

ORIGINAL RESEARCH

# The Incidence, Risk Factors, and Predictive Model of Obstructive Disease in Post-Tuberculosis Patients

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**Objective:** To assess the incidence and risk factors of tuberculosis-associated obstructive pulmonary disease (TOPD) in individuals with treatment-naive pulmonary tuberculosis (PTB) and develop a predictive model to enhance its management.

**Methods:** The incidence of TOPD among patients with treatment-naive PTB in Xinjiang, China, was followed up for one year. Patient characteristics, such as demographics, medical histories, laboratory test results, lung radiological evidence, and pulmonary function, were collected upon hospital admission and throughout follow-up visits. Risk factors associated with TOPD were evaluated by multivariate logistic regression analysis, and then a predictive model was established using LASSO regression.

**Results:** Of the 159 included patients, 69 (43.4%) developed TOPD during the follow-up period. Multivariate regression analysis identified age, body mass index, ESR, and symptom duration as significant risk factors. Subsequently, a model formula was derived from these factors to predict TOPD. Utilizing a cut-off value of 0.435, the model demonstrated a sensitivity of 89% and a specificity of 83%. **Conclusion:** In Xinjiang, the prevalence of TOPD appears notably high among treatment-naive PTB patients. Our findings, such as the risk factors and predictive model, may facilitate the early detection and improved interventions for TOPD among patients with PTB, potentially leading to better patient outcomes.

**Keywords:** tuberculosis, tuberculosis-associated obstructive pulmonary disease, risk factor, predictive model, diagnosis

# **Background**

Tuberculosis (TB) remains a serious public health threat worldwide. According to the Global Tuberculosis Report (2023), an estimated 10.6 million individuals developed TB, and 1.3 million people died from the disease in 2022. Pulmonary TB (PTB) is thought to be curable when an effective regimen is administered. However, its burden does not end with successful treatment. In fact, after successful mycobacterial eradication, many individuals will suffer from post-TB lung diseases, such as obstructive airway disease, bronchiectasis, cavitation, and TB-destroyed lung. These complications have a broad impact on TB-affected communities, including functional impairment, disability, and reduced quality of life. Meanwhile, the number of survivors after TB treatment is significant, with an estimated 155 million individuals treated between 1980 and 2019 surviving in 2020.

Chronic obstructive pulmonary disease (COPD) has many risk factors, such as age, body mass index (BMI), level of education completed, hospitalization with a respiratory problem during childhood, cigarette exposure, TB, and a family history of COPD.<sup>6</sup> Remarkably, COPD is four times more common among those with previous TB disease (25.7% vs 8.3% without previous TB).<sup>7</sup> Previous TB is also associated with airflow obstruction<sup>8</sup> and should be considered as a potentially important cause of obstructive diseases, particularly where PTB is common. Such an association has raised awareness of a special phenotype of COPD, which is known as a sequela and complication following TB, termed tuberculosis-associated obstructive pulmonary disease (TOPD). Recently, it has been recommended that long-term health consequences should be assessed, but robust evidence of TOPD remains lacking, including its prevalence and risk factors. Therefore, further investigation is required.

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The aim of the present study was to assess the prevalence of TOPD, determine its risk factors, and develop a predictive model for TOPD incidence, which could help implement early interventions and reduce the impact of TBassociated respiratory disability.

#### **Methods**

## **Ethics**

This prospective study was conducted in Shache, Shule, and Yecheng across Xinjiang, China. Ethical approval was obtained from the Ethics Committee of the Fourth Affiliated Hospital of Xinjiang Medical University (Approval No. 2020XE0118), all methods were carried out in accordance with relevant guidelines and regulations. This study strictly adhered to the principles outlined in the Declaration of Helsinki. Participants' personal information was anonymized prior to analysis. Prior to enrollment, all participants, or their guardians if the participants were younger than 18 years old, provided written informed consent.

