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ORIGINAL ARTICLE

Randomized Evaluation of Nutritional Risk Screening and HLA-DMB Gene Expression in Early Prediction of Sepsis

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SUMMARY

Background: **Sepsis, a life-threatening syndrome with escalating mortality per treatment delay, requires prognostic tools beyond current Sepsis-3 criteria. This study investigated the dual predictive capacity of Nutrition Risk Screening 2002 (NRS-2002) and Human Leukocyte Antigen-DMB (HLA-DMB), proposing a novel early-warning framework for sepsis risk stratification.**

Methods: **This case-control study enrolled 90 patients with acute infections from the Department of Pulmonary and Critical Care Medicine at Hefei Second People's Hospital. Participants were stratified into sepsis (n = 45) and non-sepsis (n = 45) groups according to Sepsis-3 diagnostic criteria. Clinical baseline characteristics, laboratory parameters, nursing-assessed nutritional risk scores, and HLA-DMB gene expression levels were systematically collected through standardized case report forms. Binary logistic regression identified independent predictors, while ROC curve analysis was employed to construct a combinatorial prediction model.**

Results: **In patients with sepsis, HLA-DMB gene expression levels were significantly lower, while NRS-2002 scores were higher; both were independent predictors of sepsis ($p < 0.001$). Restricted cubic spline analysis indicated that higher HLA-DMB levels might reduce the risk of sepsis, whereas lower NRS-2002 scores were associated with an increased risk. Furthermore, receiver operating characteristic curve analysis demonstrated that the combined predictive efficacy of HLA-DMB expression and NRS-2002 scores surpassed that of either variable alone (AUC = 0.8430).**

Conclusions: **HLA-DMB gene expression levels and NRS-2002 scores have been utilized to assess the risk of developing sepsis. Their combined evaluation has enhanced predictive accuracy, facilitating the rational allocation of medical resources in the early stages.**

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KEYWORDS

sepsis, NRS-2002, HLA-DMB, predictive capacity

LIST OF ABBREVIATIONS

NRS-2002 - Nutrition Risk Screening 2002
HLA-DMB - human leukocyte antigen-DMB
ESPEN - European Society for Clinical Nutrition and Metabolism
SOFA - sequential organ failure assessment
BMI - body mass index
WBC - white blood cell count
N% - neutrophil percentage
K^+ - serum potassium
Cr - creatinine
BUN - blood urea nitrogen
ALT - alanine aminotransferase
AST - aspartate aminotransferase
CRP - C-reactive protein
PPS - Padua prediction score
VTE - venous thromboembolism
Ors - odds ratios
Cis - confidence intervals
RCS - restricted cubic spline
ROC - receiver operating characteristic
AUC - area under the curve

INTRODUCTION

Sepsis is a life-threatening condition with extremely high mortality and disability rates. For septic patients, each hour of delayed treatment increases the risk of death by 7 - 8% [1]. Therefore, rapid and accurate prediction is not only crucial but can be a matter of life and death. However, the current diagnostic criteria for sepsis are still based on the Sepsis-3 definition established in 2016 [2], which includes organ dysfunction scoring, as well as microbiological and biochemical indicators. These assessments, while comprehensive, lack sufficient speed and practicality for early screening in emergency settings. As a result, there is an urgent clinical need for simple and rapid screening tools to predict sepsis, which would enable more rational allocation of medical resources and potentially reduce reduction in mortality.

Nutrition Risk Screening 2002 (NRS-2002) is a standardized tool recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) to assess the nutritional risk of hospitalized patients. As a simple and rapid nursing-level screening method, it serves as the first step in evaluating a patient's nutritional status upon admission. Given the close association between malnutrition and immune dysfunction, early identification of nutritional risk through NRS-2002 may facilitate the timely prediction of sepsis, enabling prompt intervention and improved clinical outcomes.

