risk model to differentiate AFB⁻ IGRA⁺ TB from PN, and validated its application by an external independent cohort.

Patients and methods

Study design and criteria for study inclusion. The patients provided a full medical history, participated in regular physical examinations, and underwent routine investigations, including acquired immunodeficiency syndrome (AIDS) serology, chest radiography, IGRA, and microbiological sputum examination, where possible.

The inclusion criteria for PN participants were as follows: i) Meeting the diagnostic criteria of 'The People's Republic of China health industry standard (WS 382-2012; http://www.nhc.gov.cn/wjw/s9494/201209/110b324c465740 169a863d57a78c18a6.shtml)'. The clinical diagnosis can be established by any of (a), (b), (c), (d) plus (e) and excluding TB, lung tumor, non-infectious interstitial lung disease, pulmonary edema, pulmonary atelectasis, pulmonary embolism, pulmonary eosinophilic infiltrates, pulmonary vasculitis, etc. The elements of (a) to (e) are as follows: a) Newly developed cough and sputum, or aggravation of existing respiratory symptoms with purulent sputum, with or without chest pain; b) fever; c) solid lung signs and/or wet rales; d) peripheral blood WBC >10x10⁹/l or <4x10⁹/l with or without leftward nuclear shift; and e) chest X-ray showing new lamellar or patchy infiltrative shadows or interstitial changes with or without pleural effusion. ii) Sputum culture with a bacterial pathogenic basis or effective antimicrobial therapy, such as marked improvement in symptoms such as cough, yellow sputum and significant uptake of chest imaging. iii) Exclusion of viral, atypical pathogens, fungal, and *Mtb* infections. The inclusion criteria for IGRA⁺ TB participants were as follows: Meeting the diagnostic criteria for TB including positive sputum, bronchoscopic lavage, brush examination, smear microscopy of biopsy specimens for antacid bacilli, isolation and culture of *Mtb*, or positive nucleic acid test and IGRA for *Mtb*, or positive pathology of lung tissue biopsy. The inclusion criteria for AFB⁻ TB participants were as follows: There were no sputum or negative bacilli smear and negative *Mtb*, but there were chest computed tomographic (CT) scans or chest X-ray evidence and symptoms responding to TB treatment.

The exclusion criteria were as follows: i) Use of antimicrobial drugs for >24 h; (2) suffering from diseases that can affect the total number and classification of blood leukocytes, including leukemia, chronic inflammatory states, etc.; iii) having a recent (within 3 months) history of glucocorticoid application or ongoing hormone use; and iv) clearly diagnosed or highly suspected pulmonary edema, pulmonary embolism, pulmonary atelectasis, bronchial asthma, viral PN, fungal PN, atypical pathogenic PN, pulmonary eosinophilic infiltrates and lung cancer.

The involved participants were divided into two groups: The discovery cohort and the external validation cohort. For the discovery cohort, participants were enrolled at Ganzhou Fifth Hospital (Ganzhou, China) between August 2018 and August 2020, including 748 AFB⁻ TB participants and 531 PN participants. As the predefined goal was to assess the ability of laboratory biomarkers to distinguish IGRA⁺ patients presenting with AFB⁻ TB, 287 participants with IGRA⁺ TB were subjected for further analysis (AFB⁻ IGRA⁺ TB). Participants with >50% of laboratory data missing were excluded, thus making a total of 89 TB and 38 PN participants with recorded biomarker values used to construct the risk model. The external validation cohort of 134 participants in the study was collected from Shenzhen Third People's Hospital (Shenzhen, China) from June 2018 to June 2019 (Fig. 1). Among them, 15 were excluded due to missing variables, therefore the external validation cohort consisted of 77 AFB⁻ IGRA⁺ TB and 42 PN participants.

Ethical approval and patient consent to participate. The study protocol was approved by the Ethics Committee and the Institutional Review Board of Ganzhou Fifth People's Hospital (registration no. 2020-10) to allow retrospective access to the records and files of patients. Written informed consent was waived by the Ethics Committee as this was an observational and retrospective analysis.

