Original Article

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Association Between Prognostic Nutritional Index and Major Amputation in Patients with Diabetic Foot Ulcers

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Abstract

Aim: Given the clinical importance of early risk stratification in patients with diabetic foot ulcers and the limited availability of simple and reliable biomarkers, there is a need for easily accessible parameters that can support clinical decision-making. Therefore, this study aimed to evaluate the association between the Prognostic Nutritional Index (PNI) and major amputation in patients hospitalized for diabetic foot ulcers.

Methods: This retrospective observational study included patients with diabetic foot ulcers evaluated by the Diabetic Foot Board between January 2020 and August 2024, who were managed conservatively or underwent minor or major amputation. Demographic characteristics, comorbid diseases, and laboratory parameters—including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), HbA1c, neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), and PNI—were compared among groups. Multivariable logistic regression analysis was performed to identify independent factors associated with major amputation, and receiver operating characteristic curve analysis was used to assess the discriminative performance of PNI.

Results: The study cohort consisted of 756 cases: 216 without amputation, 338 with minor amputation, and 202 with major amputation. Significant differences were observed between the amputation and non-amputation groups with respect to age, coronary artery disease, chronic heart failure, CRP, ESR, NLR, SII, and PNI. Age, ESR, CRP, and PNI were identified as independently associated with major amputation.

Conclusion: The PNI reflects both immunological and nutritional status and is independently associated with major amputation in patients with diabetic foot ulcers.

Keywords: Prognostic nutritional index, amputation, immunonutrition, inflammation

Introduction

The global prevalence of diabetes mellitus in adults has surpassed 800 million and continues to increase, making it a major public health concern (1). Diabetes is associated with several microvascular and macrovascular complications, including nephropathy, neuropathy, retinopathy, and peripheral arterial disease (2). Among these, the diabetic foot ulcer is one of the most devastating complications, resulting from the combined effects of neuropathy, ischemia, impaired immunity, and chronic inflammation, and leading to delayed wound healing and increased susceptibility to severe infection. Despite advances in multidisciplinary management, 15-20% of diabetic foot ulcers still require amputation, and major amputation is associated with markedly increased mortality and reduced quality of life (3).

The Prognostic Nutritional Index (PNI) is a peripheral blood–based immunonutritional marker calculated using the serum albumin level and the lymphocyte count, reflecting both nutritional and immune status. Malnutrition, systemic inflammation, and immune dysfunction play key roles in the progression of diabetic foot ulcers; therefore,

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PNI may represent the biological vulnerability to severe outcomes such as major amputation (4,5). However, evidence regarding the association between PNI and amputation in diabetic foot disease is limited, and its specific relationship with major amputation remains unclear. We hypothesized that a low PNI is independently associated with an increased risk of major amputation in patients with diabetic foot disease.

The aim of this study was to evaluate the association between PNI and major amputation in patients with diabetic foot disease. By identifying a simple and easily accessible biomarker for early risk stratification, this study may contribute to improved clinical decision-making and the timely implementation of preventive and therapeutic strategies in high-risk patients.

Materials and Methods

Compliance with Ethical Standards

The study protocol received approval from the University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital Scientific Research Ethics Committee No. 1 (approval no.: KAEK/28.08.2024.187, date: 17.09.2024). All patients who fulfilled the inclusion criteria and were managed by the board between January 2020 and August 2024 were enrolled.

Study Design

This study was designed as a retrospective observational study based on the medical records of patients with diabetic foot ulcers evaluated by the Diabetic Foot Board between January 2020 and August 2024. The Diabetic Foot Board decided on management for patients, including minor amputation, major amputation, and non-amputation treatments such as medical therapy and vascular interventions. Based on treatment outcomes, participants were grouped into three categories: minor amputation, major amputation, or non-amputation management.

Treatment decisions were made by a multidisciplinary Diabetic Foot Board consisting of vascular surgeons, infectious disease specialists, endocrinologists, orthopedic surgeons, and plastic surgeons. Decisions were based on a structured clinical assessment, including ulcer depth and extent, presence and severity of infection, vascular status (clinical examination, ankle-brachial index, and imaging findings when available), radiological evidence of osteomyelitis, and feasibility of revascularization. Major amputation was performed when the limb was considered non-salvageable due to extensive necrosis, uncontrolled infection, or irreversible ischemia.

