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ORIGINAL RESEARCH

IFN-γ and IL-6 as Key Predicting Biomarkers for Active TB Among PLWH: Results from Four Machine Learning Methods

Juan Wan[1,*], Virasakdi Chongsuvivatwong[2,*], Pei Zhang[1,*], Qiujing Li[1], Liping Zhao[1], Lingqing Zou[1], Jun Zhao[3], Jingyi Dai[1]

[1]Department of Public Laboratory, The Third People's Hospital of Kunming /Infectious Disease Clinical Medical Center of Yunnan Province, Kunming, Yunnan, People's Republic of China; [2]Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand; [3]School of Public Health, Hubei University of Medicine, Shiyan, Hubei, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jingyi Dai, Department of Public Laboratory, The Third People's Hospital of Kunming /Infectious Disease Clinical Medical Center of Yunnan Province, Kunming, Yunnan, People's Republic of China, Email daijingyidjy@163.com

Purpose: Tuberculosis remains a major cause of mortality in people living with HIV (PLWH), yet early diagnosis remains challenging. This study aimed to identify novel biomarker combinations and develop machine learning models, and to predict active TB in PLWH in a random and a chronological subset.

Patients and Methods: We enrolled 760 PLWH with pulmonary symptoms. Demographic and clinical data and cytokine profiles were analyzed. Participants were first randomly split into training and validation sets. Subsequently, the whole dataset was re-analysed using the first 609 records as the training set and subsequent 151 records as the test set. Four models were developed with 10-fold cross-validation, incorporating feature selection and hyperparameter optimization. Model performance was assessed through ROC-AUC, sensitivity, specificity, and variable importance analysis.

Results: For the randomly split datasets, with active TB patients showed significantly elevated IFN-γ (median 5.7 vs 3.9 pg/mL, $P<0.001$) and IL-6 levels (25.3 vs 13.2 pg/mL, $P<0.001$) compared to without active TB cases. These two biomarkers were strong predictors based on the gradient boosting machine (GBM) model. AUCs (95% CI) on the randomly selected training dataset, was 0.96 (0.95, 0.97). That on the randomly selected test dataset was 0.73 (95% CI: 0.65–0.81). However, on chronological order, GBM model trained from the first 609 records AUC of 0.92 (0.91, 0.94) poorly predicted the 151 final records with the AUC of 0.66 (0.58, 0.75).

Conclusion: TB might have activated the two inflammatory biomarkers among the PLWH. The best predictive machine learning method still have limitation in generalizability to predict the outcome on other data sets.

Keywords: people living with HIV, tuberculosis, predictive modeling, IFN-γ, IL-6

Introduction

Tuberculosis (TB) remains one of the leading causes of death globally, ranking second after COVID-19 among infectious agents.[1] TB is a prevalent opportunistic infection and a primary cause of hospitalization and mortality in people living with HIV (PLWH).[1,2]

Early identification of PLWH individuals TB is critical for reducing mortality and disease burden. Inadequate diagnostics remain a major obstacle to improving outcomes in HIV-TB co-infected patients.[3] Some PLWH were confirmed active TB through pathogen-based methods such as sputum smear, culture, GeneXpert MTB/Rifampicin (RIF), and targeted next-generation sequencing. Studies have shown that up to 45.8% of PLWH had undiagnosed TB at the time of death, TB burden in this population may be significantly underestimated.[4,5] Consequently, there is an immediate necessity to develop methodologies capable of precisely detecting TB infection among PLWH individuals. Plasma biomarkers possess significant potential for evaluating the inflammatory response and immune status of patients

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with active TB. Single plasma biomarkers are unlikely to differentiate between disease states in HIV-TB co-infected individuals, necessitating the use of multiple biomarkers.[6,7] PLWH infected with active TB also contributes to a higher rate of drug-resistant TB, poorer treatment outcomes, and increased mortality, further complicating TB control efforts worldwide.[8]

Machine learning (ML) has demonstrated significant potential in enhancing predictive capabilities within healthcare, as it can significantly enhance diagnostic accuracy, anomaly detection, and disease progression prediction.[9] Biomarkers' predictive power for TB in PLWH individuals is limited, with few studies establishing reliable models linking HIV and TB.[10] Therefore, developing an accurate predictive model for TB in PLWH is highly meaningful.

In this study, we developed four predictive models based on demographic and clinical data to predict active TB in PLWH with pulmonary infection and evaluated its diagnostic accuracy in both training and validation sets of patients. The aim was to assess the value of model in predicting active TB in PLWH with pulmonary infection; conducting a screening for biomarkers that possess potential predictive significance, and to compare ability of these models to predict active TB in PLWH with pulmonary infection in the random subsets and the chronological subsets.

Materials and Methods

Study Subjects

This was a retrospective study conducted at Kunming Third People's Hospital from April 1, 2020 to June 1, 2024. PLWH who was clinically diagnosed with pulmonary infection, regardless of their antiretroviral therapy status, were enrolled in the study. PLWH who met the following criteria were considered for pulmonary infection: (i) chest imaging suggestive of pulmonary infection; (ii) fever, cough, expectoration, dyspnea, or other respiratory symptoms. (iii) the specific pathogen causing the pulmonary infection has not yet been identified. Exclusion criteria comprised individuals with taking immunosuppressive medication, those was diagnosed with malignant tumor, those undergoing TB treatment for more than a week, and pregnant. A total of 760 PLWH were included in the study.

All participants underwent a clinical assessment that included routine laboratory tests, such as complete blood count, coagulation function, and serum biochemical tests, twelve cytokines and infection indicators measurements. The flowchart of the study is shown in Figure 1.

