Establishment and Validation of a Predictive Model for Non-tuberculous Mycobacterial Infections in Acid-fast Bacilli Smear-positive Patients

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Research

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Abstract

Background: To identify non-tuberculous mycobacteria (NTM) infections in initially acid-fast bacilli (AFB) smear-positive patients more quickly and effectively, we attempted to establish a predictive model and validate it in clinical practice.

Methods: A total of 125 AFB smear-positive patients with mycobacteriosis diagnosed in the Respiratory Department of Shanghai Pulmonary Hospital were retrospectively analyzed，including 64 cases of NTM and 61 cases of pulmonary tuberculosis (PTB), to identify the clinical features distinguishing NTM from PTB patients. A bivariate regression was then set up to determine the independent predictive risk factors to NTM infections. A receiver operating characteristic (ROC) curve was used to determine the model's predictive discrimination. Finally, the model was verified both internally and externally for its predictive ability.

Results: Compared with PTB, female patient, the symptom of hemoptysis, the exists of bronchiectasis, and negative test for QuantiFERON tuberculosis (QFT) were more common in NTM patients (78.1% vs. 34.4%, 37.5% vs. 11.5%, 76.6% vs. 19.7%, 82.8% vs. 8.2%, respectively, $P \leq 0.001$). The lesions on chest high-resolution computed tomography (HRCT) in NTM patients were more commonly involved in the right middle lobe (87.5% vs. 52.5%, $P<0.001$) and left lingular lobe (71.9% vs. 50.8%, $P=0.016$), and cystic change was more commonly morphological alteration (32.8% vs. 8.2%, $P=0.001$). Binary regression analysis showed that female patient, the exists of bronchiectasis, negative test for QFT and right middle lobe lesion were independent risk factors for NTM in AFB smear-positive patients ($P<0.05$). A ROC curve combining the four risk factors showed a sensitivity and specificity of 85.9% and 93.4%, respectively, and the area under the curve (AUC) was 0.963. Moreover, internal validation and external validation of the later clinical data both confirmed the effectiveness of the model.

Conclusions: The predictive model would be useful for early differential diagnosis of NTM in initially AFB smear-positive patients.

1. Background

Non-tuberculous mycobacteria (NTM) is widely distributed in nature and has been isolated from natural water, tap water and soil. Although known as an opportunistic pathogen, population-based data have shown a worldwide increase in the prevalence of human NTM infections due to a rise in the number of susceptible/immunocompromised individuals, such as acquired immune deficiency syndrome (AIDS) patients, together with the adoption of novel detection technologies [1-3]. Pulmonary infection is by far the most frequent disease caused by NTM. Actually, NTM-related lung diseases (NTM-LD) are not mandatory to be treated, while NTM-LD patients are always mis-diagnosed as other chronic lung diseases, such as tuberculosis or lung cancer [4,5]. As the symptoms are not typical, there are many difficulties in diagnosis, leading to inappropriate treatment [6]. In the clinical work, in countries with a high incidence rate of tuberculosis, such as China, once a patient shows positive results in acid-fast bacillus

(AFB) smear, he/she is likely to be diagnosed with pulmonary tuberculosis (PTB) and undergo anti-tuberculosis treatment. When species identification tests are unavailable or not conducted, patients are not suspected to have NTM infections until they are not susceptible to anti-tuberculosis drugs, often resulting in undesirable side effects and heavy financial burdens [7,8]. Therefore, early differential diagnosis of NTM is particularly important in the clinical practice, which may have a significant impact on the prognosis of the disease.

Although there have been many studies on the clinical characteristics of NTM, but few predictive models have been established, and most of them have to rely on the conventional culture results for diagnosis, the time required is about 4-8 weeks. In the present study, we retrospectively analyzed the clinical characteristics of AFB smear-positive patients with mycobacteriosis admitted to the Respiratory Department of Shanghai Pulmonary Hospital, in order to recognize the clinical characteristics of NTM-LD and establish a fast and efficient model, which can be easily and quickly applied to clinical work. The predictive model would allow us to know the diagnosis 4-8 weeks earlier than the conventional culture results, which can improve the efficiency of differential diagnosis.

2. Patients And Methods

2.1. Patient screening

A total of 443 specimens of positive mycobacterial culture from consecutive patients admitted to Respiratory Department of Shanghai Pulmonary Hospital between January 2019 to February 2020 were screened in this study. Moreover, 362 cases were obtained when 81 specimens were excluded, which were repetitive or different specimens of the same patient. In addition, 42 cases who did not meet the diagnostic criteria of mycobacterial infections according to American Thoracic Society (ATS) [9,10], and 195 AFB smear-negative cases were excluded. Therefore, 64 cases of NTM and 61 cases of PTB were included in this study. The screening process was shown in Figure 1. All aspects of the study were performed in accordance with relevant guidelines and regulations.

2.2. Diagnostic criteria

Samples of sputum, bronchoalveolar lavage fluid (BALF), lung tissue and pleural effusion cultured positive to NTM or complex group of mycobacterium tuberculosis were considered as the gold standard, as well referred to the ATS guidelines of NTM [9] and PTB [10] for diagnosis.

2.3. Variables

The variables collected in this study included general data [age, gender, body mass index (BMI) and smoking history], history of respiratory and systemic diseases, primary symptoms, arterial blood gas conducted in room air when admission, and serological indicators [QFT, T-lymphocyte subsets, C-reactive

protein (CRP), erythrocyte sedimentation rate (ESR), immunoglobulin (Ig), serological test of rheumatism, and tuberculosis antibody] were obtained through reviewing the medical records.

Microbiological test results were accessed by detecting samples of sputum, electronic bronchoscope brush and lavage, lung puncture fluid, and pleural effusion. AFB smear-positive means smear positive of any one of the above-mentioned samples, and the results of mycobacterial culture were also obtained from any one of the above-mentioned samples.

2.4. Chest high-resolution computed tomography (HRCT) scans

All chest HRCT scans were performed at Shanghai Pulmonary Hospital within 1 month of admission using Philips brilliance CT instruments or Siemens Definition AS CT instruments. Scan parameters used were set as follows: 120 kV, 200-240 mA, slice thickness 2 mm, high resolution reconstruction algorithm, and thickness 1 mm. Full-lung CT scan was performed in the supine position with breath-holding in maximum inspiration from the apex to the upper abdomen. Lung window (window level 700 HU, window width 1,200) and mediastinal window (window level 50 HU, window width 450) were adopted to observe CT images. The predominant location, the number of affected lobes, and imaging features, such as nodular, patchy, stripe shadow, consolidation, pleural thickening, cystic change, mediastinal lymphadenopathy, pleural effusion, cavity, atelectasis, calcified shadow, and satellite nodules, were assessed by two experienced radiologists, and the conclusions were jointly drawn. When the conclusions were inconsistent, the images were reviewed again until a consistent result was obtained.

