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ORIGINAL RESEARCH

Prognostic Value of Wagner Grade and Platelet Level in Diabetics with Infected Foot Ulcers After Antibiotic Therapy

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Purpose: The aim of the current study was to investigate factors potentially associated with the healing of infected foot ulcers in patients with diabetes after antimicrobial therapy with drugs selected based on antimicrobial susceptibility testing.

Patients and Methods: A retrospective study was conducted to analyze clinical data from 99 type 2 diabetes mellitus patients with foot infection admitted to our center from January 2016 to December 2020. Pathogenic characteristics, results of wound discharge testing, and relevant wound surface factors were analyzed. Etiological characteristics and the results of susceptibility testing, wound healing rates, and factors potentially associated with wound healing rates were also analyzed.

Results: Baseline data were analyzed via the *t*-test for independent samples, the Mann–Whitney *U*-test, and the chi-square test to identify variables significantly associated with prognosis. Least absolute shrinkage and selection operator regression analysis then determined that Wagner grade, essential hypertension, platelets, Gram negative bacteria, and neutrophil-to-lymphocyte ratio were of predictive value. A nomogram plot was built based on these five variables, and it yielded a standard C-index of 0.964, and an internally corrected C-index of 0.931. In multivariate logistic regression analysis Wagner grade (odds ratio [OR] 12.30, 95% confidence interval [CI] 2.471–61.194, $p = 0.002$) and platelet level (OR 0.978, 95% CI 0.960–0.996, $p = 0.018$) were significantly associated with wound healing outcomes. Restricted cubic spline analysis indicated that there was a linear relationship between wound healing and platelet levels, and that this relationship was strongest in patients classified as Wagner grade 2 with a platelet count ≤ 200 (p for nonlinearity = 0.442).

Conclusion: Wagner grade, essential hypertension, platelet count, Gram negative bacteria, and neutrophil-to-lymphocyte ratio could predict the course of healing of infected foot ulcers in type 2 diabetes mellitus patients. When the Wagner grade was 2 and the platelet level was ≤ 200, platelet level was linearly associated with healing outcome, whereby a lower platelet level predicted a worse wound healing outcome.

Keywords: diabetic foot ulcer, DFU, platelet, RCS, ulcer healing, Wagner grade

Introduction

Currently the global prevalence of diabetes is 9.3%, and it is one of the top ten causes of death in the world. It is estimated that by 2030, it will exceed 500 million.[1] Diabetes-related foot disease (DFD) is one of the most serious complications of diabetes. It is one of the main causes of hospitalization of diabetes patients, and it greatly increases the cost of treatment.[2] The main risk factors for foot ulcer in diabetes include a history of foot ulcers, a prolonged course of diabetes, peripheral vascular disease, coronary artery disease, advanced peripheral neuropathy, and end-stage renal

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disease.[1,3,4] Patients with diabetic foot ulcer (DFU) infection are at a higher risk of amputation, and their costs of treatment are increased, so they must be evaluated and treated in a timely manner.[5–7]

DFUs are difficult to treat successfully, and they are the main reason for hospitalization and lower limb amputation. In one study they occurred in 15% of all diabetes patients and were present in 84% of all diabetes-related lower limb amputees.[8] Due to a lack of consensus on treatment, many chronic wounds persist for longer than necessary.[9] As the life expectancy of aging populations increases, the prevalence of chronic wounds is likely to increase, resulting in higher social costs.[10] Therefore, improved wound healing rates and new developments in this field should be encouraged.[11]

Multidisciplinary nursing and structured medical care have proven to be the most effective ways to reduce the amputation index of DFD,[12] and the incorporation of optimized infection control management could significantly improve the prognosis of patients with DFU.[13,14] Rapid initiation of appropriate empirical antibiotic treatment, as well as early identification of pathogens and corresponding adjustment of the antibiotic regimen with respect to drug sensitivity are reportedly essential for effective treatment of ulcers and prevention of amputation.[15,16] In previous studies, factors such as low socio-economic status, smoking,[17,18] gender, kidney injury,[19] ischemia, diabetes neuropathy,[20] and high levels of glucose and triglycerides[21] have been significantly associated with the risk of foot amputation. It remains unclear which factors are most conducive to foot wound healing in diabetics however, particularly in those treated for infections after antimicrobial drug sensitivity testing.

The current study assessed the effects of clinical, biochemical, and epidemiological factors, and patient behavior-related predictors on wound prognosis in DFD patients. Understanding these factors and their effects is crucial for enabling multidisciplinary teams to develop management and treatment plans for diabetes patients aimed at preventing amputation.

