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ORIGINAL RESEARCH

Advancing Precise Syphilis Diagnosis: A Nontreponemal IgM Antibody-Based Model for Latent Syphilis Staging

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Purpose: Accurate differentiation between early and late latent syphilis stages is pivotal for patient management and treatment strategies. Nontreponemal IgM antibodies have shown potential in discriminating latent syphilis staging by differentiating syphilis activity. This study aimed to develop a predictive nomogram model for latent syphilis staging based on nontreponemal IgM antibodies.

Patients and Methods: We explored the correlation between nontreponemal IgM antibodies and latent syphilis staging and developed a nomogram model to predict latent syphilis staging based on 352 latent syphilis patients. Model performance was assessed using AUC, calibration curve, Hosmer–Lemeshow $\chi 2$ statistics, C-index, Brier score, decision curve analysis, and clinical impact curve. Additionally, an external validation set was used to further assess the model's stability.

Results: Nontreponemal IgM antibodies correlated with latent syphilis staging. The constructed model demonstrated a strong discriminative capability with an AUC of 0.743. The calibration curve displayed a strong fit, key statistics including Hosmer–Lemeshow χ^2 at 2.440 (P=0.486), a C-index score of 0.743, and a Brier score of 0.054, all suggesting favorable model calibration performance. Decision curve analysis and clinical impact curve highlighted the model's robust clinical applicability. The external validation set yielded an AUC of 0.776, Hosmer–Lemeshow χ^2 statistics of 2.440 (P=0.486), a C-index score of 0.767, and a Brier score of 0.054, further underscored the reliability of the model.

Conclusion: The nontreponemal IgM antibody-based predicted model could equip clinicians with a valuable tool for the precise staging of latent syphilis and enhancing clinical decision-making.

Keywords: syphilis, latent syphilis, latent syphilis staging, nontreponemal IgM antibody, predictive model

Introduction

Syphilis is a chronic systemic disease caused by *Treponema pallidum*, typified by periods of active clinical infection separated by intervals of latent infection.[1,2] Latent syphilis is identified by seropositivity yet lacks clinical manifestations. Based on the estimated duration of infection, latent syphilis is commonly split into two phases, the early phase pertains to a latent syphilis infection acquired within the preceding year, whereas the late phase encompasses latent syphilis infections of over one year or more in duration.[2–5] In recent years, the latent syphilis epidemic has exhibited a substantial and swift escalation, making latent syphilis a focal point in syphilis prevention and control.[6,7]

Accurate differentiation between early and late latent syphilis holds paramount importance, as this differentiation directly correlates with the infectivity status of the patients and underpins treatment and management.[8,9] Early latent syphilis typically signifies an ongoing phase of syphilis activity, carrying the potential for secondary syphilis relapse and recurrent infectivity. In contrast, individuals in the late stage of latent syphilis are generally considered noninfectious.[1,2,5,8] Hence, distinct treatment approaches are warranted for these two stages. Early latent syphilis

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demands the prompt application of efficacious antibiotic regimens, while the focus of treatment in the late stage aims at preventing complications in asymptomatic individuals.[1,9] However, within practical clinical management, patients often struggle to pinpoint the timing of infection due to the absence of clinical symptoms, this predicament poses a formidable challenge in establishing the staging of latent syphilis. Current serologic assays encompass treponemal and nontreponemal tests, both crucial for syphilis diagnosis, however, evidence indicated that these tests remain insensitive in blood and lack the ability to differentiate between early and late stages of latent syphilis.[10] An urgent imperative exists for the development of appropriate tools to enhance precise staging identification.

Nontreponemal antibodies, which target the cardiolipin, are developed in response to *T. pallidum* infection.[11] Nontreponemal antibodies are frequently linked to disease activity, with immunoglobulin(Ig) M antibodies being produced early in the infection and maintained by the constant stimulation of active *T. pallidum*.[12,13] Our previous research determined that nontreponemal IgM antibodies can function as a serological marker of syphilis activity,[14] indicating their promising role as identifiers for the staging of latent syphilis. This study aimed to explore the correlation between nontreponemal IgM antibodies and the staging of latent syphilis, and develop a predictive nomogram model utilizing nontreponemal IgM antibodies and other clinical parameters for latent syphilis staging, aiming at equipping clinicians with an effective tool to choose appropriate treatment strategies and make informed management decisions.

