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Long noncoding RNA NEAT 1 and its target microRNA-125a in sepsis: Correlation with acute respiratory distress syndrome risk, biochemical indexes, disease severity, and 28-day mortality

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Abstract

Background: Sepsis is one of the main contributors to in-hospital deaths. This study aimed to evaluate the clinical roles of long noncoding RNA (lncRNA) nuclear-enriched abundant transcript 1 (NEAT1) and microRNA (miR)-125a in sepsis.

Methods: LncRNA NEAT1 and miR-125a in plasma samples from 102 sepsis patients and 100 healthy controls (HCs) were detected by reverse transcription-quantitative polymerase chain reaction. In sepsis patients, general disease severity was assessed by acute physiology and chronic health evaluation (APACHE) II score and sequential organ failure assessment (SOFA) score. Meanwhile, acute respiratory distress syndrome (ARDS) occurrence and mortality during 28 days were recorded.

Results: LncRNA NEAT1 was increased, but miR-125a was decreased in sepsis patients compared to HCs, and in ARDS sepsis patients compared to non-ARDS sepsis patients. The receiver's operative characteristic (ROC) curves revealed that higher lncRNA NEAT1 or lower miR-125a had certain predictive value for ARDS risk. Further multivariate logistic regression revealed miR-125a but not lncRNA NEAT1 was correlated with ARDS risk independently in sepsis patients. Additionally, lncRNA NEAT1 was positively, but miR-125a was negatively correlated with APACHE II score and SOFA score in sepsis patients. Moreover, higher lncRNA NEAT1 and lower miR-125a were observed in 28-day deaths compared to 28-day survivors and were correlated with increased accumulating mortality in sepsis patients.

Conclusion: LncRNA NEAT1 high expression and miR-125a low expression correlate with increased ARDS risk, enhanced disease severity, higher 28-day mortality, and negatively associate with each other in sepsis patients.

KEYWORDS
28-day mortality, acute respiratory distress syndrome, long noncoding RNA nuclear-enriched abundant transcript 1, microRNA-125a, sepsis

1 | INTRODUCTION

Sepsis, which is characterized by dysregulation of the immune response to infection that might lead to multiple organ dysfunction, affects millions of people worldwide and causes huge hospital utilization burden, both in financial and humanistic.[1,2] Meanwhile, sepsis is one of the main contributors to in-hospital death, and the mortality risk of sepsis patients ranges from 10%-70% depending on disease severity.[3,4] Acute respiratory distress syndrome (ARDS) is one of the common complications of sepsis: According to previous studies, nearly one-quarter of sepsis patients would develop ARDS and these patients have worse outcomes.[5,6] Recently, several studies have been conducted to search for biomarkers that could predict the incidence and outcome of sepsis as well as sepsis-induced ARDS; however, the clinical application of these biomarkers in sepsis and sepsis-induced ARDS is still premature, and there is much worth digging under this category.[7,8]

Long noncoding RNA (lncRNA) nuclear-enriched abundant transcript 1 (NEAT1) is originally found to participate in the formation of paraspeckles, a kind of ribonucleoprotein bodies found in mammalian cell nuclei.[9] In recent years, it is considered that lncRNA NEAT1 is highly involved in the regulation of sepsis. For instance, lncRNA NEAT1 could regulate several pathways including the nuclear factor-κB (NF-κB) pathway and toll-like receptor 4 (TLR4) pathway to promote inflammation and injury of multiple organs such as liver, kidney, heart, and lung.[10-13] Meanwhile, it is suggested that lncRNA NEAT1 might modulate the progression of sepsis through directly targeting microRNA (miR)-125a,[14] and miR-125a not only regulates inflammation in sepsis animal models, but also serves as a potential biomarker for the prediction of sepsis risk and the indication of inflammation, disease severity as well as the incidence of ARDS in sepsis patients.[15,16] Based on the above-mentioned information, we hypothesized that lncRNA NEAT1 might interact with miR-125a to serve as a potential biomarker for the management of sepsis; however, relevant information is rare.

This study aimed to investigate lncRNA NEAT1 and miR-125a expressions as well as their potentials for reflecting ARDS risk, disease severity as well as short-term prognosis in sepsis patients, which might provide potential information for predicting the occurrence of ARDS to improve the overall prognosis of sepsis patients.

2 | MATERIALS AND METHODS

2.1 | Sepsis patients and health controls

In this study, 102 sepsis patients admitted to our hospital between January 2018 and September 2019 were consecutively enrolled. All enrolled patients were diagnosed as sepsis in accordance with The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[1] and admitted to our hospital within the previous 24 hours. Patients were excluded from this study if they were younger than 18 years, transferred from other hospitals, taking immunosuppressive drugs, concomitant with systemic autoimmune disorders (eg rheumatoid arthritis, systemic lupus erythematosus, systemic vasculitis), suffering from tumors or hematological malignancies, infected with human immunodeficiency virus, or in pregnancy or lactation. In addition, health controls (HCs) cohort consisting of 100 healthy volunteers were recruited from the Health Examination Center of our hospital, who were enterprise staff and underwent health examination between October 2019 and December 2019. All HCs were required to have age-and-gender-matched to enrolled sepsis patients, no history of sepsis, no infection, no use of immunosuppressant, and antibiotics within 1 month, and no obvious abnormality in physical examination indexes. This study was approved by the Institutional Review Board of our hospital. All participants or their guardians provided the written informed consents.