2.5 Statistical analysis

SPSS (version 26, IBM) and GraphPad Prism (version 8) were used for statistical analysis and graph drawing, respectively. Quantitative data were presented as mean ± standard deviations (SD). The independent sample Student’s t-test was used for comparisons between NTM and PTB groups. The chi-square test was used for constituent ratio comparisons. If the theoretical frequency was greater than or equal to one and less than five, the continuous correction chi-square was employed. If the theoretical frequency was less than 1, Fisher's exact test was employed. A bivariate regression was set up to determine the independent predictive risk factors to NTM in AFB smear-positive patients. A receiver operating characteristic (ROC) curve was used to determine the model’s predictive discrimination. Meanwhile, a nomogram was made with R (version 3.6.3) to visually show the predictive model. Moreover, the model was internally verified through calibration curve, and was externally verified with another ROC curve. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. General characteristics

A total of 125 AFB smear-positive patients with confirmed mycobacterial culture results were included in this retrospective study, of which 64 were NTM-LD and 61 were PTB. Table 1 shows the general characteristics of the two groups. There were no statistically significant differences in age and BMI between the two groups. The proportion of females in the NTM group was 78.1%, which was significantly higher compared with the PTB group (34.4%), $P<0.001$. Negative test for QFT was found in 82.8% of NTM patients, which was much higher compared with the PTB group (8.2%, $P<0.001$). In addition, the proportion of previous smoking was lower in the NTM group (7.8% vs. 37.7%, $P<0.001$). In this study, we found that the proportion of patients with bronchiectasis was significantly higher in NTM patients compared with the PTB patients (76.6% vs. 19.7%, $P<0.001$). Chronic obstructive pulmonary disease (COPD) and diabetes were more common in PTB patients ($P=0.003$ and $P=0.002$, respectively). The arterial blood gas results showed no obvious hypoxia in the enrolled patients, and no differences were found between the two groups.

Table 1
Clinical characteristics

	NTM (n=64)	PTB (n=61)	*P*-value
Age (yr, ±SD)	58.4±11.7	56.3±17.0	0.417
Gender			
Male	14 (21.9)	40 (65.6)	**<0.001**
Female	50 (78.1)	21 (34.4)	**<0.001**
BMI ($kg \cdot m^{-2}$)	20.38±2.87	20.70±2.76	0.551
Smoking history	5 (7.8)	23 (37.7)	**<0.001**
QFT positive	11 (17.2)	56 (91.8)	**<0.001**
QFT negative	53 (82.8)	5 (8.2)	**<0.001**
Pre-existing pulmonary diseases			
Bronchiectasis	49 (76.6)	12 (19.7)	**<0.001**
COPD	2 (3.1)	12 (19.7)	**0.003**
Interstitial Lung disease	3 (4.7)	4 (6.6)	0.649
Healed tuberculosis	5 (7.8)	2 (3.3)	0.476
Asthma	3 (4.7)	1 (1.6)	0.646
Lung cancer	2 (3.1)	1 (1.6)	1.000
DPB	2 (3.1)	0 (0)	/
Systemic diseases			
Cardiac disease	12 (18.8)	14 (23.0)	0.563
Diabetes	3 (4.7)	15 (2.5)	**0.002**
Upper airway disorder	7 (10.9)	2 (3.3)	0.190
Digestive system disease	5 (7.8)	6 (9.8)	0.690
Malignancy (including postoperative)	2 (3.1)	1 (1.6)	1.000
Connective Tissue Disease	2 (3.1)	2 (3.3)	1.000
PO_2 (mmHg)	87.8±12.5	83.6±16.2	0.113
PCO_2 (mmHg)	38.4±3.5	37.9±3.7	0.443

SaO_2 (%)	96.9±1.3	96.3±2.3	0.081

Abbreviation: QFT, quantiFERON tuberculosis；BMI, body mass index；COPD, chronic obstructive pulmonary disease; DPB, diffuse panbronchiolitis; PaO_2, oxygen partial pressure; $PaCO_2$, carbon dioxide partial pressure; SaO_2, oxygen saturation. NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

Data are presented as mean ± SD or number of patients with percentage of total in parentheses, and the data presented in bold type are statistically significant.

3.2. Clinical manifestations

Figure 2 illustrates the primary clinical symptoms of the enrolled patients. The symptoms of cough and sputum, as the most common symptoms in the two groups, had no significant difference. In addition, the symptoms of dyspnea and fever were also relatively common in the two groups, while the symptom of hemoptysis in NTM patients was significantly more common (37.5% vs. 11.5%, P=0.001), and a higher proportion of PTB patients showed symptoms of chest tightness (P=0.019). Besides, the symptoms of night sweats and chest pain were relatively rare in both groups, showing no significant difference.

3.3. Serological indicators

Table 2 shows the results of serological indicator tests. There was no significant difference between the two groups in terms of T lymphocyte and serum immunoglobulin levels, including IgG, IgA, IgM and total IgE. As systemic inflammatory indicators, CRP and ESR were both significantly lower in the NTM group compared with the PTB group (P<0.001). Meanwhile, patients in the NTM group showed a higher positive ratio of serum tuberculosis antibody and rheumatological indicators (23.8% vs.15.8%, and 26.2% vs. 13.8%, respectively), while there was no statistical difference between the two groups.

Table 2
Serological indicators

	NTM (n=64)	PTB (n=61)	*P*-value
CD4 (%)	32.1±10.2	31.5±12.9	0.824
CD8 (%)	21.0±8.1	19.6±8.0	0.439
CD4/CD8	1.75±0.84	1.87±1.05	0.563
IgG (g/L)	13.15±5.25	13.20±3.81	0.951
IgA (g/L)	2.71±1.36	3.06±1.79	0.222
IgM (g/L)	1.07±0.48	1.01±0.49	0.523
Total IgE			
>200	12.5%	14.7%	0.713
100-200	15.6%	6.6%	0.108
<100	71.9%	78.7%	0.378
CPR (IU/mL)	5.81±8.38	27.88±34.66	**<0.001**
ESR (mm/h)	36.02±26.86	65.60±35.70	**<0.001**
Tuberculosis antibody positive	23.8%	15.8%	0.273
Serological test of rheumatism [a]	26.2%	13.8%	0.091

Abbreviation: Ig, immunoglobulin; CPR, C-reactive protein; ESR, erythrocyte sedimentation rate; NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

Data are presented as mean ± SD or percentage, and the data presented in bold type are statistically significant ($P<0.05$).

[a] Serological test of rheumatism includes anti-dsDNA antibody, anti-Sm antibody, anti-snRNP antibody, anti-Ro antibodies (SSA), anti-La antibodies (SSB), anti-Jo-1 antibody, anti-Scl-70 antibody, anti-nucleosome antibody, anti-CENP-B antibody, anti-ribosomal P-protein antibody, anti-histone antibody and rheumatoid factor. Positive for one or more serological tests is considered abnormal case of rheumatism.

