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Construction and Validation of a Predictive Model for Culture Results of Mycobacterium Tuberculosis in Superficial Lymph Nodes

Qian Li, Leipeng Ren, Weitong Wu, Dangze Sun, Lin Wei, Chao Ding, Peijia Luo

Department of Thoracic Surgery, Xi'an Chest Hospital, Xi'an, Shaanxi, 710100, People's Republic of China

Correspondence: Peijia Luo, Department of Thoracic Surgery, Xi'an Chest Hospital, Xi'an, Shaanxi, 710100, People's Republic of China, Email 1171185306@qq.com

Background: To establish and validate a nomogram for predicting the culture results of Mycobacterium tuberculosis in superficial lymph nodes.

Methods: The clinical data of patients with superficial lymph node tuberculosis admitted to Xi'an City Chest Hospital from November 23, 2018, to May 30, 2024, were selected and divided into a training set and a validation set according to a ratio of 7:3. Influencing factors were identified through multivariate logistic regression analyses. Using R version 4.3.2, we developed a predictive model and generated a nomogram based on this model. The performance of the nomogram was evaluated using receiver operating characteristic (ROC) curves, calibration curve analysis (CCA), and decision curve analysis (DCA).

Results: The positive rate of superficial lymph node tuberculosis culture was 23.0% (103/446). Multivariate Logistic regression analysis showed that anti-tuberculosis treatment duration (OR=0.98, 95% CI: 0.97 ~ 0.99), initial treatment or retreatment (OR=0.12, 95% CI: 0.05 ~ 0.28), and adenosine deaminase (OR=1.12, 95% CI: 1.03 ~ 1.22) were independent factors affecting the culture results of Mycobacterium tuberculosis in superficial lymph nodes. The areas under the ROC curves were 0.86 (95% CI: 0.82–0.91) for the training set and 0.89 (95% CI: 0.84–0.95) for the validation set. The P values of calibration curves were 1.000 and 0.961, respectively, and the predicted values were in good agreement with the actual values. The threshold probabilities of clinical decision curves were 3%~64% and 1%~68%, respectively.

Conclusion: The positive rate of Mycobacterium tuberculosis culture in superficial lymph nodes is low. The increase in retreatment patients and anti-tuberculosis treatment time are obstacle factors for Mycobacterium tuberculosis culture positivity, while an increase in adenosine deaminase is a promoting factor for Mycobacterium tuberculosis culture positivity. The nomogram model established based on these factors can be used to predict the results of Mycobacterium tuberculosis culture in superficial lymph nodes.

Keywords: superficial lymph node tuberculosis, mycobacterium culture, predictive model, nomogram

Introduction

In 2022, 7.5 million cases of TB (including new and recurrent patients) were reported to the global registry. Of these, 83% were pulmonary TB while 17% were extrapulmonary TB. Extrapulmonary tuberculosis is easily misdiagnosed and missed, with a significant number of patients remaining unreported and unmanaged. The actual incidence may, therefore, be higher.[1] Lymph node tuberculosis is a specific infectious disease that affects the lymph nodes throughout the body. It is caused by the invasion of Mycobacterium tuberculosis (MTB) into the lymph nodes, leading to proliferation or granulomatous inflammation of the lymph nodes and surrounding soft tissues. As the most common type of extra-pulmonary tuberculosis (EPTB),[2,3] superficial lymph node tuberculosis predominantly occurs in the cervical region but can also affect other areas such as the axilla and groin.[4,5] Mycobacterial culture and drug susceptibility testing are important diagnostic tools and reference indicators for tuberculosis.[6] However, they have limitations in superficial lymph node tuberculosis, including long culture duration and low positive rates (10.9%~32.0%).[7,8] Due to the limited sample size in some patients, there is an urgent need for a tool to identify those with a low probability of positive tuberculosis

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culture results. This will guide doctors in making informed decisions about whether to send samples for testing, thereby saving patients' time and effort, and reducing unnecessary testing.This study retrospectively collected clinical data from patients with superficial lymph node tuberculosis, explored independent factors influencing mycobacterial culture results, and established a nomogram model to provide better reference for drug resistance screening, developing personalized testing protocols, and preventing excessive testing in the clinical setting.

Methods and Materials

Sample Source

The clinical data of patients diagnosed with superficial lymph node tuberculosis, who received treatment at Xi'an Chest Hospital from November 23, 2018, to May 30, 2024, were collected for this study. Using R software version 4.3.2, patients were randomly sampled and divided into a training set and a validation set at a ratio of 7:3. The training set was further categorized based on whether Mycobacterium culture results were positive. The diagnostic criteria for superficial lymph node tuberculosis are as follows: 1. Confirmed cases: a) Histopathology showing granulomatous inflammation with or without caseous necrosis; b) Mycobacterium culture and species identification and/or any molecular biology test [including GeneXpert MTB/RIF (Cepheid, USA), fluorescent PCR (detecting insertion sequence IS6110 of the Mycobacterium tuberculosis complex, Zhongshan Da'an Company)] detecting Mycobacterium tuberculosis; 2. Clinical diagnosis cases: Superficial lymph node enlargement accompanied by immunological tests confirming MTB infection and effective diagnostic anti-tuberculosis treatment can clinically diagnose superficial lymph node tuberculosis.[9,10] Inclusion criteria: Patients meeting the diagnostic criteria for lymph node tuberculosis, with samples obtained and sent for mycobacterial culture during diagnosis and treatment. Exclusion criteria: Missing results for mycobacterial culture, T-lymphocyte analysis, or adenosine deaminase; concurrent malignancy or HIV; severe diseases of vital organs (such as severe liver dysfunction, severe malnutrition, and systemic lupus erythematosus).The flow chart of this study is presented in Figure 1.