2.2 | Clinical data collection

Basic characteristics, such as chronic comorbidities, and primary infection sites of patients, such as age, gender, body mass index (BMI), smoke, chronic obstructive pulmonary disease (COPD), cardiomyopathy, chronic kidney failure, and cirrhosis, primary infection site including abdominal infection, respiratory infection, skin and soft tissue infection, bloodstream infection, central nervous system (CNS) infection, and other infections, were documented after admission. Biochemical indexes including serum creatinine (Scr), albumin, white blood cell (WBC), and C-reactive protein (CRP) level were collected following laboratory tests. Meanwhile, acute physiology and chronic health evaluation (APACHE) II score and sequential organ failure assessment (SOFA) score were assessed within the previous 24 hours and recorded as well. Besides, blood culture was performed prior to antibiotic therapy, and the primary infected organism was documented after the culture.

2.3 | Sample collection and determination

Sepsis patients' blood samples were extracted within 24 hours after admission, and HCs' blood samples were collected on their health examination. After collection, the blood samples were centrifuged at 4°C 1600 g for 15 minutes to collect supernatant, followed by the centrifugation at 4°C 16 000 g for 10 minutes to isolate the plasma, which was then stored at −70°C until detection. The relative expressions of lncRNA NEAT1 and miR-125a in plasma of patients and HCs were determined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The RT-qPCR was performed according to the method described in our previous study,[17] and the primers were as follows: LncRNA NEAT1, Forward (5′->3′): TGTCCCTCGGCTATGTCAGA; Reverse (5′->3′): GAGGGGACGTGTTTCCTGAG. MiR-125a, Forward (5′->3′): ACACTCCAGCTGGGTCCCTGAGACCCTTTAAC; Reverse (5′->3′): TGT CGTGGAGTCGGCAATTC. GAPDH, Forward (5′->3′): TGACCACAG TCCATGCCATCAC; Reverse (5′->3′): GCCTGCTTCACCACCTTCTTGA;

U6, Forward (5′->3′): CTCGCTTCGGCAGCACATATACTA; Reverse (5′->3′): ACGAATTTGCGTGTCATCCTTGC.

2.4 | Follow-up

Surveillance and standard care for patients were carried out as usual in hospitalization, during which the occurrence of ARDS was monitored in time. ARDS was identified based on timing (within 1 week of a known clinical insult or new or worsening respiratory symptoms), chest imaging (bilateral opacities), the origin of edema, oxygenation (partial pressure of arterial oxygen/ fraction of inspired oxygen (PaO$_2$/FIO$_2$) ≤300 mm Hg with positive end-expiratory pressure (PEEP) ≥5 cm H$_2$O), according to the Berlin definition of ARDS.[18] All patients were consecutively followed up until death in hospital or for a total of 28 days. The 28-day mortality was calculated based on patients' survival status during follow-up. Accumulating mortality was assessed from the day of admission to the day of death or last visit. Patients who lost follow-up within 28 days were censored on the date of discharge from hospital.

2.5 | Statistical analysis

Kolmogorov-Smirnov(K) test was used to determine the normality of data. Normally or approximately normally distributed data were described as mean with standard deviation, and skewed distributed data were described as median with interquartile range (IQR); categorical data were described as number (percentage). The comparison was determined by chi-square test, Student's t test, or Wilcoxon rank sum test. Correlation analysis was determined by Spearman's rank correlation test. The receiver operating characteristic (ROC) curve with the area under the curve (AUC) was used to assess the value of variables in distinguishing different subjects. Accumulating mortality was displayed by the Kaplan-Meier method, and the comparison between the two groups was determined by the log-rank test. Risk factors of ARDS in sepsis patients were analyzed by the univariate logistic regression model, and the independent risk factors of ARDS in sepsis patients were further analyzed using forward stepwise multivariate logistic regression model. All statistical analyses were performed by SPSS 24.0 software (IBM, Chicago, Illinois, USA), and the figures were plotted using GraphPad Prism 7.01 software (GraphPad Software Inc., San Diego, California, USA). P value < .05 was considered significant.

3 | RESULTS

3.1 | Clinical features of sepsis patients

The clinical features of sepsis patients are shown in Table 1. In brief, the mean age of sepsis patients was 54.2 ± 10.9 years, and there were 41 (40.2%) females as well as 61 (59.8%) males. As to