Data collection. The medical records of all participants were reviewed by experienced TB clinicians, including medical history, symptoms, clinical signs, microbiological tests, laboratory findings, chest CT chest X-rays, and treatment measures. A total of 41 laboratory biomarkers were assessed by differential statistics and odds ratio (OR) calculation for variable selection.

Statistical analyses. For laboratory results, continuous variables were preprocessed by $\log_2$-transformation before analysis. The laboratory data were verified for skewed distribution using the Kolmogorov-Smirnov test. In the present study Wilcoxon rank-sum test was suitable for skewed distribution data (16-18). P-values were adjusted by false discovery rate (FDR). Variables between two conditions were defined as statistically significant when FDR <0.2 (19).

A univariate logistic model (glm) was used to calculate the OR for each laboratory biomarker. The regression coefficient of the glm was regarded as the log OR. Variables with an FDR <0.2 or at a statistically significant level (P-value <0.05) in the glm analysis were candidates for the construction of a multivariate risk model (lrm) and nomogram, and the final variables were determined using Akaike's information criterion (AIC) as a stopping rule. The goodness of fit of the lrm model was calculated using Hosmer and Lemeshow

Table I. Demographics and baseline characteristics of AFB$^-$ IGRA$^+$ TB and PN participants.

Variables	The first cohort				The second cohort	
	TB	PN	χ^2	P-value	TB	PN
Age	n=204	n=156			n=77	n=42
Mean ± SD	46±15	47±15	0.03	0.87	29±9	41±19
≤20	13	12	-	-	10	7
21-40	56	29	-	-	57	16
41-60	96	89	-	-	10	12
≥60	39	26	-	-	0	7
Sex						
Male	150	118	3.82	0.05	52	26
Female	54	38	2.78	0.10	25	16
Types						
Infiltrative	162	-	-	-	41	-
Cavitary	19	-	-	-	3	-
Secondary	8	-	-	-	33	-
Tuberculous pleurisy/empyema	15	-	-	-	0	-
Anti-TB treatment						
Yes	163	-	-	-	-	-
No	41	-	-	-	-	-
TB-DNA (FAM)						
Positive	4	-	-	-	38	-
Negative	40	-	-	-	30	-
TB-antibody						
Positive	3	-	-	-	20	-
Negative	20	-	-	-	15	-
Treatment						
Initial	143	-	-	-	77	-
Re-treated	13	-	-	-	0	-

P-value, chi-square test. AFB$^-$, acid-fast bacillus smear-negative; IGRA$^+$, interferon-γ release assay-positive; TB, tuberculosis; PN pneumonia.

C statistics test. The performance of the nomogram was evaluated by the concordance index (C-index) and assessed by comparing nomogram-predicted vs. actual observation, and bootstrapping with 1,000 resamples to decrease the overfit bias was applied for calibration (20,21). The glm was generated by glmnet package (version 4.1-4) (22) and the nomogram was generated by DynNom package (version 5.0.2) (23). All analyses and figures were generated in R version 4.0.3 (https://www.r-project.org/).

Results

Differential laboratory biomarkers between AFB$^-$ IGRA$^+$ TB and PN. The characteristics of the AFB$^-$ IGRA$^+$ TB and PN participants are shown in Table I. No significant differences in age and sex were found in the first cohort. Males made up the majority in both cohorts. Clinical types of TB participants included 162 infiltrative pulmonary TB, 19 cavitary pulmonary TB, 8 secondary pulmonary TB, and 15 tuberculous pleurisy and empyema. Only 44 and 23 participants had received TB-DNA and TB-antibody examination, yielding 9.09% (4/44) and 13.04% (3/23) positive rates, respectively.

