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Establishment and validation of a predictive model for nontuberculous mycobacterial infections in acid-fast bacilli smear-positive patients

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Abstract

Introduction: Nontuberculous mycobacteria (NTM) and pulmonary tuberculosis (PTB) are difficult to distinguish in initial acid-fast bacilli (AFB) smear-positive patients.

Objectives: Establish a predictive model to identify more effectively NTM infections in initial AFB patients.

Methods: Consecutive AFB smear-positive patients in the Respiratory Department of Shanghai Pulmonary Hospital from January 2019 to February 2020 were retrospectively analysed. A multivariate regression was used to determine the independent risk factors for NTM. A receiver operating characteristic (ROC) curve was used to determine the model's predictive discrimination. The model was validated internally by a calibration curve and externally for consecutive AFB smear-positive patients from March to June 2020 in this institution.

Results: Presenting with haemoptysis, bronchiectasis, a negative QuantiFERON tuberculosis (QFT) test and being female were characteristics significantly more common in patients with NTM ($P \leq 0.001$), when compared with PTB. The involvement of right middle lobe, left lingual lobe and cystic change was more commonly seen on chest high-resolution computed tomography (HRCT) in patients with NTM ($P < 0.05$), compared with PTB. Multivariate regression showed female, bronchiectasis, negative test for QFT and right middle lobe lesion were independent risk factors for NTM ($P < 0.05$). A ROC curve showed a sensitivity and specificity of 85.9% and 93.4%, respectively, and the area under the curve (AUC) was 0.963. Moreover, internal and external validation both confirmed the effectiveness of the model.

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

Xianqiu Chen and Yuan Zhang contributed equally to this study.

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1 | INTRODUCTION

Nontuberculous mycobacteria (NTM) are widely distributed in nature and have 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 the most common clinical manifestation of NTM infection.[4] Actually, NTM-related lung diseases (NTM-LDs) are not mandatory to treat, as there may be circumstances where patients meet diagnostic criteria for NTM-LD but do not have progressive disease or sufficiently severe disease to warrant therapy.[4] As the symptoms are atypical, NTM-LDs are always misdiagnosed as other chronic lung diseases, such as tuberculosis or lung cancer.[5,6] The difficulties in diagnosis may lead to inappropriate treatment for other diseases.[7] In 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 as pulmonary tuberculosis (PTB) and to receive anti-tuberculosis treatment. When species identification tests are unavailable or not conducted, patients are not suspected to have NTM infections until the clinicians notice the failed treatment to anti-tuberculosis drugs, often resulting in undesirable side effects and heavy financial burden.[8,9] Therefore, early differential diagnosis of NTM is particularly important in clinical practice, which may have a great impact on the prognosis of the disease.

Although there have been many studies on the clinical characteristics of NTM, few predictive models have been established, and most of them have to rely on the conventional culture results for the diagnosis, which can take about 4–8 weeks. In the present study, we retrospectively analysed the clinical characteristics of AFB smear-positive patients with confirmed 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 in most cases, which can improve the efficiency of differential diagnosis.

2 | METHODS

2.1 | Patient screening

All specimens positive for mycobacteria culture from consecutive patients admitted to the Respiratory Department of Shanghai Lung Hospital, from January 2019 to February 2020, were selected for this study. We excluded repeated or different specimens from the same patient, cases with a negative AFB smear and cases that did not meet the American Thoracic Society (ATS) diagnostic criteria for mycobacterial infections (ATS).[4,10] Finally, cases of confirmed NTM and PTB were included in this study according to the results of mycobacterium culture. For the validation court, all the successional AFB smear-positive specimens of the Respiratory Department from 1 March 2020 to 30 June 2020 were screened for external validation. The inclusion and exclusion criteria were the same. The study was performed in accordance with the Declaration of Helsinki. The ethics committee of Shanghai Pulmonary Hospital approved the research. There were no written informed consent documents taken from the participants because of its retrospective nature.