Materials and Methods

Study Cohort and Design

From January 2020 to December 2020, 4384 patients underwent serological testing for syphilis including nontreponemal antibodies test (Toluidine red unheated serum test, TRUST) and treponemal antibodies test (Chemiluminescence immunoassay, CLIA) at Zhongshan Hospital, Xiamen University. According to CDC[13] and ECDC[15] guidelines, syphilis is diagnosed based on clinical symptoms and serological testing (including Nontreponemal tests and Treponemal tests). Specifically, patients exhibited syphilis clinical symptoms alongside positive results for both serological tests were diagnosed with syphilis. Patients with positive serological tests but without clinical evidence of syphilis were categorized as having latent syphilis, with those infected for less than one year classified as early latent syphilis, and those infected for more than a year referred to late latent syphilis. Finally, 447 patients were diagnosed with syphilis, of whom 360 patients (80.54%) were diagnosed with latent syphilis. The serum samples were collected from latent syphilis patients and stored at −80°C for nontreponemal IgM antibody testing.

According to the criteria for developing a clinical prediction model, it is essential that the sample size guarantees a minimum of 10 events for each predictor variable.[16] In our case, five variables, CLIA, TRUST, gender, age, and nontreponemal IgM antibody were used to construct the predictive model, requiring a minimum of 50 cases. Excluding 8 patients with HIV and/or autoimmune diseases or refused to participate, we included 352 patients to construct a clinical prediction model, which meets the necessary requirements. Furthermore, data from 60 patients diagnosed with latent syphilis at Zhongshan Hospital, Xiamen University, during January and February 2021, were employed for external validation.

Ethics Statement

Ethics approval for this research was granted by the Zhongshan Hospital, Xiamen University Research Ethics Committee (No: xmzsyyky2023084).

Patient Consent Statement

The research complied with the Declaration of Helsinki and national legislation, and adult patients provided informed written consent to participate, and patients under 18 years of age provided informed written consent along with informed written consent from a parent or legal guardian.

Testing for Nontreponemal IgM Antibodies

The detection of nontreponemal IgM antibodies was accomplished through a two-step indirect immunoassay with a commercially available chemiluminescence test kit as instructed by the manufacturer (Boson Biotech Co., Ltd., Xiamen, China), and employing a BHP9504-02 chemiluminescence meter (Hamamatsu Photon Techniques Inc., Hamamatsu, Japan) to quantify the obtained chemiluminescent reaction in relative light units. The ratio of the chemiluminescence signal to the cutoff value (S/CO) was used to indicate antibody levels, with S/CO ≥ 1.0 being positive and S/CO < 1.0 being negative.

Statistical Analyses

The statistical analyses were performed utilizing R software (version 3.6.0) and GraphPad Prism version 9.5.0. (GraphPad Software, San Diego, CA, USA). Statistical data were reported in the form of numbers and percentages. The Spearman correlation test was used to investigate the correlation between two variables. R package "rms", "pROC", "regplot", "boot" and "rmda" was utilized for generating, evaluating, and validating the predictive nomogram model. All tests were two-sided with a P value <0.05 deemed statistically significant.

Result

Characteristics of the Latent Syphilis Patients

The cohort of 352 latent syphilis patients was approximately equally divided between men and women, with 47.44% (167/352) being male and 52.56% (185/352) being female. Within the latent syphilis population, a predominant concentration of patients fell within the 40–59 years age range, accounting for 40.90% (144/352) of the total. This was followed by those aged 19–39 and those aged ≥60 years, both accounting for 28.98% (102/352). Notably, the ≤18 years age group represented only 1.14% (4/352) of the cohort. Among these patients, those in the early stage of latent syphilis comprised 61.36% (216/352) of total patients, and those in the late stage accounted for 38.64% (136/352) of the total. Regarding nontreponemal IgM antibodies, positive nontreponemal IgM antibodies were demonstrated in 20.74% (73/352) of patients, and 79.26% (279/352) of patients exhibited negative nontreponemal IgM antibodies.

