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ORIGINAL RESEARCH

Diagnostic Value of Neutrophil CD64 Index in Diabetic Foot Osteomyelitis

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Objective: This study aims to assess the value of the Neutrophil CD64 (nCD64) index as a novel, rapid biomarker for the diagnosis of Diabetic foot osteomyelitis (DFO), with the goal of enhancing the accuracy of early detection.

Methods: A total of 142 hospitalized patients with type 2 diabetes complicated by diabetic foot infection were included. Participants were categorized into a Non-DFO group (84 cases) and a DFO group (58 cases) based on the presence or absence of osteomyelitis. The white blood cell count (WBC), C-reactive protein (CRP), and procalcitonin (PCT), erythrocyte sedimentation rate (ESR), and the nCD64 index were compared between the two groups. Logistic regression analysis was performed to identify risk factors associated with DFO. Receiver operating characteristic (ROC) curves were generated, and the area under the curve (AUC) was calculated to evaluate the sensitivity and specificity of the nCD64 index in diagnosing DFO.

Results: The nCD64 index was significantly elevated in the DFO group compared to the Non-DFO group ($P<0.05$). Logistic regression analysis indicated that the nCD64 index and ESR were independent risk factors for DFO ($P < 0.05$). The ROC-AUC analysis indicated that both the nCD64 index and ESR were associated with DFO. The nCD64 index exhibited superior predictive value compared to ESR (AUC: 0.895 vs 0.846). Specifically, the nCD64 index demonstrated the highest sensitivity at 96.6%, while ESR showed the highest specificity at 82.1%. When combined, the predictive value of the nCD64 index and ESR was optimal, with an AUC of 0.937, sensitivity of 86.2%, and specificity of 91.7%.

Conclusion: The nCD64 index may serve as an effective new biomarker for the early detection of DFO. The combination of the nCD64 index and ESR could further enhance diagnostic accuracy.

Keywords: diabetic foot, osteomyelitis, nCD64 index, erythrocyte sedimentation rate, early diagnosis

Introduction

Diabetic foot osteomyelitis (DFO) is a condition characterized by the progression of soft tissue infections to the bone, initially affecting the cortex and subsequently involving the bone marrow.[1] It is the most prevalent type of infection associated with diabetic foot (DF),[2] leading to considerable psychological stress for patients and placing a significant burden on society. Research indicates that approximately 20% to 60% of individuals with diabetic foot infection (DFI) also develop osteomyelitis,[3] which heightens the risk of lower limb amputation.[2] Furthermore, the mortality rate within 5 years following major amputations can be as high as 50%.[4,5]

Research has demonstrated that effective antibiotic treatment can significantly reduce the rates of toe and lower limb amputation in patients suffering from DFO.[6,7] While the confirmation of infection predominantly relies on microbial culture results—which typically take 24 to 48 hours to obtain[8], the identification of rapid and effective diagnostic markers for DFO is essential. Currently, the inflammatory markers employed in diagnosing DFI and monitoring disease progression exhibit certain limitations. Commonly used in clinical settings, these markers include white blood cell count (WBC), C-reactive protein (CRP), and procalcitonin (PCT). The effectiveness of WBC in distinguishing between infectious and non-infectious inflammatory conditions is notably low;[9] it can be elevated due to factors such as injury,

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physical exercise, emotional stress, and schizophrenia.[10–12] CRP, an acute-phase protein synthesized and secreted by the liver,[13] typically begins to rise 12 to 24 hours following infection and peaks within 2 to 3 days,[14] making it a late-stage indicator of infection. Furthermore, conditions such as myocardial infarction, tumors, and acute trauma can also lead to increased CRP levels.[15–17] PCT can be detected 3 to 4 hours after the onset of infection, reaching its peak within 6 to 12 hours, with a half-life of approximately 24 hours.[18] However, PCT is not suitable for differentiating between non-infectious diseases and infectious diseases,[19] as it may be elevated in cases of severe trauma, various major surgeries, and inflammatory events like cardiogenic shock, even in the absence of bacterial infections.[20] Although studies suggest that WBC, CRP, and PCT may serve as supplementary laboratory indicators for detecting DFI,[21] other research indicates that these markers do not effectively differentiate DFO.[22] In contrast, the erythrocyte sedimentation rate (ESR) has shown potential as a diagnostic marker for DFO, albeit with moderate sensitivity (72%) and specificity (84%).[22] Consequently, the search for early warning inflammatory indicators that can facilitate prompt anti-infective treatment is of paramount importance for the effective management of DFO.