With advances of science and technology, many disease mechanisms have been increasingly elucidated at the genetic level. Sepsis, a condition characterized by immune dysregulation in response to infection, demonstrates significant alterations in mRNA expression levels of immune regulatory genes during its onset and progression. Consequently, the mRNA expression of key genes is critically important for early sepsis prediction. Our research team has previously conducted preliminary screening of candidate genes potentially associated with sepsis prognosis. Through comprehensive analysis using the Inflammatix sample database and NanoString nCounter Sprint Profiler technology platform, we have identified that HLA-DMB gene expression is closely associated with sepsis development.

Based on the aforementioned background, this study aimed to investigate the predictive roles of NRS-2002 and HLA-DMB gene expression levels in sepsis and to further evaluate the clinical value of their combined detection. The ultimate goal was to provide novel risk assessment strategies for the early identification of sepsis.

MATERIALS AND METHODS

Study design

This study enrolled hospitalized patients at the Second People's Hospital of Hefei from September 2023 through March 2024. The study protocol was approved by the hospital's Ethics Committee (approval no. 2023-Research-084) and conducted in accordance with the ethical principles of the Declaration of Helsinki. A total of 93 patients with acute infections were initially enrolled, out of whom 3 were excluded due to discharge within 48 hours, resulting in a final cohort of 90 patients included in the analysis.

Patients

Patients were stratified into a sepsis group (45 patients) and a non-sepsis group (45 patients) based on the presence of acute infection and a sequential organ failure assessment (SOFA) score ≥ 2. The inclusion criteria were as follows: 1) age ≥ 18 years; 2) confirmed diagnosis of acute infection within 48 hours of admission, defined by a) fever ($T \geq 37.3°C$), b) abnormal white blood cell count (WBC $> 10 \times 10^9$/L or $< 4 \times 10^9$/L), and c) at least one infection-related clinical manifestation such as cough, sputum production, abdominal pain, diarrhea, frequent urination, urgency, or headache; 3) completion of NRS-2002 assessment and measurement of blood HLA-DMB gene expression levels during hospitalization; and 4) availability of complete clinical data, including laboratory test results, imaging findings, and medical records. The exclusion criteria included: 1) patients without infection; 2) those who were discharged within 48 hours; 3) those with incomplete medical records; and 4) those who declined HLA-DMB gene expression testing.

NanoString nCounter gene expression analysis
Total RNA was extracted using the PreAnalytix PAXgene Blood RNA kit, with RNA quality verified by an $A_{260/280}$ ratio between 1.8 and 2.1. The target gene–specific probe mixture, consisting of capture, detection, and barcode probes, was prepared and hybridized with the sample RNA at 65°C for 12 - 16 hours. The resulting probe-RNA complexes were then immobilized on an nCounter digital array, where complementary sequences immobilized the target complexes, followed by washing to remove unbound probes. Imaging was performed using the nCounter system, where a laser scanner detected barcode probe fluorescence signals, and software quantified and normalized gene expression levels. The resulting data included raw counts and standardized expression values.

Data collection
Patient demographic and clinical data were retrieved from the hospital's electronic medical record system, including gender, age, history of comorbidities, and BMI. Laboratory parameters recorded during hospitalization included white blood cell count (WBC), neutrophil percentage (N%), serum potassium (K^+), creatinine (Cr), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and C-reactive protein (CRP), as well as ICU readmission within seven days. Additionally, nurses assessed the Padua prediction score (PPS) and NRS-2002 based on the patient's clinical status upon admission.

Diagnostic criteria for sepsis
According to the 2016 Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [3], sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction was identified by an acute increase in the SOFA score of ≥ 2 points, applied to patients with suspected or confirmed infection.

The criteria for assessing the Padua prediction score and nursing nutrition score
The PPS [4] is used to assesses the risk of VTE in hospitalized patients based on factors such as active cancer, previous VTE, reduced mobility, known thrombophilic conditions, recent trauma or surgery, age ≥ 70 years, heart or respiratory failure, acute myocardial infarction or ischemic stroke, acute infection or rheumatologic disorder, obesity (BMI ≥ 30 kg/m^2), and ongoing hormonal treatment. A score of ≥ 4 indicates high VTE risk, necessitating prophylactic anticoagulation. The NRS-2002 [5], developed by ESPEN, serves as a nutritional screening tool for hospitalized patients, evaluating recent weight loss, reduced dietary intake, BMI, disease severity, and age. A total score ≥ 3 denotes significant nutritional risk.