Correlation Between Nontreponemal IgM Antibodies and Latent Syphilis Staging

The overwhelming majority of patients diagnosed with positive IgM antibodies were identified as early latent syphilis cases (71/73, 97.26%), while a mere 2.74% (2/73) were categorized as late latent syphilis cases. Among the 279 patients diagnosed with negative IgM Antibodies, the distribution of early and late latent syphilis cases was approximately balanced (51.97% vs 48.03%) (Figure 1A). This emphasized a potential link between IgM and the staging of latent

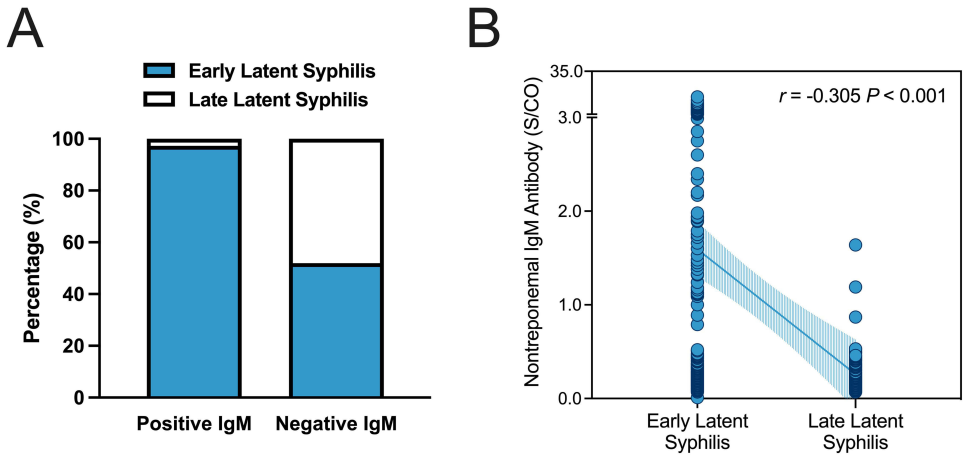

Figure 1 Relationship between Nontreponemal IgM Antibodies and the Staging of Latent Syphilis Patients. (**A**) Distribution of clinical phases among patients with different nontreponemal IgM antibodies results. (**B**) Nontreponemal IgM antibodies correlate with the staging of latent syphilis patients.

syphilis, with positive IgM Results possibly indicating an inclination toward the early stage of latent syphilis. Further correlation analysis confirmed the relationship between nontreponemal IgM antibody and latent syphilis staging, yielding a Spearman correlation coefficient of −0.305 (P<0.001). The Spearman correlation findings were visually represented using an optimal-fitting curve along with 95% confidence intervals (Figure 1B).

Construction of the Latent Syphilis Staging Model

Utilizing CLIA, TRUST, gender, age, and nontreponemal IgM antibody, we constructed a predictive nomogram model to estimate the probability of patients being in the early latent syphilis stage. As depicted in Figure 2, the five variables were annotated with a scale along the line segment, symbolizing their value ranges. The extent of the line segment conveyed their significance in determining the prediction of latent syphilis staging. The distribution of patient counts on both continuous and categorical variables is respectively depicted using density plots or box plots. In the nomogram model for latent syphilis staging, gender (P=0.015), age (P=0.006), and nontreponemal IgM antibody (P<0.001) were identified as independent predictors, while CLIA (P=0.866) and TRUST (P =0.354) were not.

The area under the curve (AUC) value served as an indicator of the model's accurate categorization across thresholds, as the AUC value approached 1, it signified superior classification performance. The receiver-operating characteristic curve displayed an AUC of 0.743 (95% CI: 0.692–0.793), signifying the robust discriminative capability of the nomogram (Figure 3A). Furthermore, the model exhibited an AUC of 0.776 (95% CI: 0.645–0.889) in the external validation dataset, further emphasizing its discriminative ability (Figure 3B).