The study flowchart is presented in Figure 1. In our study, amputations below the ankle level were classified as minor, while those above the ankle were considered major. Amputations at the ankle joint were classified as major because they significantly affect walking ability.

Patients aged >80 years were excluded to minimize confounding by advanced frailty, multimorbidity, and age-related alterations in albumin synthesis and in lymphocyte counts that could bias PNI interpretation. Individuals who had chronic liver disease affecting albumin levels or whose data were incomplete were not included in the study. Patients using medications that affect hematological parameters were excluded. Patients who declined the amputation recommended by the Diabetic Foot Board were excluded from the final analysis, as outcome classification was based on the actual treatment performed. The patients' demographic data and medical histories, including comorbidities such as rheumatologic disease, peripheral neuropathy, venous insufficiency, coronary artery disease, chronic heart failure, chronic kidney disease, arterial thrombosis, venous thrombosis, peripheral artery disease, and osteomyelitis, were recorded. Peripheral artery disease was defined based on clinical history, an ankle-brachial index of <0.9, or vascular imaging findings (6).

Laboratory parameters were obtained at hospital admission, prior to the Diabetic Foot Board evaluation, as part of a routine clinical assessment. Due to the urgent nature of some admissions, fasting status was not standardized; however, all measurements were performed in the hospital's central laboratory using standard procedures. Laboratory parameters obtained prior to the Board evaluation—including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), HbA1c, neutrophil-to-lymphocyte ratio, systemic immune-inflammation index (SII), and PNI—were recorded and compared among the groups. Prognostic Nutritional Index was calculated as [10×serum albumin (g/dL)] + [0.005×lymphocyte count (/mm^3)], and SII was calculated as (platelet×neutrophil)/lymphocyte count.

In our retrospective study design, the outcome (major amputation, minor amputation, or non-amputation treatment) was determined at the time of the Diabetic Foot Board decision. Therefore, there was no additional follow-up period beyond the board decision.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation for normally distributed data and as median (minimum-maximum) for non-normally distributed data, as appropriate. The distribution of continuous variables was assessed using the Shapiro-Wilk test, supported by visual inspection of histograms and Q-Q plots.

Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. For comparisons of continuous variables among three independent groups, the Kruskal-Wallis test was used for non-normally distributed data. When a significant difference was detected, post hoc pairwise comparisons were performed using the Mann-Whitney U test with Bonferroni correction.

Multivariable logistic regression analysis was conducted to identify factors independently associated with major amputation. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, with a p-value>0.05 indicating an adequate model fit. Multicollinearity among independent variables was assessed using the variance inflation factor (VIF), and no significant collinearity was observed (all VIF values were <2). The adequacy of the multivariable logistic regression model was evaluated based on the events-per-variable (EPV) ratio. With 202 major amputation events and 8 candidate predictors included in the model, the EPV was approximately 25, exceeding the commonly recommended minimum threshold of 10 and the more conservative threshold of 20, thereby indicating a low risk of model over-fitting.

To control for type I error in multiple pairwise comparisons among the three groups (non-amputation vs. minor amputation, non-amputation vs. major amputation, and minor amputation vs. major amputation), Bonferroni correction was applied, and the adjusted significance level was set at α=0.0167. A two-tailed p-value<0.05 was considered statistically significant.

Results

A total of 756 patients managed by the Diabetic Foot Board were enrolled in the study. Of these, 216 (28.5%) were in the non-amputation group, 338 (44.8%) were in the minor amputation group, and 202 (26.7%) were in the major amputation group.