Statistical analysis
Data were analyzed using SPSS 27.0, with figures generated via GraphPad Prism 9 and R 4.3.1. Normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed variables were reported as mean ± standard deviation (mean ± SD) and compared using independent sample *t*-tests; non-normally distributed variables were expressed as median (P50, P25 - P75) and analyzed with the Mann-Whitney U test. Categorical variables were presented as frequencies (n) and percentages (%), with group comparisons performed using chi-squared (χ^2) or Fisher's exact tests. A binary logistic regression model was employed to identify independent sepsis risk factors and construct a multivariate prediction model, using stepwise regression for variable selection and calculating odds ratios (ORs) with 95% confidence intervals (CIs). Restricted cubic spline (RCS) analysis explored nonlinear relationships between HLA-DMB gene expression, NRS-2002, and sepsis risk, assessing dose-response effects. Receiver operating characteristic (ROC) curves were generated to evaluate the area under the curve (AUC), sensitivity, specificity, and diagnostic performance of HLA-DMB, NRS-2002, and their combined model. All tests were two-tailed, with $p < 0.05$ considered statistically significant.

RESULTS

Baseline data component comparison
Table 1 compares the baseline characteristics, selected laboratory indicators, blood HLA-DMB gene expression levels, NRS-2002, and PPS between the sepsis group and the non-sepsis group. The results show that patients in the sepsis group had a significantly higher proportion of males (80% vs. 55.6%), older median age (75.6 years vs. 66.4 years), higher prevalence of hypertension (57.8% vs. 28.9%), Cr (88.8 vs. 68.4 μmol/L), CRP (69.5 vs. 42.6 mg/L), NRS-2002 (3.1 vs. 1.7), and significantly increased HLA-DMB gene expression levels with all differences reaching statistical significance ($p < 0.05$). These findings suggest that age, hypertension, Cr, CRP, NRS-2002, and HLA-DMB gene expression level may be important influencing factors for sepsis. Other indicators, such as diabetes, showed no significant difference between the two groups.

Baseline data component comparison
The positive clinical indicators and selected baseline characteristics of the patients presented in Table 1 were included in a binary logistic regression model to identify potential risk factors. The analysis was performed in a stepwise manner: Model 1 included baseline variables (gender, age, hypertension, and diabetes) and was subjected to multivariate logistic regression analysis. Model 2 was constructed by adding Cr to Model 1. Model 3 was further built upon Model 2 by including the HLA-DMB variable. Finally, Model 4 incorporated NRS-

Table 1. Comparison of baseline data between the two groups of patients.

Characteristics	Sepsis group (n = 45)	Non-sepsis group (n = 45)	χ^2	p
Baseline data				
Gender (male)	36 (80)	25 (55.6)	6.156	0.013
Age	75.6 (72, 82)	66.4 (30.5, 71.5)	4.824	< 0.001
Hypertension	26 (57.8)	13 (28.9)	7.647	0.006
Diabetes	11 (24.4)	5 (11.1)	2.736	0.098
BMI	21.8 (18.83, 24.33)	22.1 (19.45, 24.3)	0.032	0.974
Laboratory parameters				
WBC	8.4 (5.2, 11.4)	7.8 (5.7, 9.7)	0.383	0.701
N%	74.4 (69.7, 89.2)	75.5 (69.6, 83.2)	1.715	0.086
K^+	3.8 (3.5, 4.1)	3.7 (3.5, 4.0)	0.295	0.786
Cr	88.8 (60, 95.8)	68.4 (55.3, 75.5)	2.457	0.014
AST	45.1 (22.5, 49)	30.2 (23, 31.5)	1.644	0.100
CRP	69.5 (34.7, 96.9)	42.6 (15.2, 46.2)	3.389	< 0.001
Other				
PPS	2.7 (1, 3.75)	2.6 (1, 4)	0.054	0.957
NRS-2002	3.1 (2, 4)	1.7 (0.25, 3.75)	4.175	< 0.001
HLA-DMB	846.6 (404, 1,294.5)	1,531.7 (1,164, 1,921)	4.689	< 0.001

Table 2. Binary logistic regression model.