3.4. Chest HRCT imaging features

Table 4 summarizes the chest HRCT imaging features. The majority of chest HRCT in AFB smear-positive patients with mycobacteriosis was involved in bilateral lungs, accounting for 79.7% in the NTM group and 60.5% in the PTB group ($P=0.234$). There was no obvious difference in terms of the number of

affected lobes between the two groups (3.4±1.4, 3.2±1.6, *P*=0.404). Nodules and patchy shadows were the most common chest CT imaging features of the two groups, with no significant difference between the two groups. Patients in the PTB group had a higher proportion of stripe shadow, consolidation shadow, pleural thickening, mediastinal lymphadenopathy, pleural effusion, cavity and satellite nodule (*P*<0.05). However, only cystic change was more commonly detected in the NTM group (32.8% vs. 8.2%, *P*=0.001). In terms of the lesion distribution, the right middle lobe and the left lingular lobe were the more commonly involved lobes compared with the PTB group (*P*<0.001 and *P*=0.016, respectively), and the results are illustrated in Figure 3. Figure 4 shows typical chest HRCT images of NTM-LD.

3.5. Microbiological test results

Table 3 lists the microbiological test results of the enroll patients. Specimens examined included sputum, bronchoscope brush, BALF, lung puncture fluid, and pleural effusion. The positive rate of sputum AFB smear in the NTM group was lower compared with the PTB group (37.2% vs. 68.3%, *P*=0.003). Moreover, the positive rate of either AFB smear or mycobacterial culture of BALF was the highest among all types of specimens, accounting for 83.9% and 98.4% in the NTM group, and 83.0% and 95.8% in the PTB group, respectively.

Table 3
Microbiological results

Methods	**Samples**	**NTM (n=64)**	**PTB (n=61)**	***P*-value**
AFB smear- positive	Sputum	19/51, 37.2	28/41, 68.3	**0.003**
	Bronchoscope brush	31/60, 51.7	22/53, 41.5	0.280
	Bronchoalveolar lavage fluid	52/62, 83.9	39/47, 83.0	0.901
	Lung puncture fluid	0/0, 0	4/7, 57.1	/
	Pleural effusion	0/0, 0	2/6, 33.3	/
Mycobacterium culture	Sputum	21/28, 75.0	31/34, 91.2	0.169
	Bronchoalveolar lavage fluid	61/62, 98.4	46/48, 95.8	0.416
	Lung puncture fluid	0/0, 0	5/8, 62.5	/
	Pleural effusion	0/0, 0	3/5, 60.0	/

Abbreviation: AFB, acid-fast bacillus; NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

Data are presented as number of positive cases/detected cases, followed by percentage (%), and the data presented in bold type are statistically significant (*P*<0.05).

Table 4
Chest CT imaging features

	NTM (n=64)	PTB (n=61)	*P*-value
Affected lobes (n,±SD)	3.4±1.4	3.2±1.6	0.404
Location			
Bilateral	51 (79.7)	43 (70.5)	0.234
Unilateral	13 (20.3)	18 (29.5)	0.234
Patchy shadow	57 (89.1)	56 (91.8)	0.603
Nodular shadow	53 (82.8)	49 (80.3)	0.720
Cystic change	21 (32.8)	5 (8.2)	**0.001**
Stripe shadow	16 (25.0)	25 (41.0)	0.057
Pleural thickening	15 (23.4)	30 (49.2)	**0.003**
Cavity	9 (14.1)	21 (34.4)	**0.008**
Consolidation	9 (14.1)	18 (29.5)	**0.036**
Mediastinal lymphadenopathy	6 (9.4)	19 (31.1)	**0.002**
Calcified shadow	5 (7.8)	10 (16.4)	0.140
Pleural effusion	3 (4.7)	17 (27.9)	**<0.001**
Atelectasis	3 (4.7)	4 (6.6)	0.649
Satellite nodule	1 (1.6)	7 (11.5)	0.058
Isolated lesion	0 (0)	4 (6.6)	/

Abbreviation: NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

Data are presented as number of patients with percentage of total in parentheses, and the data presented in bold type are statistically significant (*P*<0.05).

3.6. Risk factor analysis and establishment of a predictive model

Based on the above-mentioned clinical characteristics, we found that female patient, negative test for QFT, bronchiectasis, the symptom of hemoptysis, right middle lobe and left lingual lobe involved in chest HRCT, and cystic change in NTM patients were seven characteristics statistically different from the PTB patients. Using binary logistics method, we found that only female patient, bronchiectasis, negative test for QFT, and right middle lobe involved in chest HRCT were independent risk factors for NTM, showing statistical difference (P<0.05). Table 5 shows the odds ratios (OR) and 95% confidence intervals (CI) of variables related to NTM. Where OR=1, the variables had no risk in NTM. A combination of these four risk factors yielded in a sensitivity and specificity of 81.3% and 96.7%, respectively, in a ROC curve, with an AUC of 0.965 (Figure 5). Meanwhile, a nomogram was made to visually show the predictive model in Figure 6.

3.7. Validation of the predictive model

At first, a calibration curve was made for internal validation of the model (Figure 7), from which we could see that the probability of NTM predicted by the model was very close to the actual observation probability. Next, we conducted external verification of the cases after the retrospective population, and Figure 8(A) shows the case screening process. A total of 116 successional AFB smear-positive specimens of inpatients of the Respiratory Department between March 1st. 2020 to June 30th. 2020 were screened for external verification. 35 AFB smear-positive cases were screened out after excluding repetitive specimens and cases with incomplete data. Another ROC curve was made to evaluate the predictive effect of the model on external data, with an AUC of 0.913 (P=0.002), illustrated in figure 8(B).

Table 5
Binary regression analysis to find the independent risk factors of NTM in AFB-smear positive patients

	***P*-value**	**OR**	**95% CI**	
			Lower	**Upper**
Female	**0.005**	9.654	1.974	47.220
Hemoptysis	0.052	6.566	1.032	41.758
Bronchiectasis	**0.010**	14.140	1.880	106.365
QFT negative	**<0.001**	97.293	14.022	675.093
Right middle lobe involved	**0.012**	15.423	1.824	130.418
Left lingular lobe involved	0.335	0.433	0.079	2.377
Cystic change	0.447	2.262	0.276	18.541

Abbreviation: NTM, Non-tuberculous mycobacteria; AFB, acid-fast bacillus, OR, odds ratios; CI, confidence interval; QFT, QuantiFERON tuberculosis.

Data presented in bold type are statistically significant (P<0.05).

4. Discussion

Previous studies have reported that NTM is more likely to infect older females [2,11]. According to this study, female patients in the NTM group accounted for 78.1%, with an average age of 58.4±11.7 years, suggesting that estrogen played a protective role against NTM [11,12]. The guidelines have shown that NTM is particularly prone to structural lung diseases, such as COPD and bronchiectasis [9,13]. This study showed that up to 76.6% of NTM patients had bronchiectasis, which was significantly higher compared with the patients with PTB, while COPD was more commonly detected in the PTB group. In systemic complications, upper airway disease showed a relatively high rate in the NTM group, although with no statistical difference in our data, which might be attributed to that it is more associated with bronchiectasis [13].

Patients with mycobacteriosis often lack specificity in clinical manifestations [6]. Fever and night sweats were not particularly common, while as systemic symptoms, they were more commonly detected in patients with PTB than NTM. Reflecting systemic inflammation, the levels of CPR and ESR were significantly higher in the PTB patients compared with the NTM patients in this study.