The patients' demographic characteristics and medical histories, including comorbidities, are summarized in Table 1. The mean age was 59.7±10.9 years in the non-amputation group, 61.5±10.9 years in the minor amputation group, and 63.7±10.0 years in the major amputation group. The age of patients in the major

Table 1. Baseline demographic and clinical characteristics of the study groups

	Non-amputation n=216 (28%)	Minor amputation n=338 (45%)	Major amputation n=202 (%27)	p-value
Age	59.7±10.9	61.5±10.9	63.7±10	**0.002**[ǂ]
Gender Male/Female	164/52	253/85	142/60	0.376[*]
Cigarette Yes/No	65/150	117/221	74/127	0.342[*]
Rheumatologic disease Yes/No	5/211	5/333	5/197	0.669[#]
Peripheral neuropathy Yes/No	66/150	127/211	66/136	0.203[*]
Venous insufficiency Yes/No	14/202	21/317	10/192	0.787[*]
Coronary artery disease Yes/No	111/105	164/173	125/77	**0.01**[*]
Chronic heart failure Yes/No	62/154	95/242	79/123	**0.019**[*]
Chronic kidney disease Yes/No	60/154	101/237	73/129	0.168[*]
Arterial thrombosis Yes/No	62/154	88/250	72/130	0.058[*]
Venous thrombosis Yes/No	7/209	9/329	8/194	0.722[*]
Peripheral artery disease Yes/No	187/29	300/38	179/23	0.724[*]
Osteomyelitis Yes/No	138/78	237/100	135/67	0.28[*]

[*]Chi-square test, [#]Fisher's exact test, [ǂ]Kruskal-Wallis test.
Age; Non-amputees vs. Minor amputees p=0.082, Non-amputees vs. Major amputees p<0.0001, Minor amputees vs. Major amputees p=0.036
Coronary artery disease; Non-amputees vs. Minor amputees p=0.532, Non-amputees vs. Major amputees p=0.031, Minor amputees vs. Major Amputees p=0.003
Chronic heart failure; Non-amputees vs. Minor amputees p=0.859, Non-amputees vs. Major amputees p=0.03, Minor amputees vs. Major amputees p=0.009

amputation group was significantly higher than that of the other two groups (non-amputation vs. Minor amputation, p=0.082; Non-amputation vs. Major amputation, p<0.0001; Minor amputation vs. Major amputation, p=0.036).

The numbers of patients with coronary artery disease in the non-amputation, minor amputation, and major amputation groups were 111 of 216, 164 of 338, and 125 of 202, respectively. The prevalence of coronary artery disease was significantly greater in the major amputation group than in the other two groups (non-amputation vs. Minor amputation, p=0.532; Non-amputation vs. Major amputation, p=0.031; Minor amputation vs. Major amputation, p=0.003).

The numbers of patients with chronic heart failure were 62 of 216, 95 of 338, and 79 of 202 in the non-amputation, minor amputation, and major amputation groups, respectively. The prevalence of chronic heart failure was significantly higher in the major amputation group than in the other two groups (Non-amputation vs. Minor amputation, p=0.859; Non-amputation vs. Major amputation, p=0.03; Minor amputation vs. Major amputation, p=0.009).

The PNI and hematological values of the groups are presented in Table 2. A comparison of hematological parameters and indices revealed that both the minor and major amputation groups exhibited significantly higher levels of CRP, ESR, neutrophil-to-lymphocyte ratio, and SII compared with the non-amputation group, whereas their PNI was significantly lower (p<0.001).

The multivariable logistic regression model is shown in Table 3. The Hosmer–Lemeshow test indicated good model calibration (χ^2=2.01, p=0.98), with no significant difference between observed and predicted outcomes. Logistic regression analysis identified advanced age, elevated CRP and ESR levels, and lower PNI as significant risk factors for major amputation.

The results of the receiver operating characteristic (ROC) analysis, including sensitivity and specificity values illustrating the predictive performance of the PNI for major amputation, are summarized in Table 4. A PNI cut-off value of 34 predicted major amputation with a

Table 2. Inflammatory markers and prognostic nutritional index across study groups