Characteristics	Model 1	Model 2	Model 3	Model 4
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Gender (male)	0.317 (0.117, 0.861) *	0.409 (0.144, 1.166)	0.435 (0.131, 1.440)	0.330 (0.080, 1.357)
Age	0.999 (0.991, 1.007)	1.000 (0.992, 1.007)	1.005 (0.997, 1.014)	1.009 (0.999, 1.019)
Hypertension	3.178 (1.201, 8.413) *	2.602 (0.949, 7.138)	2.077 (0.656, 6.573)	1.273 (0.351, 4.615)
Diabetes	1.498 (0.431, 5.207)	1.462 (0.410, 5.209)	1.324 (0.342, 5.134)	1.255 (0.281, 5.601)
Cr	——	1.013 (0.994, 1.032)	1.016 (0.990, 1.043)	1.019 (0.991, 1.049)
HLA-DMB	——	——	0.998 (0.997, 0.999) ***	0.998 (0.997, 0.999) ***
NRS-2002	——	——	——	2.161 (1.380, 3.384) ***

ORs Odds ratios, 95% CI 95% confidence intervals, *** p < 0.001, * p < 0.05.

2002 into Model 3. The results of these analyses are summarized in Table 2.

Restricted cubic spline (RCS) analysis

In the multivariate logistic regression model, both HLA-DMB and NRS-2002 were identified as significant risk factors for the occurrence of sepsis, with ORs and 95% CIs of 0.998 (0.997 - 0.998) and 2.161 (1.380 - 3.384), respectively (both p < 0.001). To further assess potential nonlinear associations between these variables and the risk of sepsis, RCS analysis was performed. The results of this analysis are presented in Figure 1.

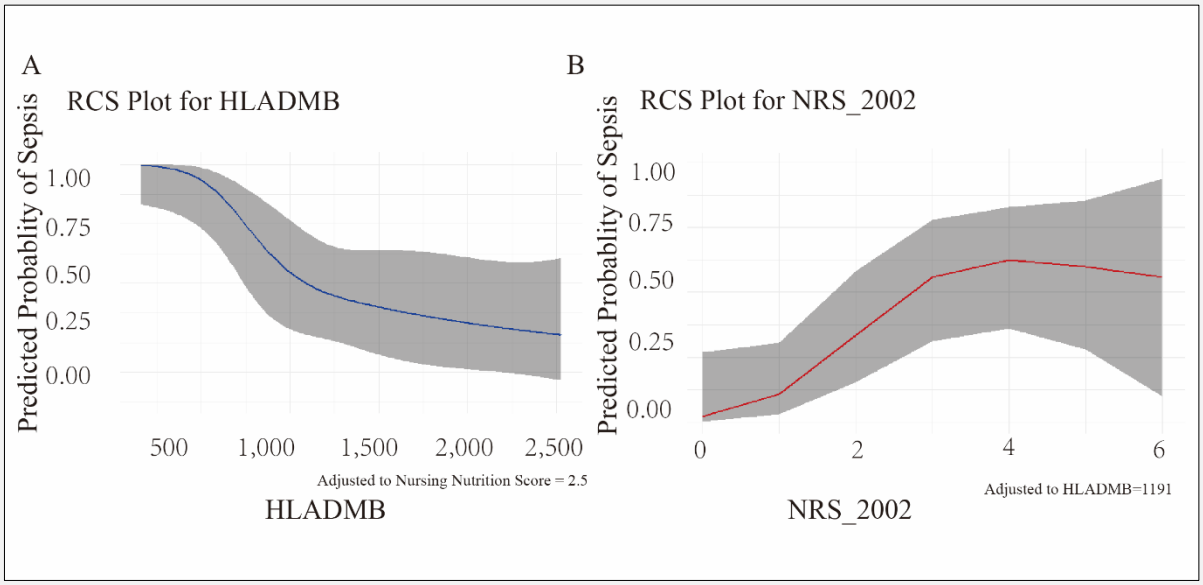

Figure 1. Restricted cubic spline analysis.