Materials and Methods

Study Setting and Data Collection

This retrospective study was conducted at the Endocrinology Department of a tertiary hospital in eastern China. All patients in the unit were managed in accordance with the established treatment plan for diabetes foot infection. In accordance with the nursing standards of the department, if feasible, wound secretion samples were collected at the bedside or debridement room immediately after admission. That testing included blood plate culture for 18–24 hours to obtain pure colonies, and the preparation of bacterial suspensions. Bacteria were identified with a VITEK2 Compact automatic bacterial identification instrument. Concurrently, MH plates were coated with bacterial suspensions and manual drug sensitivity testing was performed. In cases of suspected systemic infection, blood culture was also conducted. Patients' clinical characteristics and health status were retrospectively obtained from the hospital information system. A group of experts evaluated comorbidities in accordance with expert consensus and guidelines.

Laboratory examinations performed on samples acquired at admission included blood glucose, glycosylated hemoglobin, creatinine, routine blood examinations, inflammatory index, liver function, thyroid function, blood lipid level, and blood coagulation function. The Wagner grading method was used to assess DFD. Infection severity was assessed using the American Society of Infectious Diseases classifications, which are based on signs of local infection and evidence of systemic infection.[22] Complications including peripheral neuropathy and diabetic fundus disease were identified via physical and laboratory examinations recorded by physicians. The study only included cultures collected within the first 2 weeks of hospitalization, not during subsequent coinfections, in an effort to identify primary pathogens. The study was approved by the Helsinki local committee of the hospital (IRB 2023-KY-116). The ethics Committee of The Affiliated Taicang Hospital of Soochow University has confirmed that no need consent in this study, as a retrospective study and data analysis was performed anonymously.

Inclusion Criteria

All patients diagnosed with foot infection due to type 2 diabetes mellitus (T2DM) at the center from January 2016 to December 2020 were included if they were an inpatient and had DFU, foot disease was assessed in accordance with the Wagner classification method, and wound secretion culture and drug sensitivity testing were conducted after admission.

Exclusion Criteria

Patients were excluded if they had taken antibiotics before wound secretion culture and drug sensitivity testing, or had severe liver and kidney dysfunction and could not tolerate normal doses of antibiotics, or if there was a history of anti-infection treatment within 3 months before admission.

Follow-Up and Outcomes

Patients whose wounds healed before discharged from hospital were assigned to a healed group. Patients with an ulcer site that did not heal prior to discharge, and patients who underwent amputation were assigned to a non-healed group. All patients were followed up for 6 months to determine outcomes.

Statistical Analysis

Quantitative continuous variables are expressed as means ± the standard deviation (SD). Categorical variables are expressed as frequencies and percentages. The chi-square test and the *t*-test were used to compare categorical variables and continuous variables between groups, and the Mann–Whitney *U*-test was used to compare non-normally distributed variables. Least absolute shrinkage and selection operator (LASSO) regression analysis was used to identify factors related to diabetes prognosis, establish a clinical model and nomogram, generate a model calibration curve, calculate the C-index, and test the reliability of the model via internal verification procedures. Multivariate logistic regression was used to evaluate correlations between covariates, and statistically significant variables were analyzed. Linear relationships between variables and prognosis were then analyzed using a restricted cubic spline chart. These tests were conducted at a significance level of 5%. Statistical analyses were performed using SPSS (version 23.0; Chicago, Illinois, USA) and R (v.4.1.2; https://www.r-project.org) software.

Results

Group Assignment and Baseline Characteristics

A total of 99 patients diagnosed with diabetic foot infection were included in the study, of which 11 (11.1%) experienced adverse events such as non-healing or amputation after treatment and follow-up. Thus, 11 patients were assigned to the non-healed group and 88 were assigned to the healed group. Baseline characteristics are shown in Table 1. There were significant differences in Gram negative bacteria (GNB), Wagner grade, neutrophils, lymphocytes, eosinophils, blood

Table 1 Baseline Characteristics

Variables	Non-Healed (n=11)	Healed (n=88)	*P* value
Age, yrs	68.3 ± 10.6	62.3 ± 15.8	0.22
Male	9(81.8)	59(67.0)	0.49
BMI	23.9 ± 3.7	24.6 ± 3.3	0.50
Essential hypertension	7(63.6)	31(35.2)	0.07
Prior CAD	3(27.3)	19(21.6)	0.46
Prior stroke	2(18.2)	14(15.9)	0.56
Prior sepsis	2(18.2)	8(9.1)	0.31
Diabetic fundus disease	0(0.0)	9(10.2)	0.33
Periphery neuropathy	2(18.2)	21(23.9)	0.51
Hypohepatia (mild to moderate)	0(0.0)	4(4.5)	0.62

(*Continued*)

Table 1 (Continued).