Validation of the Latent Syphilis Staging Model

Model validation was measured by the calibration curve and Brier score. The calibration curve, generated from 1000 bootstrapped resamples, was used to assess the model's ability to provide accurate probability predictions and was further assessed employing the Hosmer–Lemeshow goodness-of-fit test. A Brier score closer to zero indicated better model calibration, reflecting a stronger alignment between predictions and observed outcomes. The C-index was employed to evaluate the predictive accuracy of the model, with a nearing value of 1 indicating a heightened predictive accuracy. The calibration curve of the constructed nomogram model was illustrated in Figure 4A. The close resemblance between the apparent curve and the bias-corrected curve showed a strong fit, emphasizing the commendable reproducibility and reliability of the predictive model. Additionally, the apparent curve and the bias-corrected curve were relatively close to the ideal curve, suggesting the favorable predictive consistency of the nomogram model. The Hosmer–Lemeshow $\chi 2$ statistics of the calibration curve was 2.440

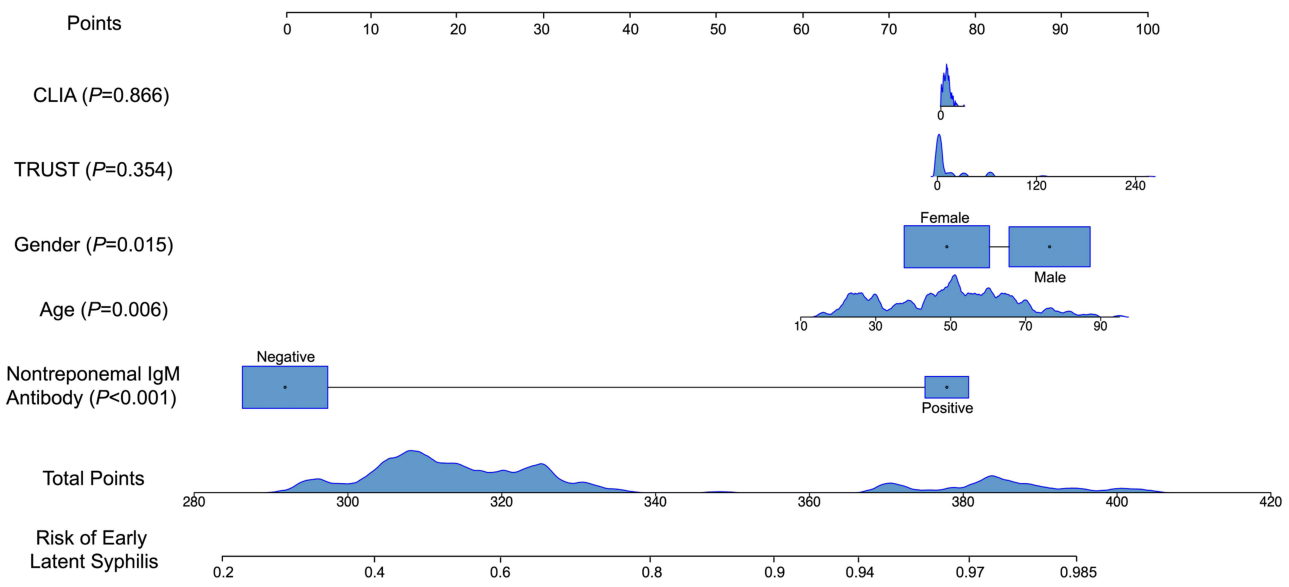

Figure 2 The Nomogram Model for Predicting Latent Syphilis Staging.