Nowadays, interferon-gamma release assays (IGRAs) are essential tools for detecting infections with mycobacterium tuberculosis, including latent tuberculosis infection. IGRAs, including both QFT and T-SPOT.TB, enable the direct observation of the response of a patient's blood cells to specific antigens derived from mycobacterium tuberculosis [14,15]. In this study, negative test for QFT was found in 81.3% of NTM patients, and it was proved to be an independent risk factor for predicting NTM, which could be used as an important reference index for differential diagnosis.

The gold standard for diagnosis of NTM infections is referred to the result of tuberculosis culture and strain identification, and a least two batches of sputum samples are required to confirm a diagnosis of pulmonary NTM [16,17]. As NTM is widely distributed in the environment, sputum samples may be contaminated during collection and examination, resulting in false positive results. BALF culture may be more sensitive than sputum culture in diagnosing nodular bronchiectatic NTM-LD [18]. We found that the positive rate of BALF tuberculosis culture was higher than the sputum culture. Because electronic bronchoscopy is relatively sterile, the result of BALF samples is also more reliable than sputum.

Meanwhile, definite diagnosis of NTM-LD cannot simply rely on isolated and cultured NTM from the respiratory tract. To make a diagnosis of NTM-LD, etiology, clinical symptoms and imaging findings are indispensable [19]. Chest HRCT is an important imaging examination for the diagnosis of NTM-LD. Our data showed that the chest HRCT manifestations of AFB smear-positive cases of mycobacteriosis were mainly multiple nodules and plaques in both lungs. The involvement of the right middle and the left lingular lobes might be some of the characteristics that distinguished NTM from PTB in the present study, and cystic change was found to be an HRCT morphological feature of NTM, which was more commonly detected in NMT patients.

In the clinical practice, for patients with cough and expectoration, especially with hemoptysis and the characteristics of mycobacteriosis in chest CT, AFB smear of sputum and other respiratory tract specimens would be the primary and relatively fast examinations to make a clear diagnosis. Patients with positive AFB smear results may be directly diagnosed as PTB and received a long-term anti-tuberculosis treatment, in the absence of specimen culture and strain identification. According to Chang's study, of the patients with AFB smear-positive sputum but no PTB, 18.5% were later diagnosed with NTM-LD, and 32% were afflicted with NTM colonization [20]. Once NTM-LD is misdiagnosed as PTB and given chemotherapy, there will be not only a high risk of adverse reactions, but also a long course of treatment, poor efficacy, and a heavy economic burden [7,8]. Despite the recent advances in the BACTEC MGIT (Mycobacteria Growth Indicator Tube) 960 System (BD, Franklin Lakes, NJ, USA), which greatly shortens the time required for culturing mycobacteria from 6-8 weeks to 2-4 weeks, the time required to confirm a diagnosis is still significant [21]. Therefore, how to determine the NTM-LD by some rapid and effective clinical features will directly affect the administration of anti-tuberculosis treatment and the prognosis of the disease for patients with AFB smear-positive results. Based on this situation, we analyzed all the clinical characteristics in AFB smear-positive patients to find the independent risk factors for NTM and then established a model to predict NTM, showing a prominent high level of sensitivity and specificity. Moreover, through the internal and external verifications of the model, we suggested that the model showed the practicality and effectiveness in the clinical practice.

In conclusion, female patients, bronchiectasis, negative test for QFT, and the right middle lobe affected were independent risk factors for fast prediction of NTM-LD in AFB smear-positive patients. Through the verification of the model, it showed prominent practicability and effectiveness in clinical practice. When patients show all the above-mentioned characteristics, it is strongly suggests the possibility of NTM infection, and it is necessary to wait for the mycobacterial culture results and meanwhile to conduct the identification and drug susceptibility test of mycobacterium, by which incorrect anti-tuberculosis treatment-related adverse reaction and the appearance of drug-resistant tuberculosis might be reduced, and the unnecessary economic burden could also be lessened. Our findings might have important clinical significance for improving the early diagnosis rate of NTM-LD and the prognosis of the disease.

However, there are some limitations in this study. First, the test of QFT may not be conducted in every hospital or center. This study lacked the identification of NTM species, and did not distinguish the positive degree of AFB from + to 4+. In addition, the total number of cases observed in this study was insufficient, and the data were acquired from a single center, which might have certain impact on the representative of the results. Even though we have drawn some firm conclusions, then established and validated a fairly effective model. We will further verify it in subsequent clinical practice and in more centers.

5. Conclusions

The factors of female patient, bronchiectasis, negative test for QFT, and findings of the right middle lobe in chest HRCT could be useful for early differential diagnosis of NTM in AFB smear-positive patients. The

predictive model based on these factors could be quickly and effectively applied in the clinical work in the respiratory department, which would allow us to know the diagnosis 4-8 weeks earlier than the conventional culture result.

Abbreviations

AFB, acid-fast bacillus

AUC, area under the curve

BALF, bronchoalveolar lavage fluid

HRCT, Chest high-resolution computed tomography

NTM, non-tuberculous mycobacteria

NTM-LD, non-tuberculous mycobacteria related lung diseases

PTB, pulmonary tuberculosis

QFT, quantiFERON tuberculosis

ROC, receiver operating characteristic

Declarations

Ethics approval and consent to participate:

Ethical approval for the study was granted by the Research Ethics Committee of the School of Medicine, Tongji University.

Consent for publication:

Not applicable.

Availability of data and materials:

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

Competing interests:

The authors declare that they have no competing interests.

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Provision of study materials or patients: Xianqiu Chen and Yuan Zhang