The significant variables between the two conditions were further explored. Using unpaired t-test and setting FDR <0.2, 13 variables with marked differences were identified (Table II). Only uric acid (UA) was elevated (fold change >1.2). Notably, five variables, including aspartate aminotransferase (AST), total bile acid (TBA), triglyceride (TG), alanine aminotransferase (ALT), and glutamyl transpeptidase (GGT), were reduced (fold change <0.83) in TB compared with PN (Fig. 2A). These data revealed variable expression differences between the two conditions, and that some variables may be useful to distinguish AFB$^-$ IGRA$^+$ TB from PN.

To find the odds that TB would progress or not be given exposure to these laboratory variables, OR was assessed for each variable by univariate logistic model. Notably, 11 variables significantly associated with TB progression (P<0.05; Table II) were identified. Among them, four variables indicated a protective effect in TB progression (OR <1), including UA, red blood cell (RBC), Hb, and ALB; while seven variables

Table II. Statistical differences and OR values of each variable in AFB⁻ IGRA⁺ TB compared with PN.

Variables	P-value	FDR	Fold change	OR	P-value of OR	2.5% CI	97.5% CI
UA	6.82E-06	2.73E-04	1.3	0.36	2.49E-05	0.22	0.57
GLU	6.94E-04	0.01	0.88	4.26	1.45E-03	1.82	10.93
AST	8.54E-04	0.01	0.82	1.78	1.47E-03	1.26	2.56
TBA	5.90E-03	0.06	0.66	1.59	0.01	1.14	2.3
TG	1.45E-02	0.11	0.82	2.66	0.02	1.2	6.65
RBC	0.02	0.11	1.11	0.28	0.02	0.09	0.72
ALT	0.02	0.13	0.82	1.31	0.03	1.03	1.67
Hb	0.03	0.13	1.05	0.37	0.03	0.15	0.89
GGT	0.03	0.14	0.72	1.33	0.04	1.02	1.74
ALP	0.04	0.14	0.86	1.92	0.05	1.02	3.71
LDH	0.04	0.14	0.83	1.6	0.06	1.01	2.71
ALB	0.04	0.14	1.05	0.4	0.05	0.16	0.98
WBC	0.06	0.19	0.93	1.49	0.06	0.98	2.29

FDR threshold, <0.2. AFB-, acid-fast bacillus smear-negative; IGRA+, interferon-γ release assay-positive; TB, tuberculosis; PN pneumonia; FDR, false discovery rate; OR, odds ratio; CI, confidence interval; UA, uric acid; GLU, glucose; AST, aspartate aminotransferase; TBA, total bile acid; TG, triglyceride; RBC, red blood cell; ALT, alanine aminotransferase; Hb, hemoglobin; GGT, glutamyl transpeptidase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; ALB, albumin; WBC, white blood cell.
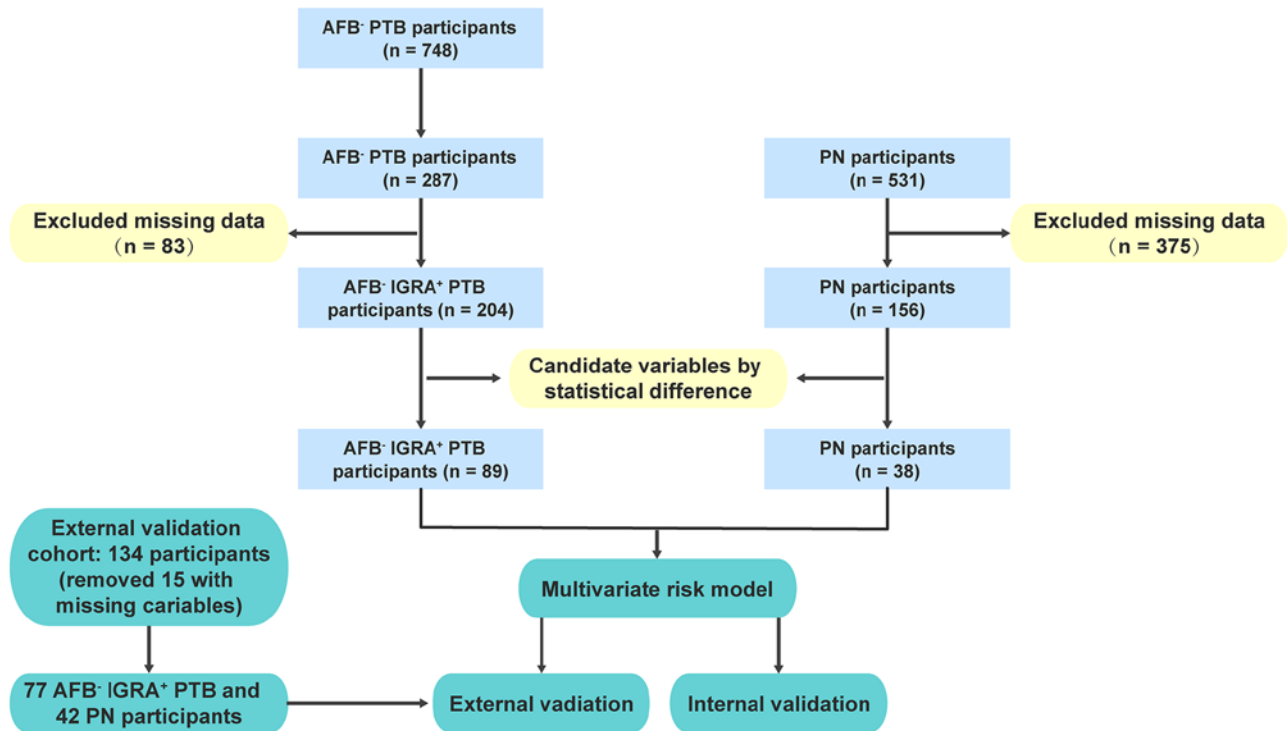

