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A routine blood test-associated predictive model and application for tuberculosis diagnosis: a retrospective cohort study from northwest China

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Abstract

Objectives: This study aimed to use the results of routine blood tests and relevant parameters to construct models for the prediction of active tuberculosis (ATB) and drug-resistant tuberculosis (DRTB) and to assess the diagnostic values of these models.

Methods: We performed logistic regression analysis to generate models of plateletcrit-albumin scoring (PAS) and platelet distribution width-treatment-sputum scoring (PTS). Area under the curve (AUC) analysis was used to analyze the diagnostic values of these curves. Finally, we performed model validation and application assessment.

Results: In the training cohort, for the PAS model, the AUC for diagnosing ATB was 0.902, sensitivity was 82.75%, specificity was 82.20%, accuracy rate was 81.00%, and optimal threshold value was 0.199. For the PTS model, the AUC for diagnosing DRTB was 0.700, sensitivity was 63.64%, specificity was 73.53%, accuracy rate was 89.00%, and optimal threshold value was −2.202. These two models showed significant differences in the AUC analysis, compared with single-factor models. Results in the validation cohort were similar.

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Conclusions: The PAS model had high sensitivity and specificity for the diagnosis of ATB, and the PTS model had strong predictive potential for the diagnosis of DRTB.

Keywords

tuberculosis, cohort study, predictive model, diagnosis, China, sputum, blood platelets, hematologic tests, albumins

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Introduction

Laboratory testing is important for the diagnosis of tuberculosis, formulation of treatment regimens, and assessment of results. The smear test is the most commonly used bacteriological method for the diagnosis of tuberculosis. However, this method has low sensitivity.[1] Culture on solid agar is the gold standard for detection of *Mycobacterium tuberculosis* (MTB), but requires 4–6 weeks to obtain results,[1] which is unsuitable for immediate confirmation of a diagnosis of infection. Rapid diagnostic methods based on nucleic acid amplification techniques have been developed; they generally demonstrate high sensitivity and offer some advantages over conventional solid culture,[2,3] particularly with respect to increased speed while maintaining accuracy for clinical management of tuberculosis and associated infection control.[3] Although diagnostic methods based on genetic testing are rapid and sensitive, the equipment, facilities, and reagents required for these methods carry increased cost, which limits their widespread application. According to a report by the World Health Organization (WHO), the mean global positivity rate for the detection of pathogen in tuberculosis patients is 57%,[4] and this ratio is lower in poor rural areas. Tuberculosis control organizations and patients in developing countries (particularly those who are sputum-negative) urgently need rapid, accurate, uncomplicated, inexpensive, and accessible methods for diagnosis of tuberculosis.

Blood is the “sentinel” for many diseases, because it can indicate both hematologic malignancies and tuberculosis infections. Routine blood tests can be widely used for the detection of active tuberculosis (ATB) infection. However, the use of routine blood test results may require considerable modification to effectively aid in the diagnosis of ATB. Previous studies showed that red blood cell (RBC) count, hemoglobin (Hb) level, platelet (PLT) count, and mean platelet volume (MPV) were reduced in patients with ATB.[5–8] However, another study showed reactive thrombocytosis, as well as increased plateletcrit (PCT) levels and platelet distribution widths (PDWs), in patients with ATB.[9] Although single routine blood markers may be useful for identifying community-acquired pneumonia (CAP) and ATB, the sensitivities and specificities of these markers are relatively low when they are used as diagnostic markers;[9] thus, they cannot aid in the early diagnosis of drug-resistant tuberculosis (DRTB). This study aimed to use combinations of widely available biomarkers to construct models for the prediction of ATB and DRTB and to assess the diagnostic values of these models.

Patients and methods

Study population

This retrospective cohort study was performed in accordance with the principles of the Declaration of Helsinki, and following approval from the ethics committee of Ankang Central Hospital (Shaanxi, China) (ECACH-2016010). Informed consent was not required because the study did not put the patients at risk.

All participants in each cohort were patients and health examiners in Ankang Central Hospital. Continuous data of laboratory examination results recorded during the period from October 2016 to June 2018 that met the selection criteria (as described in the Case definitions section) were extracted and divided into different cohorts in a temporal manner (training cohort: October 2016 to December 2017; validation cohort: January 2018 to June 2018). Data from health check-ups during the same period underwent time-based stratification (based on examination during the study period) wherein a certain amount of observation units were randomly selected to form a health control (HC) group. Briefly, sample number was established based on the Medical Reference Value Range, with an average >100. Given the complexity of the influence of healthy adults on the assessed variables, the sample number was increased based on sampling situation. During the study period, patients in the Health Examination database that met the requirements (normal routine blood results, liver function, and chest X-ray findings) were considered for inclusion as the control group. Because this was a large number of possible patients, we used the age and sex of the ATB group as standards for fuzzy matching and divided the possible controls into 21 month-based subgroups. Equal numbers of control patients were randomly selected from each month-based subgroup to form the final HC group. Using these cohorts, a diagnostic model was tentatively constructed, with its reference standard and optimal threshold value determined through normal distribution and receiver operating characteristic curve (ROC) analysis. Laboratory testing and result reporting personnel and reference standard evaluators did not know the clinical data, model reference standards, or diagnostic results of the study subjects beforehand. Examinations of patients with non-tuberculous diseases that may cause changes in routine blood tests were excluded. Sample descriptions and exclusion criteria are shown in Figure 1.