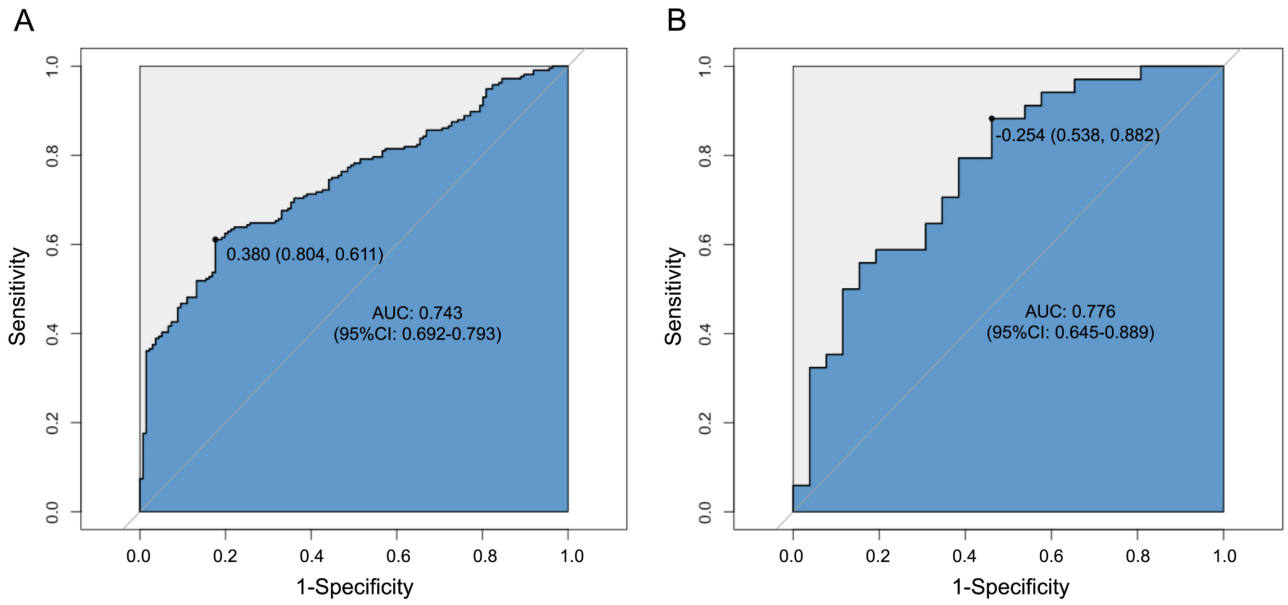

Figure 3 Receiver-Operating Characteristic Curves for Evaluating the Predictive Accuracy of the Latent Syphilis Staging Model. (**A**) Receiver-Operating Characteristic Curves for the Model in the Training Set. (**B**) Receiver-Operating Characteristic Curves for the Model in the Validation Set.

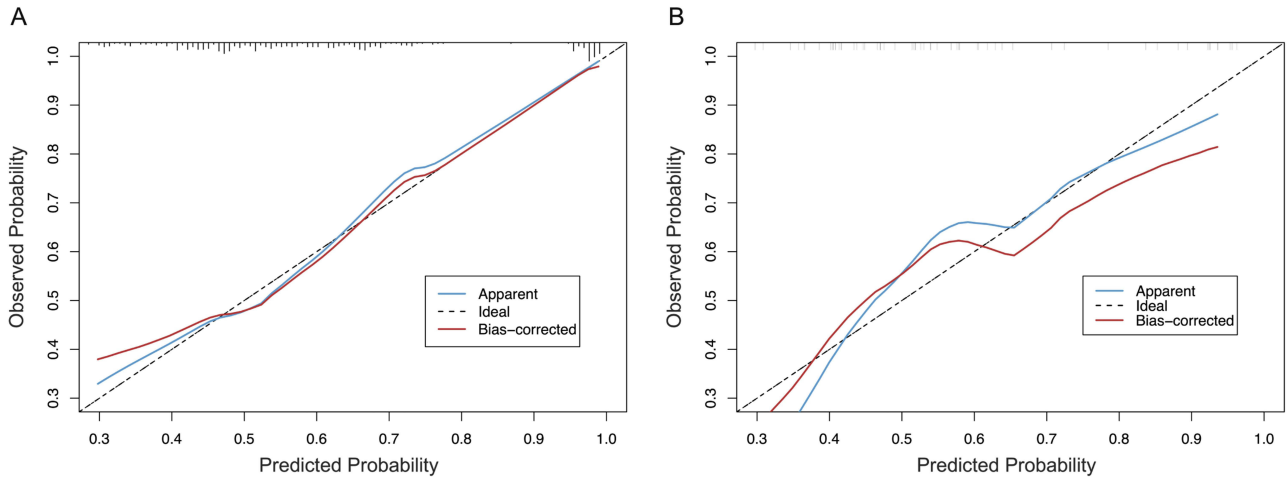

Figure 4 Calibration Curve for Validating the Latent Syphilis Staging Model. (**A**) Calibration Curve for the Model in the Training Set. (**B**) Calibration Curve for the Model in the Validation Set.

