Metformin promotes smear conversion in tuberculosis-diabetes comorbidity and construction of prediction models

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Abstract
Background: The comorbidity of tuberculosis (TB) and diabetes mellitus (DM) is a global health concern. Metformin is commonly used in DM but the potential effectiveness in comorbid patients is uncertain. This retrospective study aims to investigate the effect of metformin on TB-DM comorbidity and construct prediction models.

Methods: Patients diagnosed with TB-DM in West China Hospital were retrospectively enrolled from Nov 2013 to Sep 2019. Electronic health records of patients were extracted. Two-month smear conversion (2SC) was considered an outcome indicator of TB. Univariate and multivariate logistic regression (LR) were used to assess the role of metformin and other independent predictors. Meanwhile, prediction models were built by LR, elastic net regression, support vector machine, k-nearest neighbors, and random forest.

Results: A total of 927 individuals were recruited, among which 408 (44.01%) were metformin-exposed patients. A higher 2SC rate was observed in the metformin users. Other impact factors such as smoking, glucose, and creatinine levels were also identified. Multivariable models were then constructed using filtered variables. The support vector machine model yields the highest AUC (0.808, 95% CI: 0.767–0.849) and specificity (83.24%). LR model outperformed others in terms of sensitivity (69.71%).

Conclusion: This retrospective study of a large population from southwestern China provides strong clinical evidence for the positive effects of metformin in TB-DM. Metformin is associated with a better therapeutic outcome and promising for the adjuvant therapy of TB-DM. Furthermore, a combination of support vector machine and LR models is recommended to discriminate the patients with poor treatment outcomes.

Keywords: tuberculosis, diabetes mellitus, metformin, smear conversion, prediction model

Yili Wang, Yanbing Zhou and Liyu Chen contributed equally to this work.

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1 INTRODUCTION

Accumulating evidence suggests that comorbidity of tuberculosis (TB) and diabetes mellitus (DM) is imposing a heavy financial and public health burden worldwide.\(^1\)\(^2\) This is not only because of the increase in DM patients but also because patients with DM are 1.5–3.6 times more likely to develop active TB than those without DM.\(^3\)\(^4\) There were approximately 463 million DM patients\(^5\) and an estimated 21.9 million patients suffering from TB.\(^6\) Worse still, DM was shown to be associated with a higher proportion of anti-TB treatment failure (Odds Ratio [OR] = 1.7),\(^7\) death (OR = 1.7),\(^7\) and relapse (Risk Ratio = 3.9),\(^8\) because DM patients usually experienced delayed drug absorption and lower drug exposure, ultimately leading to anti-TB drug resistance.\(^9\) To combat the growing burden of TB-DM comorbidity, especially the challenges associated with medical treatment, developing novel drugs or exploring the new roles of current drugs is considered an available approach. Notably, the development of new drugs requires a lot of time and money, so exploring the new roles of current drugs may be a more practical consideration. Among focused current drugs, metformin is one of the candidates in the anti-TB field.\(^10\)\(^11\)

As an AMP-activated protein kinase (AMPK) activator, metformin is approved by the Food and Drug Administration (FDA) for the treatment of DM. AMPK is a key energy sensor that promotes autophagy, which is essential in the clearance of intracellular Mycobacterium tuberculosis (Mt\(b\)).\(^12\)\(^13\) Therefore, the effect of metformin on TB infection has drawn extensive attention over recent years. Mechanistically, metformin may inhibit the survival of intracellular Mt\(b\) by activating AMPK, increasing reactive oxygen species (ROS), oxidative stress, and inducing autophagy.\(^14\)\(^15\) However, the effects of metformin have shown inconsistencies in both animal models and clinical studies.