Neutrophil CD64 (nCD64) is a receptor for the Fc region of IgG, typically expressed at low levels on the surface of neutrophils. However, during bacterial infections, its expression is significantly elevated.[23] This upregulation is mediated by cytokines such as interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF).[24] When nCD64 on the cell surface binds to IgG, it triggers the aggregation of nCD64, initiating a series of reactions that activate downstream signaling cascades. This process leads to the release of additional factors by phagocytes, including Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6),[25] and enhances the oxidative destruction of target cells, facilitating phagocytosis and the clearance of pathogens. In healthy individuals, nCD64 levels are typically low. However, during bacterial infections, nCD64 expression can rapidly and significantly increase within just a few hours. This upregulation serves as a valuable tool for differentiating between resting and activated neutrophils.[26] As a marker of neutrophil activation, nCD64 upregulation not only promotes inflammatory responses but also enhances the capacity to eliminate pathogens. Importantly, nCD64 upregulation is recognized as one of the earliest steps in the host's immune response to bacterial invasion.[27]

Research has demonstrated that the nCD64 index possesses significant clinical diagnostic value for bacterial infections, particularly in the early diagnosis and treatment of these conditions.[28] The nCD64 index is relatively stable and is not influenced by medication usage or disease activity. Currently, the nCD64 index is markedly elevated in various bacterial infectious diseases such as sepsis, bronchitis, acute pancreatitis, and bacterial peritonitis, showcasing higher sensitivity and specificity.[29–32] However, there have been no reported applications of the nCD64 index in DFO. Thus, this study aims to investigate the diagnostic value of the nCD64 index in DFO to provide a foundation for clinical treatment strategies.

Materials and Methods

Study Subjects

The retrospective study consisted of 142 participants with type 2 diabetes mellitus complicated by DFI who were hospitalized at our institution between March 2019 and February 2022. Of these participants, 86 were male and 56 were female, with ages ranging from 44 to 83 years, and an average age of 68.43 ± 8.73 years. The duration of diabetes mellitus varied from 1 to 30 years, with a mean of 11.91 ± 6.75 years. The duration of DF ranged from 1 week to 17 weeks. Participants were classified into two groups based on the presence or absence of concurrent osteomyelitis: the Non-DFO group (n=84) and the DFO group (n=58). The diagnostic criteria for type 2 diabetes mellitus were based on the standards established by the World Health Organization in 1999. The diagnostic criteria for DF were established according to the standards set by the International Working Group on the Diabetic Foot (IWGDF). The diagnosis of osteomyelitis was confirmed if either of the following criteria was met (Figure 1): (1) Histopathological confirmation of bone tissue involvement; (2) Probe-to-bone (PTB) positivity and X-rays evidence of bone destruction. DFO was determined using the PTB test and conventional X-rays, which together form a diagnostic combination with high sensitivity and specificity.[33,34] Both tests must yield positive results to confirm the presence of DFO. The PTB test is considered positive when the bone is palpated through the diabetic foot ulcer (DFU) using sterile forceps. Positive X-ray