2.2 | Diagnostic criteria

Samples of sputum, bronchoalveolar lavage fluid (BALF), lung puncture tissue and fluid, and pleural effusion cultured positive to NTM or a complex group of *Mycobacterium tuberculosis* were considered as the gold standard of diagnosis. Meanwhile, the diagnosis was made by referring to the ATS guidelines of NTM[4] and PTB,[10] combining with clinical symptoms and imaging features.

2.3 | Variables

The variables collected in this study included general data, history of respiratory and systemic diseases, primary symptoms, arterial blood gas conducted in room air when admission, and serological indicators obtained through reviewing the medical records.

Microbiological test results were accessed by detecting samples of sputum, electronic bronchoscopy brush and BALF, lung puncture tissue and fluid, and pleural effusion. The methods included smear, GeneXpert and culture. AFB smear-positive means smear positive of any one of the samples, and these samples were cultured for mycobacteria at the same time. For the reason of medical costs, not all the samples, we tested conducted the test of GeneXpert at the same time.

2.4 | Chest HRCT scans

All chest high-resolution computed tomography (HRCT) scans were performed in 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. 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 drawn jointly. 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 $\pm$ standard deviation (SD). The independent sample Student's t test was used for comparison 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 1 and less than 5, the continuous correction chi-square was employed. If the theoretical frequency was less than one, Fisher's exact test was employed. A multivariate regression with forward selection method was used to determine the independent predictive risk factors for NTM in AFB smear-positive patients. Meanwhile, a receiver operating characteristic (ROC) curve was made to determine the model's predictive discrimination. A nomogram was made with R software (version 3.6.3) to visually show the predictive model. Moreover, the model was internally validated through a calibration curve and externally validated for another group of patients by a new ROC curve. A value of $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics

A total of 443 specimens positive for mycobacterial culture from consecutive patients admitted to Respiratory Department of Shanghai Pulmonary Hospital between January 2019 and February 2020 were screened, as illustrated in Figure 1. After the exclusion of duplicate specimens, AFB smear-negative cases, and cases that did not meet the diagnostic criteria of mycobacteriosis, a total of 125 AFB smear-positive patients were included in this 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, body mass index (BMI) and arterial blood gas results between the two groups. The proportion of females in the NTM group was significantly higher compared with that in the PTB group (78.1% vs. 34.4%, $P < 0.001$). Negative test for QFT was found in 82.8% of NTM patients, which was significantly higher ($P < 0.001$) compared with PTB group. In this study, we found that the proportion of patients with bronchiectasis was significantly higher in the NTM group compared with that in the PTB group (76.6% vs. 19.7%, $P < 0.001$). Haemoptysis in patients with NTM was more common (37.5% vs. 11.5%, $P = 0.001$). Table S1 shows the results of serological indicators. As systemic inflammatory indicators, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were both significantly lower in the NTM group ($P < 0.001$). Meanwhile, patients in the NTM group showed a higher positive ratio of serum tuberculosis antibody and rheumatological indicators (23.4% vs. 14.8%, and 25.0% vs. 13.1%, respectively), although there was no statistical difference between the two groups.

Table S2 lists the microbiological test results of the enrolled patients. Specimens examined included sputum, bronchoscopy brush, BALF, lung puncture tissue and 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.