Singhal et al.\(^14\) showed that the use of metformin inhibited the growth of Mt\(b\) and reduced chronic inflammation in the lungs of Mt\(b\)-infected mice. In contrast, another study did not observe the effectiveness of metformin for anti-TB treatment. Mice receiving adjuvant treatment with metformin had a similar lung bacterial burden during treatment as control mice, and the proportion of relapses was similar in both groups.\(^17\) In clinical studies, several studies have reported that metformin administration is associated with lower mortality in TB patients with DM,\(^18\)\(^19\) Ma et al.\(^20\) first explored the effect of metformin on better treatment outcomes and prognosis in a Chinese population with DM. Higher smear conversion rates and treatment success were observed in the metformin use group, significant differences were lacking, though.\(^20\) Similarly, no significant effect of metformin on sputum culture conversion and relapse after completion of TB treatment was indicated by another retrospective cohort study in Korea.\(^21\)

Collectively, it is shown that the effect of metformin on anti-TB treatment in TB-DM comorbidity is ambiguous in different studies. These controversies led us to conduct this study to explore the potential effect of metformin on TB-DM comorbid patients. We aim to clarify the function of metformin in TB-DM patients’ treatment outcomes. Additionally, predictive models by machine learning are established to help treatment and management strategies in clinical practice.

2 MATERIALS AND METHODS

2.1 Ethics statement

This study was approved by the Institutional Review Board of West China Hospital, Sichuan University, and was registered at the Clinical Trial Registry (ChiCTR1900028670) as part of the research project.

2.2 Study participants

Patients diagnosed with TB and DM in the West China Hospital of Sichuan University between December 2013 and September 2019 were retrospectively enrolled. All included cases needed to meet the following criteria: (1) adult males or females aged ≥ 18 years old; (2) patients with tuberculosis. Subjects were evaluated at the West China Hospital of Sichuan University (Chengdu, China). The diagnosis was established according to the Health Industry Standard of the People’s Republic of China – Diagnosis of Tuberculosis (WS 288–2017).\(^24\) by combing clinical manifestations, imaging data together with laboratory tests; (3) Individuals with type 2 diabetes: the diagnosis of diabetes was performed according to (WHO) criteria,\(^25\) respectively. If any of the following criteria were met, the cases would be ruled out. Exclusion criteria: (1) pregnant or lactating women; (2) patients with severe acute trauma or burns, hepatic failure, renal failure, metastatic carcinoma, and end-stage illness; (3) multidrug-resistant TB or extremely drug-resistant TB; (4) patients with incomplete electronic health records. Based on the presence or absence of metformin treatment, 927 patients were divided into 408 (44.01%) in the metformin-user group and 519 (55.99%) in the non-metformin-user group. Based on whether the smear turned negative after 2 months of intensive treatment, 927 patients were divided into 544 (58.68%) in the smear-negative conversion group and 383 (41.32%) in the persistent smear-positive group. The demographic and clinical data were compared among groups.

2.3 Data collection and processing

Electronic health records (EHR), the original record of the patient in the hospital, were collected and available information was extracted by two independent authors. A total of 68 items in four aspects, general characteristics of patients (age, gender,
etc.), clinical manifestations (fever, sweat, etc.), laboratory tests (acid-fast bacilli and culture, blood glucose, white blood cells, etc.), imaging data (lesion location, lesion size, etc.) were collected. If missing data were greater than 30%, the corresponding indicators would be removed. The remaining indicators would undergo multiple imputations by chained equations (MICE). The accuracy of multiple imputations for each included indicator was further assessed.

2.4 | The definition of TB-related prediction indicators

Two-month smear conversion (2SC) was defined as positive Acid-Fast Bacillus smears result before treatment and negative results after the 2-month intensive phase of anti-TB treatment. Relapse referred to recurrent TB according to microbiological, clinical, and epidemiological information after successful treatment of TB disease. TB-related death referred to death due to respiratory failure or severe hemoptysis associated with TB before the negative conversion of the smear.

2.5 | Feature selection and model development

Participants were randomly divided into the training set and the testing set according to the ratio of 1:1. The stepwise selection by logistic regression (LR) was performed to select risk factors, and features with \( p < 0.05 \) were finally entered model construction. Four machine learning methods (LR, support vector machine [SVM], k-nearest neighbors [KNN], and random forest [RF]) were performed to build models to offer an optimum choice in clinical practice. Elastic net regression (ENR) was also applied to select variables for the ENR model independently. The performance of each model was evaluated by sensitivity, specificity, and area under the curve (AUC). Receiver operating characteristic (ROC) curves and calibration curves were plotted to visualize the diagnostic performance. All analyses were realized by R version 4.1.0 and Python version 3.6.