## Subjects

Between November 2021 and October 2022, treatment-naive PTB patients were consecutively enrolled. All included patients were followed up for one year, with TOPD and mortality being the primary outcomes of interest. PTB diagnosis was based on a combination of symptoms, medical history, imaging findings, and microbiological evidence (including smear, nucleic acid amplification tests, and culture). TOPD is characterized by the presence of irreversible airflow obstruction (the ratio of the forced expiratory volume in the first second (FEV1) and the forced vital capacity (FVC) is less than 70%; ie, FEV1/FVC<70%).9

The inclusion criteria were individuals who were treatment-naive for PTB, aged between 15 and 75 years, and nonsmokers. Additionally, the included individuals had not used steroids or other immunosuppressive agents during the past month. The exclusion criteria for this study were as follows: individuals with underlying lung diseases such as asthma, interstitial fibrosis, or lung cancer, as well as those undergoing mechanical ventilation. Moreover, patients with other medical conditions, such as malignancies, autoimmune diseases, kidney diseases, coronary artery disease, heart failure, cerebral hemorrhage, and mental disorders, were excluded. Individuals who did not provide consent to participate in this study or who were lost to follow-up were also excluded.

#### Data Collection

The data collected encompass a wide array of variables, including the following: 1) demographics; gender, age, and BMI; 2) medical history: occupation, educational level, medical ID, date of admission, and smoking history; 3) signs: body temperature, respiration rate, and pulse rate; 4) symptoms: cough, sputum, chest tightness, chest pain, asthma, hemoptysis, night sweats, fatigue, and weight loss; 5) underlying diseases: chronic bronchitis, bronchiectasis, coronary heart disease, and hypertension; 6) laboratory examinations: complete blood count, erythrocyte sedimentation rate (ESR), and albumin.

## Statistical Analysis

Statistical analysis was performed using SPSS (version 27.0) and R packages (version 4.1.2). Quantitative data were presented as counts and percentages, while continuous data were described using the mean and standard deviation. All patients were included for training, and 30% of them were randomly selected for validation. For continuous data, the t-test or the Mann–Whitney U-test was utilized to compare data between the two groups, while the Chi-squared test was employed for categorical data. Variable selection for the predictive model was executed using LASSO regression, and then the model was constructed using multivariate logistic regression. The predictive model's accuracy was evaluated by receiver operating characteristic (ROC) analysis. The calibration was assessed by the Hosmer-Lemeshow goodness-of-fit test, and calibration plots were used for visualization. Additionally, the clinical utility of the model was assessed using decision curve analysis (DCA). A P-value of less than 0.05 was considered statistically significant.

## Results

## **Baseline Characteristics**

Of the 301 admitted PTB patients, 159 treatment-naive individuals were included in this study, with the others excluded due to loss of follow-up. Among the included patients, 85 (53.5%) were male and 74 (46.5%) were female. After admission, all patients underwent standardized anti-TB therapy with the 2HRZE/4HR regimen (an initial 2-month intensive phase with Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol, followed by a 4-month continuation phase with Isoniazid and Rifampicin). After one year of follow-up, no deaths were found, 69 patients (43.4%) were diagnosed with TOPD, and 90 patients (56.6%) were classified as the non-TOPD group as they did not exhibit airflow obstruction. The prevalence of TOPD was slightly higher among the male patients compared to the female patients. Age distribution analysis revealed that although both patients with TOPD and those without were mainly in the 40–70-year range, there was a statistically significant age difference observed between the two groups (*P*<0.05). The majority of the included patients belonged to the Uyghur ethnicity (98.7%), predominantly had a farming occupation (93.7%), and had a notably low educational level (primary school or below, 88.7%). Additionally, approximately 17% of the patients hailed from low-income households, earning less than 10,000 yuan annually. Table 1 shows the baseline characteristics of the included PTB patients.