Case definitions

Subjects in the HC group participated in voluntary health screening services during the same period, did not have any discomfort or known diseases, and had normal chest X-ray results. Subjects in the CAP group were patients who had been diagnosed with CAP at the Department of Respiratory Medicine at Ankang Central Hospital during the same period and had negative interferon gamma release assay (IGRA) findings. The diagnosis of CAP complied with American Thoracic Society guidelines:[10] recent coughing, expectoration, fever, respiratory distress, and chest pain with wet rales on lung auscultation and/or elevated inflammatory markers. In addition, chest X-ray results of these patients showed new infiltrative lesions. Subjects in the ATB group had a confirmed tuberculosis diagnosis by positive bacteriological and molecular biology tests.[11]

Bacterial loads in sputum specimens were graded as previously described:[11] +, 1–9 bacteria/50 fields; 1+, 10–49 bacteria/50 fields; 2+, 1–9 bacteria/field; 3+, 10–90 bacteria/field; and 4+, $\geq$100 bacteria/field. At least 50 fields were observed to confirm the 2+ grade and at least 20 fields were

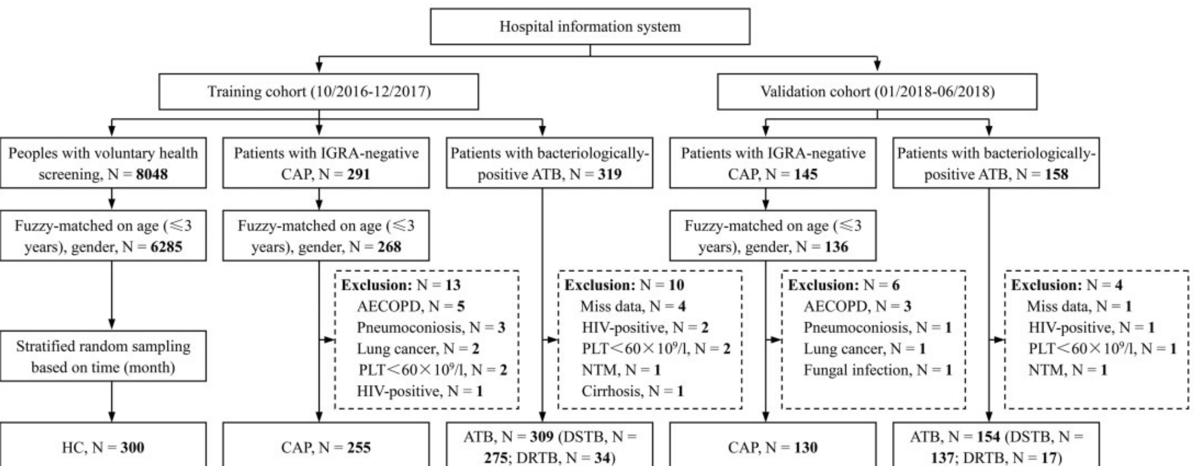

Figure 1. Description of the sample. Abbreviations: AECOPD: acute exacerbation of chronic obstructive pulmonary disease; ATB: active tuberculosis; CAP: community-acquired pneumonia; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; HC: healthy control; HIV: human immunodeficiency virus; IGRA: interferon gamma release assay; NTM: non-tuberculous mycobacteria; PLT: platelet.

observed to confirm the 3+ and 4+ grades. Three sputum specimens were examined, and the test with the highest load was recorded. Sputum culture grades were recorded as follows: +: the actual colony count was reported, as the bacterial colony growth was less than 1/4 of the slope surface area; 1+: bacterial colony growth comprised 1/4 of the slope surface area; 2+: bacterial colony growth comprised 1/2 of the slope surface area; 3+ bacterial colony growth comprised 3/4 of the slope surface area; 4+: bacterial colony growth comprised the entire slope surface area. The Roche fixed culture test was used as a standard, and no redundant tests were performed; the results of the sputum culture were regarded as more important than the results of the sputum smear, which were regarded as more important than DNA/RNA results—if a patient had been tested by more than one method, the method of highest importance was used to determine the bacteriological findings. A grade of 3+ or 4+ was considered high-grade sputum.

Measurement of complete blood counts and other items

The Sysmex XN-9000 automated blood fluid analyzer and corresponding reagents (Sysmex Corporation, Kobe, Japan) were used to determine complete blood count. Serum albumin (ALB) was measured using the Olympus AU2700 fully automated biochemical analyzer and ALB biochemical reagents from Beijing Leadman Biochemistry (Beijing, China). IGRAs were performed using an enzyme-linked immunospot assay (Oxford Immunotec, Abingdon, UK) to quantify the numbers of effector T cells against tuberculosis in the patients' blood specimens.

Measurement of tuberculosis

Sputum or body fluid specimens were first used for an Auramine O fluorescence stain test (KRJ/TTR500 automatic smear staining machine, Xiangyang Courager Medical Apparatus, Xiangyang, China) and acid-fast bacilli were observed under the microscope (SDPTOP EX30, Ningbo Sunny Instruments Co., Ltd., Ningbo, China). Next, an MTB nucleic acid amplification assay was performed (RNA constant temperature amplification, Roche LightCycler 480 II Real-time PCR Cycler; Rendu Biotechnology [Shanghai, China]; PCR-fluorescence method, Applied Biosystems 7500 Real-Time PCR System; DAAN Gene of Sun Yat-sen University [Guangzhou, China]), as well as a rapid drug-resistance gene assay (DNA microarray chip method; CapitalBio Corporation, Chengdu, China). Finally, an MTB culture test (Roche fixed culture test), bacterial species identification assay (para-nitrobenzoic acid inhibition test negative, 2-thiophene-carborylic acid hydrazide positive), and a drug sensitivity test (the ratio method) were performed. Culture media were purchased from BaSo Diagnostics Inc. (Zhuhai, China). The laboratory conformed to national P3 standards and underwent National Reference Laboratory quality control and management. All laboratory staff participated in specialized training with respect to detection methods and device operation.