Collection and assembly of data: Xianqiu Chen

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Figures

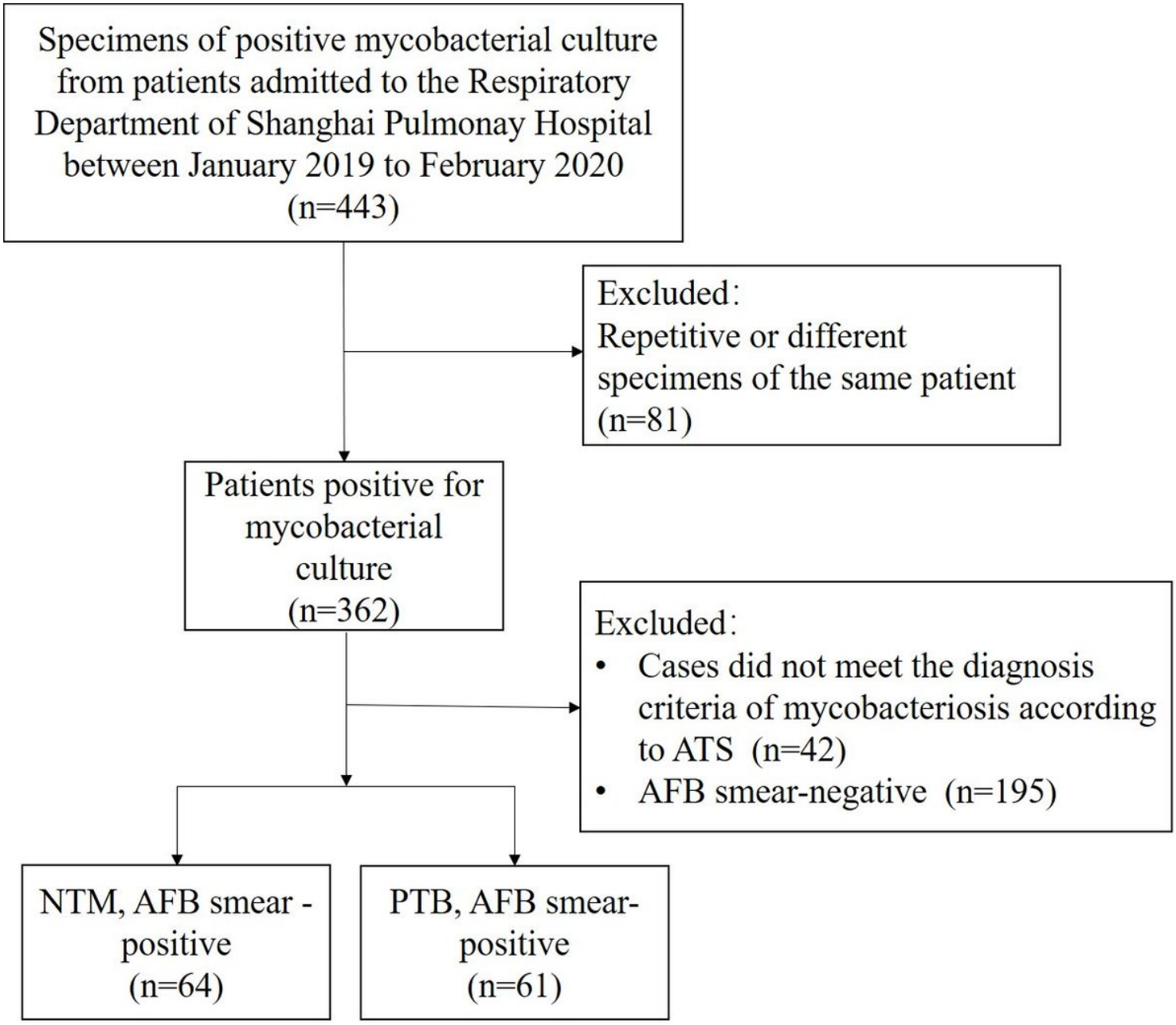

Figure 1

Study flow diagram The process of patients screening is shown in this diagram. Abbreviations: NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis; ATS, American Thoracic Society; AFB: acid-fast bacillus.

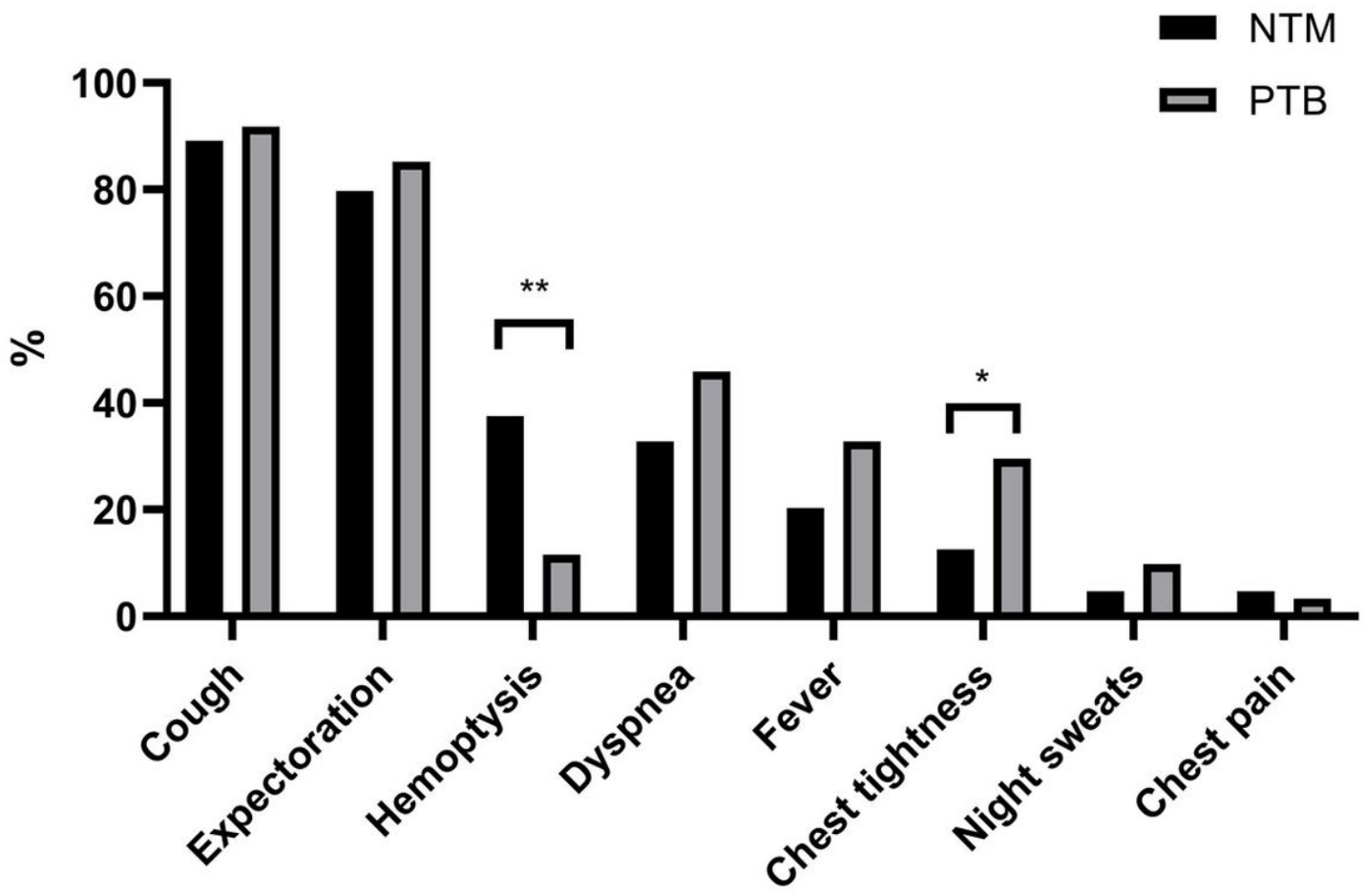

Figure 2

Primary symptoms of AFB smear-positive patients enrolled are illustrated in the figure. * Compared with the NTM group, chest tightness was more common in patients with PTB (P=0.019). ** Hemoptysis was more common in NTM patients compared with the PTB patients (P=0.001). Abbreviations: NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