(*P*=0.486). Moreover, the Brier score of 0.054 further highlighted the excellent calibration performance of the predictive model, and the C-index score of 0.743 underscored the strong predictive accuracy of the nomogram model. In addition, the corrected C-index score and corrected Brier score remained stable at 0.729 and 0.104 after 1000 iterations of bootstrap resampling.

The calibration curve of the external validation dataset was shown in Figure 4B. The Hosmer–Lemeshow $\chi 2$ statistics, C-index score, corrected C-index score, Brier score, and corrected Brier score were 2.440 (*P*=0.486), 0.767, 0.691, 0.054, and 0.104, respectively. This further underscored the strong consistency between the model's predicted probability of early latent syphilis and the actual probability of patients being in the early latent syphilis stage.

Clinical Utility of the Latent Syphilis Staging Model

The decision curve analysis and the clinical impact curve were employed to evaluate the clinical practicality by depicting estimated high-risk counts across thresholds, reflecting accurate positive case ratios.[17] The decision curve analysis in Figure 5A provided the visual representation of clinical net benefit at different risk thresholds. The blue curve represented

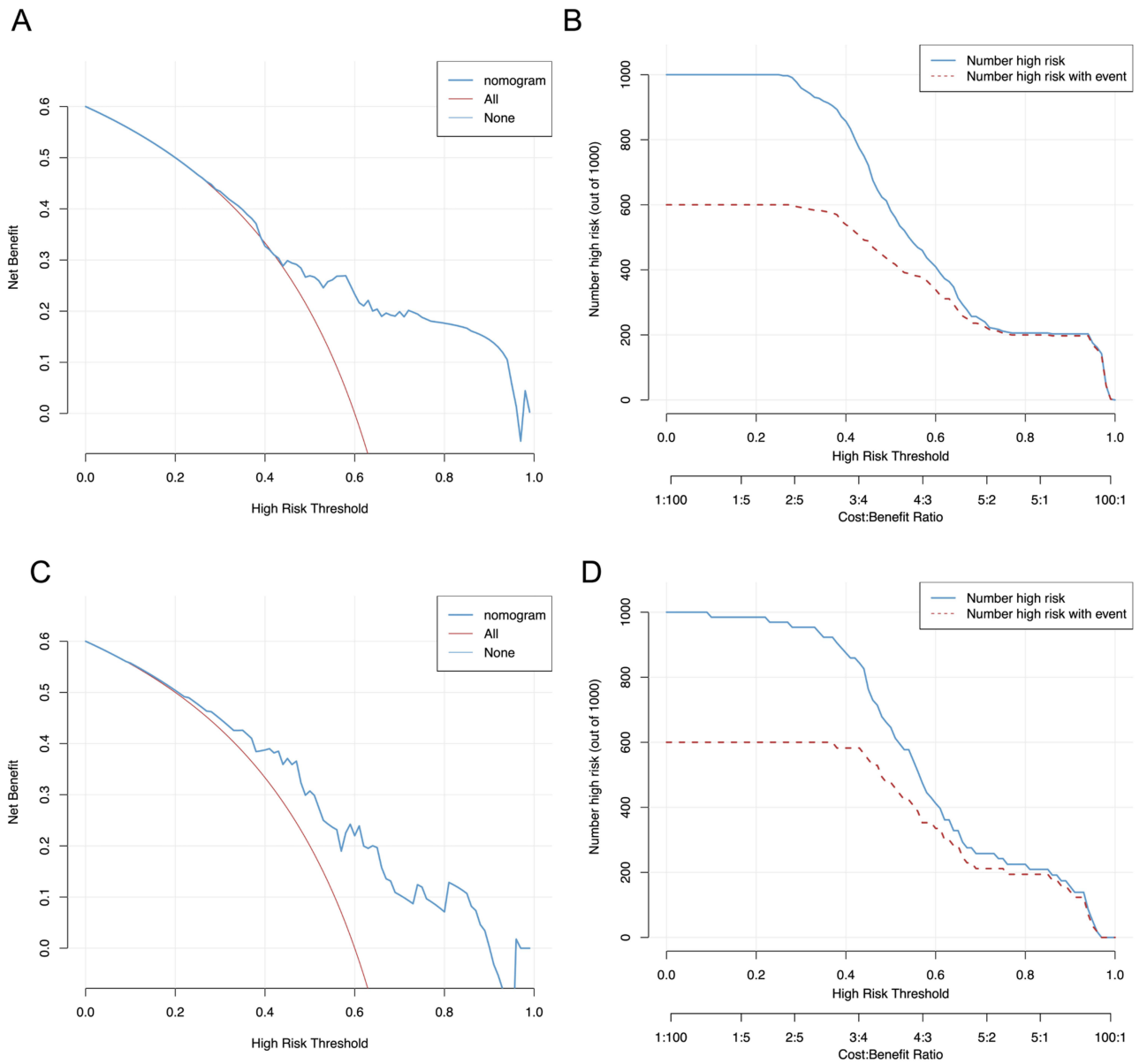

Figure 5 Decision Curve Analysis and Clinical Impact Curve for Evaluating the Clinical Utility of the Latent Syphilis Staging Model. (**A**) Decision Curve Analysis of the Model in the Training Set. (**B**) Clinical Impact Curve of the Model in the Training Set. (**C**) Decision Curve Analysis of the Model in the Validation Set. (**D**) Clinical Impact Curve of the Model in the Validation Set.

treatment solely for patients identified as early latent syphilis cases by the nomogram model, the red curve indicated treatment for all patients, and the black curve signified no treatment for any patients. The result suggested the nomogram's substantial net benefit across a broad and clinically relevant range of threshold risk, underscoring the considerable predictive value and superior clinical applicability of the predicted model.

The clinical impact curve depicted in Figure 5B further demonstrated the clinical effectiveness of the predictive model. The blue curve delineated the patient count classified with early latent syphilis across the varying thresholds based on the constructed predictive model, while the red curve portrayed the true early latent syphilis cases at the corresponding thresholds. Impressively, when the threshold risk surpassed 70% of the predicted risk, the population identified as high-risk for early latent syphilis by the predictive model demonstrated a strong alignment with the actual cases of early latent syphilis.