Statistical analyses

IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Inter-group rate comparison was performed using the χ^2 test. The Kruskal–Wallis H test was used to assess inter-group differences in continuous variables, whereas the Mann–Whitney U test was used to assess

intra-group differences in these variables. Binary univariate and multiple logistic regression analyses (Forward: LR and Enter) were used to construct regression models. The Hosmer–Lemeshow Test (HL test) was used to test the goodness of fit of the model. CAP and drug-susceptible tuberculosis (DSTB) were used as reference standards for ATB and DRTB, respectively. ROC analysis was used to determine the optimal threshold value (corresponding values for the highest sums of sensitivity and specificity); the optimal combination of area under the curve (AUC), sensitivity, and specificity was reported. AUC comparison of the two related diagnostic tests was performed using ROCKIT 0.9β software (IBM-compatible version, IBM). Adobe Photoshop CS 6 software (version 13.0×32, Adobe Systems Incorporated, San Jose, CA, USA) was used to edit images. A difference of $p < 0.05$ was considered to be statistically significant in all comparisons.

Results

Participant characteristics

Table 1 shows the demographic and laboratory characteristics of the participants; notably, there were no statistically significant differences in sex or age distribution among all groups (ATB, CAP, and HC). The clinical characteristics of the ATB group are shown in Supplementary Table 1. There were no statistically significant differences between the training and validation cohorts.

Altered routine blood test results in patients with ATB

The data in Table 1 show that patients with ATB had altered results in routine blood tests, specifically with respect to PLT and ALB levels. Differences in PLT level and PDW between DSTB and DRTB subgroups were statistically significant in the training cohort. Although PDW showed a decreasing trend and PLT level showed an increasing trend in the DRTB subgroup in the validation cohort, these measurements were not statistically significant from those in the DSTB subgroup in that cohort (Table 1).

Plateletcrit-albumin scoring (PAS) model for differentiation between CAP and ATB

Using the Forward: LR method, PCT and ALB levels were introduced in the final PAS model for differentiation between CAP and ATB (Table 2). The HL test for the goodness of fit of the PAS model yielded $\chi^2 = 4.745$ and $p = 0.784$, which indicated that the fit of the model was very good; its predictive accuracy was 81.00%. The 95% reference range for HCs was -5.233 to -0.345. In univariate comparison with ALB and PCT levels, the AUC and Youden's index of the PAS were higher (Figure 2a, Table 3); moreover, the AUC differences between the PAS model and ALB or PCT alone were statistically significant (Table 4). These findings demonstrate that the PAS model can effectively differentiate between CAP and ATB.

PDW-treatment-sputum scoring (PTS) model for differentiation between DSTB and DRTB

There were slight differences in the method used for the construction of the PTS model, compared with the PAS model, and an optimal Nagelkerke R^2 value (0.106) was reached when the Enter method was used. The HL test for the goodness of fit of the PTS model yielded $\chi^2 = 12.938$ and $p = 0.114$, which indicated that the fit of the model was moderate; its predictive accuracy was 89.00% (Table 2). In univariate comparison with PDW, the AUC and

Table 1. Demographic and laboratory characteristics of the study participants

	Training cohort (N = 864)				Validation cohort (N = 284)		
			ATB (N = 309)			ATB (N = 154)	
Characteristic	HC (N = 300)[a]	CAP (N = 255)[b]	DSTB (N = 275)[c]	DRTB (N = 34)[d]	CAP (N = 130)	DSTB (N = 137)[c]	DRTB (N = 17)[d]
Age, years, M (IQR)	50.00 (40.00–58.75)	50.00 (39.00–62.00)	51.00 (37.00–63.00)	52.00 (42.75–65.00)	50.00 (34.00–59.00)	49.00 (31.50–61.50)	51.00 (43.00–65.00)
Male sex, no. (%)	226 (75.33)	204 (80.00)	220 (80.00)	27 (79.41)	98 (75.38)	102 (74.45)	14 (82.35)
Pre-treated cases, no. (%)	NA	NA	77 (28.00)	16 (47.06)*	NA	38 (27.74)	10 (58.82)**
High-grade sputum, no.(%)	NA	NA	72 (26.18)	17 (50.00)**	NA	39 (28.47)	8 (47.06)
WBC, $\times 10^9$/l, M (IQR)	6.34 (5.36–7.37)***	5.90 (4.74–7.61)	6.94 (5.42–8.60)***	7.30 (5.83–9.52)	6.05 (4.58–7.82)	6.19 (5.06–8.44)	7.55 (5.74–9.51)
RBC, $\times 10^{12}$/l, M (IQR)	4.80 (4.49–5.12)***	4.19 (3.74–4.62)***	4.03 (3.61–4.49)	4.15 (3.71–4.68)	4.27 (3.71–4.61)	4.29 (3.79–4.85)	4.19 (3.91–4.93)
Hb, g/dl, M (IQR)	14.40 (13.50–15.68)***	12.60 (11.30–14.10)***	11.50 (10.10–13.00)***	12.40 (10.18–13.45)	12.90 (11.00–13.70)	12.20 (10.65–13.90)	12.10 (11.75–13.20)
HCT, %, M (IQR)	45.40 (42.40–48.35)***	39.00 (34.90–43.10)***	36.30 (32.50–40.20)***	39.45 (32.70–42.75)	39.40 (34.13–42.13)	38.20 (33.90–43.05)	37.40 (34.95–40.90)
PLT, $\times 10^9$/l, M (IQR)	177.00 (147.00–212.00)***	183.00 (145.00–239.00)	263.00 (196.00–336.00)***	296.00 (258.75–357.75)*	185.00 (139.75–242.00)	257.00 (212.00–338.50)***	310.00 (202.50–380.50)
MPV, fl, M (IQR)	11.90 (10.80–13.08)***	11.70 (10.60–12.80)	10.90 (9.90–12.00)***	10.50 (9.43–11.23)	11.80 (10.50–12.70)	11.20 (10.10–12.00)**	10.50 (10.00–12.20)
PCT, %, M (IQR)	0.208 (0.176–0.239)***	0.220 (0.174–0.270)	0.280 (0.220–0.350)***	0.315 (0.260–0.373)	0.220 (0.170–0.269)	0.279 (0.242–0.340)***	0.309 (0.224–0.404)
PDW, fl, M (IQR)	16.50 (16.30–16.68)***	16.10 (13.70–17.00)***	13.50 (11.40–16.10)***	12.30 (10.70–13.95)*	16.00 (13.88–17.10)	14.70 (12.40–16.25)***	13.3 (11.20–15.15)
ALB, g/l, mean ± s	44.18 ± 4.06***	39.86 ± 4.88***	30.55 ± 6.07***	31.42 ± 5.88	39.13 ± 3.82	30.90 ± 6.07***	29.97 ± 6.57