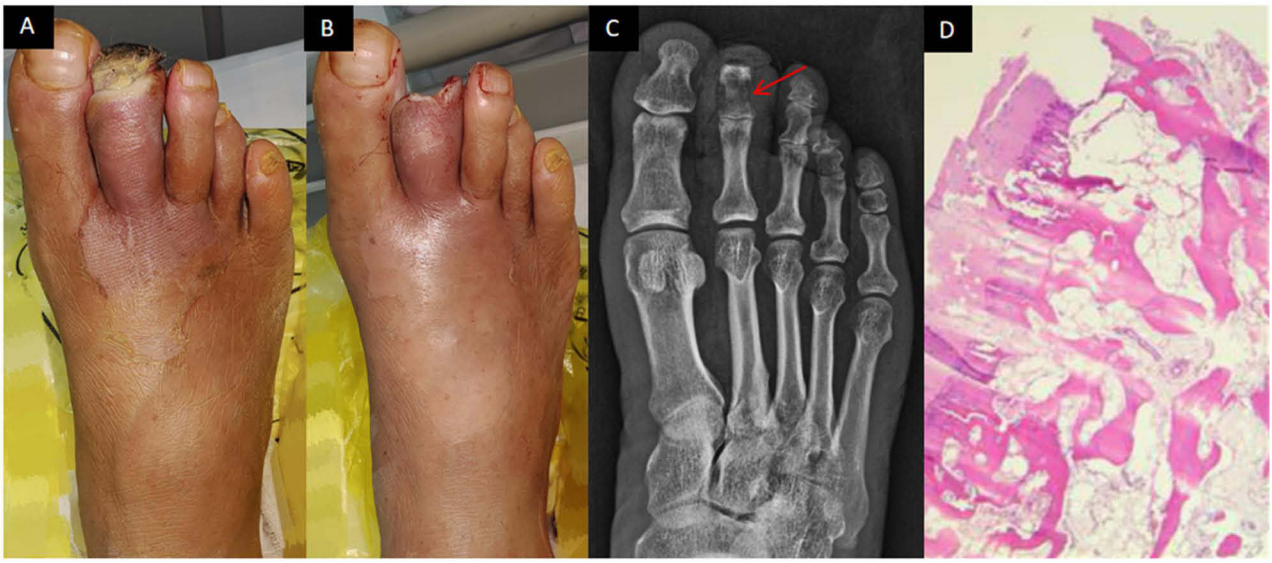

Figure 1 A male DFO patient on the right foot. (**A**) Preoperative, (**B**) Postoperative, (**C**) X-ray findings revealed bone destruction in the second toe, and (**D**) Histopathological confirmation of osteomyelitis. The red arrow indicates the location of bone destruction on the X-ray.

results are indicated by the presence of changes compatible with DFO in the affected bone, such as cortical destruction, or necrotic bone, alongside other radiographic findings documented in the literature.[35]

Inclusion Criteria for the Study Were the Following

(1) age 18 years or older; (2) Type 2 diabetes mellitus; (3) confirmed DFI as determined by clinical and laboratory evaluations; (4) Wagner Grade 3; (5) complete data availability; (6) Han ethnicity.

Exclusion Criteria for the Study Were the Following

(1) Bacterial infections in areas or organs other than the feet; (2) acute cardiovascular or cerebrovascular diseases; (3) blood disorders, connective tissue diseases, or tumors; (4) pregnant or breastfeeding; (5) taking contraceptive pills or other medications for infectious diseases; (6) used glucocorticoids or immunomodulatory drugs within the three months prior to enrollment; (7) severe liver or kidney dysfunction; (8) incomplete data.

All participants who enrolled in the study were thoroughly briefed and provided their signed consent. This research received approval from the ethics committee of Xuzhou Central Hospital (XZXY-LJ-20160310-025) and was conducted in accordance with the Declaration of Helsinki.

Data Collection

The collection of clinical data encompasses: gender, age, smoking, duration of diabetes, duration of diabetic foot, fasting blood glucose, HbA1c, blood lipid profiles, ankle-brachial index (ABI), diabetic complications, and comorbidities.

Detection of the nCD64 Index

We collected 2mL of whole blood from the participants using EDTA as an anticoagulant and analyzed it within 2 hours. Added 5 μL of nCD64 and CD45 phycoerythrin (PE)-labeled antibodies to 50 μL of whole blood, mixed thoroughly, and incubated in the dark at room temperature for 40 minutes. The subsequent step involved lysing the red blood cells, processing it, and conducting flow cytometry analysis. The mean fluorescence intensity (MFI) of nCD64 expression on neutrophils, monocytes, and lymphocytes in the experimental tube were measured by flow cytometry within 2 hours. The nCD64 index was calculated using the following formula: (neutrophil nCD64 MFI / lymphocyte nCD64 MFI) / (monocyte nCD64 MFI / neutrophil nCD64 MFI).