3.2 | Chest HRCT imaging features

Chest HRCT imaging features of enrolled patients are summarized in Table 2. The majority of chest HRCT showed that the pulmonary infection involved both lungs, accounting for 79.7% in the NTM group and 70.5% in the PTB group ($P = 0.234$). There was no obvious
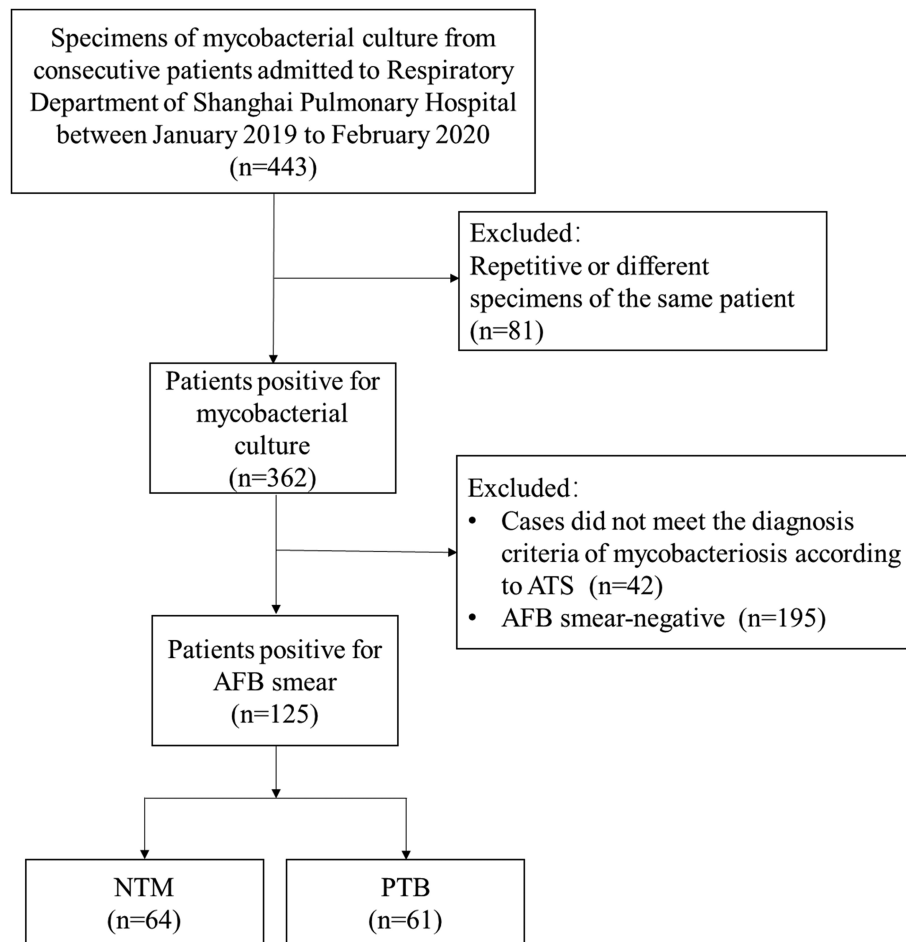
difference in the number of affected lobes between the two groups (3.4 ± 1.4, 3.2 ± 1.6, $P = 0.404$). Nodules and patchy shadows were the most common chest CT imaging features, with no significant difference between the two groups. Patients in the PTB group showed more of consolidation shadow, pleural thickening, mediastinal lymphadenopathy, pleural effusion and cavity ($P < 0.05$). However, only cystic change was more commonly seen 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 lingual lobe were more commonly involved lobes compared with the PTB group ($P < 0.001$ and $P = 0.016$, respectively). Figure S1 shows typical chest HRCT images of NTM-LD.

3.3 | Risk factor analysis and establishment of a predictive model

On the basis of the above clinical characteristics, we found that in NTM patients, being female, negative test for QFT, the existence of bronchiectasis, haemoptysis, right middle lobe and left lingual lobe involvement in chest HRCT, and cystic change were

seven characteristics statistically different from the PTB patients. Using a multivariate regression method, we found that only being female, bronchiectasis, negative test for QFT, and right middle lobe involvement in chest HRCT were independent risk factors for NTM, showing statistical difference ($P < 0.05$). Table 3 shows the odds ratios (ORs) and 95% confidence intervals (CIs) of variables related to NTM. Where OR = 1, the variables had no risk in NTM. A combination of the 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 2). Meanwhile, a nomogram was made to visually show the predictive model in Figure 3, which is a scoring model that can apply to clinical cases immediately. The figure legend shows the instruction of how to use the nomogram.

3.4 | Validation of the predictive model

For internal validation, a calibration curve was made (Figure 4), from which we could see that the probability of NTM predicted by the model was very close to the actual probability. Next, we conducted external

TABLE 1 Comparison of clinical characteristics of patients with NTM and PTB

Characteristics	NTM ($n = 64$)	PTB ($n = 61$)	P value[a]
Age (mean ± SD), year	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·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
Primary symptoms			
Cough	57 (89.1)	56 (91.8)	0.603
Expectoration	51 (79.7)	52 (85.2)	0.415
Hemoptysis	24 (37.5)	7 (11.5)	**0.001**
Dyspnea	21 (32.8)	28 (45.9)	0.134
Fever	13 (20.3)	20 (32.8)	0.114
Chest tightness	8 (12.5)	18 (29.5)	**0.019**
Night sweats	3 (4.7)	6 (9.8)	0.266

Note. 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.