Variables	Non-Healed (n=11)	Healed (n=88)	*P* value
Hypoproteinemia	1(9.1)	6(6.8)	0.57
Renal insufficiency*	3(27.3)	17(19.3)	0.39
Malignant tumor	1(9.1)	2(2.3)	0.30
Ketoacidosis	1(9.1)	14(15.9)	0.48
Gram negative bacteria	8(72.7)	33(37.5)	0.03
Laboratory data			
HbA1c, %	9.83 ± 1.62	9.38 ± 2.17	0.51
Fasting Insulin level, mIU/L median (IQR)	12.10 [5.80, 45.60]	12.10 [8.45, 16.13]	0.38
C-peptide, nmol/L	0.33± 0.16	0.35 ± 0.26	0.19
Wagner grade	3.45 ± 1.13	2.08 ± 0.78	0.002
Total Cholesterol, mmol/L	3.90 ± 0.85	4.03 ± 0.95	0.67
Triglyceride, mmol/L	1.24 ± 0.33	1.23 ± 0.55	0.95
LDL-C, mmol/L	2.09 ± 0.53	2.43 ± 0.78	0.16
HDL-C, mmol/L	1.06 ± 0.46	0.94 ± 0.27	0.41
T3, pmol/L	3.33 ± 0.93	3.56 ± 0.82	0.38
T4, pmol/L	16.24 ± 3.68	14.92 ± 2.32	0.10
TSH, mIU/L	1.49 ± 0.78	2.28 ± 1.56	0.10
WBC, 10^9/L median (IQR)	10.10 [7.80, 13.60]	7.95 [5.85, 10.70]	0.06
Neutrophil, %	83.59 ± 8.57	73.30 ± 11.77	0.006
Lymphocyte, %	9.65± 5.17	18.44 ± 10.23	0.007
Monocyte, %	5.96± 2.14	6.58 ± 2.02	0.35
Eosinophil, % median (IQR)	0.40 [0.01, 0.50]	0.85 [0.20, 1.90]	0.02
Basophil, % median (IQR)	0.20 [0.10, 0.30]	0.20 [0.10, 0.40]	0.43
RBC, 10^12/L	4.32 ± 0.69	4.20 ± 0.70	0.58
Hemoglobin, g/L	131.27 ± 19.08	126.51 ± 21.18	0.48
Hematocrit	38.83 ± 6.45	37.23 ± 5.91	0.40
RDW, %	13.13 ± 1.05	12.82 ± 1.02	0.33
PDW, %	14.75 ± 3.79	13.52 ± 2.48	0.32
Platelet count, 10^9/L	174.00 ± 78.88	213.56 ± 72.19	0.09
CRP, mg/L median (IQR)	47.00 [20.30, 200.50]	23.10 [9.00, 86.50]	0.08
BUN, mmol/L median (IQR)	5.89 [5.41, 7.89]	5.41 [4.74, 5.80]	0.02
Creatinine, umol/L median (IQR)	61.01 [50.01, 96.10]	61.02 [55.12, 74.75]	0.51
LCR median (IQR)	0.17 [0.03, 0.82]	0.70 [0.15, 2.60]	0.02

(*Continued*)

Table 1 (Continued).

Variables	Non-Healed (n=11)	Healed (n=88)	*P* value
MLR	0.87 ± 0.56	0.50 ± 0.39	0.007
NLR median (IQR)	8.98 [4.86, 19.85]	4.22 [2.81, 7.75]	0.003
ALT, U/L	25.52 ± 9.80	22.13 ± 18.40	0.55
AST, U/L	23.53 ± 7.33	22.76 ± 17.32	0.89
Total bilirubin, umol/L	11.23 ± 5.25	11.55 ± 4.62	0.83
Prothrombin time, s	13.23 ± 1.95	13.09 ± 1.33	0.75
INR	1.12 ± 0.16	1.11 ± 0.11	0.79