Figure 1. Overview of study design and analysis workflow. A total of 748 AFB⁻ TB and 531 PN participants were prospectively evaluated. Individuals with >50% of clinical data missing were excluded. Candidate variables were filtered by statistical differences. A total of 89 AFB⁻ IGRA⁺ TB participants and 38 PN participants with candidate variables were finally included in the construction of a multivariate risk model. AFB⁻, acid-fast bacillus smear-negative; TB, tuberculosis; PN, pneumonia; IGRA⁺, interferon-γ release assay-positive; PTB, pulmonary tuberculosis.

including GLU, AST, TBA, TG, ALT, GGT, and ALP, were revealed as risk factors for TB progression (OR >1).

Multivariate risk model to predict TB progression probability. Combined with the aforementioned results, and using AIC as a stopping rule, five laboratory variables (age, UA, ALB, Hb, and WBC) were finally selected to develop a multivariate risk model with 89 AFB⁻ IGRA⁺ TB and 38 PN participants. Notably, the expression distribution of variables in both groups revealed more unbalance in PN
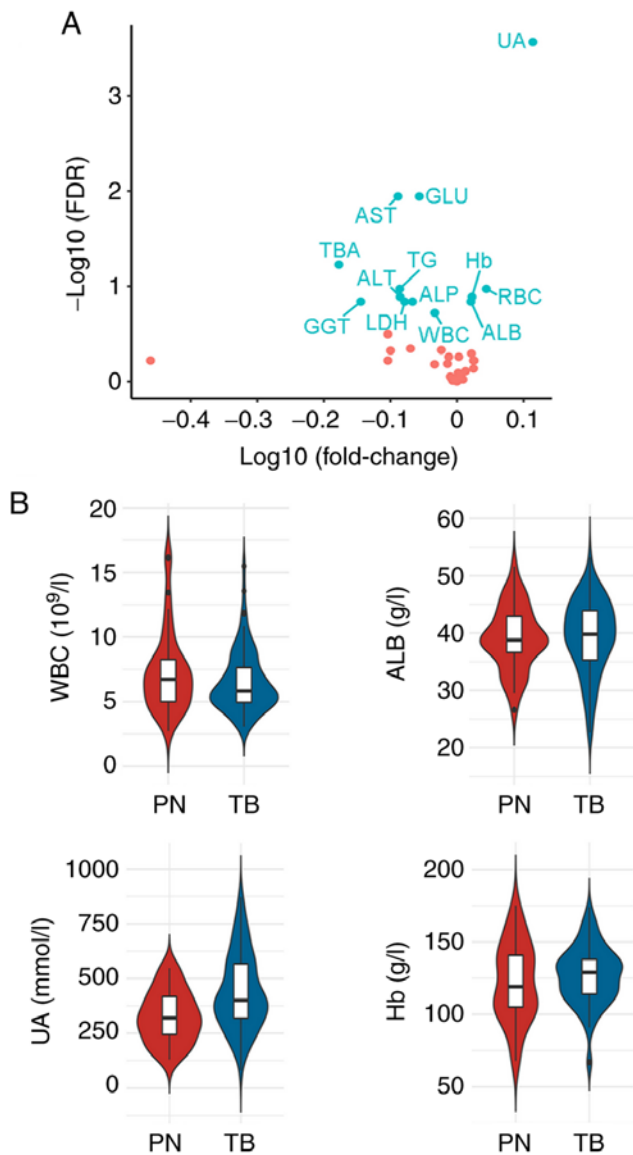
Figure 2. Statistical differential comparison between AFB⁻ IGRA⁺ TB and PN. (A) Volcano plot showing the distribution of all laboratory variables between the TB and PN. (B) Violin plots showing the values of four variables between two groups. AFB⁻, acid-fast bacillus smear-negative; IGRA⁺, interferon-γ release assay-positive; TB, tuberculosis; PN, pneumonia; UA, uric acid; GLU, glucose; AST, aspartate aminotransferase; TBA, total bile acid; TG, triglyceride; Hb, hemoglobin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; RBC, red blood cell; GGT, glutamyl transpeptidase; LDH, lactate dehydrogenase; WBC, white blood cell; ALB, albumin.

than AFB⁻ IGRA⁺ TB (Fig. 2B). The risk model yielded a C-index of 0.7 (95% CI: 0.61, 0.8), with P=0.01 (chi-square test) (data not shown). The calibration plot revealed a moderate agreement between the model prediction and the actual observation (Fig. 3A; P=0.18, Hosmer-Lemeshow test). Using the nomogram, the values for each variable were mapped to points on a scale axis ranging from 0 to 10. With a corresponding number of points assigned to given magnitudes of the variables, the risk probability was calculated by the corresponding cumulative point score for all the variables (25). It was revealed that UA had the most protective effect in TB progression, followed by Hb; while age, WBC and ALB were shown to be risk factors (Fig. 3C).

Next, an external validation cohort of 134 participants, consisting of 77 AFB⁻ IGRA⁺ TB and 42 PN participants were prospectively collected. The C-index of nomogram for predicting the external cohort was 0.77 (95% CI: 0.68, 0.86) (data not shown). The calibration plot also revealed consistent results between the prediction by nomogram and actual observation (Fig. 3B) with P=0.13 (Hosmer-Lemeshow test).

Discussion

In the present study, different profiles were analyzed between AFB⁻ IGRA⁺ TB and PN, and five laboratory variables (age, UA, ALB, Hb and WBC) were selected to construct a multivariate risk model and nomogram. Internal validation and a calibration plot showed moderate agreement between nomogram probability and actual observation, with a C-index of 0.7 (95% CI: 0.61, 0.8). A similar result in an external validation cohort (C-index: 0.77; 95% CI: 0.68, 0.86) was obtained. These findings indicated that five laboratory variables may be used to predict TB disease probability when a clinical sample is AFB⁻ IGRA⁺.