Sample processing procedure: 1. Collect patient samples and perform pre-processing according to the reagent kit instructions. Tissue samples are homogenized using a tissue grinder and resuspended in saline; Purulence samples are resuspended in an appropriate amount of saline. 2. BACTEC MGIT 960: Take 2–3 mL of the pre-processed special sample, add 1–2 times the volume of 4% NaOH solution, vortex, then let stand for 15 minutes. Add PBS buffer to 45 mL, centrifuge and discard the supernatant, add 1 mL of PBS buffer, mix well, and let stand for 10 minutes. Take 0.5 mL and add it to the culture tube, place it in the BACTEC MGIT 960 incubator for cultivation. Positive results are confirmed by acid-fast staining and MTB antigen testing.[11]

Data Collection

Clinical data of patients were collected, including: (1) demographic characteristics: gender, age, body mass index (BMI), smoking, and drinking habits. (2) Tuberculosis-related data: course of disease, duration of anti-tuberculosis treatment, whether to undergo surgery, comorbidity with tuberculosis, anti-tuberculosis treatment regimen (isoniazid, rifampicin, pyrazinamide, ethambutol standard quadruple [H-R-Z-E], non-[H-R-Z-E] regimen), initial treatment or retreatment (1. Initial treatment refers to patients who meet one of the following conditions: a. Patients who have never received tuberculosis anti-tuberculosis drug therapy; b. Patients taking conventional drugs with standard chemotherapy regimens who have not completed the course of treatment, or those who have received irregular chemotherapy for less than 1 month. 2. Retreatment refers to patients who meet one of the following conditions: a. Patients who have received anti-tuberculosis drug treatment for unreasonable or irregular tuberculosis > 1 month for a period of 1 month; b. Patients who have failed treatment and relapsed.[12] (3) Other disease data: diabetes, hepatobiliary conditions. (4) Laboratory examination data: anemia (adult male: <120g/L, adult female [not pregnant]: <110g/L; Pregnant women: <100g/L; Children 6 to 59 months old: <110 g/L, HCT <0.33), hypoproteinemia (total protein < 60.0 g/L, albumin < 25 g/L), adenosine deaminase (ADA), T lymphocyte subset analysis (total T lymphocytes CD3+, T helper CD3+CD4+, T suppressor lymphocyte CD3+CD8+, T helper/T suppressor cells).[13] (5) The number and type of lesion samples (Purulence samples and granulation tissue).

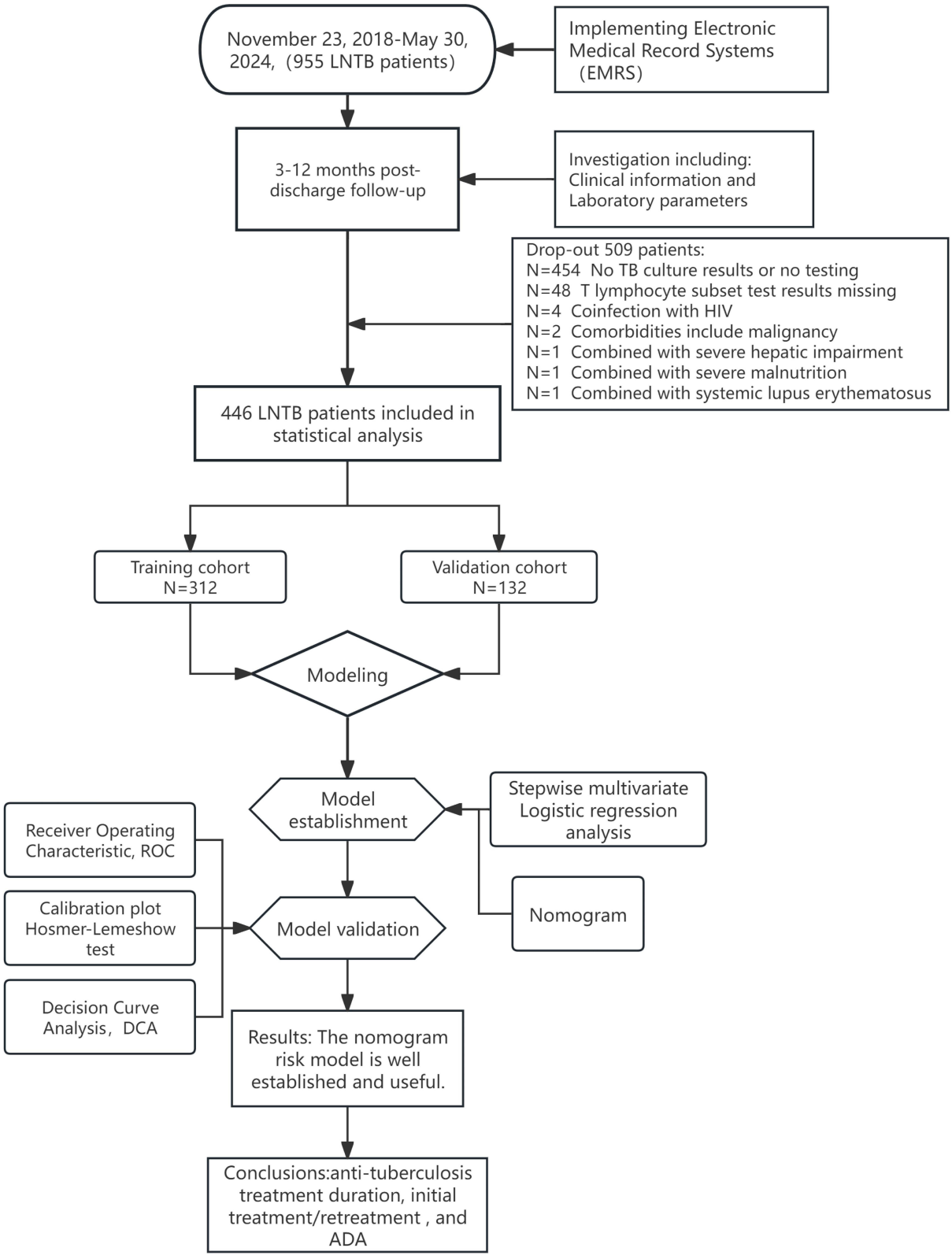

Figure 1 Study flow chart of patient inclusion.
Abbreviations: LNTB, Lymph Node Tuberculosis; TB, Tuberculosis; ADA, Adenosine deaminase; HIV, Human Immunodeficiency Virus.