	Non-amputees n=216 (28%)	Minor amputees n=338 (45%)	Major amputees n=202 (27%)	p-value	Non-amp. vs. Minor	Non-amp. vs. Major	Minor vs. Major
CRP (mg/L) median (min-max)	29 (0.2-321)	46 (0.8-407)	117 (0.7-468)	<0.001λ	0.003	<0.001	<0.001
ESR (mm/h) median (min-max)	58 (2-132)	62 (4-140)	82 (1-140)	<0.001λ	0.052	<0.001	<0.001
HbA1c (%) median (min-max)	8.2 (4-15)	8.6 (5-15)	8 (4-13)	0.09λ	-	-	-
Neutrophil/lymphocyte ratio median (min-max)	3.46 (0.1-37)	4 (0.3-107)	6.2 (1.1-51)	<0.00λ	0.211	<0.001	<0.001
Systemic immune inflammatory index median (min-max)	1186 (40-20692)	1466 (185-30391)	2651 (283-19157)	<0.001λ	0.008	<0.001	<0.001
Prognostic nutritional index median (min-max)	36 (3-49)	37 (19-49)	30 (16-74)	<0.001λ	0.698	<0.001	<0.001

λKruskal-Wallis test
CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

Table 3. Multivariable logistic regression analysis of factors associated with major amputation

	Odds ratio (OR)	95% CI (clinically meaningful)	p-value
Age (per 1 year)	1.029	1.010-1.048	**0.002**
Coronary artery disease	0.918	0.581-1.474	0.722
Chronic heart failure	0.720	0.449-1.156	0.173
Peripheral artery disease	1.078	0.75-1.230	0.800
CRP (per 10 mg/L)	1.046	1.020-1.072	<0.001
ESR (per 10 mm/h)	1.111	1.041-1.185	**0.001**
Neutrophil/lymphocyte ratio	0.971	0.921-1.024	0.277
Systemic immune inflammatory index	1.000	0.998-1.043	0.089
Prognostic nutritional index (per 5 points)	0.650	0.56-0.76	**<0.001**

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, CI: Confidence interval

Table 4. The findings of the ROC analysis with sensitivity, specificity values demonstrating the success of the prognostic nutritional index in major amputation prediction				
AUC (95% CI)	**Cut-off**	**p-value**	**Sensitivity %**	**Specificity %**
0.750 (0.711-0.789)	34	<0.001	70.3%	70.2%
ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval				

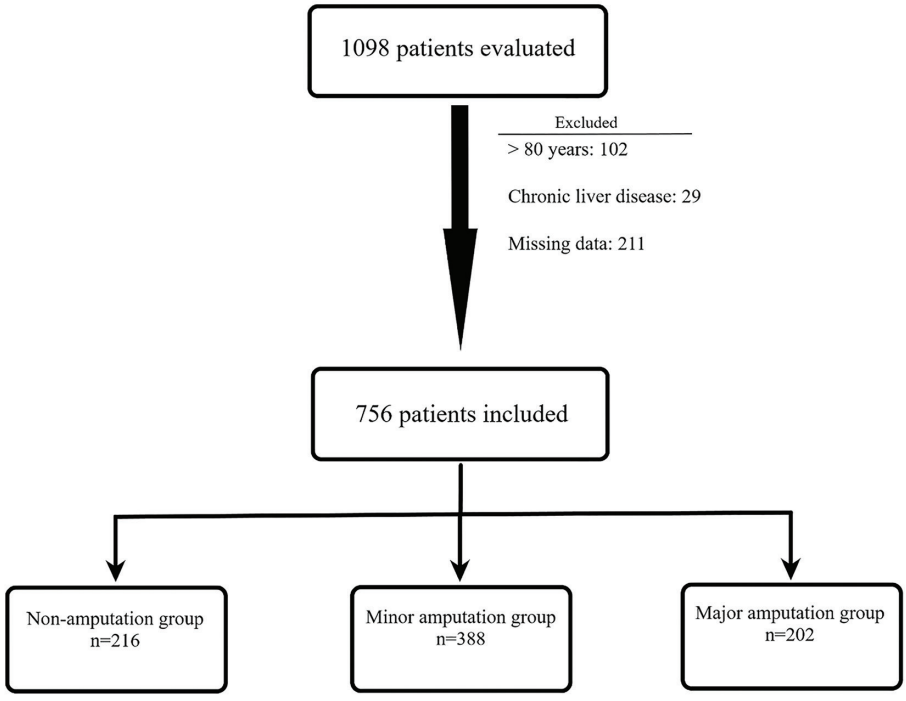


Figure 1. Study flow-chart

sensitivity of 70.3% and a specificity of 70.2% (p<0.001). The corresponding ROC curve for the PNI is depicted in Figure 2.