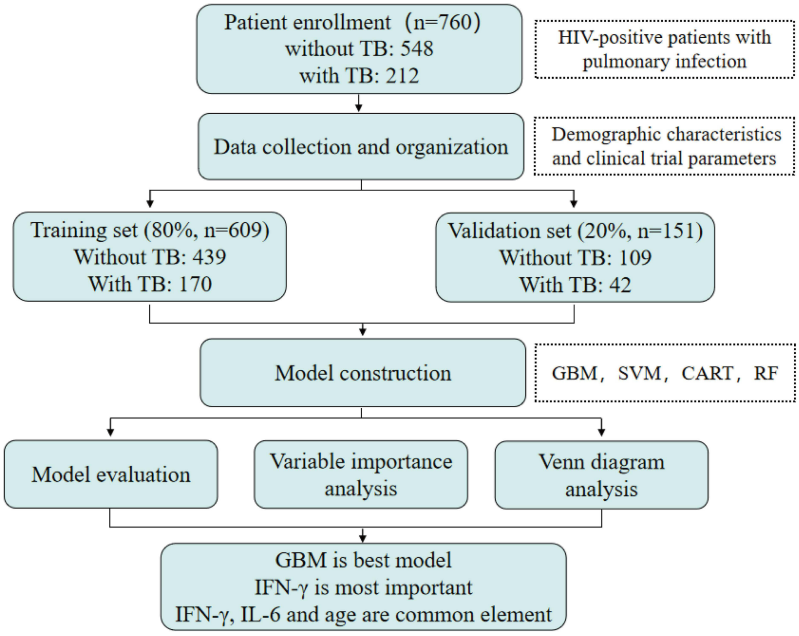

Figure 1 Research design and numbers of participants included in the study.
Abbreviations: TB, tuberculosis; GBM, gradient boosting machine; SVM, support vector machine; CART, classification and regression trees; RF, random forest; IFN-γ, interferon-γ; IL-6, interleukin-6.

Laboratory Examinations

The following routine laboratory examinations were collected at the time of admission: Complete blood count, coagulation function, serum biochemical indicators, twelve cytokines and infection indicators measurements. These included:

1. Complete blood count: white blood cell count (WBC), neutrophil count (NEU_CT), percentage of neutrophils (NEU_PT), lymphocyte count (LYM_CT), percentage of lymphocytes (LYM_PT), monocytes count (MON_CT), percentage of monocytes (MON_PT), eosinophil count (EOS_CT), percentage of eosinophils (EOS_PT), basophil count (BAS_CT), percentage of basophils (BAS_PT), red blood cells (RBC), hemoglobin (HGB), blood platelets (PLT).
2. Coagulation function tests: prothrombin time (PT), PT range of motion (PT_PT), international normalized ratio (INR), partial prothrombin time (APTT), fibrinogen (FIB), thrombin time (TT).
3. Serum biochemical tests: total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), globulin (GLOB), prealbumin (PALB), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), alpha-L-fucosidase (AFU), glutamate dehydrogenase (GLDH), urea (UREA), creatinine (CREA), uric acid (UA).
4. Twelve cytokines and infection indicators measurements: interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70), interleukin-17 (IL-17), interferon-γ (IFN-γ), interferon-α (IFN-α), tumor necrosis factor-α (TNF-α), hypersensitive C-reactive protein (hCRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR).

Additionally, six demographic characteristics were collected: gender, age, marital status, smoking history, drinking history, and drug use history.

The concentrations of the 12 cytokines were quantified using flow cytometer (DxFLEX) via multiplex bead-based flow cytometric immunoassay. All measurements were performed using PLWH blood samples, and the results are expressed in pg/mL.

Data Integration and Classification

The clinical data and laboratory test results for each participant were integrated using their hospital identification numbers. TB status was determined based on the medical records and categorized into two groups: with active TB and without active TB. The outcome of interest was whether the patient had active TB or not.

Outcome (TB) Definitions

The diagnosis of active TB in participants was made by two experienced specialists based on a comprehensive assessment of both clinical manifestations and laboratory results. A definitive diagnosis of "with active TB" was microbiologically confirmed by a positive result in any of the following tests conducted on samples collected during admission: culture, GeneXpert MTB/RIF, or Mycobacterium tuberculosis (Mtb)-DNA tests. Conversely, "without active TB" was defined as individuals with negative microbiology, IGRA, and chest radiography results, and other infectious etiology for which TB infection had been ruled out. This definition was adopted from "The People's Republic of China health industry standard for diagnosis of pulmonary TB", which can be seen at http://www.nhc.gov.cn/ewebeditor/uploadfile/2017/11/20171128164254246.pdf.

Statistical Analysis

The data analysis was conducted using R software (version 4.2.3). Continuous variables were assessed for normality using the Shapiro–Wilk test. For normally distributed continuous variables, the mean and standard deviation (SD) was reported, while non-normal distributed variables were presented as the median and interquartile range (IQR). Categorical variables were summarized using frequency counts and percentages. Age were analyzed as continuous variables, five variables were analyzed as categorical variables, including: gender, marital history, smoking history, drinking history, drug use history, and other variables were analyzed as continuous variables. To compare differences between PLWH diagnosed with active TB and those without active TB, the Pearson χ^2 test was applied to categorical variables. For continuous variables, a *t*-test was used for those following a normal distribution, and the Rank-sum test was used for non-

normally distributed continuous variables. Missing data were addressed using interpolation techniques implemented in the "MICE" package, which stands for Multivariate Imputation by Chained Equations, and can enhance the accuracy and reliability of subsequent statistical analyses. Applying synthetic minority oversampling technique (SMOTE) to mitigate model bias, prevent overfitting, and improve model performance.

In order to randomly divide the dataset to ensure the credibility of the data analysis, for the construction and evaluation of predictive models, the dataset was randomly divided into a training set (80%) and a test set (20%). The splitting procedure employed stratified sampling to ensure that the class distribution of TB diagnosis in both the training and test sets remained consistent with that of the original dataset. All random processes were controlled by setting a fixed random seed to ensure full reproducibility.

To explore temporal trends, we employed chronological subsetting, partitioning the data into sequential time periods. This facilitates the identification of evolving patterns and period-specific effects that a random split might obscure. However, it assumes temporal independence between subsets and may oversimplify continuous processes by treating time as discrete states.

Model Development and Validation

Four ML algorithms—Gradient boosting machine (GBM), support vector machine (SVM), classification and regression trees (CART), and random forest (RF)—were developed and validated using the caret package. Indicators with missing values exceeding 30% were excluded from the model, and 55 variables were retained for modeling. The patients were randomly divided into a training set (80%) and a validation set (20%). The performance of each model was evaluated using several metrics, including accuracy, sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and area under the curve (AUC) from the receiver operating characteristic (ROC) curve. Kappa statistics were used to assess agreement between model predictions and actual results. To ensure model stability and repeatability, 10-fold cross-validation with 10 repetitions was applied. We also conducted an analysis across the entire dataset.