Statistical Analysis

All statistical analyses were performed with SPSS 27.0 and R software, version 4.3.2. Normally distributed quantitative data are characterized as "Mean ± Standard Deviation [M ± SD]", with intergroup differences being assessed through the *t*-test. Skewed distribution quantitative data are represented by "Median (Quartiles) [M(Q_{25}, Q_{75})]", and intergroup differences are evaluated by the Mann–Whitney rank sum test. Categorical variables are depicted by "Number, Percentage (%)", and intergroup differences are compared by the chi-squared test. For some independent variables with less than 5% missing data, single imputation is employed. Mycobacterium tuberculosis culture outcomes are the dependent variable, and Stepwise multivariate logistic regression analyses are conducted to identify independent influencing factors. The rms package creates nomograms and calibration curves for the predictive model. The model's discriminative power and calibration are appraised by ROC curves, Hosmer-Lemeshow tests or calibration curve plots, and clinical net benefit analysis (DCA). Bilateral testing is adopted, with $P<0.05$ considered statistically significant.

Results

Patient Characteristics

A total of 446 patients were included in this study. Of these, 343 cases were negative for etiology while 103 cases were positive, making the positive rate of etiology 23.0% (103/446). According to a 7:3 ratio, there were 312 cases in the training group and 134 cases in the validation group. No significant differences were found between the training and validation sets in terms of clinical characteristics and laboratory parameters ($P>0.05$), suggesting that the data from both sets are comparable and suitable for mutual validation (Table 1).

Table 1 Comparisons of Clinical Characteristics Between Training Set and Validation Set

Variables	Total (n = 446)	Validation set (n = 134)	Training set (n = 312)	Statistic	*P*
Age, Mean ± SD	36.38 ± 14.52	36.78 ± 14.88	36.20 ± 14.38	t=0.39	0.699
BMI, Mean ± SD	21.51 ± 3.00	21.75 ± 3.15	21.40 ± 2.93	t=1.12	0.262
Gender, n(%)				χ^2=0.69	0.408
Female	280 (62.78)	88 (65.67)	192 (61.54)		
Male	166 (37.22)	46 (34.33)	120 (38.46)		
Smoking, n(%)				χ^2=0.00	0.973
No	413 (92.60)	124 (92.54)	289 (92.63)		
Yes	33 (7.40)	10 (7.46)	23 (7.37)		
Drinking, n(%)				χ^2=0.39	0.532
No	440 (98.65)	131 (97.76)	309 (99.04)		
Yes	6 (1.35)	3 (2.24)	3 (0.96)		
Course of disease (months), M (Q_{25}, Q_{75})	4.00 (1.50, 12.00)	4.00 (1.50, 10.75)	4.00 (1.50, 12.00)	Z=−0.61	0.539
Anti-tuberculosis treatment (day), M (Q_{25}, Q_{75})	31.00 (16.00, 96.00)	28.00 (18.00, 92.25)	35.00 (15.00, 101.25)	Z=−0.35	0.725
Initial treatment or retreatment, n(%)				χ^2=1.15	0.283
Initial treatment	219 (49.10)	71 (52.99)	148 (47.44)		
Retreatment	227 (50.90)	63 (47.01)	164 (52.56)		
Adenosine deaminase (ADA), M (Q_{25}, Q_{75})	10.00 (8.00, 12.00)	10.00 (8.00, 12.00)	10.00 (8.00, 12.00)	Z=−0.56	0.572
Anti-tuberculosis treatment regimen (H-R-Z-E), n(%)				χ^2=2.91	0.088
Yes	160 (35.87)	56 (41.79)	104 (33.33)		
No	286 (64.13)	78 (58.21)	208 (66.67)		
Anemia, n(%)				χ^2=0.92	0.338
No	371 (83.18)	108 (80.60)	263 (84.29)		
Yes	75 (16.82)	26 (19.40)	49 (15.71)		
Hypoproteinemia, n(%)				χ^2=0.81	0.369
No	397 (89.01)	122 (91.04)	275 (88.14)		
Yes	49 (10.99)	12 (8.96)	37 (11.86)		

(*Continued*)

Table 1 (Continued).

Variables	Total (n = 446)	Validation set (n = 134)	Training set (n = 312)	Statistic	*P*
Comorbidity with tuberculosis, n(%)				χ^2=0.23	0.635
No	224 (50.22)	65 (48.51)	159 (50.96)		
Yes	222 (49.78)	69 (51.49)	153 (49.04)		
Diabetes, n(%)				χ^2=1.44	0.229
No	435 (97.53)	133 (99.25)	302 (96.79)		
Yes	11 (2.47)	1 (0.75)	10 (3.21)		
Hepatobiliary diseases, n(%)				χ^2=0.15	0.697
No	351 (78.70)	107 (79.85)	244 (78.21)		
Yes	95 (21.30)	27 (20.15)	68 (21.79)		
Operation, n(%)				χ^2=0.75	0.385
No	51 (11.43)	18 (13.43)	33 (10.58)		
Yes	395 (88.57)	116 (86.57)	279 (89.42)		
Sample type, n(%)				χ^2=2.18	0.336
Purulence samples	111 (24.89)	37 (27.61)	74 (23.72)		
Granulation tissue	200 (44.84)	53 (39.55)	147 (47.12)		
Purulence samples + Granulation tissue	135 (30.27)	44 (32.84)	91 (29.17)		
Number of samples, n(%)				χ^2=2.80	0.094
1	311 (69.73)	86 (64.18)	225 (72.12)		
2	135 (30.27)	48 (35.82)	87 (27.88)		
Total T lymphocytes CD3+, M (Q_{25}, Q_{75})	1109.00 (849.50, 1457.75)	1101.50 (799.50, 1499.00)	1119.50 (860.75, 1456.50)	Z=−0.23	0.821
T helper CD3+CD4+, M M (Q_{25}, Q_{75})	640.50 (469.00, 836.75)	632.50 (480.00, 816.75)	641.00 (465.50, 839.25)	Z=−0.20	0.844
T suppressor lymphocyte CD3+ CD8+, M (Q_{25}, Q_{75})	416.50 (303.25, 584.75)	413.00 (270.75, 587.25)	422.00 (310.75, 579.00)	Z=−0.70	0.486
T helper/T suppressor cells, M (Q_{25}, Q_{75})	1.53 (1.15, 2.02)	1.56 (1.22, 2.07)	1.51 (1.14, 2.01)	Z=−0.81	0.417

Notes: The normal range of each index: body mass index: 18.5~23.9kg/m^2, adenosine deaminase: 0~15u/l, total T lymphocytes CD3+: 690~2540 cells/ul, T helper lymphocytes CD3+CD4+: 410~1590 cells/ul, T inhibitory lymphocyte CD3+CD8+190~1140 cells/ul, T helper/T inhibitory cells: 1.40~2.00 cells/ul.