The decision curve analysis and clinical impact curve for the external validation set were presented in Figure 5C and D, additionally demonstrating the model's valuable guidance in informing clinical practice.

Discussion

To the best of our knowledge, this research represents the initial attempt to construct a predictive model for staging latent syphilis, in the hope of offering novel insights into the field of latent syphilis research and laying a promising foundation for future investigations. The incidence of latent syphilis has exhibited a notable and concerning upward trend in recent years. In China, the proportion of latent syphilis cases in the overall syphilis cases has escalated from 14.2% in 1995 to 73.6% in 2016.[7] Our study likewise revealed that an overwhelming majority of syphilis cases were attributable to latent syphilis, which accounts for 80.54% (360/447) of all cases. Such a large proportion highlights the imperative for an intensified focus on addressing latent syphilis within public health strategies and clinical management.

The distinction between early and late latent syphilis is of paramount significance, due to its intricate connection with syphilis activity and direct implications for patient management and treatment strategies. Failure to accurately manage active syphilis infections can result in the progression of severe complications, including cardiovascular disease and central nervous system disorders.[18] Therefore, since patients with early latent syphilis are in the active syphilis infection stage, they should be better managed clinically to minimize the risk of syphilis transmission and to impede disease progression toward more severe manifestations. In accordance with the World Health Organization (WHO) guidelines for syphilis treatment, late latent syphilis necessitates prolonged antimicrobial therapy courses compared to early latent syphilis.[4,19] For individuals with undetermined infection duration, treatment often adopts an approach consistent with late latent syphilis,[1,8] which might lead to overtreatment. Overtreatment may pose unnecessary treatment risks to certain populations, and may also have public health consequences, such as causing stock-outs of the drug in resource-limited or hyperendemic areas.[20] Hence, there is an urgent demand for a precise tool to effectively determine the staging of latent syphilis.