Statistical Analysis

Data analysis was performed using SPSS 25.0, while Prism 9.0 was used for plotting. For data that adhered to a normal distribution, independent samples *t*-tests were conducted to assess statistical significance between groups, with results presented as mean ± standard deviation ($\bar{x} \pm s$).

Nonparametric continuous variables were analyzed using the Mann–Whitney *U*-test and reported as median (interquartile range). Categorical data were reported as frequency (n) and relative percentage (%), with chi-square (χ^2) tests employed for analysis. Variables identified as associated with the occurrence of DFO in univariate analysis were subsequently subjected to multivariate analysis using a logistic regression model. ROC curves were generated to evaluate the effectiveness of various inflammatory indicators in diagnosing DFO. All tests were two-tailed, and a p-value of less than 0.05 was considered statistically significant.

Results

Comparison of Baseline Data Between the Non-DFO Group and DFO Group

The duration of diabetic foot ulcer, HbA1c, and ABI showed statistically significant differences between the Non-DFO group and DFO group (all P<0.05). Compared with the Non-DFO group, the DFO group had a longer duration of diabetic ulcers, higher average blood glucose levels, and poorer lower limb vascular conditions (Table 1).

Table 1 Comparison of the Clinical Characteristics Between the Non-DFO Group and DFO Group

Variables	Non-DFO (%)	DFO (%)	P-value
n	84	58	
Gender (man/woman)	48/36	38/20	0.316
Age (years)	68.73±8.73	68.00±8.78	0.628
Smoking	35 (41.7)	23 (38.9)	0.106
Duration of Diabetes (year)	12.55±6.65	10.98±6.85	0.175
Duration of Diabetic Foot Ulcer (week)	2 [1.11, 4.00]	3 [1.86, 4.00]	0.019*
FPG (mmol/L)	11.53±3.66	12.11±4.34	0.389
HbA1c (%)	10.57±2.44	11.89±2.94	0.004**
TC (mmol/L)	4.45±1.00	4.19±0.92	0.126
TG (mmol/L)	1.26±0.59	1.16±0.54	0.284
LDL-C (mmol/L)	2.92±0.83	2.81±0.72	0.414
HDL-C (mmol/L)	0.94±0.27	0.89±0.20	0.175
ABI	0.73±0.31	0.57±0.30	0.002**
Hypertension (%)	31 (36.9)	24 (41.4)	0.591
Coronary Artery Disease (%)	32 (38.1)	21 (36.2)	0.819
Cerebral Infarction (%)	18 (21.4)	15 (25.9)	0.539
Diabetic Retinopathy (%)	44 (52.4)	29 (50.0)	0.780
Diabetic Nephropathy (%)	39 (46.4)	22 (37.9)	0.315
Diabetic Peripheral Neuropathy (%)	39 (46.4)	27 (46.6)	0.988

(*Continued*)

Table 1 (Continued).

Variables	Non-DFO (%)	DFO (%)	P-value
Site of Ulcer			0.205
Forefoot	57 (67.9)	46 (79.3)	
Midfoot	16 (19.0)	5 (8.6)	
Hindfoot	11 (13.1)	7 (12.1)	

Notes: *p<0.05, **p<0.01, ***p<0.001.
Abbreviations: FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; TC, serum total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ABI, ankle-brachial index; WBC, white blood cell; CRP, C-reactive protein; PCT, procalcitonin; ESR, erythrocyte sedimentation rate.

Comparison of Inflammatory Indicators Between the Non-DFO Group and DFO Group

The nCD64 index and ESR levels were significantly higher in the DFO group compared to Non-DFO group (all P<0.05) (Table 2, Figure 2A and B). Clinically, we also observed that infections in DFO group were more severe than those in the Non-DFO group.

Risk Factors for DFO

The duration of diabetic foot ulcer was included as it reflected the chronicity and potential progression of the underlying foot pathology. HbA1c was chosen as a marker of long-term glycemic control, given that poor glycemic control was a well-known risk factor for complications in diabetes, including the development of DFI. The ABI was included to assess the vascular of the lower limbs, as peripheral arterial disease was a common comorbidity in patients with DFI and could significantly impact wound healing and infection risk. The WBC count was incorporated as a general marker of inflammation and infection. ESR was included as a traditional marker of systemic inflammation, which could be elevated in the presence of DFI. Utilizing the occurrence of DFO as the dependent variable, and the duration of diabetic foot ulcer, HbA1c, ABI, WBC, the nCD64 index, and ESR as independent variables, logistic regression analysis indicated that both the nCD64 index and ESR are independent risk factors for DFO (all P<0.05), as shown in Tables 3 and 4.