## Univariate Analysis

No significant differences in height, weight, duration, BMI, albumin, white blood cell count, neutrophil count, lymphocyte count, lesion site, or pleural effusion were observed between the TOPD and non-TOPD groups (Table 2). However, significant differences were found for ESR (TOPD: 45.28 mm/h; non-TOPD: 40.87 mm/h; P<0.05), symptom duration (TOPD: 2.2 months; non-TOPD: 1.75 months; P<0.05), cavitation presence (TOPD: 22%; non-TOPD: 10%; P<0.05), acid-fast bacilli smear positivity (TOPD: 30.4%; non-TOPD: 14.4%; P<0.05), cough (TOPD: 25.6%; non-TOPD: 43.4%; P<0.01), sputum production (TOPD: 25.6%; non-TOPD: 42.0%; P<0.05), and dyspnea (TOPD: 27.8%; non-TOPD: 14.5%; P<0.05). Additionally, after one year of follow-up, the average FEV1% predicted decreased by 16.24%, and the FEV1/FVC ratio decreased by 12.3% in the TOPD group compared to their initial values.

Table I The Clinical Characteristics of the Patients Included in This Study

Variable		Cases (n)	Patients		P
			Non-TOPD	TOPD	
Sex	Male	85 (53.5%)	46 (51.1%)	39 (56.5%)	0.498
	Female	74 (46.5%)	44 (48.9%)	30 (43.5%)	
Ethnicity	Han	2 (1.3%)	2 (2.2%)	0 (0%)	0.213
	Uyghur	157 (98.7%)	88 (97.8%)	69 (100%)	
Marital status	Married	130 (81.8%)	71 (78.9%)	59 (85.5%)	0.284
	Other	29 (18.2%)	19 (21.1%)	10 (14.5%)	
Age, years	0-40	30 (17.8%)	26 (28.9%)	4 (5.8%)	<0.05
	41–65	85 (53.4%)	43 (47.8%)	42 (60.9%)	
	65	30 (27.8%)	21 (23.3%)	23 (33.3%)	
Occupation	Farmer	149 (93.7%)	81 (90%)	69 (100%)	0.063
	Worker	I (0.6%)	1 (1.1%)	0 (0%)	
	Student	5 (3.1%)	4 (4.4%)	0 (0%)	
	Other	4 (2.5%)	4 (4.4%)	0 (0%)	
Education	Primary school or below	141 (88.7%)	76 (84.4%)	65 (94.2%)	0.219
	Junior school	6 (3.8%)	5 (5.6%)	I (I.4%)	
	High school	10 (6.3%)	7 (7.8%)	3 (4.3%)	
	College	2 (1.3%)	2 (2.2%)	0 (0%)	
Family income (yuan)	>10,000	132 (83.0%)	72 (80%)	60 (87.0%)	0.247
	≤10,000	27 (17.0%)	18 (20%)	9 (13%)	

**Abbreviation**: TOPD, tuberculosis-associated obstructive pulmonary disease.

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Table 2 Comparison of Clinical Data of Patients with and without TOPD

Variable	Non-TOPD	TOPD	P	
Height (cm)	165.11±6.31	164.46±5.66	0.504	
Weight (kg)	62.01±10.18	59.94±9.90	0.200	
Duration (months)	1.75±1.17	2.20±1.44	0.040	
Body mass index (kg/m <sup>2</sup> )		22.74±3.55	22.12±3.33	0.263
Erythrocyte sedimentation rate	40.87±27.07	45.28±24.88	0.360	
Albumin (g/L)	39.28±4.98	39.66±2.83	0.457	
White blood cell count (10 <sup>9</sup> /L)		12.00±48.01	15.02±67.12	0.745
Neutrophil count (%)	· · ·			0.632
Lymphocyte count (10 <sup>9</sup> /L)		1.86±0.57	1.82±0.59	0.687
Chest imaging				
Unilateral	Left	16 (10.1%)	9 (13%)	0.791
	Right	21 (13.2%)	17 (10.7%)	
Bilateral	53 (33%)	43 (27%)		
Cavitation		9 (10%)	15 (22%)	0.040
Pleural effusion		5 (5.5%)	4 (5.8%)	0.948
Microbiological examination				
Acid-fast bacilli smear		13 (14.4%)	21 (30.4%)	0.044
Mycobacterial culture	25 (27.8%)	18 (26.1%)		
Nucleic acid amplification te	52 (57.8%)	30 (43.5%)		
Pulmonary function				
FEVI% pred (First)		86.41±26.28		
FVC% pred (First)		91.91±28.22		
FEVI/FVC (First)		0.730±0.04		
FEV1% pred (Second)		70.17±22.50		
FVC% pred (Second)		87.73±24.58		
FEVI/FVC (Second)		0.610±0.07		
FEV1% predicted (Change be		16.24±31.47		
FEVI/FVC (Change between		0.123±0.08		
Symptoms				
Cough	23 (25.6%)	34 (43.4%)	0.002	
Sputum production	23 (25.6%)	29 (42.0%)	0.028	
Wheezing	25 (27.8%)	10 (14.5%)	0.045	
Chest tightness	12 (13.3%)	8 (11.6%)	0.743	
Loss of appetite	15 (16.7%)	8 (11.6%)	0.367	
Night sweat	40 (44.4%)	28 (40.1%)	0.625	