It has been reported that patients with TB tend to exhibit increased levels of CRP, erythrocyte sedimentation rate (ESR), and UA, and low levels of Hb (25). An increased UA level was observed in 28.2% of men and 37.5% of women prior to chemotherapy, and more often during the first 2 months of treatment both in men and women, which suffered from multiple drug-resistant pulmonary TB (26). In the present study, serum UA was revealed to be at a significantly higher level in TB (FDR <0.001), with an OR value of 0.36 (P=2.5E-05) compared with PN (Table II), indicating that it may be a specific protective factor in patients with TB. Reduced plasma ALB concentrations have been reported in TB (27) and may be used as a diagnostic and prognostic marker in pretreated HIV and TB patients. WBC was revealed to be significantly increased in patients with TB compared with healthy controls, and the WBC significantly decreased during TB treatment (28,29). In the present study, WBC was statistically significant and a significant risk factor (OR=1.49), but with no higher counts in fold change compared with PN.

To predict the risk of TB for each AFB⁻ IGRA⁺ patient, a nomogram was used to provide a more accurate profile. With five variables, the nomogram had good predictive accuracy with a C-index of 0.7. External validation was essential to confirm it can be applied to patients outside of the cohort. Thus, a second participant cohort from another center was recruited, and then assessed on the nomogram, and the result obtained was consistent with the actual observation (C-index of 0.77).