Results

Basic Characteristics of the Study Population

A total of 760 PLWH were enrolled in this retrospective study, conducted from April 1, 2020 to July 1, 2024. Of these, 212 (28.7%) were diagnosed with active TB, while 548 (71.3%) were not, 572 (75.3%) were male, and median (IQR) was 48.0 (39.0, 57.0) years old. Additionally, 574 (75.5%) were married, 308 (40.5%) had a history of smoking, 391 (51.4%) had a history of alcohol consumption, and 647 (85.1%) had a history of drug use. The cohort was randomly split into a training set (80% of the participants, n=609) and a validation set (20% of the participants, n=151) (Supplemental Table 1). In the training set, 170 patients (27.9%) were diagnosed with active TB. Of the TB patients, 79.4% were male, and the mean age was 45.1 (SD=12.9) years old. Additionally, 72.9% of TB patients were married, 36.5% had a history of smoking, 46.5% had a history of alcohol consumption, and 82.4% had a history of drug use (Table 1).

Significant differences between with active TB and without active TB patients were observed in various parameters, including age, NEU_PT, LYM_CT, LYM_PT, RBC, HGB, PT, PT_PT, APTT, FIB, ALB, GLOB, PALB, GGT, ALP, GLDH, UREA, CREA, IL-6, IL-8, IFN-γ, hCRP, PCT and ESR (each $P<0.05$). These characteristics are summarized in Tables 1–3. Most of the inflammatory biomarkers are increased in the active TB cases.

The results of overall baseline analysis are shown in Supplemental Table 2. Significant differences between with active TB and without active TB patients were observed in various parameters, including age, IL-6, IFN-γ, hCRP, ESR, NEU_PT, LYM_CT, LYM_PT, HGB, PALB, GGT, ALP and UREA (each $P<0.001$). These characteristics are summarized in Supplemental Table 2.

Construction and Performance Validations of the ML Models

To develop and validate TB predictive model, the cohort was randomly split into a training set (80%) and a validation set (20%). The training set comprised 170 patients with active TB and 439 without, while the validation set included 42

Table 1 Basic Characteristics of Study Population

Variables	Training Set (n=609)			Validation Set (n=151)		
	Without Active TB (n=439)	**With Active TB (n=170)**	**P value**	**Without Active TB (n=109)**	**With Active TB (n=42)**	**P value**
Gender			0.245			0.699
Male, n (%)	329.0 (74.9)	135.0 (79.4)		77.0 (70.6)	31.0 (73.8)	
Female, n (%)	110.0 (25.1)	35.0 (20.6)		32.0 (29.4)	11.0 (26.2)	
Age, mean (SD)	49.8 (14.3)	45.1 (12.9)	**< 0.001**	51.3 (13.6)	43.1 (11.1)	**< 0.001**
Marital history, n (%)	340.0 (77.4)	124.0 (72.9)	0.241	82.0 (75.2)	28.0 (66.7)	0.289
Smoking history, n (%)	182.0 (41.5)	62.0 (36.5)	0.26	51.0 (46.8)	13.0 (31.0)	0.078
Drinking history, n (%)	231.0 (52.6)	79.0 (46.5)	0.173	64.0 (58.7)	17.0 (40.5)	0.044
Drug use history, n (%)	378.0 (86.1)	140.0 (82.4)	0.244	95.0 (87.2)	34.0 (81)	0.333

Notes: Data in parentheses represent percentage (%) and standard deviation (SD). Bolded values represent P<0.001.

Table 2 Complete Blood Count, Coagulation Function, Serum Biochemical Indicators in PLWH with Pulmonary Infection