Characteristics Selection in the Training Set

In the training set, significant differences were observed between the culture-positive and culture-negative group in terms of variables such as age, course of disease, duration of antituberculosis treatment, initial treatment or retreatment, adenosine deaminase levels, and antituberculosis treatment regimens (P<0.05). However, there was no statistically significant difference in gender, BMI, smoking, drinking, anemia, hypoproteinemia, combined with other diseases such as tuberculosis, diabetes, history of liver and gallbladder disease, surgery, sample quantity and sample type, and T lymphocyte subsets (P>0.05) (Table 2).

Table 2 Characteristics Selection in Training Set

Variables	Total (n = 312)	Culture Negative (n = 242)	Culture Positive (n = 70)	Statistic	*P*
Age, Mean ± SD	36.20 ± 14.38	34.95 ± 13.06	40.53 ± 17.68	t=−2.45	**0.016**
Bmi, Mean ± SD	21.41 ± 2.94	21.48 ± 2.86	21.19 ± 3.21	t=0.71	0.478
Gender, n(%)				χ^2=0.74	0.391
Female	192 (61.54)	152 (62.81)	40 (57.14)		
Male	120 (38.46)	90 (37.19)	30 (42.86)		
Smoking, n(%)				χ^2=2.18	0.140
No	289 (92.63)	227 (93.80)	62 (88.57)		
Yes	23 (7.37)	15 (6.20)	8 (11.43)		
Drinking, n(%)				–	1.000
No	309 (99.04)	239 (98.76)	70 (100.00)		
Yes	3 (0.96)	3 (1.24)	0 (0.00)		

(*Continued*)

Table 2 (Continued).

Variables	Total (n = 312)	Culture Negative (n = 242)	Culture Positive (n = 70)	Statistic	*P*
Course of disease (months), M (Q_{25}, Q_{75})	4.00 (1.50, 12.00)	6.00 (2.00, 14.00)	1.50 (1.00, 2.00)	Z=−6.67	**<0.001**
Anti-tuberculosis treatment (day), M (Q_{25}, Q_{75})	35.00 (15.00, 101.25)	46.50 (18.00, 128.75)	19.00 (12.00, 29.75)	Z=−5.96	**<0.001**
Initial treatment or retreatment, n(%)				χ^2=65.57	**<0.001**
Initial treatment	148 (47.44)	85 (35.12)	63 (90.00)		
Retreatment	164 (52.56)	157 (64.88)	7 (10.00)		
Adenosine deaminase (ADA), M (Q_{25}, Q_{75})	10.00 (8.00, 12.00)	9.00 (8.00, 12.00)	11.00 (9.25, 13.75)	Z=−3.55	**<0.001**
Anti-tuberculosis treatment regimen (H-R-Z-E), n(%)				χ^2=4.87	**0.027**
Yes	104 (33.33)	73 (30.17)	31 (44.29)		
No	208 (66.67)	169 (69.83)	39 (55.71)		
Anemia, n(%)				χ^2=0.14	0.707
No	263 (84.29)	205 (84.71)	58 (82.86)		
Yes	49 (15.71)	37 (15.29)	12 (17.14)		
Hypoproteinemia, n(%)				χ^2=0.30	0.585
No	275 (88.14)	212 (87.60)	63 (90.00)		
Yes	37 (11.86)	30 (12.40)	7 (10.00)		
Comorbidity with tuberculosis, n(%)				χ^2=2.09	0.148
No	159 (50.96)	118 (48.76)	41 (58.57)		
Yes	153 (49.04)	124 (51.24)	29 (41.43)		
Diabetes, n(%)				χ^2=0.94	0.333
No	302 (96.79)	236 (97.52)	66 (94.29)		
Yes	10 (3.21)	6 (2.48)	4 (5.71)		
Hepatobiliary diseases, n(%)				χ^2=3.56	0.059
No	244 (78.21)	195 (80.58)	49 (70.00)		
Yes	68 (21.79)	47 (19.42)	21 (30.00)		
Operation, n(%)				χ^2=2.52	0.113
No	33 (10.58)	22 (9.09)	11 (15.71)		
Yes	279 (89.42)	220 (90.91)	59 (84.29)		
Sample type, n(%)				χ^2=2.49	0.288
Purulence samples	74 (23.72)	53 (21.90)	21 (30.00)		
Granulation tissue	147 (47.12)	119 (49.17)	28 (40.00)		
Purulence samples + granulation tissue	91 (29.17)	70 (28.93)	21 (30.00)		
Number of samples, n(%)				χ^2=0.56	0.453
1	225 (72.12)	177 (73.14)	48 (68.57)		
2	87 (27.88)	65 (26.86)	22 (31.43)		
Total T lymphocytes CD3+, M (Q_{25}, Q_{75})	1119.50 (860.75, 1456.50)	1131.00 (880.25, 1487.00)	1049.00 (744.00, 1363.50)	Z=−1.31	0.189
T helper CD3+CD4+, M M (Q_{25}, Q_{75})	641.00 (465.50, 839.25)	642.50 (477.75, 851.75)	613.50 (458.25, 814.50)	Z=−1.20	0.229
T suppressor lymphocyte CD3+ CD8+, M (Q_{25}, Q_{75})	422.00 (310.75, 579.00)	436.00 (311.00, 590.25)	379.50 (308.50, 545.75)	Z=−1.25	0.210
T helper/T suppressor cells, M (Q_{25}, Q_{75})	1.51 (1.14, 2.01)	1.50 (1.15, 1.97)	1.60 (1.09, 2.17)	Z=−0.54	0.589

Notes: Bold text indicates statistical significance with P<0.05, representing the variables that have been selected.

Risk Factors for Positive Tuberculosis Culture in the Training Set

The six variables that were statistically significant in the analysis of differences between groups were further included in the multivariate analysis. Stepwise multivariate logistic regression analysis showed that anti-tuberculosis treatment duration (OR=0.98, 95% CI: 0.97 ~ 0.99, P=0.002), initial treatment/retreatment (OR=0.19, 95% CI: 0.07 ~ 0.51, P<0.001), and adenosine deaminase (OR=1.12, 95% CI: 1.03 ~ 1.21, P=0.005) were independent risk factors affecting the culture results of Mycobacterium tuberculosis in superficial lymph nodes (Table 3).