2.6 | Statistical analysis

Categorical variables were compared using a chi-squared test or Fisher exact test, while continuous variables were compared by t test or Mann–Whitney U test. Univariate and multivariate LR were applied to identify independent risk factors of time-independent outcomes (2-month smear conversion), while Cox regression was used to explore independent risk factors of time-dependent outcomes (relapse and relapse time, death and overall survival, etc.). Odds ratio (OR) and hazard ratio (HR) with a 95% confidence interval (CI) were calculated. The difference was considered statistically significant when the \( p \)-value was <0.05. All analyses were carried out by SPSS version 21.0.

3 | RESULTS

3.1 | Case selection and characteristics

In total, 2318 patients with TB-DM comorbidity were consecutively included in this work from West China Hospital of Sichuan University between December 2013 and September 2019. Patients with incomplete EHR and negative Acid-Fast Bacillus smear before treatment initiation were excluded. Eventually, 927 participants were recruited. Among them, 408 (44.01%) cases were metformin users while the remaining 519 (55.99%) were patients who had not taken metformin.

The general demographic characteristics, clinical manifestations, imaging data, and laboratory data of the enrolled patients, are shown in Table 1. In terms of general characteristics, no significant difference between these two groups was found, except for gender proportion (male/total, 69.85 vs. 76.88%, \( p = 0.016 \)). The most common comorbidity was hypertension (38.62%), followed by rheumatoid arthritis, a higher proportion of which was observed in non-metformin users (9.56 vs. 15.41%, \( p = 0.008 \)). No meaningful findings were observed in the aspect of clinical manifestations and imaging data. For laboratory tests, decrease cholesterol levels were observed (4.0 vs. 4.1 mmol/L, \( p = 0.048 \)) in metformin users.

Comparisons of clinical characteristics between the smear-negative conversion group and the persistent smear-positive group are shown in Table 2. No significant differences concerning age, gender, and nationality were observed between these two groups. The proportion of receiving metformin treatment was significantly higher in the smear-negative conversion group than in the persistently positive group (56.10% vs. 26.90%, \( p < 0.001 \)).

3.2 | Independent risk factors for TB-related prediction indicators

For 2SC, metformin usage was shown to be an independent protective factor. Metformin users showed higher odds of 2SC than these non-metformin users (OR = 3.672, 95% CI: 2.745–4.914, \( p < 0.001 \)). Moreover, loss of weight (OR = 1.534, 95% CI: 1.046–2.248, \( p = 0.028 \)), glucose (GLU) levels (OR = 1.047, 95% CI: 1.014–1.080, \( p = 0.004 \)), and \( \gamma \)-glutamyl transferase (GGT) (OR = 1.002, 95% CI: 1.000–1.004, \( p = 0.017 \)) were identified as independent protective factors, whereas smoking (OR = 0.675, 95% CI: 0.509–0.894, \( p = 0.006 \)), pulmonary cavity/bulla (OR = 0.710, 95% CI: 0.535–0.943, \( p = 0.018 \)), glycaed hemoglobin (HbA1c) (OR = 0.941, 95% CI: 0.886–1.000, \( p = 0.049 \)), and creatinine levels (OR = 0.998, 95% CI: 0.997–1.000, \( p = 0.013 \)) were independent risk factors of 2SC. With relapse as the outcome variable, multivariate LR revealed that hemoglobin (OR = 0.995, 95% CI: 0.990–1.000, \( p = 0.056 \)) and interleukin 6 (OR = 1.001, 95% CI: 1.000–1.002, \( p = 0.073 \)) were potentially related indicators, but lacked of statistical significance. The data on death time were missed so much that the indicator was excluded from the following analysis.
**TABLE 1  Baseline characteristics of metformin user and non-metformin user group**