Variables	Training Set			Validation Set		
	Without Active TB (n=439)	**With Active TB (n=170)**	**P value**	**Without Active TB (n=109)**	**With Active TB (n=42)**	**P value**
WBC (10^9/L)	4.8 (3.4, 7.0)	4.9 (3.2, 7.0)	0.931	5.2 (3.6, 7.0)	5.9 (4.2, 7.5)	0.365
NEU_CT (10^9/L)	3.1 (2.0, 4.8)	3.6 (2.1, 5.4)	0.172	3.5 (2.0, 5.4)	4.2 (2.3, 6.3)	0.393
NEU_PT (%)	68.7 (55.5, 80.8)	74.2 (64.5, 83.5)	**< 0.001**	70.3 (58.1, 80.1)	71.6 (58.8, 87.0)	0.207
LYM_CT (10^9/L)	0.9 (0.5, 1.5)	0.7 (0.4, 1.1)	**< 0.001**	1.0 (0.6, 1.4)	0.7 (0.5, 1.2)	0.035
LYM_PT (%)	19.1 (11.6, 30.3)	15.2 (9.2, 21.8)	**< 0.001**	19.3 (12.0, 29.3)	15.8 (6.6, 24.5)	0.038
MON_CT (10^9/L)	0.4 (0.2, 0.6)	0.4 (0.2, 0.5)	0.616	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.359
MON_PT (%)	8.0 (5.5, 10.4)	8.1 (6.0, 10.1)	0.538	7.8 (5.9, 10.4)	7.6 (4.6, 10.9)	0.871
EOS_CT (10^9/L)	0.01 (0.0, 0.1)	0.0 (0.0, 0.1)	0.123	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.161
EOS_PT (%)	0.9 (0.2, 2.8)	0.7 (0.2, 2.2)	0.222	0.7 (0.2, 1.8)	0.5 (0.0, 1.4)	0.168
BAS_CT (10^9/L)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.032	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.607
BAS_PT (%)	0.4 (0.2,0.6)	0.3 (0.2,0.6)	0.038	0.4 (0.2,0.6)	0.3 (0.2,0.5)	0.344
RBC (10^12/L)	3.8 (0.9)	3.5 (0.8)	0.003	3.7 (0.8)	3.6 (0.8)	0.653
HGB (g/L)	119.5 (27.1)	109.1 (25.5)	**< 0.001**	117.7 (25.7)	110.9 (23.4)	0.137
PLT (10^9/L)	177.0 (116.5, 245)	192.5 (126, 263)	0.086	192.5 (143, 268.2)	225.5 (151.8, 327.8)	0.163
PT(s)	14.0 (13.3, 14.7)	14.2 (13.5, 14.9)	0.018	14.1 (13.2, 14.8)	14.1 (13.3, 14.8)	0.782
PT_PT (%)	78.8 (71.8, 90)	77.8 (69.3, 86.1)	0.036	81.1 (13.4)	79.2 (13.7)	0.444
INR	1.2 (1.1, 1.2)	1.2 (1.1, 1.3)	0.053	1.2 (1.1, 1.2)	1.2 (1.1, 1.2)	0.642
APTT (s)	36.2 (32.4, 40.9)	37.8 (33.8, 42.5)	0.004	36.9 (6.4)	37.9 (5.1)	0.386
FIB (g/L)	3.6 (2.8, 4.8)	4 (3.1, 4.9)	0.024	4.0 (1.5)	4.2 (1.3)	0.479
TT (s)	17.8 (16.6, 18.9)	17.8 (17, 18.7)	0.557	17.7 (16.6, 19)	17.4 (17, 18.3)	0.777
TBIL (umol/L)	8.9 (6.4, 13.0)	8.3 (6.5, 10.9)	0.468	9.3 (6.7, 13.3)	8.3 (5.4, 12.2)	0.239
DBIL (umol/L)	3.3 (2.3, 5.0)	3.5 (2.6, 5.0)	0.213	3.7 (2.5, 5.3)	3.2 (2.2, 4.5)	0.617
AST (U/L)	30.0 (21.0, 45.8)	31.5 (21.0, 48.0)	0.962	25.0 (20.0, 38.0)	26.5 (17.2, 42.8)	0.654
ALT (U/L)	21.0 (13.8, 36.0)	21.0 (14.0, 32.0)	0.359	19.0 (12.0, 27.0)	17.0 (12.0, 25.0)	0.758
ALB (g/L)	31.3 (7.1)	29.9 (6.5)	0.036	32.3 (6.7)	29.1 (6.0)	0.008
GLOB (g/L)	31.9 (27.0, 37.7)	32.9 (28.4, 38.5)	0.048	32.1 (7.5)	34.9 (7.4)	0.041
PALB (mg/L)	157.1 (112.8, 219.0)	138.6 (95.4, 201.3)	0.002	164.6 (120.7, 206.5)	124 (87.3, 188.0)	0.021
GGT (U/L)	53.0 (30.0,99.0)	81.0 (43.0, 155.0)	**< 0.001**	50.0 (28.5, 96.8)	86.5 (39.2, 146.2)	0.032
ALP (U/L)	95.0 (77.0, 129.0)	108.0 (84.0, 156.0)	0.003	95.5 (71.0, 121.8)	107.5 (84.0, 125.0)	0.158
AFU (U/L)	22.0 (17.0, 27.0)	21.0 (16.0, 30.0)	0.588	22.0 (7.2)	23.7 (7.9)	0.207
GLDH (U/L)	6.7 (3.4, 10.2)	6.5 (3.7, 9.5)	0.566	5.9 (3.4, 8.9)	4.2 (1.9, 6.9)	0.018
UREA (mmol/L)	4.7 (3.3, 6.6)	4.3 (3.0, 5.3)	0.003	4.4 (3.4, 5.5)	3.8 (2.7, 5.0)	0.083

(*Continued*)

Table 2 (Continued).

Variables	Training Set			Validation Set		
	Without Active TB (n=439)	**With Active TB (n=170)**	**P value**	**Without Active TB (n=109)**	**With Active TB (n=42)**	**P value**
CREA (umol/L)	63.0 (51.0, 81.0)	60.0 (48.0, 73.0)	0.008	61.0 (44.0, 78.0)	55.5 (46.0, 66.0)	0.305
UA (umol/L)	305.0 (238.0, 408.5)	324.0 (225.5, 445.2)	0.455	276.0 (224.0, 355.0)	309.0 (201.0, 467.0)	0.265

Notes:Values are medians (interquartile ranges) unless stated otherwise. Bolded values represent P<0.001.

Abbreviations: WBC, white blood cell count; NEU_CT, neutrophil count; NEU_PT, percentage of neutrophils; LYM_CT, lymphocyte count; LYM_PT, percentage of lymphocytes; MON_CT, monocytes count; MON_PT, percentage of monocytes; EOS_CT, eosinophil count; EOS_PT, percentage of eosinophils; BAS_CT, basophil count; BAS_PT, percentage of basophils; RBC, red blood cells; HGB, hemoglobin; PLT, blood platelets; TBIL, total bilirubin; DBIL, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; GLOB, globulin; PALB, prealbumin; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; AFU, alpha-L-fucosidase; GLDH, glutamate dehydrogenase; UREA, urea; CREA, creatinine; UA, uric acid; PT, prothrombin time; PT_PT, PT range of motion; INR, international normalized ratio; APTT, partial prothrombin time; FIB, fibrinogen; TT, thrombin time.