Prediction of DFO by nCD64 Index and ESR

The area under the ROC curve analysis revealed that both the nCD64 index and ESR are associated with DFO; however, the nCD64 index demonstrated superior predictive value compared to ESR (area under the curve: 0.895 vs 0.846). While ESR was also a useful marker, it was less precise than the nCD64 index in predicting DFO. Specifically, the nCD64 index exhibited the highest sensitivity at 96.6%, while ESR displayed the highest specificity at 82.1%. This high sensitivity means that the nCD64 index was highly effective in identifying patients who have DFO. When combined, the predictive

Table 2 Comparison of the Inflammatory Indicators Between the Non-DFO Group and DFO Group

Variables	Non-DFO	DFO	P-value
WBC (109 /L)	11.16±1.35	11.99±3.12	0.062
CRP (mg/L)	90.88±23.38	93.84±16.98	0.383
PCT (ng/mL)	0.30±0.03	0.31±0.06	0.530
ESR (mm/h)	45.14±15.70	78.29±23.27	0.000***
nCD64 Index	1.99±0.93	3.71±0.92	0.000***

Note: ***p<0.001.

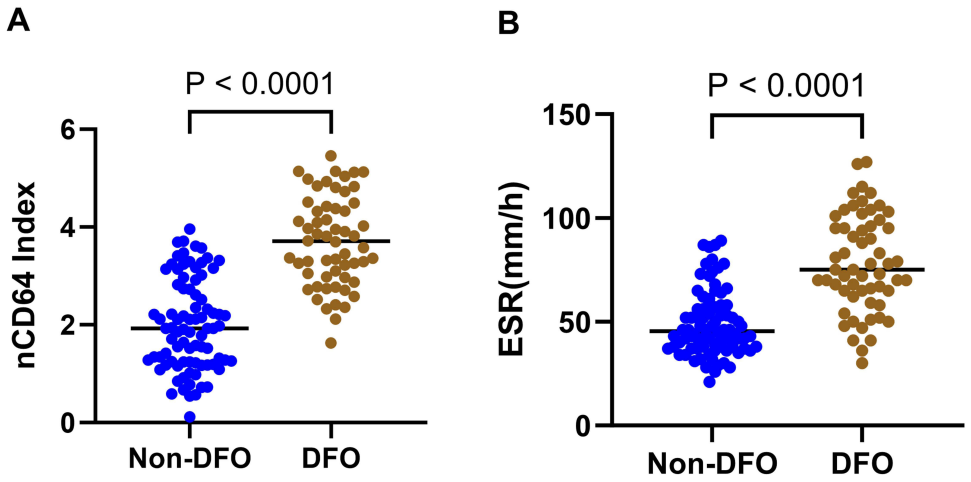

Figure 2 Comparison of the nCD64 Index and ESR between the Non-DFO Group and DFO Group. (**A**) Comparison of the nCD64 index between the Non-DFO group and DFO group. (**B**) Comparison of ESR between the Non-DFO group and DFO group.

value of the nCD64 index and ESR was optimal, with an area under the curve of 0.937, sensitivity of 86.2%, and specificity of 91.7%, as presented in Table 5 and Figure 3. This balance between sensitivity and specificity was crucial in clinical decision-making, as it ensured that the majority of true cases were identified while minimizing the risk of misdiagnosis.