**Abbreviations**: FEVI, forced expiratory volume in the first second; FVC, forced vital capacity; TOPD, tuberculosis-associated obstructive pulmonary disease.

# Multivariate Regression Analysis

Multivariate regression analysis revealed the following: 1) Compared to patients younger than 40 years, those aged 40–65 years and those older than 65 years had significantly higher odds ratios (ORs), indicating that older age is significantly associated with the occurrence of TOPD. 2) A positive ESR assay result indicated an increased risk of TOPD (OR=1.236). 3) A longer symptom duration was associated with an increased risk of TOPD (OR=1.036). 4) A higher BMI was associated with a reduced occurrence of TOPD (OR=0.982), although this reduction was not statistically significant. The detailed results are presented in Table 3.

# Development and Assessment of the TOPD Predictive Model

To refine the variable selection, LASSO regression analysis was conducted on 25 variables, and four variables (age, BMI, ESR, and symptom duration; Table 3) were identified under Log ( $\lambda$ )=0.047 (Figure 1). The predictive model for TOPD

**Table 3** The Results of Multivariate Logistic Regression Analysis, LASSO Regression Analysis, and Variable Assignment for the Predictive Model of TOPD

Variable	Subgroup	β	OR (95% CI)	P	Regression Coefficients	Assigned Value
Age, years	<40	NA	NA	NA	0.088	<40=I
	40–65	0.336	1.4 (1.121, 1.748)	0.003		41-65=2
	>65	0.328	1.388 (1.131, 1.704)	0.002		≥65=3
Body mass index		-0.018	0.982 (0.961, 1.004)	0.106	-0.0005	22.47±3.46
Erythrocyte sedimentation rate, mm/h	>20	0.212	1.236 (1.028, 1.485)	0.026	0.1275	<20=1
						≥20=2
Symptom duration		0.036	1.036 (0.978, 1.0980	0.228	0.015	1.94±1.31
Lesion					NA	0=0, I=1, 2=2

Abbreviations: CI, confidence interval; OR, odds ratio; TOPD, tuberculosis-associated obstructive pulmonary disease.

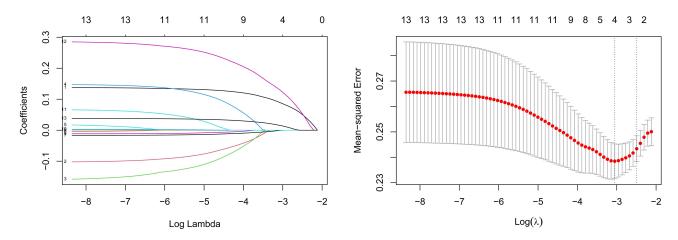
was developed using a nomogram (Figure 2), with the following formula: Age (years) $\times$ 0.66+BMI (kg/m<sup>2</sup>) $\times$ (-0.07)+ESR (mm/h) $\times$ 1.15+Symptom duration (days) $\times$ 0.18.