Table 3 Infection Indicators in PLWH with Pulmonary Infection

Variables	Training Set			Validation Set		
	Without Active TB (n=439)	**With Active TB (n=170)**	**P value**	**Without Active TB (n=109)**	**With Active TB (n=42)**	**P value**
IL-1β (pg/mL)	2.1 (1.1, 4.2)	2.6 (1.2, 5.2)	0.111	2.0 (1.1, 4.2)	2.5 (1.2, 5.4)	0.275
IL-2 (pg/mL)	1.6 (1.1, 2.6)	1.6 (1.2, 2.6)	0.803	1.5 (1.0, 2.0)	1.6 (1.0, 2.1)	0.808
IL-4 (pg/mL)	1.4 (0.8, 2.1)	1.3 (0.8, 2.1)	0.298	1.4 (0.8, 2.1)	1.3 (0.9, 1.8)	0.513
IL-5 (pg/mL)	1.5 (1.0, 2.3)	1.4 (0.9, 2.1)	0.568	1.5 (1.1, 2.2)	1.4 (0.9, 2.1)	0.614
IL-6 (pg/mL)	13.2 (5.2, 34.8)	25.3 (9.3, 69.5)	**< 0.001**	11.8 (5.0, 37.5)	28.1 (10.0, 62.0)	0.009
IL-8 (pg/mL)	40.5 (23.0, 73.8)	56.4 (26.7, 107.7)	0.005	40.7 (23.6, 62.3)	60.2 (32.1, 76.9)	0.135
IL-10 (pg/mL)	7.3 (4.8, 11.8)	7.1 (4.4, 11.7)	0.607	6.0 (4.3, 9.0)	7.0 (4.8, 11.6)	0.232
IL-12p70 (pg/mL)	2.3 (1.5, 3.6)	2.1 (1.3, 3.4)	0.091	2.0 (1.4, 2.8)	2.4 (1.6, 3.8)	0.104
IL-17 (pg/mL)	9.8 (4.5, 16.1)	8.1 (4.5, 14.5)	0.364	6.8 (4.2, 13.5)	11.5 (5.9, 16.5)	0.078
IFN-γ (pg/mL)	3.9 (2.1, 6.5)	5.7 (2.9, 11.2)	**< 0.001**	2.7 (1.8, 4.5)	7.6 (4.2, 17.9)	**< 0.001**
IFN-α (pg/mL)	4.1 (2.2, 12.0)	3.7 (2.1, 7.1)	0.119	3.4 (2.0, 6.7)	3.5 (1.9, 6.7)	0.774
TNF-α (pg/mL)	2 (1.3, 3.8)	1.8 (1.3, 3.3)	0.242	1.7 (1.3, 2.5)	1.8 (1.2, 3.0)	0.673
hCRP (mg/L)	17.2 (3.5, 57.2)	35.6 (10.1, 100.9)	**< 0.001**	22.6 (4.6, 60.8)	39 (13.5, 87.8)	0.089
PCT (ng/mL)	0.1 (0.1, 0.3)	0.1 (0.1, 0.4)	0.037	0.1 (0.0, 0.2)	0.2 (0.1, 0.4)	0.034
ESR (mm/h)	51.5 (19.0, 83.5)	68.0 (40.0, 107.5)	**< 0.001**	53.0 (22.0, 82.8)	65.5 (46.0, 104.8)	0.068

Notes: Values are medians (interquartile ranges) unless stated otherwise. Bolded values represent P<0.001.

Abbreviations: IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-17, interleukin-17; IFN-γ, interferon-γ; IFN-α, interferon-α; TNF-α, tumor necrosis factor-α; hCRP, hypersensitive C-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate.

patients with active TB and 109 without. Four ML models—GBM, SVM, CART, and RF—were developed using demographic characteristics and clinical parameters (55 variables in total). The GBM model demonstrated superior predictive accuracy compared to the other models (Table 4). In training set, the GBM model achieved the following performance metrics: 96.6% accuracy (95% CI: 95.5% to 97.6%), 98.8% specificity (95% CI: 98.0% to 99.6%), 93.7% sensitivity (95% CI: 91.6% to 95.8%), 95.5% NPV (95% CI: 93.9% to 97.0%), 98.4% PPV (95% CI: 97.2% to 99.5%), and 0.96 AUC (95% CI: 0.95 to 0.97). In validation set: the model showed 78.2% accuracy (95% CI: 70.7% to 84.5%), 84.4% specificity (95% CI: 77.6% to 91.2%), 61.9% sensitivity (95% CI: 47.2% to 76.6%), 85.2% NPV (95% CI: 78.5% to 91.9%), 60.5% PPV (95% CI: 45.9% to 75.1%), and 0.73 AUC (95% CI: 0.65 to 0.81). The distributions of each variable in the training and validation sets are presented in Supplemental Table 1.

Table 4 Comparison of Four Models of the Training Set and the Verification Set

Model	GBM		SVM		CART		RF	
	Training Set	Validation Set	Training Set	Validation Set	Training Set	Validation Set	Training Set	Validation Set
Accuracy	96.6% (95.5% to 97.6%)	78.2% (70.7% to 84.5%)	90.8% (89.1% to 92.4%)	70.9% (62.9% to 78.0%)	89.5% (87.6% to 91.2%)	71.5% (63.6% to 78.6%)	93.4% (91.8% to 94.7%)	74.2% (66.4% to 80.9%)
Specificity	98.8% (98.0% to 99.6%)	84.4% (77.6% to 91.2%)	93.5% (91.7% to 95.4%)	75.2% (67.1% to 83.3%)	90.1% (87.9% to 92.4%)	75.2% (67.1% to 83.3%)	97.1% (95.8% to 98.3%)	77.1% (69.2% to 85.0%)
Sensitivity	93.7% (91.6% to 95.8%)	61.9% (47.2% to 76.6%)	87.3% (84.4% to 90.1%)	59.5% (44.7% to 74.4%)	88.6% (85.9% to 91.4%)	61.9% (47.2% to 76.6%)	88.4% (85.7% to 91.2%)	66.7% (52.4% to 80.9%)
NPV	95.5% (93.9% to 97.0%)	85.2% (78.5% to 91.9%)	90.7% (88.6% to 92.9%)	82.8% (75.4% to 90.3%)	91.4% (89.2% to 93.5%)	83.7% (76.4% to 91.0%)	91.8% (89.8% to 93.8%)	85.7% (78.8% to 92.6%)
PPV	98.4% (97.2% to 99.5%)	60.5% (45.9% to 75.1%)	91.0% (88.5% to 93.5%)	48.1% (34.5% to 61.7%)	87.1% (84.2% to 90.0%)	49.1% (35.6% to 62.5%)	95.8% (93.9% to 97.6%)	52.8% (39.4% to 66.3%)
AUC	0.96 (0.95 to 0.97)	0.73 (0.65 to 0.81)	0.90 (0.88 to 0.92)	0.67 (0.59 to 0.76)	0.89 (0.88 to 0.91)	0.69 (0.60 to 0.77)	0.93 (0.91 to 0.94)	0.72 (0.64 to 0.80)
Kappa	0.93 (0.91 to 0.95)	0.46 (0.30 to 0.62)	0.81 (0.78 to 0.85)	0.32 (0.17 to 0.48)	0.79 (0.75 to 0.82)	0.34 (0.19 to 0.50)	0.86 (0.83 to 0.89)	0.41 (0.25 to 0.56)

Notes:Data in parentheses represent 95% CI.
Abbreviations: NPV, negative predictive value; PPV, positive predictive value; AUC, area under the ROC curve; Kappa, auxiliary indexes to evaluate the predictive effect of model. GBM, gradient boosting machine; SVM, support vector machine; CART, classification and regression trees; RF, random forest.