TABLE 1 Clinical features

Items	Sepsis patients (N = 102)
Demographics	
Age (y), mean ± SD	54.2 ± 10.9
Gender, No. (%)	
Female	41 (40.2)
Male	61 (59.8)
BMI (kg/m^2), mean ± SD	22.6 ± 3.6
Smoke, No. (%)	35 (34.3)
Complications	
COPD, No. (%)	19 (18.6)
Cardiomyopathy, No. (%)	47 (46.1)
Chronic kidney failure, No. (%)	16 (15.7)
Cirrhosis, No. (%)	21 (20.6)
Primary infection site	
Abdominal infection, No. (%)	40 (39.2)
Respiratory infection, No. (%)	22 (21.6)
Skin and soft tissue infection, No. (%)	21 (20.6)
Bloodstream infection, No. (%)	10 (9.8)
CNS infection, No. (%)	4 (3.9)
Other infections, No. (%)	5 (4.9)
Primary organism	
G−, No. (%)	57 (55.9)
G+, No. (%)	23 (22.5)
Anaerobes, No. (%)	13 (12.7)
Fungus, No. (%)	7 (6.9)
Mycoplasmas, No. (%)	4 (3.9)
Total culture negative, No. (%)	19 (18.6)
Biochemical indexes	
Scr (mg/dL), median (IQR)	1.8 (1.2-2.4)
Albumin (g/L), median (IQR)	25.8 (21.7-33.2)
WBC (10^9/L), median (IQR)	18.0 (11.6-27.4)
CRP (mg/L), median (IQR)	97.6 (52.1-138.1)
Disease severity	
APACHE II score, mean ± SD	13.3 ± 6.0
SOFA score, mean ± SD	6.1 ± 2.7

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G-, Gram-negative bacteria; G+, Gram-positive bacteria; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

primary infection site, 40 (39.2%) patients had abdominal infection, 22 (21.6%) patients had respiratory infection, 21 (20.6%) patients had skin and soft tissue infection, 10 (9.8%) patients had bloodstream infection, 4 (3.9%) had CNS infection, and 5 (4.9%) patients had other infections. Regarding biochemical indexes, the median levels of Scr, albumin, WBC, and CRP were 1.8 (1.2-2.4) mg/dL,
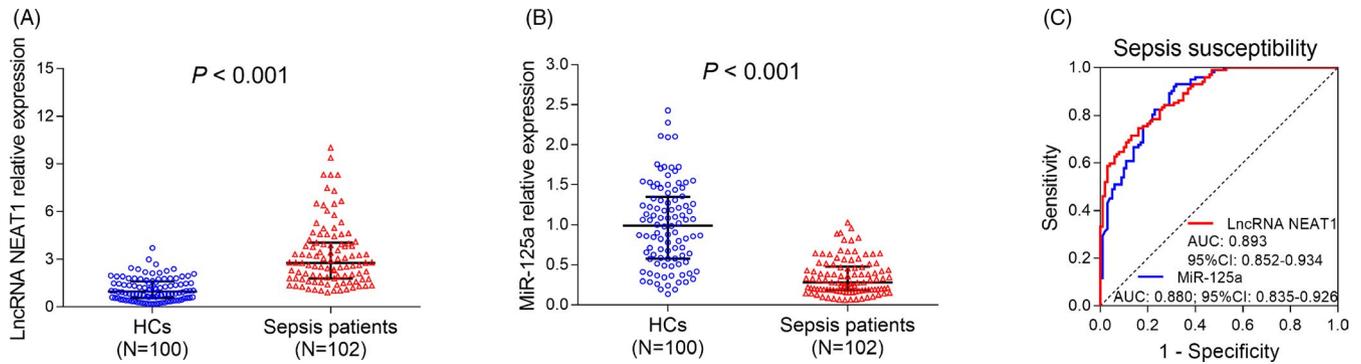
FIGURE 1 lncRNA NEAT1 and miR-125a in discriminating sepsis patients from HCs. A, LncRNA NEAT1 relative expression in sepsis patients and HCs. B, MiR-125a relative expression in sepsis patients and HCs. C, Correlation of lncRNA NEAT1 and miR-125a with sepsis by ROC curves. LncRNA: long noncoding RNA; NEAT1: nuclear-enriched abundant transcript 1; miR-125a: microRNA-125a; HCs: healthy controls; ROC: receiver operating characteristic; AUC: area under curve; CI: confidence interval
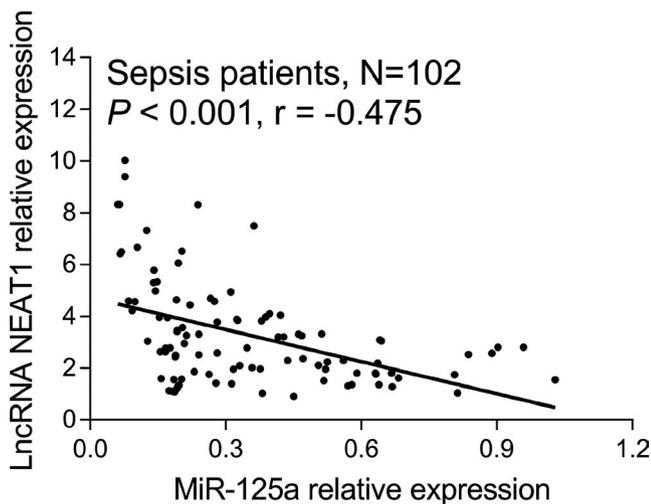
FIGURE 2 Correlation analysis between lncRNA NEAT1 and miR-125a in sepsis patients. LncRNA: long noncoding RNA; NEAT1: nuclear-enriched abundant transcript 1; miR-125a: microRNA-125a

25.8 (21.7-33.2) g/L, 18.0 (11.6-27.4) $\times 10^9$/L, and 97.6 (52.1-138.1) mg/L, respectively. As to disease severity assessment, the mean APACHE II score and SOFA score were 13.3 ± 6.0 and 6.1 ± 2.7, respectively.