| Characteristics                                      | All included patients (n = 927) | Different groups | p Value |
|-------------------------------------------------------|--------------------------------|------------------|---------|
| **General characteristics**                           |                                |                  |         |
| Age (percent_{25}, percent_{75})                      | 60.00 (51.00–70.00)            |                  | 0.518   |
| Nationality, Han/others                               | 851/76                         |                  | 0.283   |
| Sex, male/female                                      | 684/243                        |                  | 0.016*  |
| BMI, n                                                | 13/54/678/119/63               |                  | 0.459   |
| Smoking, n (%)                                        | 466 (50.27)                    |                  | 0.500   |
| TB subtype (pulmonary/extra-pulmonary/both), n        | 728/149/50                     |                  | 0.192   |
| Hepatitis B, n (%)                                    | 86 (9.28)                      |                  | 0.846   |
| HIV, n (%)                                            | 5 (0.54)                       |                  | 0.659   |
| Hypertension, n (%)                                   | 358 (38.62)                    |                  | 0.460   |
| RA, n (%)                                             | 119 (12.84)                    |                  | 0.008** |
| **Clinical manifestations**                           |                                |                  |         |
| Fever, n (%)                                          | 217 (23.41)                    | 98 (24.02)       | 0.697   |
| Sweat, n (%)                                          | 107 (11.54)                    | 45 (11.03)       | 0.665   |
| Fatigue, n (%)                                        | 120 (12.94)                    | 50 (12.25)       | 0.579   |
| Loss of weight, n (%)                                 | 161 (17.37)                    | 75 (18.38)       | 0.470   |
| Cough, n (%)                                          | 405 (43.69)                    | 186 (45.59)      | 0.301   |
| Produce sputum, n (%)                                 | 366 (39.48)                    | 162 (39.71)      | 0.902   |
| Joint pain, n (%)                                     | 78 (8.41)                      | 37 (9.07)        | 0.525   |
| Polydipsia, n (%)                                     | 44 (4.75)                      | 23 (5.64)        | 0.258   |
| Polyphagia, n (%)                                     | 19 (2.05)                      | 10 (2.45)        | 0.444   |
| Polyuria, n (%)                                       | 45 (4.85)                      | 21 (5.15)        | 0.713   |
| **Imaging examinations**                              |                                |                  |         |
| Cavity/pulmonary bulla, n (%)                         | 714 (77.02)                    | 323 (79.17)      | 0.169   |
| Pulmonary nodule/calcification, n (%)                 | 444 (47.90)                    | 197 (48.28)      | 0.834   |
| Pulmonary shadow of X-ray, n (%)                      | 310 (33.44)                    | 138 (33.82)      | 0.827   |
| Pulmonary effusion/pneumatosis, n (%)                 | 588 (63.43)                    | 249 (61.03)      | 0.178   |
| Inflammation of the lung that involves the pleura and lymph gland, n (%) | 476 (51.35) | 205 (50.25) | 0.551 |
| **Laboratory data, median (percent_{25}, percent_{75})** |                                |                  |         |
| HbA1c (%)                                             | 8.00 (6.90–9.80)               | 8.10 (6.90–9.78) | 0.295   |
| FBG (mmol/L)                                          | 8.00 (6.65–10.00)              | 8.00 (6.61–10.00)| 0.741   |
| Erythrocyte sedimentation rate (mm/h)                | 45.00 (26.00–72.00)            | 44.50 (26.00–71.00)| 0.755  |
| Hemoglobin (g/L)                                      | 126.00 (108.00–140.00)         | 126.00 (105.00–142.00)| 0.496  |
| Platelet (10^12/L)                                    | 188.00 (133.00–257.00)         | 189.00 (134.25–263.75)| 0.929  |
| White blood cell (10^9/L)                             | 6.59 (5.13–8.53)               | 6.53 (5.05–8.25) | 0.203   |
| Neutrophil percent (%)                                | 69.70 (61.00–77.20)            | 69.50 (61.33–77.68)| 0.662  |
| Lymphocyte percent (%)                                | 20.10 (12.90–27.80)            | 20.00 (12.83–27.60)| 0.973  |
| Monocyte percent (%)                                  | 6.70 (5.30–8.40)               | 6.70 (5.20–8.50) | 0.692   |
| Eosinophil percent (%)                                | 1.60 (0.70–3.00)               | 1.70 (0.70–3.20) | 0.682   |
| Basophil percent (%)                                  | 0.30 (0.20–0.50)               | 0.30 (0.20–0.50) | 0.951   |
| TBIL (μmol/L)                                         | 10.00 (7.00–14.10)             | 10.15 (7.10–14.25)| 0.824   |
3.3 Features selection and model performance