Importance and Venn Diagram Analysis of Variables of Four Model

The importance of biomarkers in predicting active TB was assessed through, variable importance analysis across the four models (Figure 2). It revealed that IFN-γ was consistently the most important variable for predicting TB in four models. In the GBM model, the top ten most important variables, ranked by importance, were: IFN-γ (100.0), age (85.8), IL-6 (74.4), TT (72.4), IL-10 (63.8), GGT (54.5), ALT (47.5), TBIL (39.2), IL-17 (35.9), and BAS-CT (35.1) (Figure 2). The ranking of variables importance for all four models is provided in Supplemental Table 3.

The common element of the top ten important variables across all four models was analyzed using a Venn diagram, which revealed that IFN-γ, IL-6 and age were consistently identified as key biomarkers across all models, and both IFN-γ, IL-6 and age are key biomarkers for predicting active TB in PLWH (Figure 3).

Reanalysis of Chronological Dataset

The dataset was reanalysed using the first 609 records as the training set and subsequent 151 records as the test set. In training set, the GBM model achieved the following performance metrics: 92.7% accuracy (95% CI: 92.7% to 92.7%), 96.2% specificity (95% CI: 94.7.0% to 97.6%), 88.2% sensitivity (95% CI: 85.4% to 91.0%), 91.5% NPV (95% CI: 89.5% to 973.6%), 94.5% PPV (95% CI: 92.4.% to 96.6%), and 0.92 AUC (95% CI: 0.91 to 0.94). In validation set: the model showed only 70.4% accuracy (95% CI: 70.1% to 70.7%), 76.1% specificity (95% CI: 68.1% to 84.1%), 55.8% sensitivity (95% CI: 40.0% to 70.7%), 81.4% NPV (95% CI: 73.8% to 88.9%), 48.0% PPV (95% CI: 34.2% to 61.8%), and 0.66 AUC (95% CI: 0.58 to 0.75). Comparison of four models of the training set and the verification set is presented in randomly split datasets (Supplemental Table 4). On chronological order, GBM model trained from the first 609 records AUC of 0.92 (0.91, 0.94) poorly predicted the 151 final records with the AUC of 0.66 (0.58, 0.75).

Discussion

In this retrospective study, we developed and validated four predictive models based on demographic characteristics and clinical parameters to predict active TB in PLWH with pulmonary infection patients. Our results show that GBM model

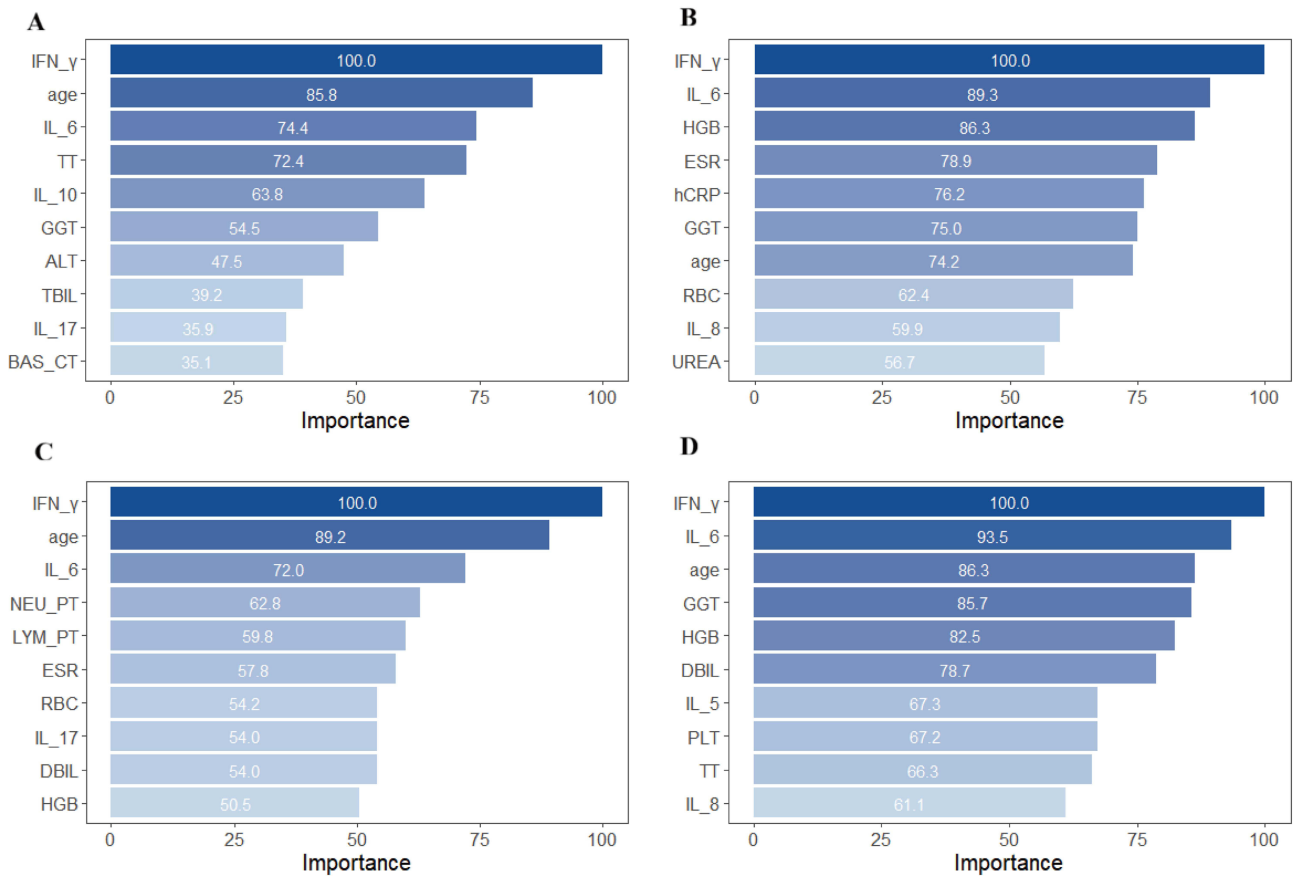

Figure 2 Importance of variables for top ten biomarkers of four models of GBM (**A**), SVM (**B**), CART (**C**), and RF (**D**); higher values indicating greater importance in the model.
Abbreviations: ALT, alanine aminotransferase; BAS_CT, basophil count; DBIL, direct bilirubin; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; HGB, hemoglobin; hCRP, hypersensitive C-reactive protein; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-17, interleukin-17; IFN-γ, interferon-γ; LYM_PT, percentage of lymphocytes; NEU_PT, percentage of neutrophils; PLT, blood platelets; RBC, red blood cells; TT, thrombin time; TBIL, total bilirubin; UREA, urea.