3.2 | LncRNA NEAT1 and miR-125a relative expressions in sepsis patients and HCs

LncRNA NEAT1 was increased in sepsis patients (median value: 2.783 (1.790-4.058)) compared to HCs (median value: 0.968 (0.563-1.614)) ($P < .001$) (Figure 1A). Meanwhile, miR-125a was decreased in sepsis patients (median value: 0.281 (0.184-0.479)) compared to HCs (median value: 0.992 (0.577-1.348)) ($P < .001$) (Figure 1B). ROC curves showed that both increased lncRNA NEAT1 (AUC: 0.893, 95% CI: 0.852-0.934) and decreased miR-125a (AUC: 0.880. 95% CI: 0.835-0.926) were correlated with sepsis risk (Figure 1C).

3.3 | Correlation between lncRNA NEAT1 and miR-125a in sepsis patients

Correlation analysis between lncRNA NEAT1 and miR-125a was conducted, and a negative correlation was observed between lncRNA NEAT1 and miR-125a in sepsis patients ($P < .001, r = −0.475$) (Figure 2).

3.4 | Differences in clinical features of ARDS sepsis patients and non-ARDS sepsis patients

During the follow-up period, 26 (25.5%) sepsis patients developed ARDS. The comparisons of clinical features between ARDS sepsis patients and non-ARDS sepsis patients were conducted and are shown in Table 2. In brief, for demographic features, both mean age ($P = .022$) and smoking behavior ($P = .015$) were increased in ARDS sepsis patients compared to non-ARDS sepsis patients. Regarding complications of sepsis, the proportion of patients with COPD ($P < .001$) was increased in ARDS sepsis patients compared with non-ARDS sepsis patients. Meanwhile, the proportion of patients who had respiratory infection ($P = .003$) was higher in ARDS sepsis patients compared to non-ARDS sepsis patients, but the primary organism that caused infection was similar between ARDS sepsis patients and non-ARDS sepsis patients (all $P > .05$). As to biochemical indexes, the median CRP level ($P < .001$) was elevated in ARDS sepsis patients compared with non-ARDS sepsis patients. For disease severity assessment, both APACHE II score ($P = .003$) and SOFA score ($P = .037$) were higher in ARDS sepsis patients compared to non-ARDS sepsis patients.

3.5 | LncRNA NEAT1 and miR-125a relative expressions in ARDS and non-ARDS sepsis patients

LncRNA NEAT1 was elevated in ARDS sepsis patients (median value: 3.863 (2.512-5.941)) compared to non-ARDS sepsis patients (median

TABLE 2 Comparison of clinical features between non-ARDS sepsis patients and ARDS sepsis patients

Items	Non-ARDS sepsis patients (n = 76)	ARDS sepsis patients (n = 26)	P value
Demographics			
Age (y), mean ± SD	52.8 ± 11.1	58.4 ± 9.0	.022
Gender, No. (%)			.110
Female	34 (44.7)	7 (26.9)	
Male	42 (55.3)	19 (73.1)	
BMI (kg/m^2), mean ± SD	22.5 ± 3.6	23.1 ± 3.6	.497
Smoke, No. (%)	21 (27.6)	14 (53.8)	.015
Complications			
COPD, No. (%)	8 (10.5)	11 (42.3)	<.001
Cardiomyopathy, No. (%)	33 (43.4)	14 (53.8)	.357
Chronic kidney failure, No. (%)	10 (13.2)	6 (23.1)	.230
Cirrhosis, No. (%)	17 (22.4)	4 (15.4)	.447
Primary infection site			
Abdominal infection, No. (%)	33 (43.4)	7 (26.9)	.137
Respiratory infection, No. (%)	11 (14.5)	11 (42.3)	.003
Skin and soft tissue infection, No. (%)	16 (21.1)	5 (19.2)	.843
Bloodstream infection, No. (%)	9 (11.8)	1 (3.8)	.237
CNS infection, No. (%)	2 (2.6)	2 (7.7)	.251
Other infections, No. (%)	5 (6.6)	0 (0.0)	.180
Primary organism			
G-, No. (%)	43 (56.6)	14 (53.8)	.809
G+, No. (%)	16 (21.1)	7 (26.9)	.536
Anaerobes, No. (%)	8 (10.5)	5 (19.2)	.251
Fungus, No. (%)	5 (6.6)	2 (7.7)	.846
Mycoplasmas, No. (%)	3 (3.9)	1 (3.8)	.982
Total culture negative, No. (%)	14 (18.4)	5 (19.2)	.927
Biochemical indexes			
Scr (mg/dL), median (IQR)	1.8 (1.2-2.4)	1.6 (1.2-2.0)	.629
Albumin (g/L), median (IQR)	26.7 (22.5-34.0)	25.0 (20.1-28.0)	.116
WBC (10^9/L), median (IQR)	16.0 (11.3-26.9)	23.8 (13.0-29.1)	.124
CRP (mg/L), median (IQR)	77.2 (46.8-126.6)	137.7 (92.3-230.3)	<.001
Disease severity			
APACHE II score, mean ± SD	12.3 ± 5.7	16.3 ± 5.7	.003
SOFA score, mean ± SD	5.8 ± 2.4	7.0 ± 3.2	.037

Note: Comparison was determined by Student's *t* test, chi-square test, or Wilcoxon rank sum test.