To facilitate clinical application, predictive models for 2SC were further built. According to the ratio of 1:1, the 927 participants were randomly divided into the training set (464 patients) and the testing set (463 patients). A total of eight variables (metformin usage, loss of weight, GLU, GGT, pulmonary cavity/bulla, smoking, creatinine levels, and HbA1c) were selected by univariate and multivariate LR analysis. Based on the above features, four machine learning methods (LR, SVM, KNN, and RF) were used for constructing TB-related predictive models. For ENR models, variables including metformin usage, body mass index (BMI), smoking, loss of weight, polyphagous, erythrocyte sedimentation rate (ESR), basophil, alanine aminotransferase (ALT), GLU, creatinine, triglyceride (TG), and cholesterol were selected for modeling.
| Characteristics | All included patients (n = 927) | Persistent smear-positive (n = 383) | Smear-negative conversion (n = 544) | p Value |
|-----------------|-------------------------------|-----------------------------------|-----------------------------------|---------|
| General characteristics | | | | |
| Age (percent 25–percent 75) | 60.00 (51.00–70.00) | 61.00 (50.00–71.00) | 60.00 (51.00–70.00) | 0.570 |
| Nationality, Han/others | 851/76 | 350/33 | 501/43 | 0.716 |
| Sex, male/female | 684/243 | 274/109 | 410/134 | 0.198 |
| BMI, n | 13/54/678/119/63 | 6/21/294/41/21 | 13/54/678/119/63 | 0.249 |
| Smoking, n (%) | 466 (50.27) | 210 (54.83) | 251 (46.14) | 0.009** |
| TB subtype (pulmonary/extra-pulmonary/both), n | 728/149/50 | 289/75/19 | 439/174/31 | 0.049* |
| Hepatitis B, n (%) | 86 (9.28) | 32 (8.04) | 54 (9.90) | 0.491 |
| HIV, n (%) | 5 (0.54) | 1 (0.30) | 4 (0.70) | 0.654 |
| Hypertension, n (%) | 358 (38.62) | 142 (37.10) | 216 (39.70) | 0.451 |
| RA, n (%) | 119 (12.84) | 50 (13.10) | 69 (12.70) | 0.921 |
| Metformin treatment, n (%) | 408 (44.00) | 103 (26.90) | 305 (56.10) | <0.001*** |
| Clinical manifestations | | | | |
| Fever, n (%) | 217 (23.41) | 96 (25.10) | 121 (22.20) | 0.345 |
| Sweat, n (%) | 107 (11.54) | 43 (11.20) | 64 (11.80) | 0.835 |
| Fatigue, n (%) | 120 (12.94) | 46 (12.00) | 74 (13.60) | 0.489 |
| Loss of weight, n (%) | 161 (17.37) | 53 (13.80) | 108 (19.90) | 0.018* |
| Cough, n (%) | 405 (43.69) | 172 (44.90) | 233 (42.80) | 0.545 |
| Produce sputum, n (%) | 366 (39.48) | 156 (40.70) | 210 (38.60) | 0.539 |
| Joint pain, n (%) | 78 (8.41) | 35 (9.10) | 43 (4.60) | 0.549 |
| Polydipsia, n (%) | 44 (4.75) | 19 (5.00) | 25 (4.60) | 0.876 |
| Polyphagia, n (%) | 19 (2.05) | 10 (2.60) | 9 (1.70) | 0.351 |
| Polynia, n (%) | 45 (4.85) | 20 (5.20) | 25 (4.60) | 0.757 |
| Imaging examinations | | | | |
| Cavity/pulmonary bulla, n (%) | 714 (77.02) | 286 (74.70) | 428 (78.70) | 0.155 |
| Pulmonary nodule/calcification, n (%) | 444 (47.90) | 167 (43.60) | 277 (50.90) | 0.033* |
| Pulmonary shadow of X-ray, n (%) | 310 (33.44) | 119 (31.10) | 191 (35.10) | 0.204 |
| Pulmonary effusion/pneumatosis, n (%) | 588 (63.43) | 246 (64.20) | 202 (62.90) | 0.678 |
| Inflammation of the lung that involves the pleura and lymph gland, n (%) | 476 (51.35) | 191 (49.90) | 285 (52.40) | 0.463 |
| Laboratory data, median (percent 25–percent 75) | | | | |
| HbA1c (%) | 8.00 (6.90–9.80) | 8.00 (6.90–10.20) | 8.00 (6.90–9.70) | 0.457 |
| FBG (mmol/L) | 8.00 (6.65–10.00) | 8.00 (6.62–9.65) | 8.00 (6.65–10.00) | 0.241 |
| Erythrocyte sedimentation rate (mm/h) | 45.00 (26.00–72.00) | 46.00 (27.00–73.00) | 43.00 (25.25–71.00) | 0.244 |
| Hemoglobin (g/L) | 126.00 (108.00–140.00) | 126.00 (109.00–139.00) | 126.00 (106.25–141.00) | 0.820 |
| Platelet (10^9/L) | 188.00 (133.00–257.00) | 187.00 (135.00–253.00) | 189.50 (131.00–261.00) | 0.883 |
| White blood cell (10^9/L) | 6.59 (5.13–8.53) | 6.51 (5.05–8.68) | 6.64 (5.18–8.32) | 0.800 |
| Neutrophil percent (%) | 69.70 (61.00–77.20) | 69.50 (61.00–77.20) | 69.75 (61.00–77.28) | 0.954 |
| Lymphocyte percent (%) | 20.10 (12.90–27.80) | 20.00 (13.00–27.60) | 20.15 (12.83–28.00) | 0.895 |
In the training set, the SVM model exhibited the highest AUC (0.808, 95% CI: 0.767–0.849) and specificity (83.24%). The sensitivity of this model was 65.60%. The second highest AUC was observed in the KNN model (0.719, 95% CI: 0.673–0.766), with the highest sensitivity of 66.18% and specificity of 65.59%. The AUC of the LR model and ENR model were 0.696 (95% CI: 0.649–0.743)
and 0.711 (95% CI: 0.664–0.757), respectively. The AUC curves of models in the training and testing set are exhibited in Figures 1 and 2, respectively. The overfitting phenomenon appeared during the construction of RF models whose AUC, sensitivity, and specificity were all equal to 1 or 100%, so it was excluded in the above comparison.