In the training group, the model achieved an ROC curve area of 0.70 (95% confidence interval (CI): 0.61–0.77; Figure 3 and Supplementary Table 1). The optimal cut-off value for predicting TOPD risk was 0.435, with a sensitivity of 72% (47/69) and a specificity of 62% (67/90). In the validation group, the model achieved an area under the ROC curve of 0.90 (95% CI: 0.81–0.99; Figure 3). It predicted 16 out of 18 actual cases of TOPD, resulting in a sensitivity of 89%; and it correctly identified 24 out of 29 non-cases, with a specificity of 83%.

The calibration curves for both the training and validation sets showed strong consistency, with no significant differences (*P*>0.05) between the predicted and actual values, indicating good predictive accuracy (<u>Supplementary Figure 1</u>). The DCA results demonstrated higher clinical applicability and a broader effective prothrombin range (<u>Supplementary Figure 2</u>). The clinical impact curve confirmed a high clinical prediction efficiency (<u>Supplementary Figure 3</u>).

#### **Discussion**

In Xinjiang, China, TOPD has a high prevalence and is common in patients with PTB. An advanced age, abnormal ESR, and longer symptom duration were identified as key factors that significantly increase the risk of TOPD following PTB treatment. Conversely, a higher BMI showed a trend towards reducing this risk. These interesting findings could help physicians better assess and stratify patients with different risks for TOPD. In addition, a nomogram predictive model was developed and validated with high accuracy, demonstrating a sensitivity of 89% and a specificity of 83% for TOPD prediction. Further analyses, including calibration curves, DCA, and the clinical impact curve affirmed the model's robust



**Figure 1** LASSO regression modeling for optimization of predictive variables (Log $\lambda$  = 0.047).

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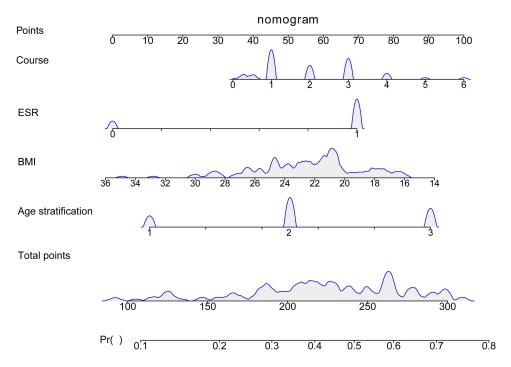


Figure 2 Clinical model column chart for tuberculosis-associated obstructive pulmonary disease (TOPD). The predictive model for TOPD was developed using a nomogram, and the formula is as follows: Age (years)×0.66+BMI (kg/m²)×(-0.07)+ESR (mm/h)×1.15+Symptom duration (days)×0.18.

Abbreviatiuons: BMI, body mass index; ESR, erythrocyte sedimentation rate.

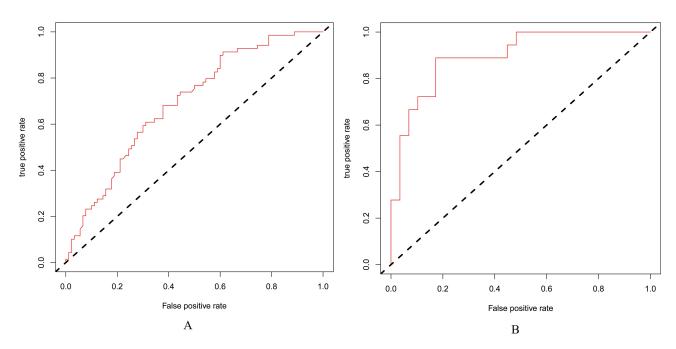


Figure 3 Receiver operating characteristic (ROC) curves for the training and validation groups. The area under the ROC curve: (A) training group, 0.70 (95% CI: 0.61–0.77); (B) validation group, 0.90 (95% CI: 0.81–0.99).

predictive performance and clinical applicability. This predictive tool supports early identification and improved intervention for at-risk individuals, enhancing clinical decision-making and emphasizing the need for personalized management strategies to mitigate chronic respiratory sequelae.