Note: Renal insufficiency*: eGFR (30–60%).
Abbreviations: ALT, Alanine transaminase; AST, Aspartate aminotransferase; BUN, blood urine nitrogen; CAD, Coronary Artery Disease; CRP, Hypersensitive C-reactive protein; HbA1c, Hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; INR, International relations ratio; IQR, interquartile range; LCR, Lymphocyte to CRP ratio; LDL-C, low-density lipoprotein cholesterol; MLR, monocyte to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; PDW, platelet distribution width; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell; TSH, Thyroid Stimulating Hormone.

urine nitrogen, lymphocyte-to-C-reactive protein ratio, monocyte-to-lymphocyte ratio, and neutrophil-to-lymphocyte ratio (NLR) between the non-healed group and the healed group (all $p < 0.05$).

LASSO Regression Analysis and Nomogram Generation

Associated factors that may affect healing after anti-infection treatment in patients with diabetic foot infection were included in the LASSO regression model ($p < 0.1$ at baseline). At the minimum value of lg (lambda), five factors—Wagner grade, essential hypertension, platelets, GNB, and NLR—emerged as factors that may be related to the prognosis of anti-infection treatment of diabetic foot infection (Figures 1 and 2). Based on the results of LASSO regression, these five factors were used to establish a predictive nomogram. The lower the sum of the specified points in the nomogram,

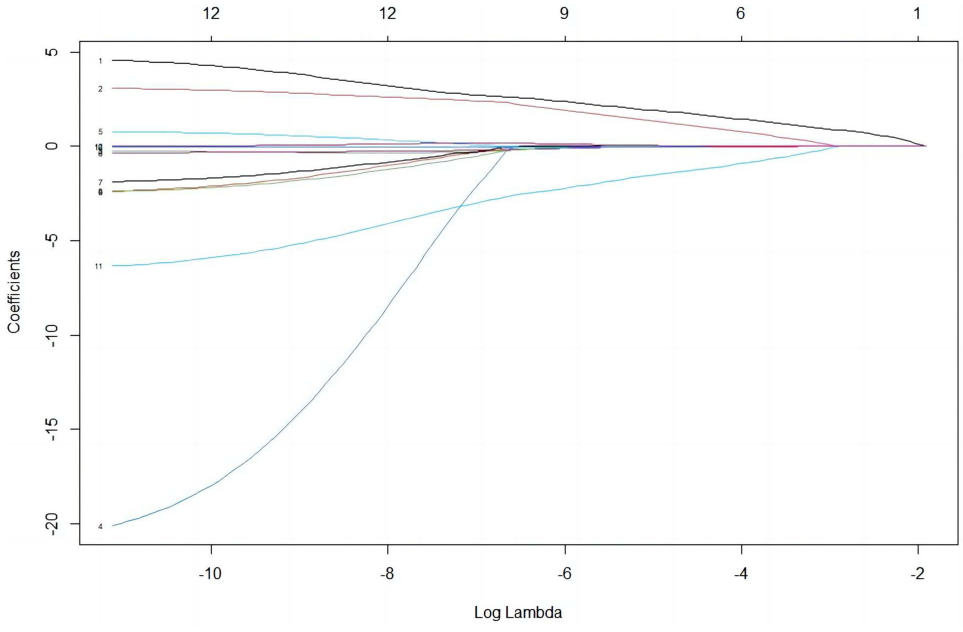

Figure 1 LASSO coefficient profiles of the 12 factors. The horizontal axis represented the log(λ) value of the independent variable, the horizontal axis represented the number of variables with non-zero coefficient, the vertical axis represented the coefficient of the independent variable, and each curve represented the variation trajectory of the coefficient of each independent variable.