Table 3 Stepwise Multivariate Logistic Analysis in the Training Set

Variables	β	S.E	Z	P	OR (95% CI)
Intercept	−1.05	0.51	−2.07	**0.038**	0.35 (0.13 ~ 0.95)
Initial treatment					1.00 (Reference)
Retreatment	−2.13	0.44	−4.86	**<0.001**	0.12 (0.05 ~ 0.28)
Anti-tuberculosis treatment (day)	−0.02	0.01	−3.09	**0.002**	0.98 (0.97 ~ 0.99)
Adenosine deaminase	0.12	0.04	2.78	**0.005**	1.12 (1.03 ~ 1.22)

Note: Bold text indicates statistical significance with P<0.05.

Construction, Evaluation, and Validation of the Nomogram

The nomogram prediction model (Figure 2) is developed through the analysis of three variables that exhibit statistically significant differences, determined by multivariate logistic regression analysis. Each variable's score can be determined using the score scale provided above the model. The likelihood of positive outcomes for superficial lymph node Mycobacterium tuberculosis culture can be inferred from the corresponding value on the total score scale along the prediction axis.

The training set's ROC curve has an area of 0.86 (95% CI: 0.82–0.91), sensitivity of 80% (75–85%), and specificity of 84% (76–93%). The area under the ROC curve for the validation set is 0.89 (95% CI: 0.84–0.95), with a sensitivity of 70% (61–79%) and specificity of 97% (91–100%), (Figure 3). According to the Hosmer-Lemeshow test, P-values are 1.000 (>0.05) for the training set and 0.961 (>0.05) for the validation set. In addition, CCA demonstrates a high level of consistency between the predicted and actual occurrences of Positive Mycobacterium Tuberculosis Culture in both sets (Figure 4).

Clinical Net Benefit: The clinical decision curve (DCA) plots threshold probability on the x-axis and net benefit rate on the y-axis. When the probability values of the DCA curves for both the training and validation populations (Figure 5) fall within the ranges of 3% to 64% and 1% to 68%, respectively, the predictive model demonstrates substantial clinical utility and offers a high net benefit to patients.

Discussion

Detection methods such as GeneXpert MTB/RIF, MTB fluorescent PCR, mycobacterial culture, and drug susceptibility testing can identify drug resistance in lymph node tuberculosis samples. These tests determine the sensitivity of MTB to anti-tuberculosis drugs, identifying drug-resistant MTB strains and guiding the development of effective chemotherapy regimens. In this study, the mycobacterial culture positive rate was 23.0% (103/446). However, the positive detection rate was influenced by numerous factors, which compromised both the culture success rate and the detection efficiency of

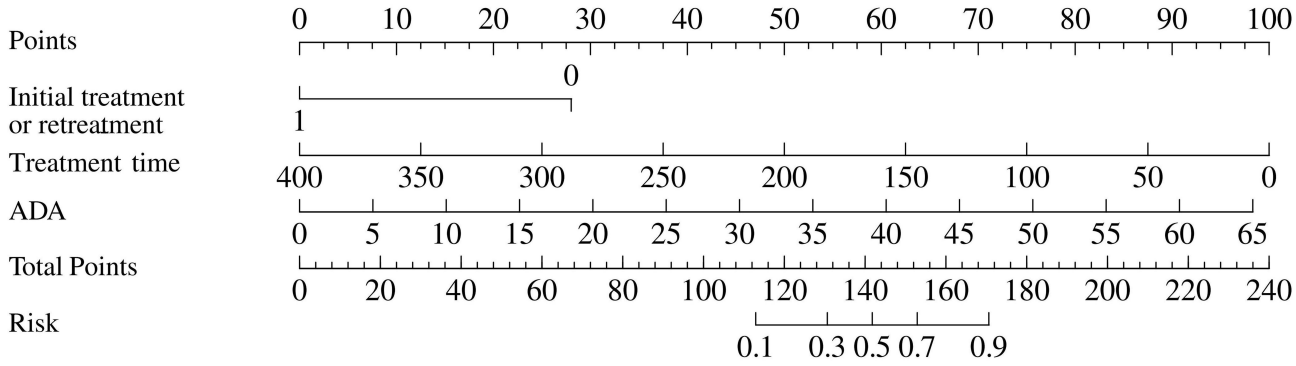

Figure 2 Nomogram to predict culture results of Mycobacterium in patients with superficial lymph nodes tuberculosis.
Notes: Initial treatment=0, retreatment=1; Treatment time: Anti-tuberculosis treatment duration; ADA, Adenosine deaminase levels.