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; ARDS, acute respiratory distress syndrome; BMI, body mass index; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G-, Gram-negative bacteria; G+, Gram-positive bacteria; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

value: 2.581 (1.573-3.824)) (*P* = .002) (Figure 3A). Meanwhile, miR-125a was reduced in ARDS sepsis patients (median value: 0.189 (0.141-0.266)) compared to non-ARDS sepsis patients (median value: 0.353 (0.194-0.524)) (*P* = .001) (Figure 3B). ROC curves showed that lncRNA NEAT1 (AUC: 0.707, 95% CI: 0.595-0.820) and miR-125a (AUC: 0.720. 95% CI: 0.605-0.836) had certain predictive values for ARDS risk in sepsis patients (Figure 3C).

3.6 | Factors correlated with ARDS risk in sepsis patients

Univariate logistic regression revealed that higher lncRNA NEAT1 level (*P* = .002, OR = 1.436), higher age (*P* = .026, OR = 1.052), smoking behavior (*P* = .017, OR = 3.056), COPD (*P* = .001, OR = 6.233), respiratory infection (*P* = .004, OR = 4.333), higher CRP (*P* < .001,
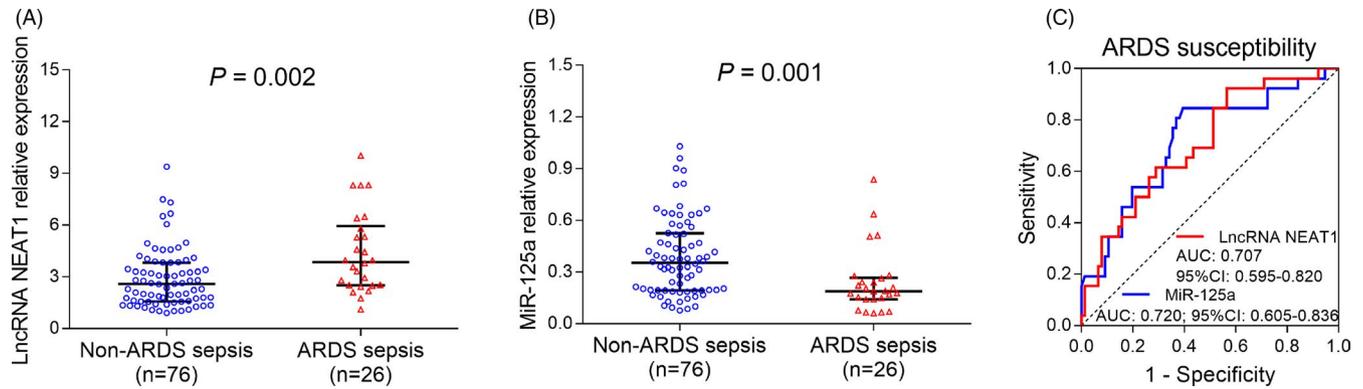
FIGURE 3 Predictive values of lncRNA NEAT1 and miR-125a on ARDS risk in sepsis patients. A, LncRNA NEAT1 relative expression in ARDS sepsis patients and non-ARDS sepsis patients. B, MiR-125a relative expression in ARDS sepsis patients and non-ARDS sepsis patients. C, Predictive values of lncRNA NEAT1 and miR-125a on ARDS risk by ROC curves. LncRNA: long noncoding RNA; NEAT1: nuclear-enriched abundant transcript 1; miR-125a: microRNA-125a; ARDS: acute respiratory distress syndrome; ROC: receiver operating characteristic; AUC: area under curve; CI: confidence interval

OR = 1.018), higher APACHE II score ($P = .005$, OR = 1.120), and higher SOFA score ($P = .042$, OR = 1.187) were correlated with increased ARDS risk, while higher miR-125a level ($P = .007$, OR = 0.018) was correlated with decreased ARDS risk in sepsis patients. Subsequently, forward stepwise multivariate logistic regression showed that higher miR-125a ($P = .016$, OR = 0.006), smoking behavior ($P = .017$, OR = 5.217), COPD ($P = .006$, OR = 9.533), respiratory infection ($P = .024$, OR = 5.052), and higher CRP ($P = .001$, OR = 1.021) were correlated with ARDS risk independently in sepsis patients (Table 3).

3.7 | ROC curve analysis of miR-125a, smoke, COPD, respiratory infection, and CRP for ARDS risk in sepsis patients

ROC curves illustrated that miR-125a (AUC: 0.720, 95% CI: 0.605-0.836), smoking behavior (AUC: 0.631, 95% CI: 0.503-0.759), COPD (AUC: 0.659, 95% CI: 0.527-0.791), respiratory infection (AUC: 0.639, 95% CI: 0.507-0.771), and CRP (AUC: 0.752, 95% CI: 0.633-0.872) all possessed predictive value on ARDS risk to a certain extent. Meanwhile, the combination of ARDS independent predictive factors (AUC: 0.902, 95% CI: 0.840-0.964) had great predictive value on ARDS risk in sepsis patients (Figure 4).