The present study still had several limitations: First, in low-income and rural settings, not all patients received all routine laboratory tests, leading to numerous missing values in the first cohort of participants. In order to analyze more biomarkers, participants with >50% of missing data were excluded, with 41 laboratory variables and a small number of participants remaining (89 AFB⁻ IGRA⁺ TBA and 38 PN), resulting in a small sample size. Second, although internal and external validation exhibited good performance, further investigations are required to optimize the nomogram in larger cohorts and more types of pulmonary TB.
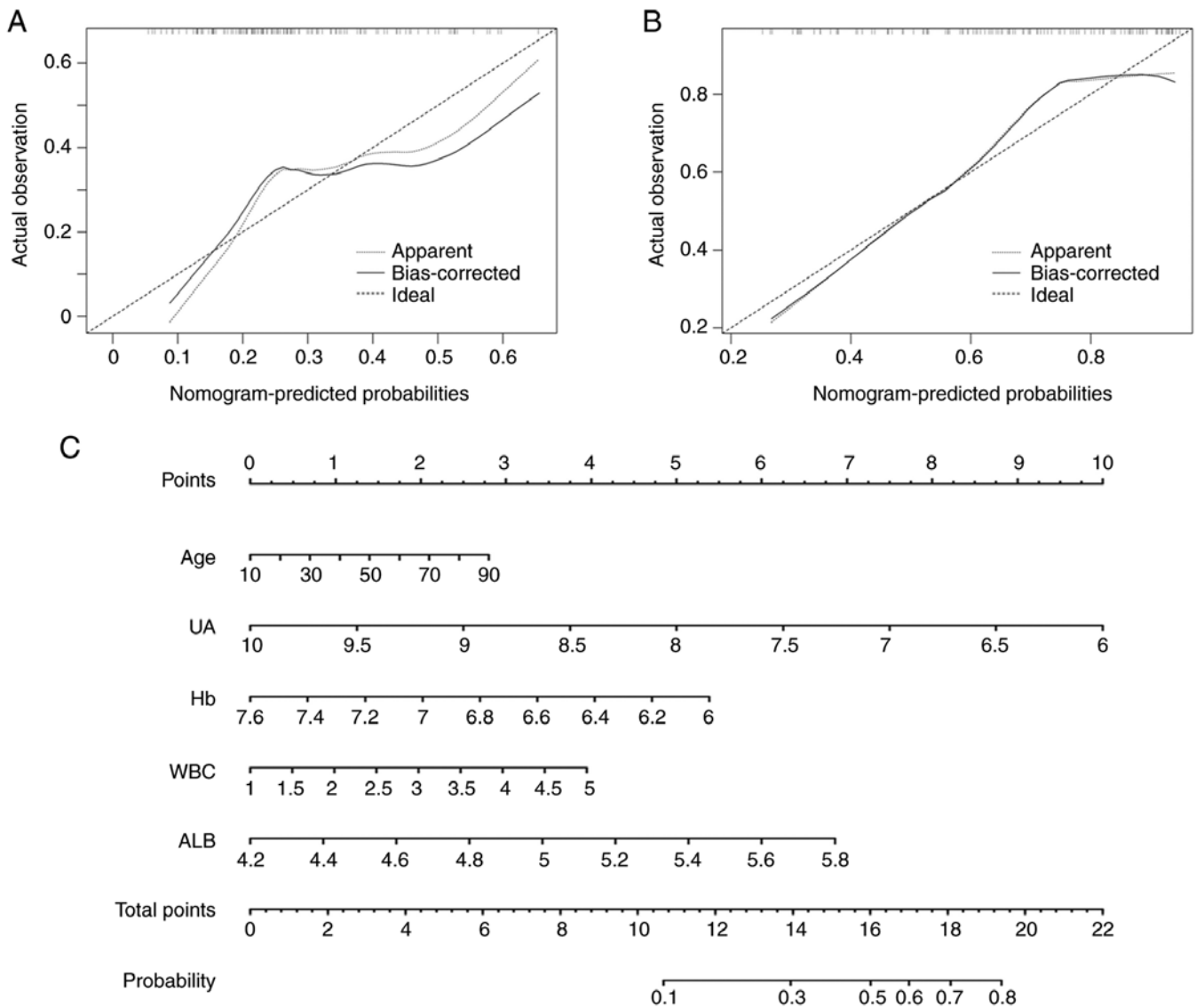
Figure 3. Multivariate risk model to predict tuberculosis probability. (A) Calibration curve of the multivariate risk model. The dotted line represents the ideal fit; circles represent nomogram-predicted probabilities; and the solid line represents bias-corrected nomogram-predicted probabilities. (B) Calibration curve of external validation cohort using a nomogram. (C) Nomogram predicting risk based on five variables. Total point values were independently calculated for each cause of outcome and then applied to the corresponding probability scale at the bottom. Values of four variables (UA, Hb, WBC and ALB) were log$_2$-transformed. UA, uric acid; Hb, hemoglobin; WBC, white blood cell; ALB, albumin.