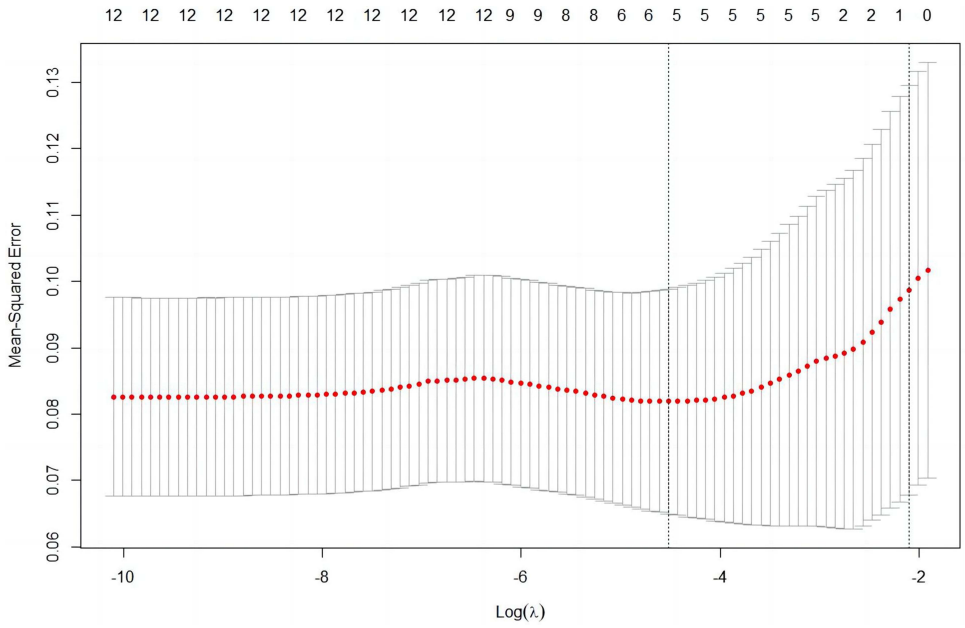

Figure 2 Ten-fold cross-validation for tuning parameter selection in the LASSO model. The dotted vertical lines were drawn at the best value of log(λ) by using the minimum criteria and 1-SE criteria. Solid vertical lines represented partial likelihood deviance ± SE. The intersection point of the left dotted line and the abscissa axis (bottom) showed the optimal value of log(λ), the corresponding value in the abscissa axis showed the number of variables with non-zero coefficient identified at the optimal log(λ).

the higher the foot wound healing rate in DFD patients. The C-index was 0.964, calculated as the discrimination value of the nomogram, indicating good predictive power with a bias-corrected C-index of 0.931. Nonsignificant Hosmer-Lemeshow test results using the calibration curve ($p = 0.935$) indicated that the prediction matched well with observed healing rates (Figure 3).

Validation of Clinical Application of the Nomogram

Decision curve analysis was performed to investigate the net benefit of the predictive model (Figure 4). The results suggested that the nomogram could provide greater net benefit than other models when the threshold probability ranged from 30% to 60%.

Restricted Cubic Spline and Multivariate Logistic Regression Analysis

Multivariate logistic regression analysis was used to compare the variables included in the LASSO regression analysis (Table 2). Healing rate was significantly associated with Wagner grade (odds ratio [OR] 12.30, 95% confidence interval [CI] 2.471–61.194, $p = 0.002$) and platelet count (OR 0.978, 95% CI 0.960–0.996, $p = 0.018$). Restricted cubic spline modelling indicated a linear association between platelet count and non-healing rate (p for non-linearity = 0.442, Figure 5).

Discussion

In the current study Wagner grade, essential hypertension, NLR, GNB, platelet count, and other factors influenced the foot infection healing rate in patients with T2DM. These factors were used to establish a clinical predictive model, and internal validation confirmed the reliability of the model. In multivariate logistic analysis Wagner grade and platelet count were the main factors that affected the prognosis of T2DM patients with foot infection. When the peripheral serum platelet count was <200, it was linearly associated with foot wound prognosis with respect to healing. The lower the platelet count, the worse the prognosis.

In a previous study Gram positive bacteria (GPB) played a leading role in diabetes foot infection in more developed countries, but the prevalence of GNB isolates in low-income people was significantly higher.[23] In general, the most common GPB identified via pathogen examination were *Staphylococcus aureus, Streptococcus*, and *Enterococcus*, and