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; DPB, diffuse panbronchiolitis; NTM, nontuberculous mycobacteria; PaCO$_2$, carbon dioxide partial pressure; PaO$_2$, oxygen partial pressure; PTB, pulmonary tuberculosis; QFT, QuantiFERON tuberculosis; SaO$_2$, oxygen saturation; SD, standard deviation.

[a]The independent sample Student's *t* test was used for comparison between NTM and PTB groups. The chi-square test was used for constituent ratio comparisons.

verification for the cases other than previous patients in our institution, and Figure 5A shows the cases screening process. A total of 116 successional AFB smear-positive specimens of inpatients of the Respiratory Department between 1 March 2020 and 30 June 2020 were screened for external verification. Thirty-five AFB smear-positive cases were screened out after excluding repetitive specimens and cases with incomplete data. See Table S3 for

TABLE 2 Comparison of chest CT features of patients with NTM and PTB

	NTM (*n* = 64)	PTB (*n* = 61)	*P* value[a]
Number of affected lobes (mean ± 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
RUL	39 (60.9)	44 (72.1)	0.185
RML	56 (87.5)	32 (52.5)	**<0.001**
RLL	45 (70.3)	40 (65.6)	0.570
LUL	27 (42.2)	35 (57.4)	0.090
LI	46 (71.9)	31 (50.8)	**0.016**
LLL	32 (50.0)	33 (54.1)	0.647
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)	—

Note. 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).

Abbreviations: LI, left lingual lobe; LLL, left lower lobe; LUL, left up lobe; NTM, nontuberculous mycobacteria; PTB, pulmonary tuberculosis; RLL, right lower lobe; RML, right middle lobe; RUL, right up lobe; SD, standard deviation.

[a]The chi-square test was used for constituent ratio comparisons, and the independent sample Student's *t* test was used for comparison of number of affected lobes.

TABLE 3 Multivariate regression analysis to find the independent risk factors of NTM in AFB-smear positive patients

			95% CI	
Factors	**P value**	**OR**	**Lower**	**Upper**
Female	**0.005**	9.654	1.974	47.220
Haemoptysis	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

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

Abbreviations: AFB, acid-fast bacillus; CI, confidence interval; NTM, nontuberculous mycobacteria; OR, odds ratio; QFT, QuantiFERON tuberculosis.
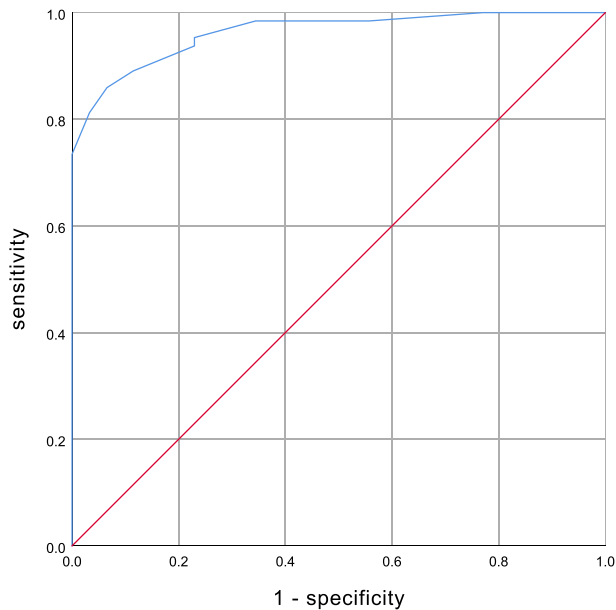
FIGURE 2 A ROC curve to predict NTM in AFB smear-positive patients. A combination of female patient, bronchiectasis, negative test for QuantiFERON tuberculosis (QFT), right middle lobe lesion in chest CT yielded a ROC curve, with a sensitivity and specificity of 85.9% and 93.4%, respectively. The area under the curve (AUC) is 0.963, $P < 0.001$. Abbreviations: AFB, acid-fast bacilli; CT, computed tomography; NTM, nontuberculous mycobacteria; ROC, receiver operating characteristic

the summary of the validation cases. Another ROC curve was made to evaluate the predictive effect of the model on external data, with an AUC of 0.913 ($P = 0.002$), as illustrated in Figure 5B.