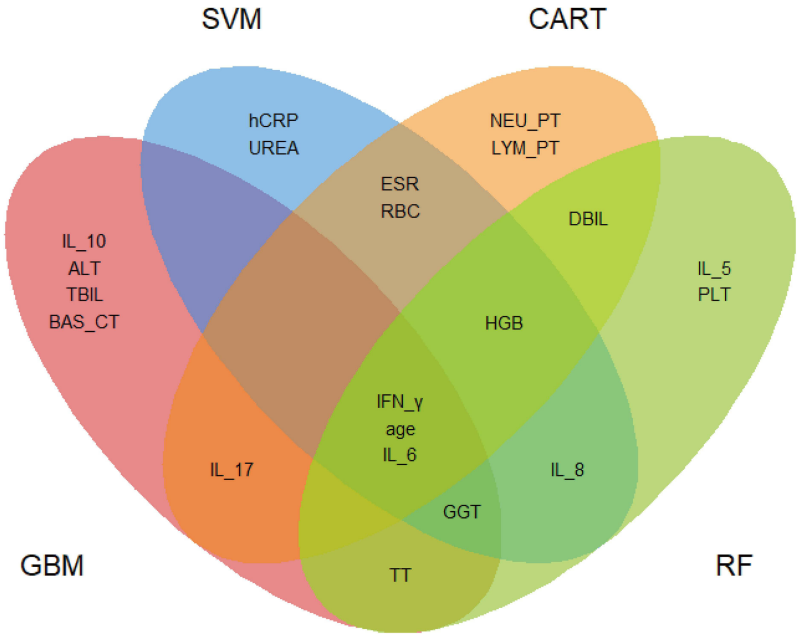

Figure 3 Intersection of top ten predictive variables in four.
Abbreviations: ALT, alanine aminotransferase; BAS_CT, basophil count; DBIL, direct bilirubin; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; HGB, hemoglobin; hCRP, hypersensitive C-reactive protein; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-17, interleukin-17; IFN-γ, interferon-γ; LYM_PT, percentage of lymphocytes; NEU_PT, percentage of neutrophils; PLT, blood platelets; RBC, red blood cells; TT, thrombin time; TBIL, total bilirubin; UREA, urea.

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offers robust predictive value in random dataset. Additionally, serum cytokines, particularly IFN-γ and IL-6 demonstrated stable significance in both the variable importance analysis and Venn diagram. However, inability of GBM model to have good prediction in chronological validation dataset.

GBM model is a good predictor of active TB in PLWH. It performed well in AUC, specificity and sensitivity. At present, the GBM model has been widely used in disease prediction, including the diagnosis of gastrointestinal stromal tumors,[11] the prediction of survival in patients with colorectal cancer,[12] the detection of coronary artery disease.[13] GBM model has high predictive performances at the individual scale, and it was demonstrated significant predictive efficacy and found extensive applications in disease prediction.[12]

Various ML algorithms, including the GBM model, have been developed and applied to tackle many problems in TB-related research.[14] There exist several models utilized for the diagnosis of TB. A retrospective study developed an ML model based on leukocyte volume, conductivity, and light scatter parameters, which demonstrated strong potential for distinguishing active TB and Latent TB infection.[15] Liu et al developed a diagnostic model for active TB that showed a remarkable potential for efficient and accurate diagnosis.[16] Queiroz et al used a neural network-based ML approach to identify TB in advanced PLWH using plasma concentrations of interleukin 15 and interleukin 10.[17] Ye et al employed ML to identify blood-specific factors in patients with bone and joint TB, constructing a diagnostic model that could aid clinicians in future diagnosis.[18] Zhang et al built logistic regression, RF, SVM, and k-nearest neighbor models using four-cytokines to identify TB among PLWH.[19] Despite these advances, to the best of our knowledge, the predictive potential of biomarkers for TB in PLWH individuals has been limited, with few studies establishing reliable models linking HIV and TB.[10]

In our study, we found that value of IFN-γ and IL-6 are higher in patients with active TB compared with patients without active TB. Our research outcomes are in accordance with the finding documented in the current literature.[19,20] Multiple possible explanations exist for the strong predictive value of these two cytokines. IFN-γ and IL-6 are elevated in TB patients, which may be related to the body's inflammatory response. When people are infected with Mtb, IFN-γ in the body will increase to help macrophages in getting rid of the Mtb infection.[21,22] IFN-γ is involved in reactive oxygen species production and Mtb clearance by inducing gene expression in the GTPase family,[23] and IFN-γ can limit the replication of Mtb within macrophages by significantly reducing the use of iron by intracellular Mtb.[24,25] Although HIV-associated immune dysfunction, PLWH often exhibit indeterminate or blunted IFN-γ responses. In TB patients of PLWH, IFN-γ levels may tend to increase compared to without active TB patients. The literature also indicates that IFN-γ is an important predictor of TB in PLWH,[19] this aligns with our research findings. When people are infected with Mtb, the infection triggers dysregulation of IL-6 production in the body, leading to an excessive IL-6 production in epithelium.[26] IL-6 induces the expression of various proteins responsible for acute inflammation, excessive production of IL-6 indicates that the body is undergoing an autoimmune and inflammatory response.[27] IL-6-induced immune dysregulation is a key contributor to the pathogenesis of TB.[28,29] Many studies have shown IFN-γ and IL-6 serve as pivotal biomarkers not only in the diagnosis of TB but also throughout its treatment progress, severity assessment, and prognosis, playing an indispensable role in each of these aspects.[30–38]