Abbreviations: ALB: serum albumin; ATB: active tuberculosis; CAP: community-acquired pneumonia; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; Hb: hemoglobin; HC: healthy control; HCT: hematocrit; IQR: interquartile range; M: median; MPV: mean platelet volume; NA: not applicable; PCT: plateletcrit; PDW: platelet distribution width; PLT: platelet; RBC: red blood cell; WBC: white blood cell.

[a b c d] These letters represent the results of comparisons between HC and ATB, CAP and HC, ATB and CAP, DSTB and DRTB, respectively.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 2. Univariate and multivariate analysis of the groups and derivation of routine blood test-associated predictive models

	CAP vs. ATB		DSTB vs. DRTB	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Univariate analysis				
Pre-treated cases	NA	NA	2.286 (1.109, 4.710)	0.025
High-grade sputum	NA	NA	2.819 (1.367, 5.816)	0.005
WBC	1.051 (0.996, 1.108)	0.068	1.018 (0.910, 1.140)	0.751
RBC	0.745 (0.573, 0.970)	0.029[a]	1.249 (0.724, 2.157)	0.424
Hb	0.970 (0.961, 0.979)	< 0.001[a]	1.006 (0.988, 1.025)	0.499
HCT	0.928 (0.900, 0.957)	< 0.001[a]	1.045 (0.982, 1.111)	0.168
PLT	1.011 (1.009, 1.014)	< 0.001[a]	1.003 (1.000, 1.006)	0.056
MPV	0.741 (0.664, 0.828)	< 0.001[a]	0.805 (0.634, 1.023)	0.077
PCT × 10	2.956 (2.314, 3.777)	< 0.001	1.376 (0.967, 1.959)	0.077
PDW	0.817 (0.771, 0.866)	< 0.001[a]	0.867 (0.762, 0.987)	0.031
ALB	0.738 (0.702, 0.775)	< 0.001	1.024 (0.965, 1.086)	0.431

Multivariate analysis	Odds ratio (95% CI)	*P* value	β-coefficient		Odds ratio (95% CI)	*P* value	β-coefficient
Intercept	3357.313	< 0.001	8.119	Intercept	0.554	0.514	–0.590
PCT × 10	2.616 (1.917, 3.570)	< 0.001	9.617	PDW	0.853 (0.746, 0.975)	0.020	–0.159
ALB	0.746 (0.708, 0.785)	< 0.001	–0.293	Pre-treated cases	1.980 (0.929, 4.218)	0.077	0.683
				High-grade sputum	2.654 (1.248, 5.644)	0.011	0.976
Routine blood test-associated models	PAS = 8.119 + 9.617 × PCT – 0.293 × ALB			PTS = –0.590 + 0.683 × (1 if pre-treated cases; 0 if new cases) + 0.976 × (1 if high-grade sputum; 0 if low-grade sputum) – 0.159 × PDW			

Abbreviations: ALB: serum albumin; ATB: active tuberculosis; CAP: community-acquired pneumonia; CI: confidence interval; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; Hb: hemoglobin; HCT: hematocrit; MPV: mean platelet volume; NA: not applicable; PAS: plateletcrit-albumin scoring; PCT: plateletcrit; PDW: platelet distribution width; PLT: platelet; PTS: PDW-treatment-sputum scoring; RBC: red blood cell; WBC: white blood cell.

[a]Not included in final model owing to lack of significance in multivariate regression.