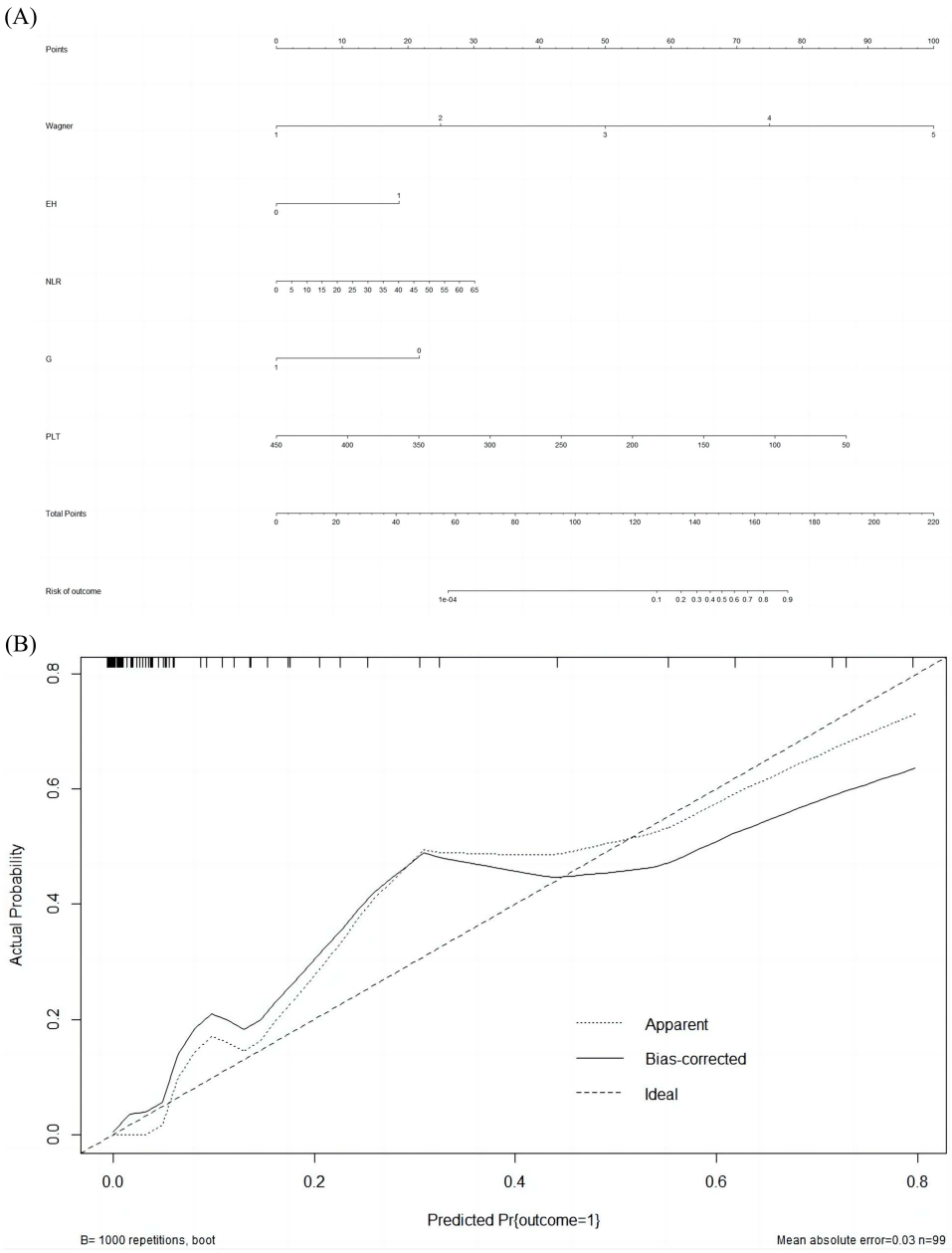

Figure 3 (**A**) Nomogram to pridict outcome of wound healed; (**B**) Calibration curve.
Abbreviations: EH, Essential hypertension; G, Gram negative bacteria; NLR, Neutrophil to lymphocyte ratio; PLT, Platelet count.

the most common GNB were *Pseudomonas, Escherichia coli, Klebsiella*, and *Proteus*. Differences in these pathogen types may be affected by environmental hygiene, personal hygiene, the use of different footwear, and other factors.[23,24] In the present study GPB was dominant in the samples collected, but patients with GPB had poorer prognoses. GNB were more commonly associated with severe sepsis and septic shock than other microorganisms,[25] and GNB were more likely to develop drug resistance.[26] Although the most effective antibiotic treatment was selected based on drug sensitivity testing of the pathogen in the current study, the development of resistance during the treatment process cannot be ruled out.

In previous studies the NLR has been associated with various infections and bacteremia.[27–30] The NLR has been used to predict the prognosis of sepsis patients with inconsistent results. A recent study indicated that NLR may be a useful prognostic biomarker in sepsis patients, and higher NLR values may indicate unfavorable prognoses in these patients.[31]