4 | DISCUSSION

According to this study, female patients in the NTM group accounted for 78.1%, with an average age of 58.4 ± 11.7 years, consistent with previous studies that NTM was more likely to infect older females.[2,11] Guidelines have shown that NTM is particularly prone to structural lung diseases, such as chronic obstructive pulmonary disease (COPD) and bronchiectasis.[9,12] This study showed that up to 76.6% of NTM patients had bronchiectasis, which was significantly higher compared with patients with PTB, although COPD was more commonly seen 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 often associated with bronchiectasis.[12] Therefore, in our study, female patients or patients with bronchiectasis were more likely to have an NTM infection.

Nowadays, interferon-gamma release assays (IGRAs) are essential tools for detecting infections with *M. tuberculosis*, mostly 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 *M. tuberculosis*.[13,14] However, the results are susceptible to variability by different factors at multiple levels, including assay manufacturing, preanalytical processing (e.g., blood volume, sample transport temperature and incubation time) or analytical testing.[15] In this study, 8.2% and 82.8% of PTB and NTM patients, respectively, had negative QFT tests. Meanwhile, 17.2% of NTM patients presented positive QFT results in our investigation. As we know, IGRAs are specific for *M. tuberculosis* and not encoded in the genomes of any Bacillus Calmette–Guerin (BCG) vaccine strains or most species of NTM, other than *M. marinum*, *M. kansasii*, *M. szulgai* and *M. flavescens*.[16] However, not all NTMs have been studied for cross-reactivity. So, most of NTM infection could have negative results. In our study, a negative test for QFT is more common in NTM patients, it was also found in other studies,[16,17] and through our multivariate regression analysis, it was found an independent risk factor for predicting NTM, which might be an important factor for differential diagnosis.