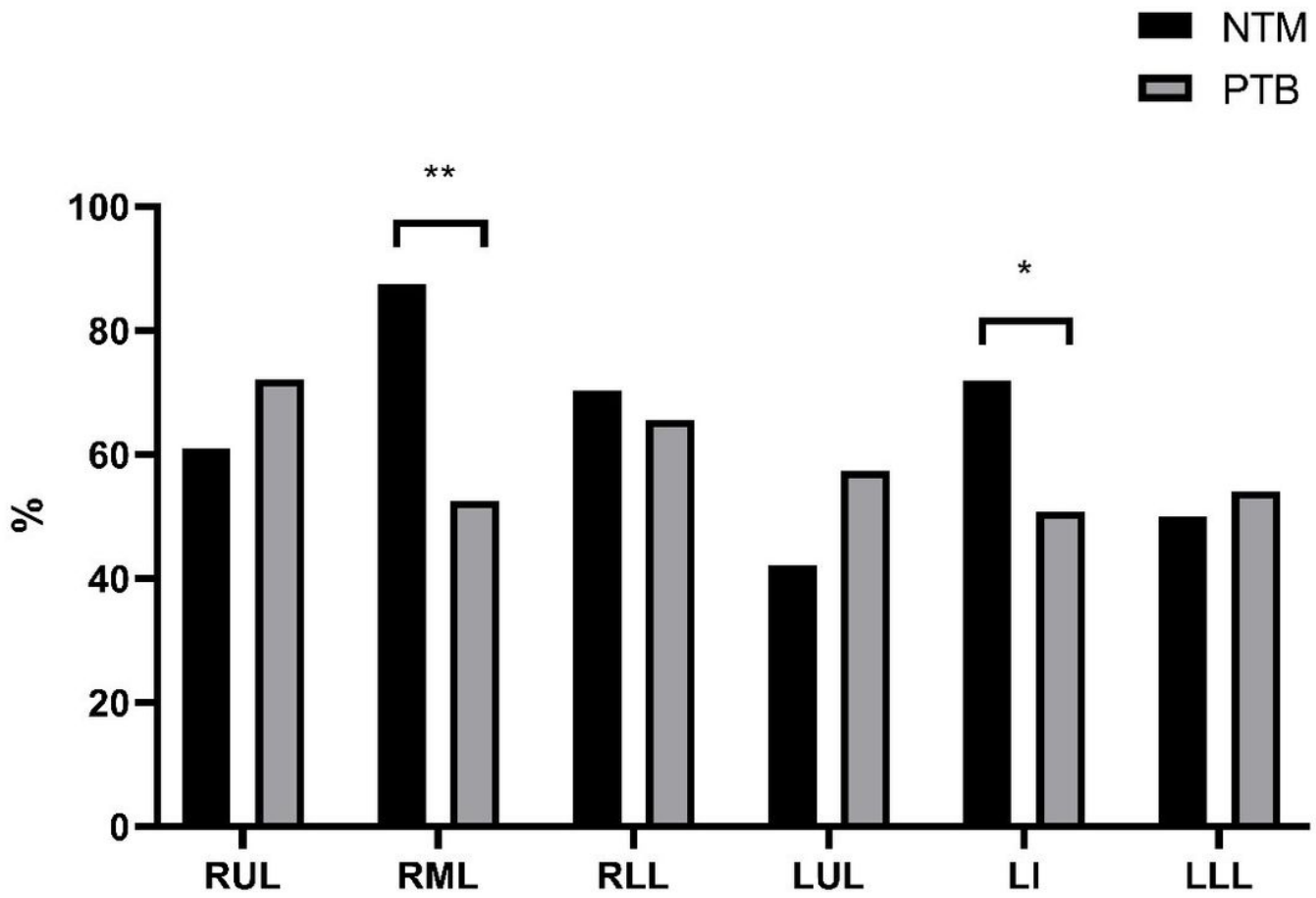

Figure 3

The affected lobes on chest CT of AFB smear-positive patients are shown in the figure. Compared with the PTB group, the left lingular lobe and the right middle lobe were more commonly involved (* P=0.016; ** P<0.001). Abbreviations: RML, right middle lobe; LUL, left up lobe; RLL, right lower lobe; RUL, right up lobe; LI, left lingular lobe; LLL, left lower lobe; NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

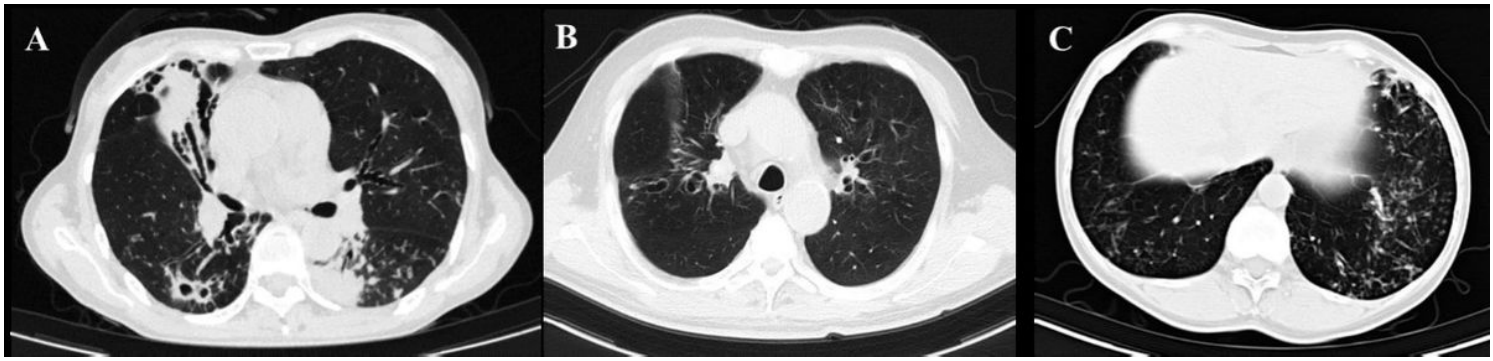

Figure 4

Chest CT images of typical NTM. (A) A typical chest CT image of NTM-LD, showing columnar and cystic bronchiectasis in the middle lobe of the right lung, multiple small nodules in both lungs, and multiple thin-walled cavity in the right lower lobe. (B) A typical chest CT image of NTM-LD, showing multiple cystic changes in both lungs. (C) A typical chest CT image of NTM-LD, showing tree-in-bud pattern and small bronchiectasis in both lungs, especially in left lung.