In a previous Chinese cohort, 21.3% of residents with post-TB were found to have airflow obstruction. <sup>10</sup> After adjusting for confounding factors, post-TB was confirmed to significantly increase the odds of developing airflow

obstruction. <sup>10</sup> During our follow-up study, the incidence of TOPD among treatment-naive PTB patients was notably high at 43.4%. This rate exceeds both the global prevalence of COPD in the general population (approximately 10.3%) and the reported incidence of obstructive disorders following TB (17.8%). <sup>11</sup> Importantly, all patients in our cohort were nonsmokers, thereby eliminating smoking as a major confounding factor for COPD. This underscores the suggestion that post-TB itself may constitute a significant risk factor for TOPD, particularly among non-smokers. Previous research also supports our findings, with a systematic review indicating a positive correlation between PTB and chronic airflow obstruction in nearly 90% of studies, <sup>12</sup> and a meta-analysis demonstrating a threefold higher odds of COPD development after PTB. <sup>13</sup>

An advanced age emerged as a crucial risk factor contributing to the development and progression of TOPD. Individuals aged 40–65 years and those 65 years and above exhibited significantly higher ORs for TOPD compared to those under 40 years old. These findings highlight age as a pivotal determinant for TOPD, with a substantial proportion of patients (53.4%) falling within the 41–65-year age range and 27.8% aged 65 and above. This age distribution parallels findings observed for COPD, where an advancing age escalates disease risk. Numerous studies consistently underscore age as a primary factor influencing COPD prevalence, particularly among individuals older than 70 years. Physiologically, aging exacerbates airway inflammation and fibrosis, which worsen disease severity. Our logistic regression analysis corroborates these findings, showing significantly elevated ORs for TOPD in the 40–65-year and 65+-year age groups, underscoring age as a critical risk factor for both the onset and progression of TOPD. Furthermore, the predominance of males among TOPD patients may be attributed to higher-risk behaviors and occupational exposures, aligning with our observation linking TB history to COPD onset in adults older than 40 years.

BMI plays a protective role against TOPD, with a higher BMI associated with a reduced risk of TOPD occurrence. However, it is acknowledged that the BMI has a dual impact on TOPD. Studies have consistently shown that a low BMI significantly predicts airway obstruction and COPD risk, while a higher BMI may mitigate risks associated with TB and post-TB impairment. Our multivariate analysis confirmed a negative correlation between BMI and TOPD risk, suggesting that a higher BMI protects against the onset of TOPD, consistent with findings from a previous systematic review. Conversely, our study highlights an increased risk of TOPD among individuals with a low BMI, which underscores how a low BMI can exacerbate unfavorable outcomes and adversely affect treatment. These insights support the pivotal role of BMI in predicting TOPD occurrence, providing dual perspectives: 1) the protective effects of a higher BMI on TOPD incidence, and 2) how a lower BMI is associated with an increased risk of developing TOPD.

An elevated ESR can predict TOPD risk. Previously, Oh et al<sup>21</sup> demonstrated a strong correlation between elevated ESR levels and TOPD risk. ESR is closely associated not only with the development, severity, and prognosis of COPD but also with assessing the inflammatory status and therapeutic efficacy in PTB, highlighting its pivotal role in monitoring the development of TOPD. For example, the ESR and serum EPO levels can reflect the severity of COPD in elderly patients.<sup>22</sup> Moreover, an elevated ESR at the end of treatment can be used as a marker to identify spinal TB patients with a poor prognosis.<sup>23</sup> Overall, the ESR serves as a critical tool for evaluating the inflammatory status, therapeutic efficacy, and prognosis in PTB patients.