The gold standard for the diagnosis of NTM infections is referred to the result of mycobacterium culture and strain identification, and at least two batches of sputum samples are required to confirm a diagnosis of pulmonary NTM.[18,19] 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.[20] We found that the positive rate of BALF mycobacterium culture was higher than the rate of sputum. Because electronic bronchoscopy is relatively sterile, the result of BALF samples is also more reliable than sputum. Meanwhile, a definite diagnosis of NTM-LD cannot simply rely on isolated and cultured NTM from the respiratory tract. To make a diagnosis of NTM-LD, aetiology, symptoms and imaging findings are indispensable.[21] 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 mycobacterium were mainly multiple nodules and plaques in both lungs. Meanwhile, the involvement of the right middle and left lingual lobes were more prominent in HRCT of NTM patients, similar to the previous literatures.[22,23] The reason may due to the lingula and its homologue, the middle lobe, have in common long, narrow, and, in the upright position, dependent bronchi, which is a
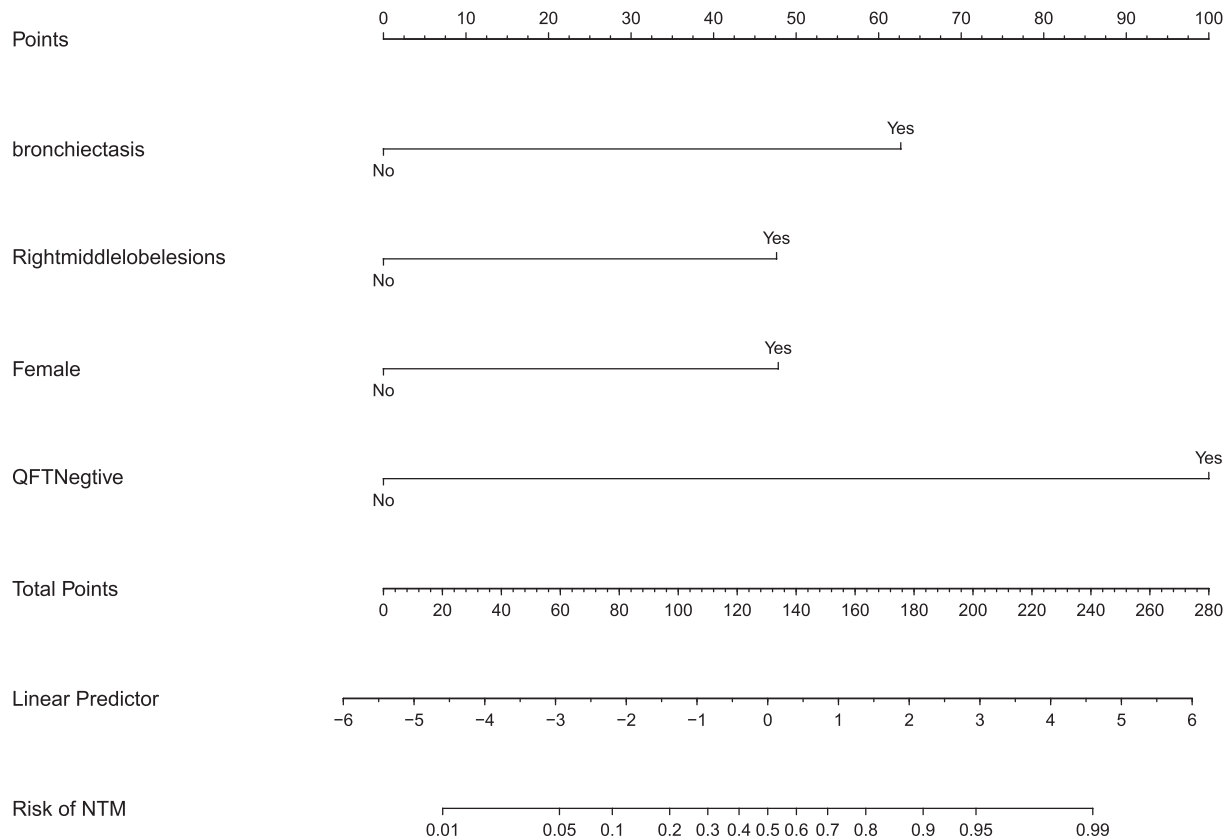
FIGURE 3 Nomogram of the predictive modal. A nomogram of the predictive model to predict NTM in AFB smear-positive patients using points of four binary variables: bronchiectasis, right middle lobe lesions, female and negative test for QFT. Draw a line perpendicular from the corresponding axis of each risk factor until it reaches the top line labelled 'points'. Sum up the number of points for all risk factors then draw a line descending from the axis labelled 'total points' until it intercepts each of the survival axes to determine risk of NTM. Abbreviations: NTM, nontuberculous mycobacteria; PTB, pulmonary tuberculosis; QFT, QuantiFERON tuberculosis
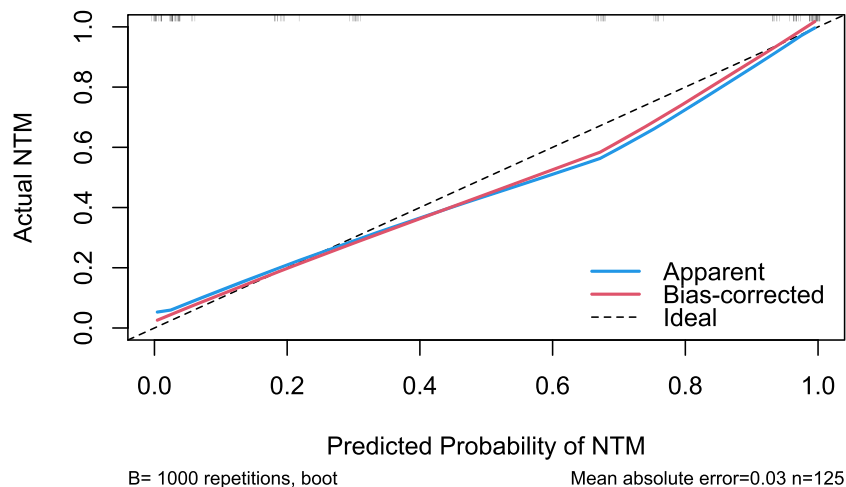