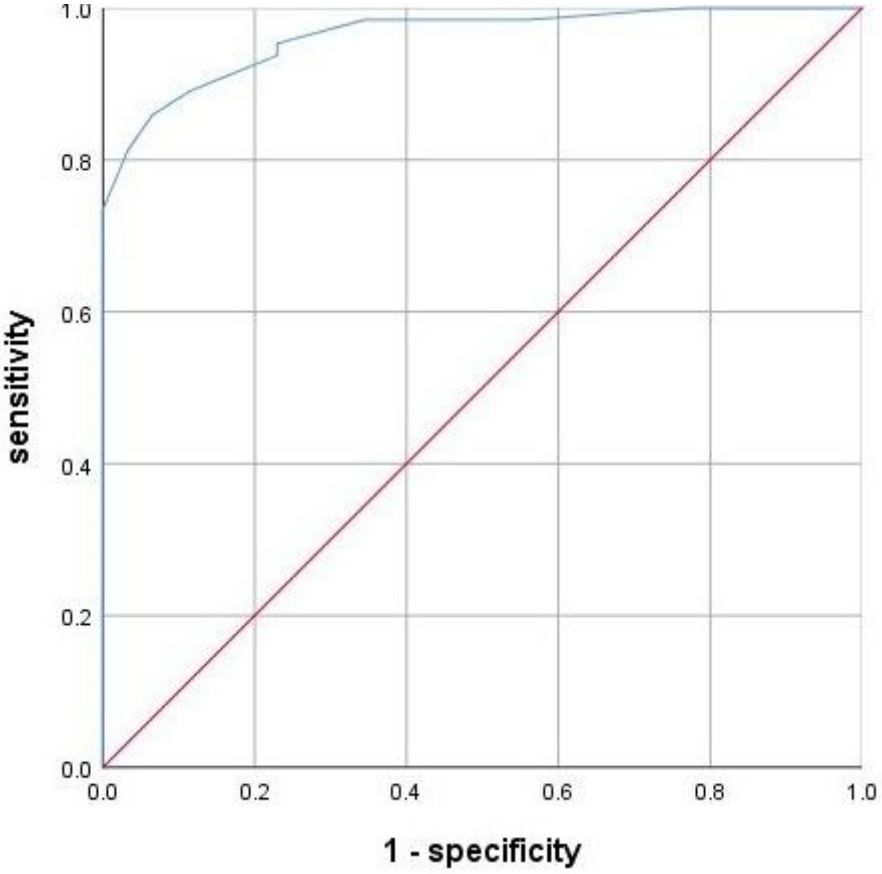

Figure 5

ROC curve to predict NTM in AFB smear-positive patients Figure 5. A combination of female patient, bronchiectasis, negative test for QFT, right middle lobe lesion in chest CT yielded a sensitivity and specificity of 85.9% and 93.4%, respectively, with an AUC of 0.963, P<0.001.

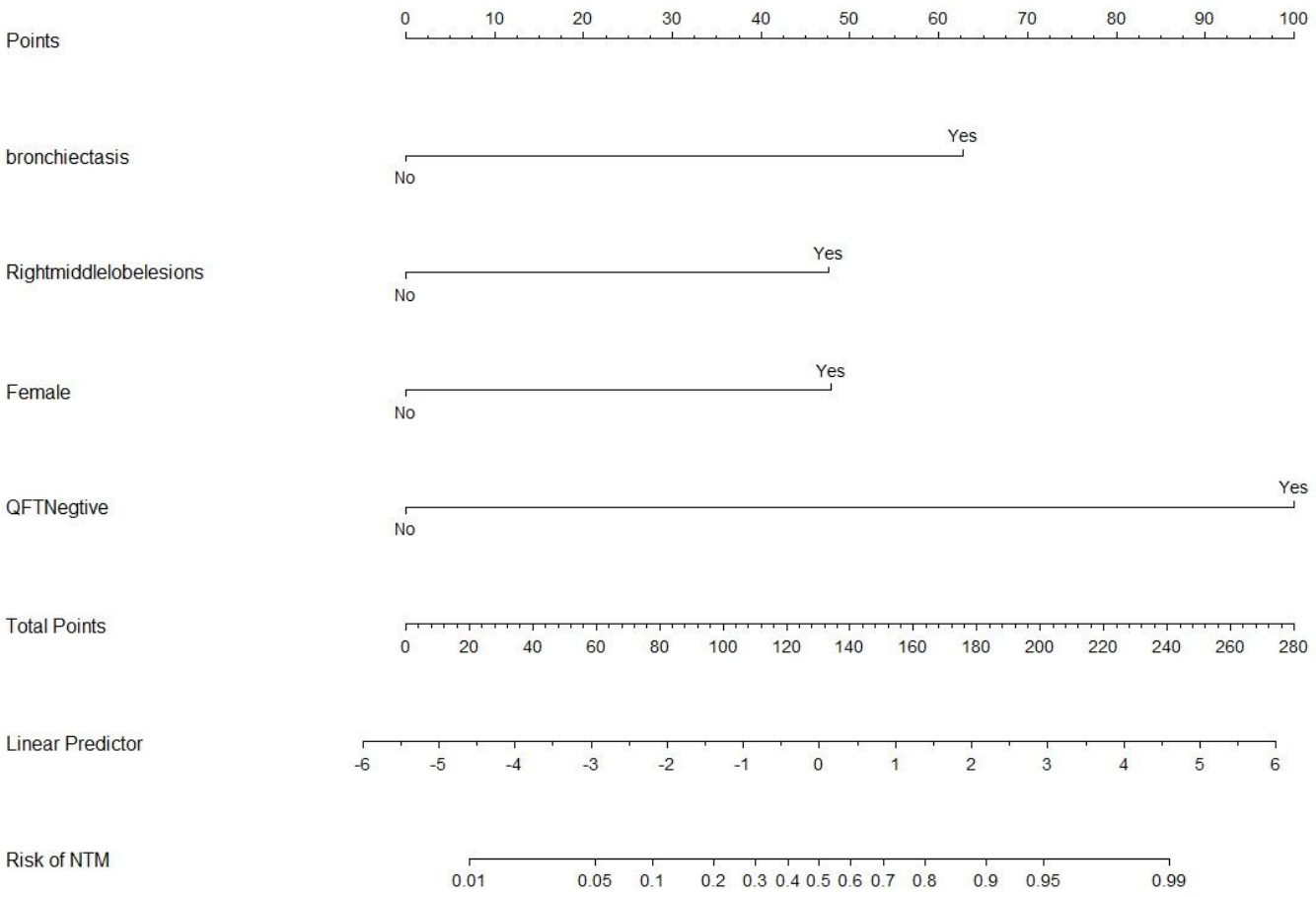

Figure 6

Nomogram of the predictive modal A nomogram of the predictive model to predict NTM in AFB smear-positive patients using points of four characteristics: bronchiectasis, right middle lobe lesions, female patient and negative test for QFT. Abbreviations: QFT, quantiFERON tuberculosis; NTM, non-tuberculous mycobacteria; PTB, pulmonary tuberculosis.