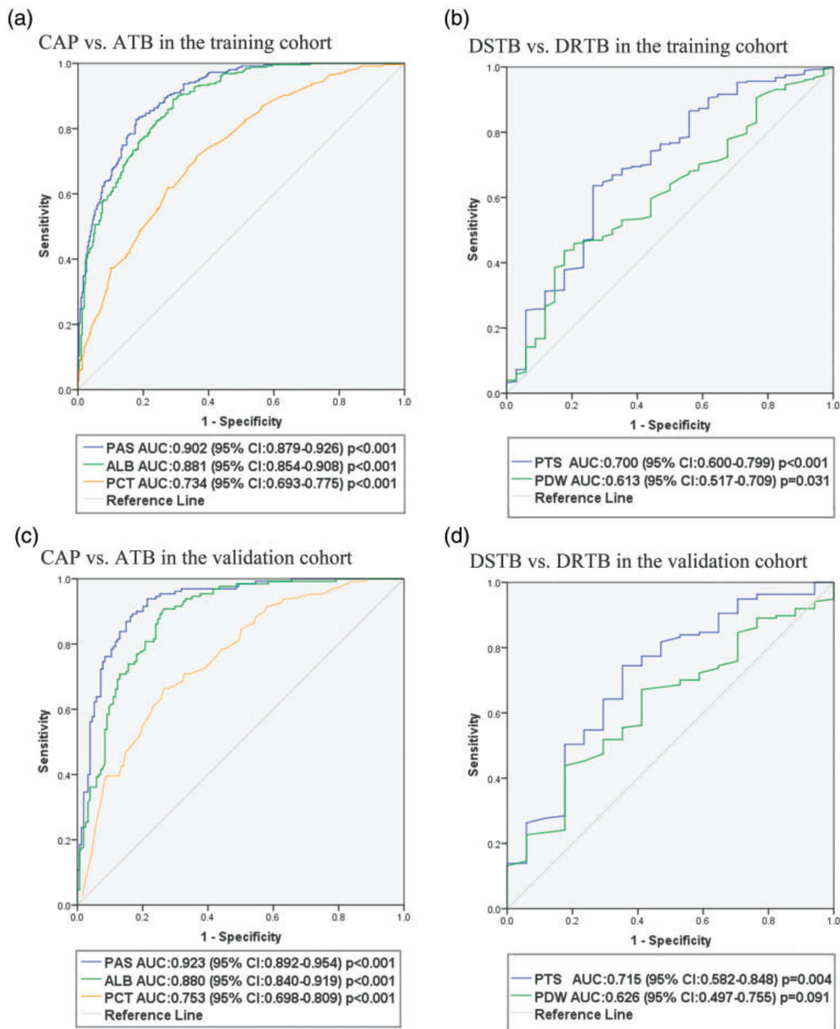

Figure 2. Receiver operating characteristic curve analyses of PAS and PTS models for distinguishing different diseases in different cohorts. A complement value of 1 was used for PAS and PTS, PCT underwent square root transformation, and PDW underwent common logarithmic transformation. Abbreviations: ATB: active tuberculosis; ALB: serum albumin; AUC: area under curve; CAP: community-acquired pneumonia; CI: confidence interval; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; PAS: plateletcrit-albumin scoring; PCT: plateletcrit; PDW: platelet distribution width; PTS: PDW-treatment-sputum scoring.

Youden's index of the PTS were higher (Figure 2b, Table 3); moreover, the AUC differences between the PTS model and PDW alone were statistically significant (Table 4). These findings demonstrate that the PTS model might effectively differentiate between DSTB and DRTB.

Validation of models for diagnosis of ATB and DRTB

In the validation cohort, the AUC, sensitivity, and specificity of the PAS model were 0.923, 93.85%, and 78.57%, respectively (Figure 2c, Table 3). The AUC, sensitivity,

Table 3. Performance of parameters for discriminating between CAP and ATB or between DSTB and DRTB

	Youden index (%)	Cut-off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Training cohort						
CAP vs. ATB						
PAS	64.95	0.199	82.75	82.20	79.32	85.23
ALB	59.89	33.82	89.02	70.87	71.61	88.66
PCT	35.12	0.259	71.37	63.75	61.90	72.96
DSTB vs. DRTB						
PTS	37.17	-2.202	63.64	73.53	95.11	20.00
PDW	25.99	14.45	43.64	82.35	95.24	15.30
Validation cohort						
CAP vs. ATB						
PAS	72.42	0.512	93.85	78.57	78.71	93.80
ALB	64.15	34.73	90.77	73.38	74.21	90.40
PCT	40.18	0.244	66.15	74.03	68.25	72.15
DSTB vs. DRTB						
PTS	39.16	–0.815	74.45	64.71	94.44	23.91
PDW	26.15	15.24	43.80	82.35	95.24	15.38

Abbreviations: ATB: active tuberculosis; ALB: serum albumin; CAP: community-acquired pneumonia; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; PAS: plateletcrit-albumin scoring; PCT: plateletcrit; PDW: platelet distribution width; PTS: PDW-treatment-sputum scoring.

Table 4. Results of comparison with the area under curve for models

	Z value	95% CI for difference	*P* value	Correlation coefficient
Training cohort				
CAP vs. ATB				
PAS vs. ALB	3.1695	0.0084, 0.0354	0.0015	0.8667
PAS vs. PCT	8.7185	0.1304, 0.2060	<0.0001	0.4075
ALB vs. PCT	6.0540	0.0994, 0.1945	<0.0001	0.0554
DSTB vs. DRTB				
PTS vs. PDW	−3.3606	−0.4940, −0.1300	0.0008	−0.6079
Validation cohort				
CAP vs. ATB				
PAS vs. ALB	4.0922	0.0235, 0.0668	<0.0001	0.8393
PAS vs. PCT	6.2232	0.1164, 0.2234	<0.0001	0.3413
ALB vs. PCT	3.6360	0.0589, 0.1968	0.0003	−0.0263
DSTB vs. DRTB				
PTS vs. PDW	−2.4761	−0.5661, −0.0659	0.0133	−0.6118

Abbreviations: ATB: active tuberculosis; ALB: serum albumin; CAP: community-acquired pneumonia; CI: confidence interval; DRTB: drug-resistant tuberculosis; DSTB: drug-susceptible tuberculosis; PAS: plateletcrit-albumin scoring; PCT: plateletcrit; PDW: platelet distribution width; PTS: PDW-treatment-sputum scoring.