FIGURE 4 Calibration curve. Calibration curve depicts the calibration of the predictive model in terms of the agreement between the predicted probabilities of NTM and actual NTM. The y-axis represents the actual NTM rate. The x-axis represents the predicted probabilities of NTM. The diagonal dotted line represents a prediction by an ideal model. The blue solid line represents the performance of the model. The line of bias corrected is generated automatically by the software after correcting the deviation to prevent overfitting. This line shall be most focused, of which a closer fit to the diagonal dotted line represents a better prediction. It was showed in this calibration curve that the probability of NTM predicted by the model was very close to the actual probability. Abbreviation: NTM, nontuberculous mycobacteria
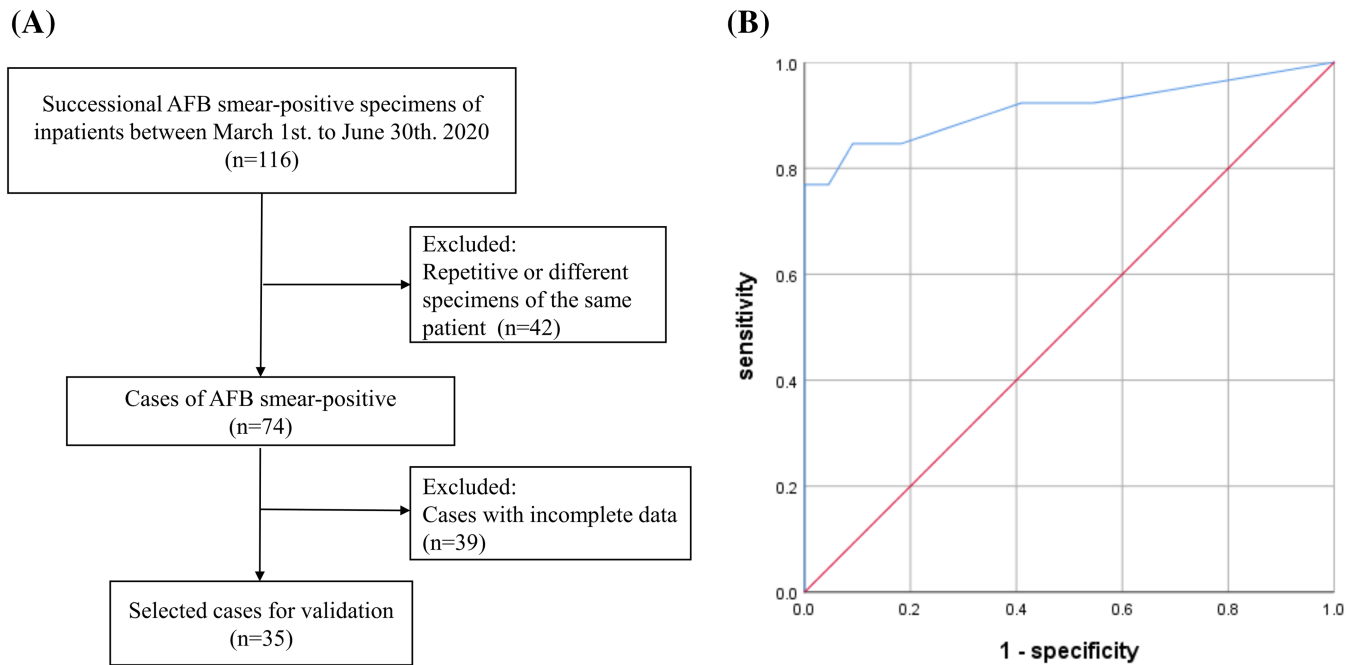
(A)