Symptom duration is a critical determinant of TOPD incidence, with a longer duration significantly increasing TOPD risk. Timely diagnosis and treatment are essential in PTB management to mitigate disease progression, reduce lung damage, and improve treatment outcomes. Typically, a prediagnostic period of more than 14 days is defined as a diagnostic delay. Our findings reveal that patients who developed TOPD experienced an average illness duration of 2.2 months, compared to 1.75 months for those without TOPD, underscoring the role of diagnostic delays in the progression of TOPD. A prolonged symptom duration heightens not only the risk of lung damage but also complicates pulmonary conditions. Persistent inflammation and immune responses may accelerate airway remodeling, thus impacting lung function. Additionally, symptom duration directly influences PTB treatment outcomes. For example, significant associations have been observed between a delayed diagnosis and increased TB severity, as well as between diagnostic delays and a poorer prognosis. Addressing these delays is essential, as it could improve TB management by reducing lung function impairment and ultimately enhancing long-term patient outcomes and quality of life.

The development of a predictive model for TOPD represents a significant advancement in clinical practice, offering a robust tool for early identification and targeted intervention among treatment-naive PTB patients. The model demonstrates high accuracy in predicting TOPD risk, making it an invaluable asset for clinicians. Clinically, the

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predictive model's utility extends beyond risk stratification. It empowers healthcare providers to initiate timely interventions aimed at mitigating the progression of TOPD, thereby potentially reducing long-term respiratory disability and improving overall patient outcomes. Moreover, validation of the model through rigorous statistical methods and its performance in both the training and validation cohorts underscore its reliability and generalizability. Calibration plots and DCA further validate its clinical applicability, demonstrating that its use translates into tangible benefits for patient care. Clinicians can utilize the nomogram derived from this model to calculate individualized risk scores, enhancing shared decision-making processes with patients regarding treatment strategies and follow-up plans. In short, the predictive model for TOPD addresses a critical gap in the management of PTB sequelae. However, future research should focus on validating and refining this model across diverse populations and healthcare settings to maximize its impact on global TB control efforts and to enhance patient care pathways.

Nevertheless, this study has several shortcomings, such as a small sample size, short follow-up duration, the absence of air pollution variables, and a notable proportion of participants lost to follow-up. These factors may have introduced significant bias, leading to inaccurate findings. To make an accurate predictive model, all patients were included in the training set. Therefore, external validation is lacking in this study, and only interval validation was provided. Additionally, the predictive model was developed based on this small sample size. Further optimization and validation across multiple populations are required to enhance the accuracy and generalizability of the predictive tool.

## **Conclusions**

This multicenter study sheds light on the clinicopathological characteristics of TOPD in treatment-naive PTB patients across Xinjiang, China. Through rigorous investigation and analysis, significant risk factors such as an advanced age, elevated ESR, and prolonged symptom duration were identified as key determinants of TOPD development. A predictive model incorporating these factors demonstrated high sensitivity and specificity for TOPD incidence, providing a valuable tool for early identification and targeted intervention. However, future research efforts should focus on refining the predictive model across diverse populations and healthcare settings to optimize management strategies and improve outcomes for PTB patients at risk of developing TOPD.

#### **Abbreviations**

TB, Tuberculosis; TOPD, tuberculosis-associated obstructive pulmonary disease; PTB, pulmonary tuberculosis; ESR, erythrocyte sedimentation rate; COPD, Chronic obstructive pulmonary disease; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; ROC, receiver operating characteristic; DCA, decision curve analysis; ORs, odds ratios; CI, confidence interval.

# **Data Sharing Statement**

The datasets generated and/or analysed during the current study are not publicly available due to limitations of ethical approval involving the patient data and anonymity but are available from the corresponding author on reasonable request.

# Ethics Approval and Informed Consent

Ethical approval was obtained from the Ethics Committee of the Fourth Affiliated Hospital of Xinjiang Medical University (Approval No. 2020XE0118), all methods were carried out in accordance with relevant guidelines and regulations. This study strictly adhered to the principles outlined in the Declaration of Helsinki. Prior to enrollment, all participants provided written informed consent.

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## **Disclosure**

The authors declare that they have no competing interests.

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