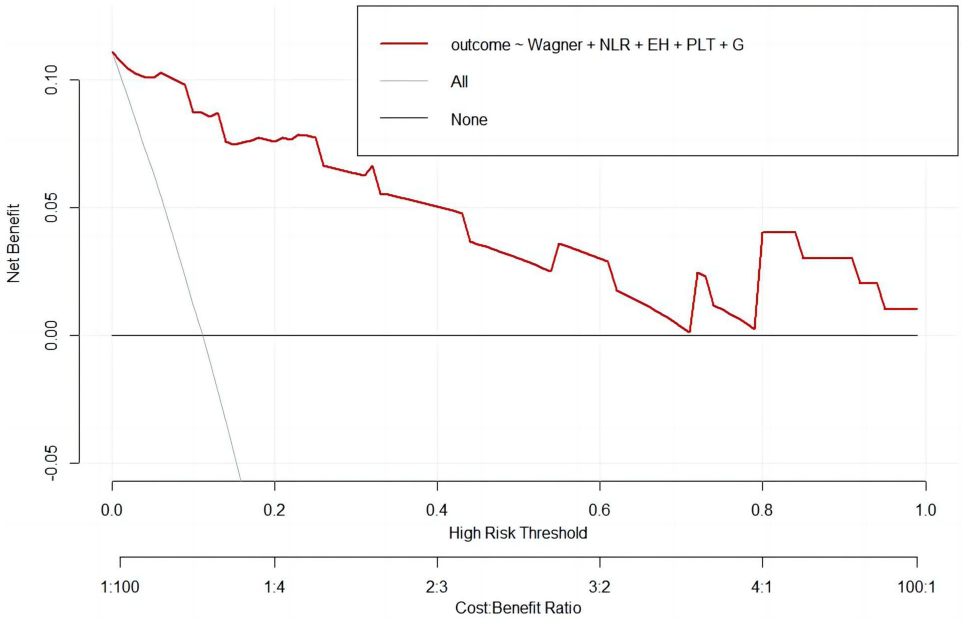

Figure 4 Decision curve analysis.
Abbreviations: EH, Essential hypertension; G, Gram negative bacteria; NLR, Neutrophil to lymphocyte ratio; PLT, Platelet count.

In the current study NLR was related to the prognosis of foot infection in diabetes patients, and in the predictive model higher NLR predicted a poorer prognosis. Although the positive association with a poorer prognosis was not strong, there was a trend in that direction.

The prognosis of foot infection in diabetes was clearly predicted by Wagner grade. In a previous study Wagner grade was significantly associated with amputation risk.[32] Different Wagner grades have different predictive value for the prognosis of foot infection in diabetes patients. For example, the amputation rates in patients with Wagner grades 4 and 5 were significantly higher than those in patients with lower grades.[33–35] Wagner grades ≤ 2 have been associated with significantly lower amputation rates,[36–38] thus it can be concluded that increased wagner grade predicts poor outcomes. In the present study Wagner grade was an independent predictor of prognosis in diabetes patients with foot infection, and the higher the Wagner grade the worse the prognosis.

The healing of infected foot wounds in diabetic patients is determined by many factors, and involves keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets.[39] The wound healing process is regulated by a complex mixture of growth factors and cytokines released from platelet-α particles. As well as preventing blood loss, platelets can promote tissue regeneration, enhance collagen synthesis, and trigger angiogenesis and immune responses by releasing growth factors and cytokines.[40] They can also promote the migration of keratinocytes within a wound, resulting in morphometric

Table 2 Multivariate Logistic Regression Analysis for Predictors of Healed Wound for Diabetes Foot Patients

Variables	*p* value	OR	95% CI	
			Lower	Upper
Wagner grade	0.002	12.296	2.471	61.194
Essential hypertension	0.093	6.52	0.733	57.969
Neutrophil to lymphocyte ratio	0.443	1.048	0.93	1.18
Platelet count	0.018	0.978	0.961	0.996
Gram negative bacteria	0.058	0.113	0.012	1.081

Abbreviations: OR, odds ratio; CI, confidence interval.