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┌─────────────────────────────────┐
│ Successional AFB smear-positive  │
│ specimens of inpatients between  │
│ March 1st. to June 30th. 2020    │
│          (n=116)                 │
└─────────────────────────────────┘
        │
        │        ┌──────────────────────────┐
        │───────▶│ Excluded:                │
        │        │ Repetitive or different  │
        │        │ specimens of the same    │
        │        │ patient  (n=42)          │
        │        └──────────────────────────┘
        ▼
┌─────────────────────────────────┐
│ Cases of AFB smear-positive      │
│          (n=74)                  │
└─────────────────────────────────┘
        │
        │        ┌──────────────────────────┐
        │───────▶│ Excluded:                │
        │        │ Cases with incomplete    │
        │        │ data (n=39)              │
        │        └──────────────────────────┘
        ▼
┌─────────────────────────────────┐
│ Selected cases for validation    │
│          (n=35)                  │
└─────────────────────────────────┘
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(B)

FIGURE 5 External validation of the predictive model. (A) Screening process of cases for validation. (B) A ROC curve to evaluate the predictive effect of the model on external data, with an AUC of 0.913, $P < 0.001$. Abbreviations: AFB, acid-fast bacillus; QFT, QuantiFERON tuberculosis

feature predisposing it to infection.[24] But only the right middle lobe involvement was proved to be an independent risk factor for NTM in our study.

In clinical, for patients with cough and expectoration, especially with haemoptysis and the characteristics of mycobacterium in chest CT, a rapid test of AFB smear of sputum would be the primary test to make a clear diagnosis. Patients with positive AFB smear results may be directly diagnosed as PTB and receive a long-term anti-tuberculosis treatment, in the absence of mycobacterium 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.[25] 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.[26] With the rapid development of molecular diagnostics of tuberculosis, GeneXpert has been used in the diagnosis of tuberculosis infection. Unfortunately, only a few patients in this study were tested for GeneXpert, and it was not really popularized. Therefore, developing a diagnostic model based on available clinical features that could help diagnose NTM-LD in a rapid and effective way would directly affect the administration of anti-tuberculosis treatment and the prognosis of the disease for patients with AFB smear-positive results. On the basis of this situation, we analysed many accessible clinical characteristics and tests in AFB smear-positive patients, and compared NTM with PTB patients, to find out the possible risk factors for NTM. Through multivariate regression analysis, we established a predictive model combining four independent risk factors, which showed a prominent high level of sensitivity and specificity. Moreover, through the internal verification of this model by a calibration curve and external verification for another group of patients with confirmed mycobacterial diseases in our institution, the model showed efficiency and ease of application.

In conclusion, female patients, bronchiectasis, negative test for QFT, and the involvement of the right middle lobe in chest HRCT were independent risk factors for fast prediction of NTM-LD in AFB smear-positive patients. If a patient shows all the above characteristics, it would suggest a high possibility of NTM infection. It is necessary to wait for the mycobacterial culture results and to conduct the identification and drug susceptibility test of mycobacterium. In that way, the adverse reaction related to anti-tuberculosis treatment and the number of drug-resistant tuberculosis would be reduced, and the unnecessary economic burden could also be lessened in most cases. Our findings may have important 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, not all patients in our institution had a QFT result, so as the total number of cases observed in this study was insufficient. And the data were acquired from a single centre, which might have a certain impact on the representative of the results. This study lacked the identification of NTM species, and did not distinguish the positive degree of AFB from + to 4+. Even though we have drawn some firm conclusions, established and validated a fairly effective model. We will furtherly verify it in subsequent clinical practice and in more centres.

CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

ETHICS STATEMENT
The authors are accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved. The ethics committee of Shanghai Pulmonary Hospital approved the research (No. K20-429).

AUTHOR CONTRIBUTIONS
(I) All authors did the conception and design. (II) H. Li and J. Xu did the administrative support. (III) X. Chen and Y. Zhou did the provision of study materials or patients. (IV) X. Chen did the collection and assembly of data. (V) X. Chen and Y. Zhou did the data analysis and interpretation. (VI) All authors did the manuscript writing. (VII) All authors did the final approval of manuscript.

DATA AVAILABILITY STATEMENT
All data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.