Discussion

We found a significant association between lower PNI levels and the risk of major amputation in patients with diabetic foot ulcers. Diabetic foot ulcers are recognized as one of the late complications associated with diabetes. Over time, patients with diabetes may develop peripheral motor and sensory neuropathies. This neuropathy leads to a reduced perception of pain and temperature, causing minor wounds or pressure points on the foot to go unnoticed. With the addition of other contributing factors, diabetic foot ulcers develop (7). While some individuals with diabetic foot ulcers are managed with medical treatments, approximately 20% undergo lower extremity amputation, either minor or major (8). Although losing a limb is challenging for any patient, predictably, major amputations have more severe consequences. In this context, identifying factors influencing major amputation is crucial.

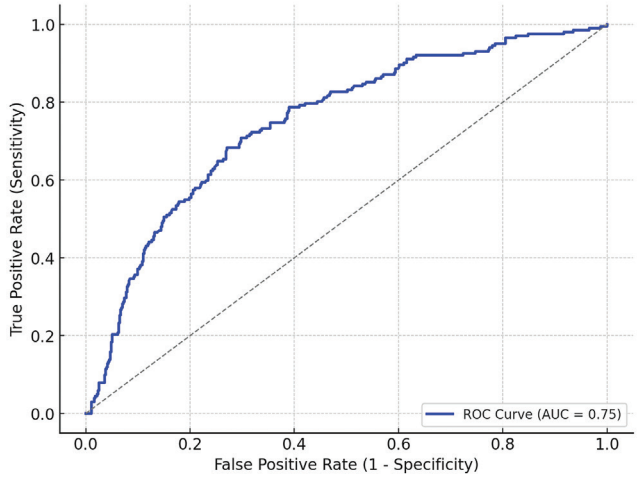


Figure 2. ROC curve of prognostic nutritional index
ROC: Receiver operating characteristic

The relationship between PNI and diabetes complications has been previously investigated. Recent studies have indicated that the PNI serves as a risk factor for both the progression of diabetic nephropathy and increased mortality (9,10). A different study demonstrated a relationship between PNI and increased mortality risk in elderly patients with chronic kidney disease (11).

Prognostic Nutritional Index is calculated using a formula that includes serum albumin levels and lymphocyte count (12). Albumin is a marker of a patient's nutritional status. Low albumin levels indicate malnutrition and increased disease severity (13). Malnutrition in individuals with diabetes mellitus is associated with an increased risk of macrovascular complications. Additionally, protein plays a crucial role in the tissue healing process, and its deficiency can lead to delayed wound healing (14). Moreover, protein deficiency due to malnutrition can impair immune response mechanisms (15). Therefore, the association between hypoalbuminemia—a marker of malnutrition—and amputation risk may be explained by its impact on the immune system and its contribution to vascular complications. Although albumin is a component of PNI and low albumin plausibly contributes to poor wound healing, albumin was not analyzed separately in this study; therefore, causal statements about albumin alone cannot be made from our data. Another parameter used to calculate PNI is the lymphocyte count. A low lymphocyte count may indicate dysfunction or suppression of the immune system (16). In the diabetic foot, bacterial infections often lead to increases in leukocyte and neutrophil counts, while lymphocytes and albumin tend to decrease as negative acute-phase reactants. These examples indicate that decreases in albumin and lymphocytes and, consequently, in PNI increase the risk of amputation.

A study evaluating the relationship between PNI and total amputation (both minor and major) found that the predictive value of PNI for forecasting amputation was 39 (17). In our study, the PNI had a predictive cut-off of 34 for major amputation. The discrepancy likely reflects differences in outcome definition (any amputation vs. major only) and patient characteristics. In clinical practice, a PNI<34 could identify patients who may benefit from early nutrition consultation and who should be prioritized for revascularization or antibiotic therapy. This finding suggests that PNI may be used not only as a prognostic marker in clinical decision-making but also as a risk stratification tool to guide the intensity of treatment.