Table 3 Univariate Logistic Regression of Risk Factors of DFO

Variable	B	SE	Wald	P-value	OR	95% CI	
Duration of Diabetic Foot Ulcer (weeks)	0.289	0.133	4.760	0.029	1.336	1.030	1.732
ABI	−1.702	0.578	8.661	0.003	0.182	0.059	0.566
HbA1c (%)	0.184	0.066	7.669	0.006	1.202	1.055	1.369
WBC (10^9 /L)	0.165	0.079	4.359	0.037	1.179	1.010	1.376
ESR (mm/h)	0.072	0.012	35.144	0.000	1.074	1.049	1.100
nCD64 Index	1.861	0.307	36.667	0.000	6.431	3.521	11.746

Table 4 Multivariate Logistic Regression of Risk Factors of DFO

Variable	B	SE	Wald	P-value	OR	95% CI	
Model 1							
nCD64 Index	1.818	0.309	34.534	0.000	6.162	3.360	11.301
Model 2							
nCD64 Index	1.581	0.321	24.307	0.000	4.859	2.592	9.108
ESR	0.054	0.014	15.116	0.000	1.056	1.027	1.085
Model 3							
nCD64 Index	1.743	0.363	23.056	0.000	5.712	2.805	11.634
ESR	0.055	0.015	13.265	0.000	1.056	1.026	1.088

Notes: Model 1, enrolled CD64 Index and ABI. Model 2, enrolled CD64 Index, ESR, and WBC. Model 3, enrolled CD64 Index, Duration of Diabetic Foot Ulcer (weeks), HbA1c, ESR, WBC, and ABI.

Table 5 ROC Curve Parameters of nCD64 Index and ESR for Predicting DFO

Variable	Cut-off	AUC (95% CI)	Sensitivity	Specificity	Youden Index
nCD64 Index	2.325	0.895 (0.846–0.944)	0.966	0.690	0.656
ESR (mm/h)	65	0.846 (0.780–0.912)	0.759	0.821	0.580
nCD64 Index+ESR	-	0.937 (0.898–0.975)	0.862	0.917	0.779

Discussion

In cases of DFO, the risk of both severe and minor amputations is significantly higher compared to isolated soft tissue infections.[36,37] Early diagnosis facilitates the selection of appropriate antibiotics and can lead to reduced amputation rates. Bone tissue culture and drug susceptibility testing are regarded as the gold standard for diagnosing and guiding antibiotic treatment. However, these procedures can be time-consuming and may not deliver timely insights necessary for early diagnosis and intervention. Therefore, it is essential to identify a rapid and accurate method for diagnosing DFO.

Patients with DFO exhibited elevated levels of WBC and CRP, suggesting that these markers may have predictive value for the occurrence of DFO.[38,39] However, WBC can be influenced by factors such as trauma and stress, implying that WBC may not serve as a reliable biological marker for bacterial infection. Additionally, CRP demonstrated poor accuracy in detecting DFO.[40] In this study, there was no statistically significant difference in WBC and CRP between the Non-DFO group and the DFO group. On the other hand, PCT is a widely used clinical indicator for predicting bacterial infections, offering higher sensitivity and specificity compared to WBC and CRP.[41,42] Some research has identified PCT as an important marker for predicting osteomyelitis,[43,44] although other studies have reported conflicting results.[22] In our study, there was also no statistically significant difference in PCT levels between the Non-DFO group and the DFO group.

Among the various blood parameters, there are currently no indicators that are highly specific for DFO. However, ESR is regarded as the most useful and valuable marker. The cutoff for ESR in diagnosing DFO is not fixed, with different studies suggesting varying recommended values. The IWGDF recommends a cutoff of 70 mm/h for DFO,[45] while other studies have proposed cutoffs of 60 mm/h and 49 mm/h as potential thresholds.[39,46] In this study, the cutoff was established at 65 mm/h.

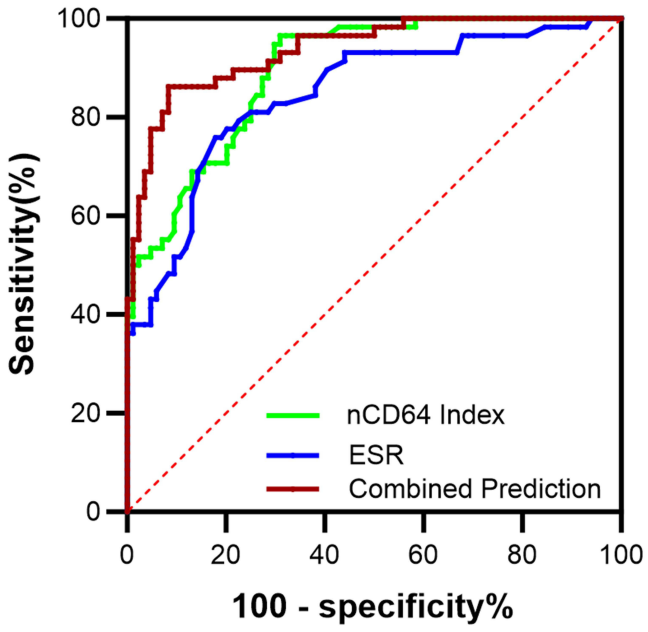

Figure 3 ROC curves of Inflammatory indicators for the diagnosis of DFO.