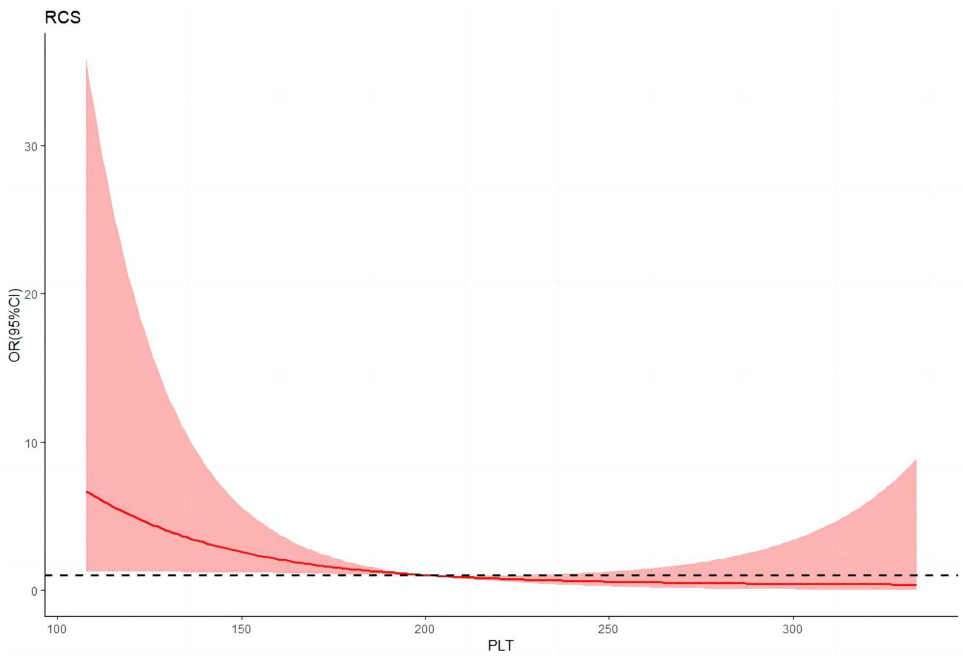

Figure 5 Association between the PLT level and wound healed rate in T2DM patients, allowing for nonlinear effects, with 95% CIs. The RCS function with 3 knots for PLT level, adjusted for Wagner grade, was performed. Curves show ORs compared with the chosen reference PLT of 200*10^9/L.

and mitotic effects, ensuring formation of a skin barrier.[41] Platelets and their derivatives also have specific effects on the treatment of chronic wounds, such as diabetic foot infections and leg ulcers.[42] These considerations may explain the linear relationship between wound healing of foot infections and peripheral plasma platelet count in diabetics, which is stronger when the platelet count is <200.

Infection plays an important role in the development of DFU. Combined infection accelerates plantar injury, leading to chronic ulcers, and even systemic infection and osteomyelitis. In the worst cases there is a risk of amputation. Early judgment of the degree of infection is of great significance with respect to predicting future developments and formulating a treatment plan. Long-term use of antibiotics to select resistant microorganisms can reportedly make treatment more difficult and increase the risk of amputation. In the current study the risk of amputation was greater in patients with poor drug treatment compliance. Adherence to prescribed treatment significantly improves the health and quality of life of diabetes patients.[43,44] Drug compliance and effectiveness are crucial for the effective treatment of foot infection in diabetics. Therefore, in the present study, in order to reduce drug resistance all antibiotics were selected in strict accordance with the results of pathogen drug sensitivity tests. Relevant research is currently lacking, therefore in conjunction with reducing drug resistance, other factors that affect prognosis should be investigated. The primary aims are to improve prognoses, reduce amputation risks, improve survival rates and quality of life, and reduce patients' medical expenses. The current study provides a foundation for future research.

The current study had some limitations. It was a single center retrospective study, which may have affected some results by way of insufficient data volume. Secondly, there were differences in the use of antibiotics by select patients before drug sensitivity test results were available, and this may have affected the final results.

Conclusion

The clinical model described herein can predict healing of infected foot ulcers in T2DM patients. The model incorporates Wagner grade, essential hypertension, platelet count, GNB, and NLR. There was a linear relationship between healing outcome and plasma platelet level, and when the Wagner grade was 2 and the platelet level was ≤ 200, a lower platelet level indicated a worse wound healing prognosis.

Abbreviations

BUN, blood urine nitrogen; CAD, coronary artery disease; CI, confidence interval; DFU, Diabetic Foot Ulcer; EH, essential hypertension; GNB, Gram negative bacteria; GPB, Gram positive bacteria; HIS, Hospital Information System; LASSO, Least absolute shrinkage and selection operator; MLR, monocyte to lymphocyte ratio; NLR, neutrophily to lymphocyte ratio; PVD, peripheral vascular disease; PLT, platelet; RCS, restricted cubic spline; T2DM, type 2 diabetic mellitus.

Ethical Approval

The study was carried out in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Affiliated Taicang Hospital of Soochow University (IRB: 2023-KY-116). As a retrospective study and data analysis was performed anonymously, this study was exempt from the informed consent from patients.

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Disclosure

The authors report no conflicts of interest in this work.

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