In the testing set, the AUC of the SVM model was 0.579 (95% CI: 0.526–0.631), while the specificity and sensitivity reached 62.11% and 51.01%, respectively. The AUC of the KNN model was slightly lower (0.675) than that of the training set, but the sensitivity (66.91%) and specificity (62.44%) were similar. The LR model had the highest AUC (0.704, 95% CI: 0.655–0.753) with a sensitivity of 69.71% but relatively low specificity (61.90%). All models' performance is listed in Table 3.

### DISCUSSION

Investigating the potential role of metformin as an adjuvant treatment for TB-DM is well warranted. In the study, a total of 927 TB-DM patients were eventually enrolled and 68 characteristics from four aspects of each subject were collected. With rigorous screening and data procession, our results suggested that metformin usage was a significant protective factor of the 2SC rate. Based on the above findings, five prediction models were further developed for predicting TB treatment outcomes.

Data analysis of baseline characteristics revealed that metformin users had a lower incidence of rheumatoid arthritis and lower cholesterol levels compared with non-metformin users. A cohort study suggested that adherence to metformin treatment was associated with

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**Figure 1** The receiver operating characteristic (ROC) curves of the (A) elastic net regression; (B) k-nearest neighbors; (C) logistic regression; and (D) support vector machine models in the training set.
a decreased risk of developing autoimmune disease in women. A clue to potential mechanisms may lie in the modulating immune and anti-inflammatory function of metformin. As a reversible inhibitor of nicotinamide adenine dinucleotide (NADH) dehydrogenase activity of the respiratory chain, metformin results in the inhibition of ATP production. Then the AMPK pathway is activated and exerts anti-inflammatory effects through different downstream pathways. One of the pathways is the nuclear factor-κB (NF-κB) signal pathway, a key regulator of inducible gene expression in the immune system. The inhibition of NF-κB leads to a subsequent reduction in cytokines involved in inflammatory response (IL-1β, IL-6, TNF-α, etc.), ultimately reaching anti-inflammation properties. As for lower cholesterol levels in metformin users, some studies observed similar results. Acetyl-CoA carboxylase (ACC) is a critical precursor for the synthesis of fatty acids, while metformin could induce ACC phosphorylation and inactivation, resulting in the reduction of triglyceride and cholesterol levels.