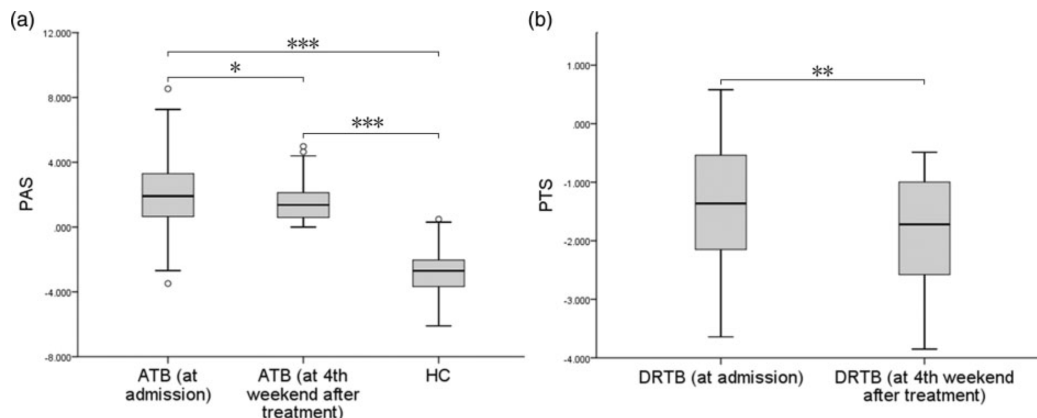

Figure 3. Changes in model values in the ATB group over time. (a) Box plots showing values of the PAS model in ATB and HC groups at different time points. (b) Box plots showing values of the PTS model in the DRTB subgroup at different time points. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Abbreviations: ATB: active tuberculosis; DRTB: drug-resistant tuberculosis; HC: healthy control; PAS: plateletcrit-albumin scoring; PTS: PDW-treatment-sputum scoring.

and specificity of the PTS model were 0.715, 74.45%, and 64.71%, respectively (Figure 2d, Table 3). These findings showed that the performances of the PAS and PTS models in the validation cohort were similar to those in the training cohort.

Application of models for evaluation of therapeutic efficacy

Quantitative changes in the PAS and PTS may reflect the efficacy of anti-tuberculosis treatment. After 4 weeks of treatment, 309 patients fulfilled the evaluation criteria (1. Acceptable routine blood and ALB examination, and 2. Acceptable acid staining smear findings, as described in the Case definitions section of this paper), of which 34 were from the DRTB subgroup (Supplementary Table 2). Our results showed that the PAS and PTS values were both reduced after 4 weeks of anti-tuberculosis treatment (Figure 3a–b). However, the PAS values remained higher than those in the HC group. These findings indicate that the models may be clinically useful for monitoring the therapeutic efficacy of anti-tuberculosis treatment.

Discussion

Previous studies have assessed the value of hematological parameters for diagnosis of ATB.[5–9] The simplicity and low cost of ALB tests can be used as a biomarker to assess disease severity, drug effects, and prognosis estimation.[12,13] The results of the prior studies supported the feasibility of using the PAS and PTS models for tuberculosis diagnosis, drug resistance prediction, and efficacy assessment.

In the present study, PCT level was the first independent variable to be included in the PAS model; thus, this variable carried greater weight in the model. In addition, white blood cell and RBC counts contributed minimally to diagnosis of ATB. The PCT reflects changes in PLT level and MPV; thus, it is typically closely associated with changes in the PLT. Possible mechanisms associated with an elevated PLT level in a patient with ATB are: (1) stimulation of inflammatory cells to synthesize

interleukin-6 and granulocyte-macrophage colony-stimulating factor after macrophages have ingested MTB, thereby promoting the proliferation, differentiation, and maturation of megakaryocytes and increasing PLT synthesis;[14,15] (2) increased PLT reactivity in the acute inflammatory phase; and (3) the gradual increase in numbers of microcytes at early stages is regarded by the machine as an increase in PLT number, thus resulting in a high PLT level.[16] Our results showed that the PLT level was significantly increased in patients with ATB, and that changes in PLT level were not rapid. Notably, MPV may be reduced by feedback regulatory mechanisms in megakaryocytes, which are not affected by PLT level in patients with ATB; in addition, the number of megakaryocytes may increase when PLT level increases. Therefore, mean megakaryocyte polyploidy and MPV would decrease. Further, the depletion of dense granules and reduction in intracellular PLT-active substances would reduce PLT function. These observed changes conflict with the results reported by Lee et al.[8] and Sahin et al.,[9] but are similar to the results reported by Huang et al.[6] In addition, these observed changes support the inverse relationship between MPV and PLT level that was proposed by O'Malley et al.[17] Reduction of MPV in patients with ATB may occur for two additional major reasons. First, although different diseases are characterized by cavitary lesions, patients with ATB are more prone to hemoptysis. This is consistent with the findings of the study by Karabulut et al.,[18] which demonstrated that patients with low MPV have an increased tendency to experience recurrent bleeding. Second, owing to feedback dysfunction, patients with primary thrombocytosis and aplastic anemia are more prone to thrombus formation and other poor outcomes than patients with ATB.

The other independent variable in the PAS model was ALB. We found that its diagnostic contribution was better than that of other routine blood parameters, with the exception of PCT level. Possible mechanisms associated with reduction in ALB level in a patient with ATB are: (1) reduced nutrient uptake due to gastrointestinal dysfunction and reduced appetite, which results in reduced anabolism—Bisaso et al.[19] reported that patients with comorbid tuberculosis had a 44.2% reduction in baseline ALB secretion rate; (2) MTB uses host proteins for its own metabolism, which increases catabolism.[20,21] Because the half-life of ALB is 15–20 days,[22,23] it may be more valuable in the identification of CAP. Our results showed a significant decline in ALB level in patients with ATB; this may be an important factor in the development of tuberculosis.