Alongside PNI, other indicators of systemic inflammation and general physiological susceptibility—such as advanced age, heightened ESR, and elevated CRP—were independently correlated with major amputation in our study. These findings indicate that both immunonutritional status and inflammatory burden influence limb outcomes in diabetic foot disease. As individuals age, the prevalence of atherosclerosis and peripheral artery disease rises, resulting in compromised blood circulation and protracted tissue healing. The lack of a notable difference between groups in peripheral artery disease may indicate the substantial baseline prevalence of vascular disease within this specific diabetic foot cohort, coupled with the extensive diagnostic criteria employed in standard clinical practice. Also, age-related immunosenescence leads to impaired immune function, thereby increasing susceptibility to infection. Because of this, issues like deep, infected ulcers and osteomyelitis can happen, which may mean that a limb needs to be cut off. It is well known that ESR and CRP, two inflammatory markers, are often much higher in these situations. Recent research indicates that elevated inflammatory markers, such as ESR and CRP, correlate with a heightened risk of major amputation (18). A recent study examining predictive factors for amputation in diabetic feet revealed that elevated CRP levels and advanced age are significant risk factors for amputation (19). Our results align with existing literature, demonstrating that elevated ESR and CRP levels, along with advanced age, correlate with a heightened risk of major amputation. While inflammatory markers like CRP and ESR may be linked to parts of PNI, the multivariable regression analysis indicated that PNI was still linked to major amputation even after controlling for age and inflammatory factors. This indicates that PNI encompasses supplementary prognostic data beyond singular inflammatory markers.

Interestingly, HbA1c levels did not significantly differ among groups. This finding suggests that acute inflammatory burden and vascular status may play a more immediate role in amputation decisions than long-term glycemic control. While poor glycemic control contributes to ulcer development, acute infection severity and ischemia may be more decisive in determining major amputation.

Study Limitations

Several limitations should be acknowledged. First, the study's results may not apply to other situations since it was done at only one center. Second, the study's retrospective design carries an inherent risk of selection bias. Third, the lack of a validated system for classifying ulcer severity, like the Wagner or University of Texas classification systems, is a big problem. Ulcer depth, infection severity, and ischemia are well-established determinants of amputation risk, and the lack of standardized severity stratification may have led to residual confounding. Furthermore, comprehensive data concerning revascularization status and procedural outcomes were excluded from the analysis, potentially exacerbating unmeasured confounding. Consequently, PNI ought to be regarded as a supplementary biomarker

rather than a replacement for established clinical staging systems. Lastly, the study did not look at mortality outcomes, which made it harder to figure out what would happen to patients in the long term.

Despite these limitations, this study has several strengths, including a well-defined patient cohort, a thorough immunonutritional assessment using PNI, and the evaluation of clinically significant outcomes such as major amputation. These factors offer significant insights into the prognostic value of PNI in patients with diabetic foot disease and may inform future risk stratification and management approaches.

Conclusion

The Prognostic Nutritional Index reflects both immune function and nutritional status and is associated with major amputation in patients with diabetic foot ulcers. Evaluation of immunonutritional status may provide additional insight into limb prognosis in patients with diabetic foot ulcers. The PNI, as an accessible and cost-effective marker, may serve as a complementary tool for risk stratification and multidisciplinary decision-making.

Ethics

Ethics Committee Approval: The study protocol received approval from the University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital Scientific Research Ethics Committee No. 1 (approval no.: KAEK/28.08.2024.187, date: 17.09.2024).

Informed Consent: Informed consent was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.C., E.D.K., Concept: B.C., E.D.K., Design: B.C., E.D.K., Data Collection or Processing: B.C., E.D.K., I.E.T., S.N.K., Analysis or Interpretation: B.C., E.D.K., I.E.T., S.N.K., Literature Search: B.C., E.D.K., I.E.T., S.N.K., Writing: B.C.

Conflict of Interest: The authors declare that they have no conflict of interest related to this study.

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