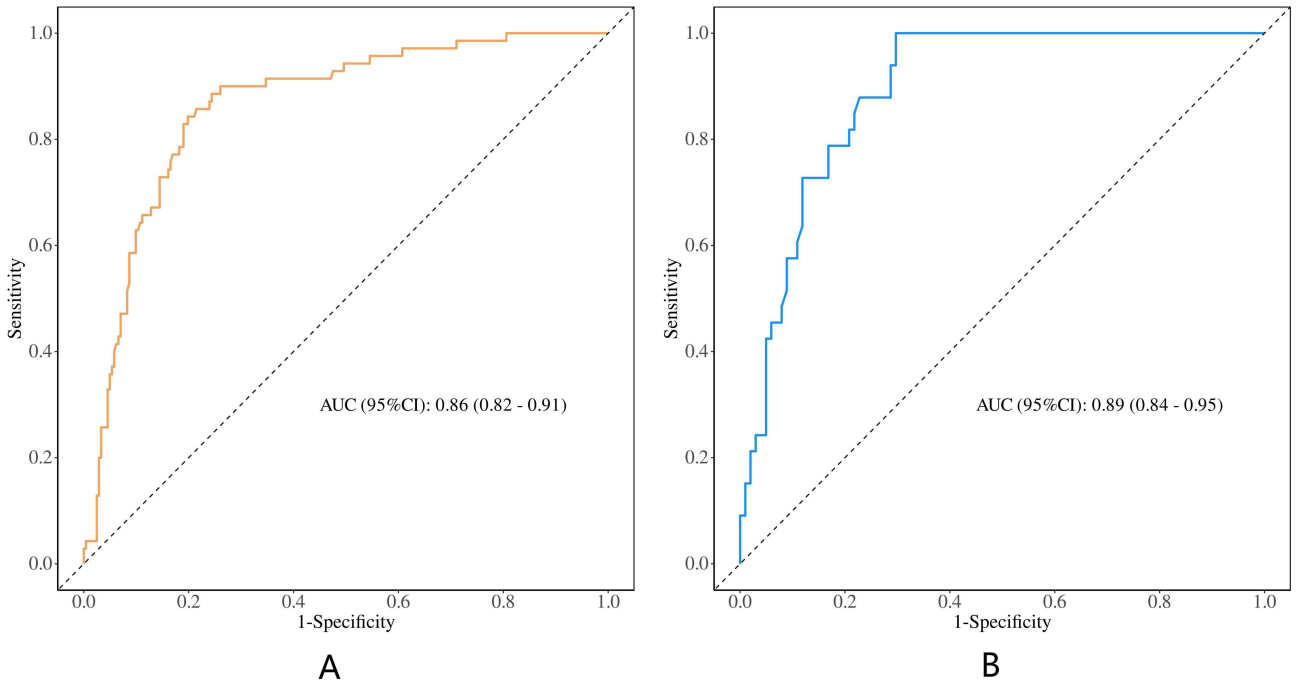

Figure 3 The AUCs of nomogram in the training set (**A**) and validation set (**B**).

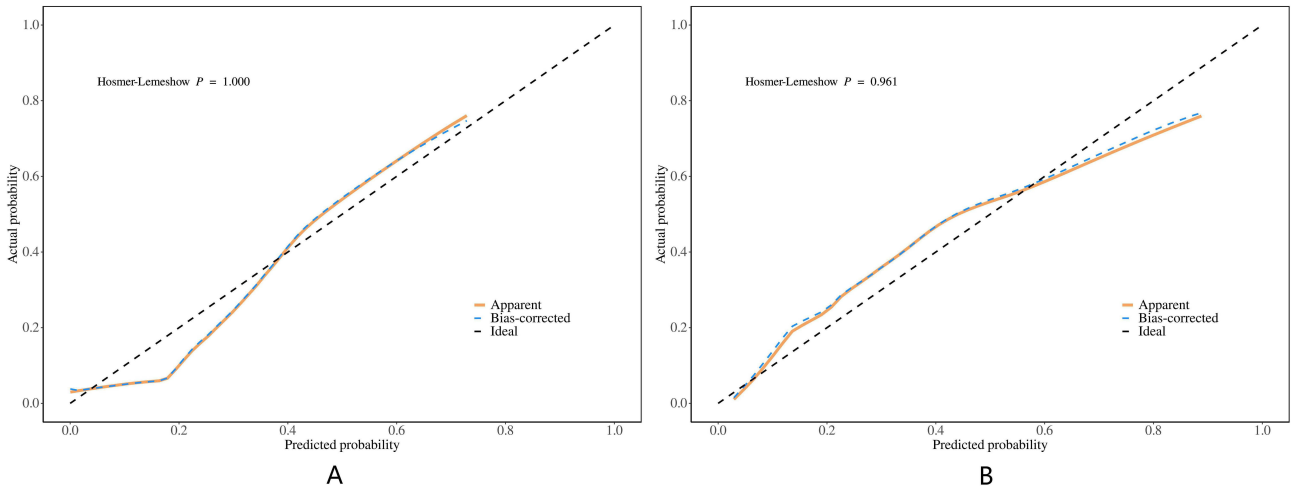

Figure 4 Calibration curves of the nomogram in the training set (**A**) and validation set (**B**).

Mycobacterium tuberculosis.[14] Consequently, investigating the factors affecting Mycobacterium tuberculosis culture in superficial lymph nodes and establishing predictive models is crucial for tailoring diagnosis and treatment plans.

In this study, three variables—anti-tuberculosis treatment duration, initial treatment/retreatment status, and adenosine deaminase level—were included to construct a nomogram prediction model. This model demonstrated good predictive power for the culture outcomes of Mycobacterium tuberculosis in superficial lymph nodes. Multivariate logistic regression showed that the probability of positive culture in patients experiencing a relapse was only 12% compared to those undergoing initial treatment. Dowling WB also found that patients without a history of tuberculosis had a significantly higher rate of positive mycobacterial cultures compared to those with a history of tuberculosis (OR=4.5, p=0.008). He also found that among patients with a history of tuberculosis, the more distant the previous episode, the more likely the current tuberculosis culture is to be positive, aligning with our study.[15] Anti-tuberculosis

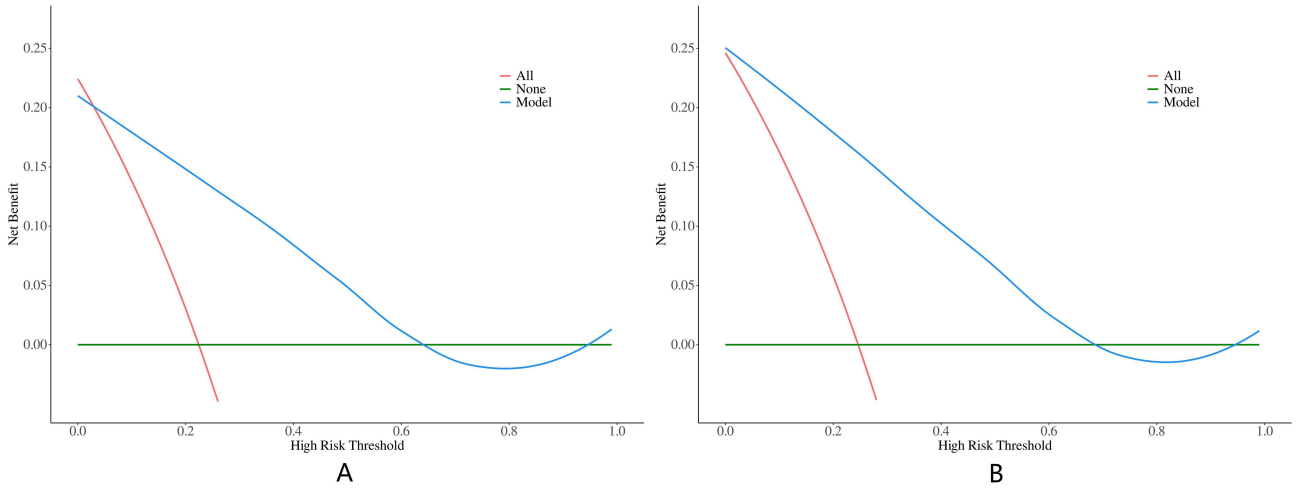

Figure 5 DCA of the nomogram in the training set (**A**) and validation set (**B**).