High bacterial loads, previous cases treated, and PDW were variables included in the final PTS model. High pre-treatment bacterial loads showed that the number of proliferating bacteria was high. MTB antigens continuously stimulated proliferation of $CD8^{+}$ T lymphocytes[24,25] and production of interferon gamma, causing hematopoietic stem cell dysfunction, reduction in myeloid progenitor cells, and significant stress on the hematopoietic system.[26,27] In the present study, the proportions of patients in DSTB and DRTB subgroups with high bacterial loads were significantly different; this is consistent with the findings of the study by Atif et al.,[28] in which a high bacterial load was a risk factor for poor outcomes in tuberculosis infection.

The WHO reported that, worldwide, an average of 19% of multidrug-resistant tuberculosis (MDR-TB) or rifampicin-resistant tuberculosis (RR-TB) patients had been previously treated for MTB infection; in contrast, only 4.1% of newly diagnosed patients overall had previously been treated for MTB infection.[4] This suggests that

previous treatment is an important factor in eliciting drug resistance. In the present study, the proportion of MDR/RR-TB patients who had been previously treated was 61% (19 of 31), which was similar to the results recently reported by Huang et al.[29]

PDW is a parameter that reflects variation in PLT volume. There are two possible reasons for reduction in PDW. First, MTB infection and associated toxins directly inhibit bone marrow hematopoiesis, thereby reducing megakaryocyte counts. This affects the synthesis and release of large PLTs, which may be more apparent in patients with DRTB. Second, MTB infection may be associated with other chronic infections.[30,31] In the present study, we observed changes in the reduction of PDW between the DSTB and DRTB subgroups in the training cohort, which supports the possibility of a positive correlation with the MPV.[32] Additionally, we observed an increase in PLT level, suggesting that increases in PLT level may be partly associated with the development of drug resistance in MTB. This increase in PLT level is consistent with the findings of a recent study by Fox et al.,[33] which showed upregulation of PLT-associated mediators and reduced intracellular killing of MTB by monocytes. In our validation cohort, we did not observe any meaningful changes in PDW or PLT level. This may be owing to small sample size, poor test performance, and false negative results. Screening of the variables showed that the differences in PLT level were marginal and had minimal effects on the results when included in the Enter model. Therefore, PLT level was not included in the PTS model. This limitation was addressed by combining with other variables in the final PTS model.

In the present study, we observed that PAS and PTS values significantly declined after 4 weeks of anti-tuberculosis treatment. This suggests that the model has value in monitoring of therapeutic progress in patients with tuberculosis. However, both PAS and PTS values in patients with tuberculosis remained higher than values in HCs, possibly due to the following factors: (1) the improvement of parameters measured in routine blood tests is a relatively slow process, as reported by Weber et al.;[7] and (2) the negative effects of anti-tuberculosis drugs on bone marrow hematopoiesis during the adaptation period may slow improvement of these parameters.

Our study had several limitations. First, it was an observational study, and the effects of non-randomization and confounders could not be excluded. Although we minimized a subset of potential confounding factors during sampling (e.g., age and sex), other comorbid factors (i.e., megaloblastic anemia) may cause residual confounding. This may reduce the usefulness of the study results and underestimate the benefits of the PTS model. Second, clinical symptoms and IGRAs, which show great variability, as well as other expensive and less common factors, were not included in this study. Third, the PTS model explained a relatively low (10.6%) proportion of the difference between DSTB and DRTB, and further clarification of the unknown variables is needed. Fourth, PTS had a low diagnostic value, but a high positive predictive value. In the future, the sample size should be increased to confirm the findings of the present study. Finally, owing to the unbalanced economic development in northwestern China, there is a high rate of delayed treatment and a high rate of severe disease in patients with ATB. Therefore, findings on the basis of samples collected from this region cannot be generalized to patients in different environments.

Despite these limitations, this study had several key strengths. To the best of our knowledge, this is the first report of models that were established using a combination of routine blood tests and relevant parameters for diagnosis and monitoring of

tuberculosis. This study showed the potential for PTS to predict DRTB without the use of genetic or drug sensitivity tests. Thus, we suspect that both models will be useful for the assessment of anti-tuberculosis treatment results, and may be appropriate for application within primary tuberculosis control institutions. Finally, our findings may provide guidance for general clinical practice.

In conclusion, the PAS model had high sensitivity and specificity for the diagnosis of ATB, while the PTS model could be used for DRTB diagnosis. The two models are superior to other models using routine blood parameters, and require less expensive tests that are more widely available than the specialized diagnostic approaches currently used for detection and monitoring of tuberculosis infections. Thus, these tests may be useful for broader clinical applications in diagnosis of ATB and screening of DRTB.

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Supplementary material

Supplemental material is available for this article online.

References

1. Small PM and Pai M. Tuberculosis diagnosis-time for a game change. *N Engl J Med* 2010; 363: 1070–1071.
2. Pai M, Kalantri S and Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part II. Active tuberculosis and drug resistance. *Expert Rev Mol Diagn* 2006; 6: 423–432.
3. Roetzer A, Diel R, Kohl TA, et al. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. *PLoS Med* 2013; 10: e1001387.
4. WHO Global tuberculosis report 2016. Geneva: World Health Organization (2016, http://www.who.int/iris/handle/10665/250441). Accessed 19 August 2018.
5. Rohini K, Surekha Bhat M, Srikumar PS, et al. Assessment of hematological parameters in pulmonary tuberculosis patients. *Indian J Clin Biochem* 2015; 31: 332–335.
6. Huang S, Yi FM, Zhou R, et al. The utility of platelet, mean platelet volume, and red cell distribution width in the diagnosis of active Crohn's disease and intestinal tuberculosis. *Saudi Med J* 2013; 34: 1161–1166.
7. Weber SF, Bélard S, Rai S, et al. Immune thrombocytopenia secondary to tuberculosis: a case and review of literature. *Int J Tuberc Lung Dis* 2017; 21: 466–470.
8. Lee MY, Kim YJ, Lee HJ, et al. Mean platelet volume in mycobacterium tuberculosis infection. *Biomed Res Int* 2016; 2016: 7508763.
9. Sahin F, Yazar E and Yıldız P. Prominent features of platelet count, plateletcrit, mean platelet volume and platelet distribution width in pulmonary tuberculosis. *Multidiscip Respir Med* 2012; 7: 38.
10. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community–zacquired pneumonia in adults. *Clin Infect Dis* 2007; 44: S27–S72.
11. The State Bureau of Quality and Technical Supervision, Ministry of Health of the People's Republic of China. Diagnostic criteria and principles of management of infectious