3.8 | Correlation in lncRNA NEAT1 and miR-125a with clinical features in sepsis patients

LncRNA NEAT1 was positively correlated with Scr ($P = .011$, $r = 0.250$), WBC ($P = .012$, $r = 0.248$), CRP ($P < .001$, $r = 0.414$), APACHE II score ($P = .019$, $r = 0.233$) and SOFA score ($P < .001$, $r = 0.357$), but negatively correlated with albumin ($P = .008$, $r = -0.262$) in sepsis patients. As to miR-125a, it was negatively associated with Scr ($P = .015$, $r = -0.239$), WBC ($P = .036$, $r = -0.208$), CRP ($P < .001$, $r = -0.352$), APACHE II score ($P < .001$,

$r = -0.376$), and SOFA score ($P < .001$, $r = -0.378$), while positively associated with albumin ($P < .001$, $r = 0.455$) in sepsis patients (Table 4).

3.9 | LncRNA NEAT1 and miR-125a relative expressions in 28-day survivors and 28-day deaths

LncRNA NEAT1 was increased in 28-day deaths (median value: 3.512 (2.048-6.511)) compared to 28-day survivors (median value: 2.581 (1.565-3.825)) ($P = .003$) (Figure 5A), but miR-125a was reduced in 28-day deaths (median value: 0.204 (0.126-0.316)) compared to 28-day survivors (median value: 0.355 (0.189-0.563)) ($P = .001$) (Figure 5B). ROC curves revealed that lncRNA NEAT1 (AUC: 0.700, 95% CI: 0.579-0.820), miR-125a (AUC: 0.723, 95% CI: 0.618-0.828), APACHE II score (AUC: 0.773, 95% CI: 0.673-0.873), Scr (AUC: 0.718, 95% CI:0.616-0.821), albumin (AUC: 0.673, 95% CI: 0.504-0.770), WBC (AUC:0.655, 95% CI: 0.543-0.767), and CRP (AUC: 0.732, 95% CI: 0.622-0.843) had certain degree of predictive values; meanwhile, SOFA score (AUC: 0.817, 95% CI: 0.722-0.911) had good predictive value for 28-day mortality risk in sepsis patients (Figure 5C,D). These data indicated that the predictive values of lncRNA NEAT1 and miR-125a on mortality risk were numerically comparable to those of regular single biochemical indexes (including Scr, albumin, WBC, and CRP), but was weaker than those of comprehensive disease severity indexes (including APACHE II score and SOFA score).

3.10 | Correlation analyses of lncRNA NEAT1 and miR-125a with accumulating mortality in sepsis patients

According to the median value of lncRNA NEAT1 in sepsis patients, they were separated into lncRNA NEAT1 high expressed

TABLE 3 Analysis of factors predicting ARDS in sepsis patients

Items	Logistic regression model		95%CI	
	P value	OR	Lower	Higher
Univariate logistic regression				
Higher lncRNA NEAT1	.002	1.436	1.141	1.806
Higher miR-125a	.007	0.018	0.001	0.332
Higher age	.026	1.052	1.006	1.101
Male	.114	2.197	0.827	5.840
Higher BMI	.493	1.044	0.923	1.181
Smoke	.017	3.056	1.217	7.671
COPD	.001	6.233	2.141	18.148
Cardiomyopathy	.359	1.520	0.621	3.719
Chronic kidney failure	.236	1.980	0.640	6.123
Cirrhosis	.450	0.631	0.191	2.083
Primary infection site (Respiratory vs others)	.004	4.333	1.583	11.860
Primary organism				
G-	Reference	-	-	-
G+	.786	0.790	0.144	4.331
Other infection organisms except for G–/G+	.388	1.524	0.586	3.961
Higher Scr	.555	0.905	0.649	1.261
Higher albumin	.197	0.968	0.921	1.017
Higher WBC	.206	1.028	0.985	1.072
Higher CRP	<.001	1.018	1.009	1.028
Higher APACHE II	.005	1.120	1.035	1.213
Higher SOFA score	.042	1.187	1.006	1.400
Forward stepwise multivariate logistic regression				
Higher miR-125a	.016	0.006	0.000	0.383
Smoke	.017	5.217	1.349	20.171
COPD	.006	9.533	1.902	47.777
Primary infection site (Respiratory vs others)	.024	5.052	1.243	20.530
Higher CRP	.001	1.021	1.008	1.034

Note: Factors predicting ARDS were analyzed by univariate or forward stepwise multivariate logistic regression model.