therapy is fundamental for all superficial lymph node TB cases. Multivariate logistic regression indicated that the likelihood of a positive mycobacterial culture decreases by 2% for each additional day of anti-tuberculosis therapy. Therefore, theoretically, samples from treatment-naïve patients who have not yet started anti-tuberculosis therapy are most likely to be culture-positive for mycobacteria. However, without anti-tuberculosis therapy, inadequate drug concentrations at the lesion site could lead to poor healing at the puncture or surgical site, and some patients may experience worsening or recurrent infections around the lesion.[16] Therefore, finding a balance between obtaining positive results and ensuring efficacy, as well as determining the optimal preoperative anti-tuberculosis treatment period, requires further study and exploration. Adenosine deaminase (ADA) is an enzyme critical for the conversion of adenosine to inosine and plays a significant role in lymphocyte differentiation and monocyte maturation into macrophages. When the body is infected by tuberculosis bacteria, the host resists through cellular immunity, leading to a significant increase in local lymphocytes and enhanced activity level. Consequently, the content of ADA in tuberculous pleural and abdominal effusion is significantly increased.[17] However, there are few studies on ADA related to extrapulmonary tuberculosis (lymph node tuberculosis) by scholars. Hence, this study also included this indicator to explore the relationship between positive Mycobacterium tuberculosis culture results and ADA levels in superficial lymph node tuberculosis. The results suggest that an increase in adenosine deaminase levels contributes to positive culture results of Mycobacterium tuberculosis in superficial lymph nodes.

The nomogram developed in this study exhibited robust predictive performance, with an area under the curve (AUC) of 0.86 in the training set and 0.89 in the validation set. Both the calibration curves from the model and validation sets showed good agreement, suggesting that the predicted probability of tuberculosis positivity aligned well with the observed actual probability. The maximum Youden index was 0.645, corresponding to a model cutoff of 0.344 with an accuracy of 0.81 (95% CI: 0.76–0.85). Evaluating the positive probability is fundamental to implementing diagnostic measures. Currently, Mycobacterium tuberculosis (MTB) nucleic acid detection via molecular biological methods exhibits high sensitivity (78.0–97.0%) and specificity (74.0–90.0%),[18–20] being rapid and unaffected by gender, age, or infection site.[21–23] There is a trend towards replacing mycobacterium culture, as most doctors opt for an encompassing range of tests upon obtaining samples, leading to a propensity for over-testing. This not only increases the economic burden on patients but also delays the time for them to get the results, especially for samples with a low likelihood of positive test results. In the present study, we integrated demographic characteristics of patients with superficial lymph node tuberculosis and clinical data pertaining to tuberculosis and comorbidities. We also included laboratory test outcomes, as well as the quantity and types of samples. Through binary logistic regression analysis, we developed a positive probability prediction nomogram model, thus making the predictive model practical.[24] In clinical practice, we calculate the probability of a positive mycobacterium culture result using some basic clinical and examination data. When this probability exceeds 0.344, it indicates an increased likelihood of a positive Mycobacterium tuberculosis

culture result in superficial lymph nodes.Therefore, It is recommended that patients with a high predicted probability of positive culture should undergo both molecular tuberculosis testing and bacteriological culture. These patients should be advised to wait for the results and follow up after discharge. Additional drug susceptibility tests should be conducted to obtain complete first-line and second-line anti-tuberculosis drug sensitivity test results, which will guide treatment.For patients with a low predicted probability of positive culture and limited lesion samples, molecular tuberculosis drug sensitivity testing should be prioritized. This approach saves time and money for the patient and optimizes medical resources. However, this strategy is not applicable to patients with recurrent episodes and a high suspicion of drug-resistant tuberculosis.The aforementioned indicators are readily accessible, facilitating further promotion in primary hospitals.

However, our study has limitations, including that it is a retrospective single-center study, and the data for both the validation and training sets are from the same hospital, which may affect the model's generalizability. Prospective, multicenter, large-scale studies are needed for further validation. Detailed stratification and trend testing analyses of anti-tuberculosis treatment duration and adenosine deaminase were not performed; due to the short acquisition time of some data, treatment information for patients at later follow-ups was not available. The data on T lymphocyte subsets may not be statistically significant in relation to the positive results of Mycobacterium tuberculosis culture. In the future, we plan to further evaluate a potential nonlinear correlation between these variables by increasing our sample size and employing a non-linear trend analysis method, specifically the Restricted Cubic Spline (RCS) test. These identified shortcomings could potentially impact the accuracy of our research findings.

Conclusion

In summary, the duration of anti-tuberculosis treatment, initial treatment or retreatment status, and adenosine deaminase level were the influencing factors for the culture results of Mycobacterium tuberculosis in superficial lymph nodes. The prediction model established in this study has high accuracy and can provide a reference for predicting the culture results of Mycobacterium tuberculosis in superficial lymph nodes.