To address the limitations of Expert (which directly detects TB and drug resistance) and IGRA (which requires complex antigen stimulation and processing), the use of IFN-γ and IL-6 can help evaluate immune status, differentiate infection, or monitor treatment. This approach offers faster speed, accessibility, lower cost, and easier specimen collection. It holds particular clinical value in patients with false-negative IGRA results.[39] IFN-γ and IL-6 provides different information than current tests that pathogen-based detection (eg, GeneXpert) and immune-based assays (eg, IGRA). By employing multi-factor combinatorial strategies, it can improve diagnostic accuracy, especially in complex or resource-limited settings.[40]

In our study, we also found that value of age is lower in patients with active TB compared with patients without active TB. It is consistent with the literature reports prevalence of TB is most common in the 15–44 age range.[41] People in this age group tend to have a high prevalence rate of TB infection. Coupled with their participation in a wider range of social activities and high-risk behaviors for TB, they deserve more attention. Both hCRP, an acute-phase protein, and ESR, which reflects levels of acute-phase proteins such as fibrinogen, were elevated in cases of TB infection.[42–44] Our study likewise observed this association. An increase in HGB may occur during TB infection. This is supported by the findings of our study.[45] NEU_PT, commonly used as an inflammatory biomarker, tends to increase following TB infection.[46] In

contrast, LYM_CT and LYM_PT, which reflect the host's immune status, are often observed to decrease in TB infection, as reported in previous studies.[47] The decrease in PALB levels, which correlates with chronic consumption and inflammatory activity, is often significantly reduced in TB patients due to inflammatory consumption and loss of appetite—a finding consistent with our observations.[48] Similarly, elevations in GGT and ALP, sensitive indicators of hepatobiliary disease, may occur in TB as a result of the infection itself or drug-induced liver injury from anti-TB medications,[44,49] which aligns with our study results. Our findings are consistent with the observation that UREA levels may decrease in TB infection, potentially due to its utilization as a nitrogen source by Mtb.[50]

Our study focuses on predicting TB in PLWH with pulmonary infections. The dataset does not include other infectious diseases, the identified biomarkers might simply reflect general inflammation rather than signatures specific to TB. However, other inflammatory mediators such as CRP, NEU-PT and others were not a significant predictor for TB. Thus, the two cytokines of IL-6 and IFN-γ probably had the highest link to TB process than other cytokines. The difference in roles of different cytokines needs to be clarified by future research.

Our study offers several strengths. We are the first to utilize ML models to predict TB in PLWH with pulmonary infection. The optimal model was selected through rigorous analysis using various ML techniques and demonstrated superior predictive performance. The sufficient sample size in this study provides a robust foundation for model development and validation. However, the study has several limitations. First, all participants were PLWH presenting with pulmonary infection symptoms, their baseline levels of IFN-γ and IL-6 may differ systematically from those in HIV-negative individuals. Therefore, our diagnostic model may not be directly applicable to HIV-negative individuals. Secondly, while the GBM model exhibited good performance in both the training and the validation sets, it has not been tested on external cohorts. The single-center nature of the study may also limit the generalizability of the findings to a global population. Finally, our study cannot distinguish active TB or Latent tuberculosis infection (LTBI). Without active TB group may include some individuals with LTBI, which could affect the performance of our model.

That the GBM model has good predictions was consistently reported by the previous investigator.[15,18,19] Their conclusions on high predictive values of the biomarkers were however based solely on predicting the random subsets. Theoretically, the relationship between variables in the random subset would be closed to that in the training (random) subset. This way of splitting created a flaw of overvaluing the predicting ability. The predictivity of the model in the training subset declined in the chronological subset indicated that in reality, the relationship between the set of variables had been changed over time. We strongly advocate this chronological approach in future work to avoid misleading results.

Conclusion

In conclusion, our findings indicate GBM model effectively predicts active TB in PLWH, based on the data from blood samples even without sputum and alveolar lavage fluid samples. This predictive model provides an alternative approach for diagnosing active TB in PLWH. IFN-γ and IL-6 are valuable biomarkers for predicting active TB in this population. These biomarkers may play a crucial role in detecting active TB among PLWH and offer valuable insights for monitoring and preventing TB in this high-risk group.

Abbreviations

AFU, alpha-L-fucosidase; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, partial prothrombin time; AST, aspartate aminotransferase; BAS_CT, basophil count; BAS_PT, percentage of basophils; CREA, creatinine; DBIL, direct bilirubin; EOS_CT, eosinophil count; EOS_PT, percentage of eosinophils; ESR, erythrocyte sedimentation rate; FIB, fibrinogen; GGT, gamma-glutamyltransferase; GLDH, glutamate dehydrogenase; GLOB, globulin; hCRP, hypersensitive C-reactive protein; HGB, hemoglobin; IFN-α, interferon-α; IFN-γ, interferon-γ; IL-10, interleukin-10; IL-12p70, interleukin-12p70; IL-1β, interleukin-1β; IL-17, interleukin-17; IL-2, interleukin-2; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; INR, international normalized ratio; LYM_CT, lymphocyte count; LYM_PT, percentage of lymphocytes; MON_CT, monocytes count; MON_PT, percentage of monocytes; NEU_CT, neutrophil count; NEU_PT, percentage of neutrophils; PALB, prealbumin; PCT, procalcitonin; PLT, blood platelets; PT, prothrombin time; PT_PT, PT range of motion; RBC, red blood cells; TBIL, total bilirubin; TNF-α, tumor necrosis factor-α; TT, thrombin time; UA, uric acid; UREA, urea; WBC, white blood cell count.

Ethical Approval and Consent to Participate

Ethics approval was obtained from the Ethics Committee of Kunming Third People's Hospital (No. KSLL2023071153). This study was conducted in compliance with the Declaration of Helsinki.

The need for informed consent to participate was waived by the Ethics Committee of Kunming Third People's Hospital because of the retrospective nature of the study. The names and identification numbers of all patients included in this study were anonymized to ensure confidentiality and protect the identities of the participants.

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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 report no conflicts of interest in this work.

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