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; ARDS, acute respiratory distress syndrome; BMI, body mass index; CI, confidence interval;COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; G-, Gram-negative bacteria; G+, Gram-positive bacteria; lncRNA NEAT1, long noncoding RNA nuclear-enriched abundant transcript 1; miR-125a, microRNA-125a; OR, odds ratio; Scr, serum creatinine; SOFA, sequential organ failure assessment; WBC, white blood cell.
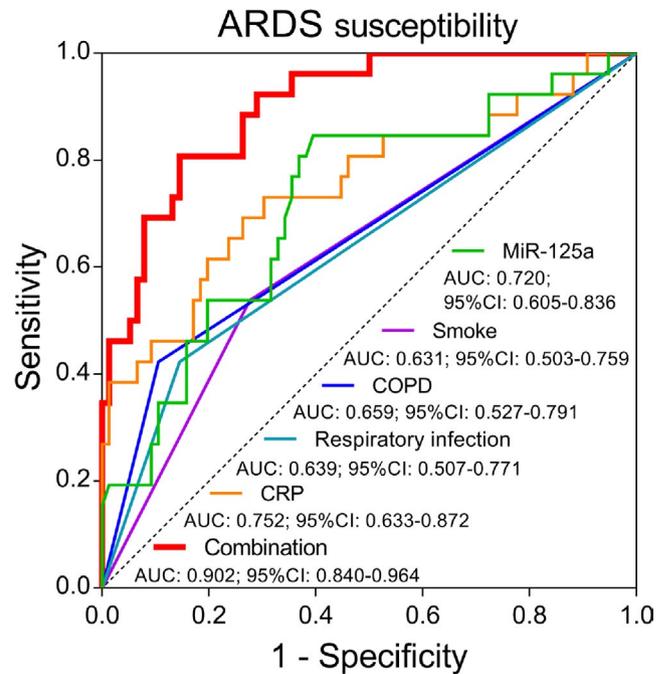
FIGURE 4 ROC curve analysis. ARDS: acute respiratory distress syndrome; miR-125a: microRNA-125a; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; AUC: area under curve; CI: confidence interval

TABLE 4 Correlation of lncRNA NEAT1/miR-125a with major biochemical indexes and disease severity in sepsis patients

Items	LncRNA NEAT1		miR-125a	
	P value	r	P value	r
Scr	.011	0.250	.015	−0.239
Albumin	.008	-0.262	<.001	0.455
WBC	.012	0.248	.036	−0.208
CRP	<.001	0.414	<.001	−0.352
APACHE II score	.019	0.233	<.001	−0.376
SOFA score	<.001	0.357	<.001	−0.378

Note: Correlation was determined by Spearman's rank correlation test.

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; CRP, C-reactive protein; lncRNA NEAT1, long noncoding RNA nuclear-enriched abundant transcript 1; miR-125a, microRNA-125a; Scr, serum creatinine; SOFA, sequential organ failure assessment; WBC, white blood cell.

patients and lncRNA NEAT1 low expressed patients. Accumulating mortality was increased in lncRNA NEAT1 high expressed patients compared to lncRNA NEAT1 low expressed patients ($P = .024$) (Figure 6A). Meanwhile, sepsis patients were also divided into miR-125a high expressed patients and miR-125a low expressed patients according to the median value of miR-125a in them. Accumulating mortality was higher in miR-125a low expressed patients compared to miR-125a high expressed patients ($P = .022$) (Figure 6B).
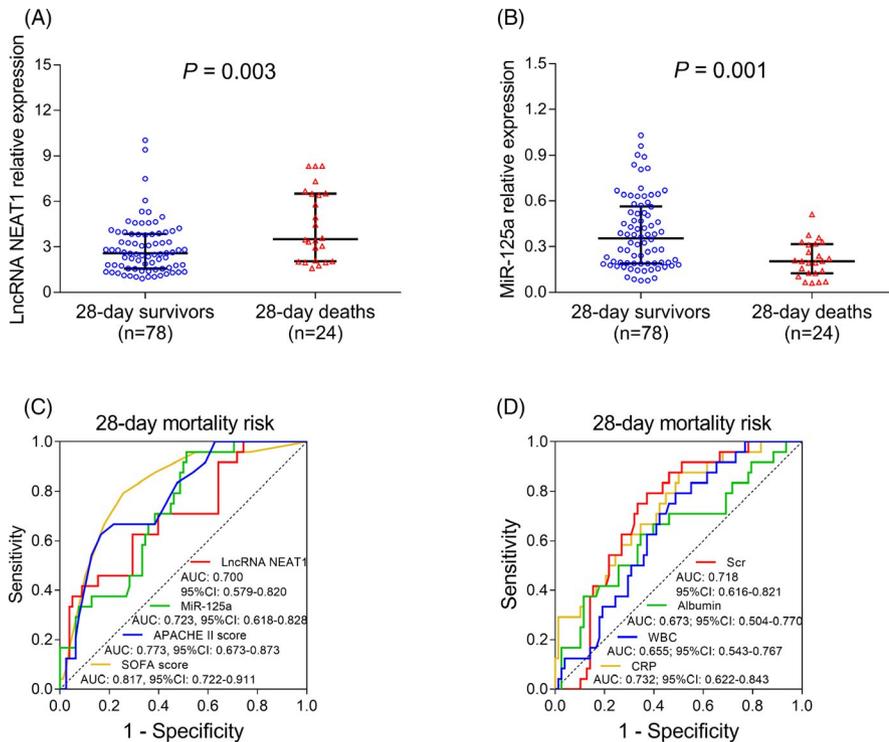
FIGURE 5 Predictive values of lncRNA NEAT1 and miR-125a on 28-d mortality risk in sepsis patients. A, LncRNA NEAT1 relative expression in 28-d deaths and 28-d survivors; B, MiR-125a relative expression in 28-d deaths and 28-d survivors; C, Predictive values of lncRNA NEAT1, miR-125a, APACHE II score, and SOFA score on 28-d mortality risk by ROC curves; D, Predictive values of Scr, albumin, WBC, and CRP on 28-d mortality risk by ROC curves. LncRNA: long noncoding RNA; NEAT1: nuclear-enriched abundant transcript 1; miR-125a: microRNA-125a; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; Scr: serum creatinine; WBC: white blood cell; CRP: C-reactive protein; ROC: receiver operating characteristic; AUC: area under curve; CI: confidence interval
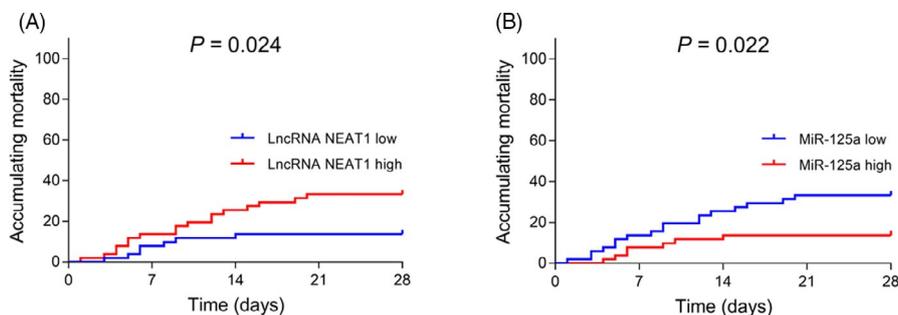
FIGURE 6 Comparison of accumulating mortality between lncRNA NEAT1/miR-125a high expressed patients and low expressed patients. A, Comparison of accumulating mortality between lncRNA NEAT1 high expressed patients and lncRNA NEAT1 low expressed patients; B, comparison of accumulating mortality between miR-125a high expressed patients and miR-125a low expressed patients. LncRNA: long noncoding RNA; NEAT1: nuclear-enriched abundant transcript 1; miR-125a: microRNA-125a