In addition, the association between prior TB and lung cancer has been undefined. Several large cohort studies provided evidence supporting an association between prior TB and risk of lung cancer (30,31). In a systematic review and meta-analysis published in 2011, a previous diagnosis of TB was associated with increased lung cancer risk [relative risk (RR)=1.76 (95% CI=1.49 to 2.08)] with little variation by smoking status (32). Similarly, a pooled analysis from the International Lung Cancer Consortium found a lung cancer RR of 1.48 (95% CI=1.17 to 1.87) associated with a history of TB, controlling for smoking status. However, there was no attempt to differentiate TB from lung cancer with the risk model of the present study. In future projects, applicability of this model in other diseases will be further investigated. Following improvement of this model, such as increasing its applicability, it is anticipated that it may help clinicians to reduce the cost and time to diagnose AFB$^-$ IGRA$^+$ TB in low-income, high-burdened, and resource-constrained rural area settings.

In conclusion, the present study identified a five-variable signature to distinguish AFB$^-$ IGRA$^+$ TB from PN patients. A risk model was built to differentiate AFB$^-$ IGRA$^+$ TB from PN, and was validated in an external independent cohort, which could be applied in low-income and resource-constrained rural area settings.

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Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors' contributions

DX, JZ, FX, QY, KH, WX, HZo and HZh contributed to the study conception and design. Primary clinical case information, data collection, and analysis were performed by DX, JZ, QY and FX. The first draft of the manuscript was written by DX, FX, and HZh. KH, WX and HZo conducted the literature search, as well as the screening and quality assessment of the clinical data. DX and HZh confirm the authenticity of all the raw data. All authors contributed to this manuscript and have consented to its submission. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee and the Institutional Review Board of Ganzhou Fifth People's Hospital (registration no. 2020-10). Written informed consent was waived by the Ethics Committee as this was an observational and retrospective analysis study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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