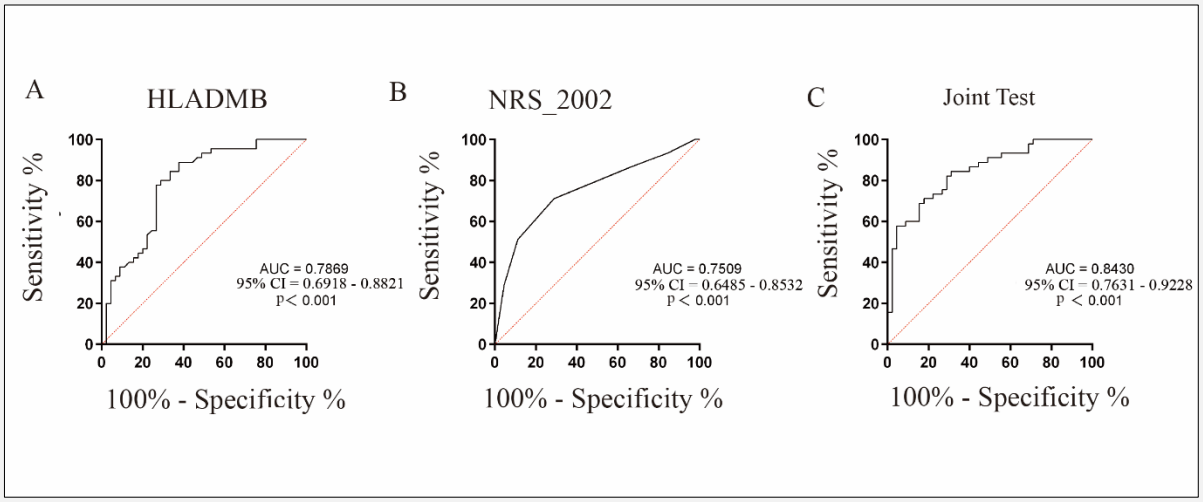

Figure 2. Diagnostic performance of HLA-DMB expression and nutritional risk screening for early sepsis prediction.

ROC curves are shown for A HLA-DMB expression, B NRS-2002 score, and C the combined model. AUC values with 95% CIs and p-values are indicated.

Figure 1A demonstrates that as HLA-DMB levels increase from low values (< 500), the predicted probability of sepsis decreases significantly. When HLA-DMB reaches approximately 1,000, this downward trend levels off but continues to show a slight decline, suggesting that HLA-DMB may serve as a protective factor, with higher HLA-DMB levels associated with a reduced risk of sepsis. Figure 1B illustrates that the relationship between NRS-2002 and the predicted probability of sepsis initially increases and then plateaus or slightly declines. Specifically, when NRS-2002 ranges from 0 to 4, the probability of sepsis increases markedly with rising NRS-2002. Beyond NRS-2002 of 4, this increasing trend flattens and may even slightly decrease, indicating that low levels of NRS-2002 may contribute to an elevated risk of sepsis occurrence.

ROC curve

Based on the identification of HLA-DMB and NRS-2002 as independent risk factors for sepsis in patients with acute infection, we developed a clinical prediction model. ROC curves were constructed to assess the predictive performance of these factors, as shown in Figure 2A and Figure 2B. The results demonstrated that both HLA-DMB and NRS-2002 had good predictive value for the occurrence of sepsis in patients with acute infection, with areas under the curve (AUC) of 0.7869 (95% CI: 0.6918 - 0.8821) and 0.7509 (95% CI: 0.6485 - 0.8532), respectively. Moreover, the combination of HLA-DMB and NRS-2002 further improved predictive performance, yielding an AUC of 0.8430 (95% CI: 0.7631 - 0.9228) (Figure 2C).