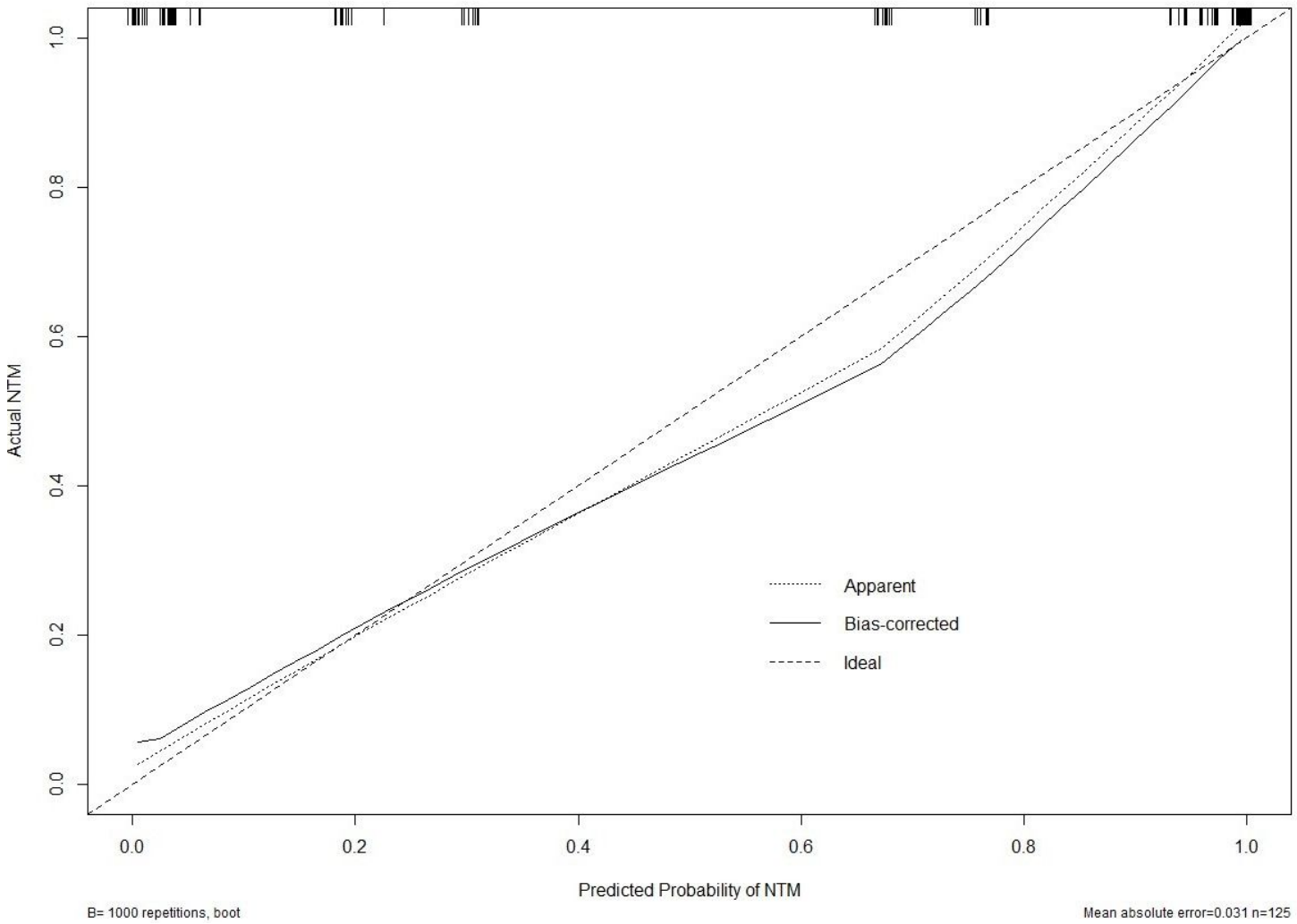

Figure 7

Calibration curve of predicted probability of NTM. The probability of NTM predicted by the model was very close to the actual observation probability. When the predicted probability was above 0.3, the model slightly over-estimated the probability of NTM.

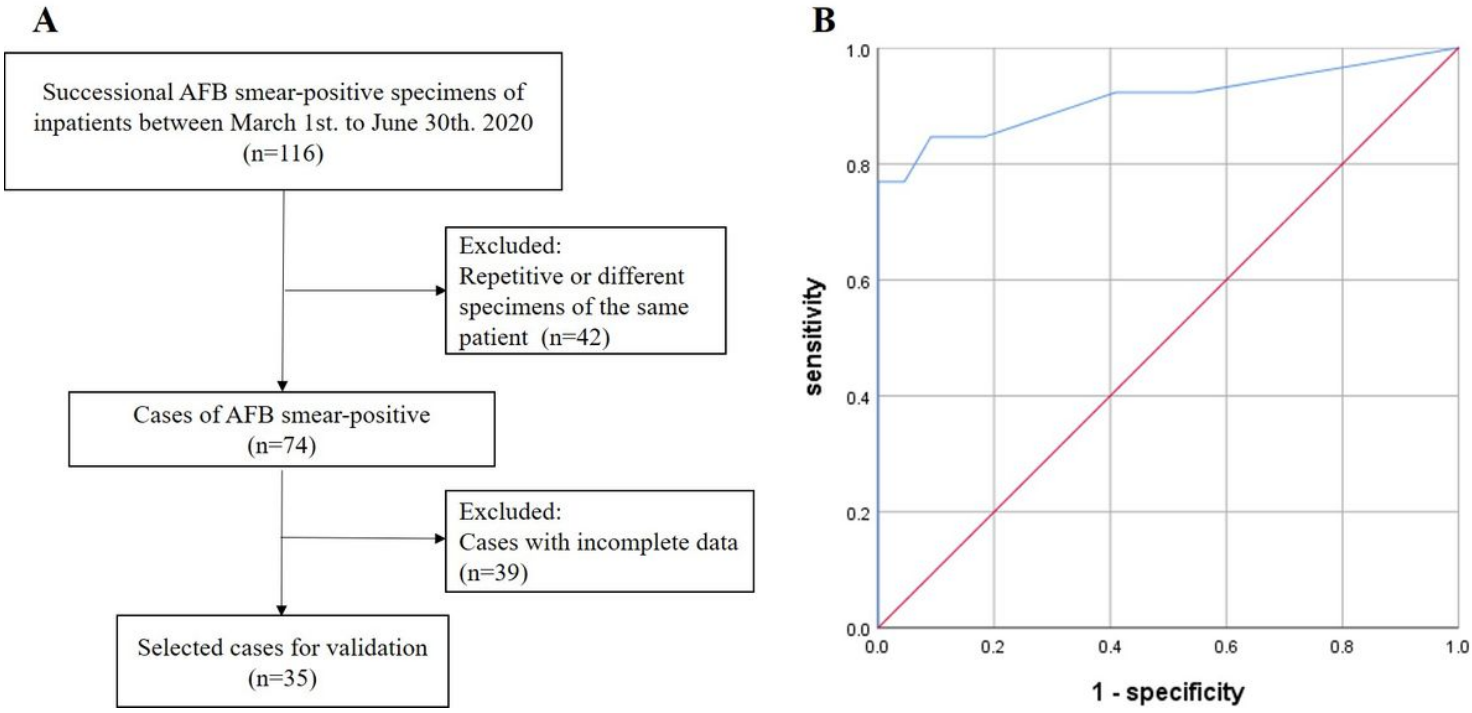

Figure 8

External validation of the predictive model. (A) Screening process of cases for validation. (B) ROC curve to evaluate the predictive effect of the model on external data, with an AUC of 0.913, P<0.001. Abbreviations: QFT, quantiFERON tuberculosis; AFB, acid-fast bacillus.