Our prior investigation identified that nontreponemal IgM antibodies could serve as a viable serologic indicator for active syphilis.[14] Hence, we postulated that nontreponemal IgM antibodies might help in distinguishing the stages of latent syphilis by differentiating syphilis activity. In line with this hypothesis, we assessed nontreponemal IgM antibodies in a cohort of 352 latent syphilis patients. The outcomes demonstrated that a significant majority of individuals with positive nontreponemal IgM antibodies were classified as early latent syphilis cases, while only a minute fraction were late latent syphilis cases, implying that positive nontreponemal IgM results may correlate with an inclination towards the early stage of latent syphilis. The subsequent Spearman correlation analysis definitively elucidated the association between nontreponemal IgM antibodies and the staging of latent syphilis, indicating that nontreponemal IgM antibodies could serve as a crucial tool for constructing predictive models for latent syphilis staging.

Our predictive nomogram model offers a practical and effective tool for clinicians to estimate the probability of patients being in the early latent syphilis stage. By incorporating CLIA, TRUST, gender, age, and nontreponemal IgM antibodies, the model demonstrated a strong discriminative capability, as evidenced by the AUC value of 0.743. The diagnosis of latent syphilis relies on serological testing, which unfortunately cannot differentiate between early and late latent stages. Our established model similarly revealed that gender, age, and nontreponemal IgM antibodies were independent predictive factors for latent syphilis staging, while CLIA and TRUST do not hold such distinction. The calibration curve analysis, the C-index score, and the Brier score further supported the reliability and reproducibility of our nomogram model by providing compelling evidence supporting our predictive model's commendable reproducibility and reliability. The clinical decision curve and the clinical impact curve accentuated the robust clinical applicability of our predictive model, signifying its substantial utility for clinical practice. The external validation set further emphasized the reliability of these results. These findings collectively reaffirm the favorable performance of our predictive nomogram model.

The predictive model has gained widespread recognition as an essential component of contemporary medical decision-making.[21] The predictive model specifically developed for the staging of latent syphilis has the potential to serve as a valuable and practical tool for clinicians to empower clinical decision-making. By accurately assessing the stage of disease progression, clinicians can tailor treatment strategies effectively, preventing further advancement and enhancing treatment efficacy, thus improving patient quality of life. For patients with latent syphilis, particularly those

unsure of their infection timing, we recommend clinicians conduct nontreponemal IgM antibodies testing and utilize the established predictive model. This approach ensures accurate determination of disease stage, enabling precise therapeutic interventions and optimizing healthcare resource allocation.

Several limitations should be acknowledged in the present study. First, 360 patients with latent syphilis were identified during our study, but only 352 patients were included in the construction of the model. HIV can have an impact on the course of syphilis, but due to the small number of HIV-positive patients in the study, we were unable to investigate the differences caused by HIV in the latent syphilis group, so we excluded the HIV-positive patients for the stability of the data. Second, since nontreponemal antibodies target the cardiolipin antigen, certain underlying diseases such as autoimmune diseases may cause false-positive results in nontreponemal IgM tests, which could partially affect the results. Therefore, this study excluded patients with autoimmune diseases, which may potentially lead to biased outcomes. Third, the development and validation of the prediction model relied on single-center data, although the sample sizes for both the training and validation sets met the required standards for building the predictive model, they were relatively limited. To enhance its robustness, additional validation and optimization through a prospective study involving a larger, multicenter sample will be necessary in the future.

Conclusion

In Conclusion, our study indicated a close correlation between nontreponemal IgM antibodies and the staging of latent syphilis. The developed predictive nomogram model based on nontreponemal IgM antibody offered a valuable clinical tool for accurate latent syphilis staging identification and therefore aiding clinicians in making informed treatment decisions. However, further validation and prospective studies are warranted to validate the model's applicability across diverse patient populations and healthcare settings.

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Disclosure

The authors report no conflicts of interest in this work.

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