DISCUSSION

Sepsis is a critical public health issue associated with high mortality and long-term morbidity [6]. Therefore, rapid and accurate prediction of sepsis risk is essential for clinical decision-making and can be lifesaving. Our study identified that advanced age, male gender, history of hypertension [7], renal impairment [8], and increased CRP levels were significantly associated with a higher risk of developing sepsis. These findings highlight the need for clinicians to pay particular attention to patients with hypertension, renal impairment, and elevated inflammatory markers for early identification and execution of proactive intervention strategies that improve clinical outcomes.

HLA-DMB is part of MHC-II molecules, which plays a crucial role in antigen processing and presentation, thus regulating the immune response [9]. In this study, HLA-DMB levels were negatively correlated with the risk of sepsis, suggesting a potential protective role. Further analysis using RCS revealed a nonlinear relationship between HLA-DMB levels and sepsis risk, with the risk decreasing as HLA-DMB levels increased, until reaching a plateau around a level of approximately 1,000. Yaojun Peng's findings were consistent with ours, showing that HLA-DMB was significantly downregulated in sepsis compared to healthy controls [10]. Christophe Lelubre's findings also indicate a negative correlation between HLA-DMB and sepsis [11]. HLA-DMB was a key component of the MHC class II antigen presentation pathway, ensuring the effective delivery of antigens to $CD4^+$ T cells. In septic patients, peripheral blood HLA-DMB expression is markedly reduced and negatively associated with poor prognosis. This may be attributed to impaired function of antigen-presenting cells and decreased activity of transcriptional regulatory factors under immunosuppressive conditions [12].

NRS-2002 is a comprehensive clinical assessment tool designed to evaluate nutritional status and identify nutritional risk among patients [13]. It quantitatively assesses factors such as weight changes, BMI, nutritional intake status, severity of disease, and age (aged ≥ 70 years) to determine the risk of malnutrition [14]. Studies showed that an NRS-2002 score of ≥ 3 indicated a risk of malnutrition. Patients at nutritional risk were more likely to develop sepsis, which might have been associated with impaired immune function, poor nutritional status, and greater disease severity. Therefore, NRS-2002 could serve as a supplementary indicator for predicting the risk of sepsis occurrence [15]. RCS analysis further demonstrated a nonlinear relationship: the risk of sepsis increased substantially with a score ranging from 0 to 4, whereas it plateaued or slightly declined when scores exceeded 4. Previous research has shown that malnutrition significantly elevates the risk of infectious complications [16], including sepsis [17], likely due to impaired immune function that compromises the body's ability to resist pathogen invasion. Additionally, patients with high nutrition scores often have greater disease severity and organ dysfunction, further exacerbating their risk of sepsis. Thus, clinicians should closely monitor patients with high nutrition scores and promptly provide targeted nutritional interventions to mitigate their sepsis risk.

In this study, a clinical prediction model combining HLA-DMB and NRS-2002 was developed to predict sepsis risk. ROC curve analysis revealed that the combined predictive performance (AUC = 0.8430) was notably superior to that of either HLA-DMB (AUC = 0.7869) or NRS-2002 (AUC = 0.7509) alone, suggesting that integrating assessments of immune function and nutritional status could provide a more comprehensive and accurate prediction of sepsis risk. Such an integrated approach holds promise as a valuable tool for the early identification and precise management of high-risk patients in clinical practice. Nonetheless, this study was a single-center retrospective analysis, and future prospective multicenter studies with larger sample sizes are needed to validate the effectiveness and generalizability of this predictive model and to further explore its practical utility in clinical decision-making.

CONCLUSION

This study indicates that HLA-DMB and NRS-2002 are independent predictors for sepsis risk, and their combined application further improves predictive accuracy, facilitating early clinical identification and timely intervention for high-risk patients.

Availability of Data and Materials:
The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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Declaration of Interest:
The authors have no conflicts of interest to declare.

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