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nCD64 has demonstrated high sensitivity and specificity for bacterial infections and is regarded as a reliable diagnostic marker for such conditions.[47,48] Expression of nCD64 begins within 1 to 3 hours after infection, with detectable upregulation occurring within 3 to 6 hours post-infection, highlighting its potential for early infection detection.[30] Additionally, nCD64 maintains relatively consistent expression in blood samples for over 30 hours and requires only a small blood volume for testing, making this method notable for its accuracy, speed, and simplicity. However, there is currently no consensus among institutions regarding the measurement of the nCD64 index. Some research defined the nCD64 index as the median fluorescence intensity of neutrophil nCD64 divided by that of lymphocyte nCD64. In monocytic cells, CD64 is produced in large quantities under both physiological and pathogenic conditions, while its expression in lymphocytes is comparatively low. Given this expression pattern, the nCD64 index employed in this study demonstrated enhanced efficacy. The detection of nCD64 index requires specialized equipment and skilled operators. Provided that these conditions are met, the test could be readily accessible in community and hospital laboratories. The findings indicated that the nCD64 index in the DFO group was significantly higher than those in the Non-DFO group, suggesting that the nCD64 index can effectively reflect the condition of DFO.

Logistic regression analysis indicated that the nCD64 index and ESR were independent risk factors for the occurrence of DFO. Both indices were significantly associated with the development of DFO, and their levels notably increased in the context of bacterial infections, highlighting their role in the inflammatory response process. The underlying mechanism may involve the elevation of the nCD64 index and ESR above normal levels, which promotes the adhesion and migration of leukocytes to endothelial cells. This leads to increased infiltration of leukocytes into non-infected areas of the foot. Excessive inflammation can damage the normal tissues and cells in the foot, with inflammatory factors such as IL-1 and IL-6 aggravating injuries to the foot wound and impacting the bone, ultimately resulting in osteomyelitis. Additionally, higher levels of the nCD64 index and ESR are correlated with more severe disease. During this period, the number of leukocytes in the foot wound tissue continues to rise, releasing additional cytokines and intensifying the inflammatory response, which leads to a rapid deterioration of the wound.

According to the area under the ROC curve, both the nCD64 index and ESR were linked to the occurrence of DFO, with the nCD64 index demonstrating superior predictive value compared to ESR. The nCD64 index exhibited the highest sensitivity (96.6%), while the ESR showed the greatest specificity (82.1%). Given the controversies surrounding DFO treatment, early antibiotic therapy is essential for limb preservation, making it crucial to select antibiotics with strong bone penetration. Failing to choose appropriate antimicrobial agents early on may result in disease progression and adverse outcomes, such as amputation. Therefore, it is vital to enhance both the sensitivity and specificity of DFO diagnosis. The combined assessment of the nCD64 index and ESR improved specificity and the Youden index, indicating that a joint diagnostic approach can enhance the overall diagnostic efficiency for DFO.

The limitations of this study include its retrospective design and the fact that it is based on a single-center research project. Additionally, the sample size is relatively small, highlighting the need for further research with larger sample sizes and prospective designs to facilitate deeper exploration.

Conclusion

In summary, early identification and timely intervention of DFO continue to pose significant challenges. The findings of this study suggest that the nCD64 index may be an effective new biomarker for the early detection of DFO. Combining the nCD64 index with ESR could further enhance diagnostic accuracy.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Approval and Informed Consent

This research received approval from the ethics committee of Xuzhou Central Hospital ((XZXY-LJ-20160310-025) and was conducted in accordance with the Declaration of Helsinki.

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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.

Disclosure

The authors declare no conflicts of interest.

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