Ethics Statement

The Declaration of Helsinki was followed in conducting this study. The Ethics Committee of Xi'an Chest Hospital approved the research protocol (R2023-004-01). To protect privacy, no personally identifiable information is provided. Due to the retrospective nature of this study and the fact that all patients were anonymous participants, this study obtained an informed consent waiver from the Ethics Committee of Xi'an Chest Hospital. In addition, during the implementation of this study, this study was carried out in accordance with national legislative and institutional requirements.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

References

1. Wang M, Wang N, Ni N, et al. A Questionnaire Survey on Extrapulmonary Tuberculosis Control and Prevention - China, 2021. *China CDC Wkly.* 2023;5(8)176–179. doi:10.46234/ccdcw2023.032

2. An H, Wang Z, Chen H, et al. Clinical efficacy of short-course chemotherapy combined with topical injection therapy in treatment of superficial lymph node tuberculosis. *Oncotarget*. 2017;8(66):109889–109893. doi:10.18632/oncotarget.22492
3. Sarfaraz S, Iftikhar S, Salahuddin N. Frequency, clinical characteristics, risks, and outcomes of Paradoxical upgrading reactions during anti-tuberculosis treatment in tuberculous lymphadenitis.Pak. *J Med Sci*. 2020;36(1):S27–S32.
4. Rai DK, Kumar R, Ahmad S. Clinical characteristics and treatment outcome in Tubercular lymphadenitis patients-A prospective observational study. *Indian J Tuberc*. 2020;67(4):528–533. doi:10.1016/j.ijtb.2020.07.021
5. Heffernan C, Egedahl ML, Barrie J, et al. The prevalence, risk factors, and public health consequences of peripheral lymph node-associated clinical and subclinical pulmonary tuberculosis. *Int J Infect Dis*. 2023;129:165–174. doi:10.1016/j.ijid.2023.01.026
6. Ganchua SKC, White AG, Klein EC, et al. Lymph nodes The neglected battlefield in tuberculosis. *PLoS Pathog*. 2020;16(8):e1008632. doi:10.1371/journal.ppat.1008632
7. Carroll NM, Richardson M, Engelke E, de Kock M, Lombard C, van Helden PD. Reduction of the rate of false-positive cultures of Mycobacterium tuberculosis in a laboratory with a high culture positivity rate. *Clin Chem Lab Med*. 2002;40(9):888–892. doi:10.1515/CCLM.2002.157
8. Loizos A, Soteriades ES, Pieridou D, et al. Lymphadenitis by non-tuberculous mycobacteria in children. *Pediatr Int*. 2018;60(12):1062–1067. doi:10.1111/ped.13708
9. Pang P, Duan W, Liu S, et al. Clinical study of tuberculosis in the head and neck region-11 years' experience and a review of the literature. *Emerg Microbes Infect*. 2018;7(1):4. doi:10.1038/s41426-017-0008-7
10. Senior Department of Tuberculosis. The 8th Medical Center of Chinese PLA General Hospital, Editorial Board of Chinese Journal of Antituberculosis, Tuberculosis Control Branch of China Intrnational Exchange and Promotive Association for Medical and Health Care. Expert consensus on the diagnosis and treatment of superficial lymph node tuberculosis. *Chin J Antitubercul*. 2023;45(6):531–542. doi:10.19982/j.issn.1000-6621.20230120
11. Mishra H, Reeve BWP, Palmer Z, et al. Xpert MTB/RIF Ultra and Xpert MTB/RIF for diagnosis of tuberculosis in an HIV-endemic setting with a high burden of previous tuberculosis: a two-cohort diagnostic accuracy study. *Lancet Respir Med*. 2020;8(4):368–382. doi:10.1016/S2213-2600(19)30370-4
12. World Health Organization. *Use of Targeted Next-Generation Sequencing to Detect Drug-Resistant Tuberculosis Rapid Communication, 2023*. Geneva: Word Health Organization; 2023.
13. Sharif N, Ahmed D, Mahmood RT, et al. Comparison of different diagnostic modalities for isolation of Mycobacterium Tuberculosis among suspected tuberculous lymphadenitis patients. *Braz J Biol*. 2021;83:e244311. doi:10.1590/1519-6984.244311
14. Davies LRL, Wang C, Steigler P, et al. Age and sex influence antibody profiles associated with tuberculosis progression. *Nat Microbiol*. 2024;9(6):1513–1525. doi:10.1038/s41564-024-01678-x
15. Dowling WB, Whitelaw A, Nel P. Tracing TB: are there predictors for active TB disease in patients with Xpert Ultra trace results. *Int J Infect Dis*. 2022;114:115–123. doi:10.1016/j.ijid.2021.10.056
16. Yang S, Wang LL, Li TX, et al. Progress in research of epidemiology of extra pulmonary tuberculosis. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2021;42(1):171–176. doi:10.3760/cma.j.cn112338-20200814-01067
17. Fenhua J, Daohui W, Hui L, Xiaodong X, Wen H. Diagnostic value of combined pleural interleukin-33, adenosine deaminase and peripheral blood tuberculosis T cell spot detection TB for tuberculous pleurisy. *BMC Infect Dis*. 2021;21(1):861. doi:10.1186/s12879-021-06575-w
18. Fantahun M, Kebede A, Yenew B, et al. Diagnostic accuracy of Xpert MTB/RIF assay and non-molecular methods for the diagnosis of tuberculosis lymphadenitis. *PLoS One*. 2019;4(9):e0222402. doi:10.1371/journal.pone.0222402
19. Chen HK, Liu RS, Wang YX, et al. Xpert MTB/RIF assay for the diagnosis of lymph node tuberculosis in children: a systematic review and meta-analysis. *J Clin Med*. 2022;11(15):4616. doi:10.3390/jcm11154616
20. Mohindra S, Nayak HK, Mohindra N, et al. Diagnostic yield of endoscopic ultrasound-guided fine-needle aspiration of tubercular lymphadenitis using combination of cytology and Gene Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) genes. *Indian J Gastroenterol*. 2021;40(6):630–635. doi:10.1007/s12664-020-01136-6
21. Kim SB, Shin B, Lee JH, et al. Pleural fluid ADA activity in tuberculous pleurisy can be low in elderly, critically ill patients with multi-organ failure. *BMC Pulm Med*. 2020;20(1):13. doi:10.1186/s12890-020-1049-6
22. Muzanyi G, Mulumba Y, Mubiri P, Mayanja H, Johnson JL, Mupere E. Predictors of recurrent TB in sputum smear and culture positive adults: a prospective cohort study. *Afr Health Sci*. 2019;19(2):2091–2099. doi:10.4314/ahs.v19i2.33
23. Nguyen MH, Levy NS, Ahuja SD, Trieu L, Proops DC, Achkar JM. Factors associated with sputum culture-negative vs culture-positive diagnosis of pulmonary tuberculosis. *JAMA Network Open*. 2019;2(2):e187617. doi:10.1001/jamanetworkopen.2018.7617
24. Song M, Zhang M, Han J, Fu W. Construction and validation of a nomogram to identify the risk of cavitation in pulmonary tuberculosis. *Infect Drug Resist*. 2024;17:2803–2813. doi:10.2147/IDR.S459330