pulmonary tuberculosis (GB15987-1995). http://www.nhfpc.gov.cn/zhuz/s9491/201212/34117.shtml. Accessed 6 September 2018.

12. Alvarez-Uria G, Midde M, Pakam R, et al. Diagnostic and prognostic value of serum albumin for tuberculosis in HIV infected patients eligible for antiretroviral therapy: data from an HIV cohort study in India. *Bioimpacts* 2013; 3: 123–128.
13. Sudfeld CR, Isanaka S, Aboud S, et al. Association of serum albumin concentration with mortality, morbidity, CD4 T-cell reconstitution among Tanzanians initiating antiretroviral therapy. *J Infect Dis* 2013; 207: 1370–1378.
14. Ogawa T, Uchida H, Kusumoto Y, et al. Increase in tumor necrosis factor alpha- and interleukin-6-secreting cells in peripheral blood mononuclear cells from subjects infected with Mycobacterium tuberculosis. *Infect Immun* 1991; 59: 3021–3025.
15. Unsal E, Aksaray S, Köksal D, et al. Potential role of interleukin 6 in reactive thrombocytosis and acute phase response in pulmonary tuberculosis. *Postgrad Med J* 2005; 81: 604–607.
16. Adeiga AA, Akinosho RO and Onyewuche J. Evaluation of immune response in infants with different nutritional status: vaccinated against tuberculosis, measles and poliomyelitis. *J Trop Pediatr* 1994; 40: 345–350.
17. O'Malley T, Langhome P, Elton RA, et al. Platelet size in stroke patients. *Stroke* 1995; 26: 995–999.
18. Ekber Karabulut A, Çevik Y, Emektar E, et al. Analysis of mean platelet volume and red blood cell distribution width in recurrent epistaxis. *Turk J Emerg Med* 2018; 18: 67–70.
19. Bisaso KR, Owen JS, Ojara FW, et al. Characterizing plasma albumin concentration changes in TB/HIV patients on anti retroviral and anti -tuberculosis therapy. *In Silico Pharmacol* 2014; 2: 3.
20. Bircher J, Benhamou JP and Mcintyre N. *Oxford textbook of clinical hepatology*. New York: Oxford University Press, 1991.
21. Kaysen GA, Dubin JA, Muller HG, et al. Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients. *Kidney Int* 2002; 61: 2240–2249.
22. Nicholson JP, Wolmarans MR and Park GR. The role of albumin in critical illness. *Br J Anaesth* 2000; 85: 599–610.
23. Beeken WL, Volwiler W, Goldsworthy PD, et al. Studies of I-131-albumin catabolism and distribution in normal young male adults. *J Clin Invest* 1962; 41: 1312–1333.
24. Levine B, Mizushima N and Virgin HW. Autophagy in immunity and inflammation. *Nature* 2011; 469: 323–335.
25. Prezzemolo T, Guggino G, La Manna MP, et al. Functional signatures of human CD4 and CD8 T cell responses to mycobacterium tuberculosis. *Front Immunol* 2014; 5: 180.
26. Gravano DM, Al-Kuhlani M, Davini D, et al. CD8+ T cells drive autoimmune hematopoietic stem cell dysfunction and bone marrow failure. *J Autoimmun* 2016; 75: 58–67.
27. McCabe A and MacNamara KC. Macrophages: key regulators of steady-state and demand-adapted hematopoiesis. *Exp Hematol* 2016; 44: 213–222.
28. Atif M, Sulaiman SA, Shafie AA, et al. Treatment outcome of new smear positive pulmonary tuberculosis patients in Penang, Malaysia. *BMC Infect Dis* 2014; 14: 399.
29. Huang H, Ding N, Yang T, et al. Cross-sectional whole-genome sequencing and epidemiological study of multidrug-resistant Mycobacterium tuberculosis in China. *Clin Infect Dis* Epub ahead of print 15 October 2018. DOI: 10.1093/cid/ciy883.
30. Ekici-Günay N, Çakır I and Çelik T. Is there clinical value in counting nucleated red blood cells and platelet indices in primary immunodeficiency disease? *Turk J Pediatr* 2017; 59: 657–663.
31. Zhao D, Qiu G, Luo Z, et al. Platelet parameters and (1, 3)-β-D-glucan as a diagnostic and prognostic marker of invasive fungal disease in preterm infants. *PLoS One* 2015; 10: e0123907.
32. Bessman JD, Williams LJ and Gilmer PR Jr. Platelet size in health and hematologic disease. *Am J Clin Pathol* 1982; 78: 150–153.
33. Fox KA, Kirwan DE, Whittington AM, et al. Platelets regulate pulmonary inflammation and tissue destruction in tuberculosis. *Am J Respir Crit Care Med* 2018; 198: 245–255.