Our study revealed metformin usage was a significant protective factor for 2SC. Current evidence showed that metformin might exert anti-TB effects through several pathways. From the perspective of the host, metformin activates the AMPK pathway and reduces the secretion of pro-inflammatory cytokines. Metformin could enhance autophagy and increase mitochondrial reactive oxygen species concentration. In vitro experiments showed that metformin increased the expression of β-defensin in human lung epithelial cells and Mtb-infected macrophages, which played critical roles in intracellular killing and long-term inhibition of Mtb. On the other hand, Mtb infection promotes a shift in cellular metabolism from oxidative phosphorylation to aerobic glycolysis. Whereas metformin reverses the change in glucose metabolism, thereby limiting the growth of Mtb.

Furthermore, we established five prediction models, including SVM, RF, LR, ENR, and KNN. Among these, the LR model yields
higher sensitivity and AUC, but it showed relatively lower specificity. Thus, LR has sufficient power to predict the occurrence of poor outcomes in TB-DM patients based on the diagnostic performance of this model. The SVM model harbored the highest specificity, which indicated the ability to ensure the consistency between the predicted results and the actual results while facing the problem of some missed cases with poor outcomes. Although we tried to adjust the hyper-parameters to realize the improvement of both sensitivity and specificity, this expectation had not been fulfilled. Therefore, we recommend combining these two models. That is, potential cases with poor outcomes were screened by the LR model and then identified through the SVM model.

Despite the significance of drugs in the prevention and proper treatment of diseases, medications may lead to adverse drug reactions. Indeed, probable adverse reactions caused by metformin treatment were also considered, including Metformin-associated lactic acidosis (MALA), hypoglycemia, and gastrointestinal adverse reactions. However, no MALA was observed among the study subjects, which is consistent with the low incidence of MALA reported in the literature (1–5 cases per hundred thousand people).36 Metformin-induced hypoglycemic events (5 cases) were mentioned only in a few cases before admission and gastrointestinal adverse reactions (20 cases) were also rarely represented during follow-up. Due to the low incidences of mild adverse reactions, and most of them appeared particularly common with combination therapy, in-depth evaluation was not pursued in this analysis.

To our knowledge, this study included the largest number of TB-DM samples and systematically collected multiple clinical and socio-demographic information. On the premise of adequate test efficacy, we concluded that metformin usage was a protective factor for 2SC. These strong effects provide supporting evidence for the clinical control of TB-DM comorbid patients. Moreover, five different models were developed for application in different scenarios, which realized the cycle from clinical practice to research and then back to the origin. Nevertheless, the limitations of our current study need to be pointed out. This study had the inherent defects of the retrospective study. Although we performed multiple imputations to deal with missing data and compared the distribution between the interpolation and the original value, unexpected bias could not be completely avoided. We also attempted to consider metformin dosage, nonetheless, many missing data restricted us from performing such an analysis. Moreover, patients from a single center may limit the generalizability of the findings, so a prospective study is needed to further explore the roles of metformin in the adjunctive therapy of TB-DM comorbid patients.

Collectively, this study suggested that metformin usage is an independent protective factor for 2SC, and five different predictive models were developed to provide information for clinical decision-making. Metformin is expected to be applied in the adjuvant therapy of TB-DM and plays a positive role in the whole process of TB treatment.

**AUTHOR CONTRIBUTIONS**

Stud design: Binwu Ying and Ping Feng. Data collection: Yili Wang, Liyu Chen, Yanbing Zhou, Yuhui Cheng, Hongli Lai. Data analysis: Yanbing Zhou, Yili Wang, Liyu Chen, Mengyuan Lyu, Jiongjiong Zeng, Yao Zhang. Manuscript writing: Yili Wang.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data analyzed during the current study are available from the corresponding author on reasonable request. The data are not publicly available due to privacy or ethical restrictions.

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