4 | DISCUSSION

LncRNA NEAT1 is reported to be a critical regulator is the progression of sepsis. According to previous studies, the down-regulation of lncRNA NEAT1 decreases several pro-inflammatory cytokines by modulating let-7a/TRL4 pathway, high-mobility group box 1/receptors for advanced glycation end product (HMGB1/RAGE) pathway and NF-κB pathway in sepsis cell model.[11,13] Meanwhile, inhibition of lncRNA NEAT1 alleviates cell apoptosis but promotes proliferation in sepsis-evoked acute lung injury cell model[13]; interestingly, similar results are also reported in sepsis-induced acute kidney injury, myocardial injury, and brain injury cell models.[10-12] Moreover, it is suggested that lncRNA NEAT1 could regulate the progression of sepsis through directly targeting miR-125a,[14] and the later one critically modulates immunity by promoting the alternative activation (M2) polarization

of macrophages and development of neutrophils,[19,20] thus participating in the incidence and progression of sepsis. Therefore, lncRNA NEAT1 might regulate inflammation, multiple organ injury, and target miR-125a to aggravate the progression of sepsis.

Various studies have been conducted to identify lncRNAs that are correlated with sepsis.[21-24] However, whether lncRNA NEAT1 could interact with miR-125a to correlate with sepsis risk was still unclear. In the present study, we found that lncRNA NEAT1 was up-regulated, while miR-125a was down-regulated in sepsis patients compared to HCs. Notably, both lncRNA NEAT1 high expression and miR-125a low expression had strong correlations with sepsis risk. Possible explanations might be that (a) lncRNA NEAT1 high expression might modulate several signaling pathways (including the NF-κB pathway and HMGB1/RAGE pathway[11,13]) to exacerbate inflammation and organ injury when the host was infected and (b) decreased

miR-125a expression might hinder the M2 polarization of macrophages and the development of neutrophils (mentioned above) to promote inflammation and to suppress the clearance of invading microbiome of the immune system.[19,25]

ARDS is one of the common complications of sepsis that might worsen the prognosis of sepsis patients.[6] Currently, several lncRNAs have been identified to be potential biomarkers for the occurrence of ARDS in sepsis patients.[26,27] However, whether lncRNA NEAT1 might interact with miR-125a to predict ARDS risk in sepsis patients was not clear. In the present study, increased lncRNA NEAT1 and decreased miR-125a were observed in ARDS sepsis patients compared to non-ARDS sepsis patients, and both of them showed a certain degree of predictive values on elevated ARDS risk in sepsis patients. Possible explanations for these data might be that (a) higher lncRNA NEAT1 expression might improve apoptosis of lung epithelial cells[13] to exacerbate lung injury, which promoted ARDS risk of sepsis patients; (b) both increased lncRNA NEAT1 and decreased miR-125a might improve inflammation (mentioned above[11,19]), which enhanced injury of lung epithelial cells and elevated ARDS risk in sepsis patients. Meanwhile, univariate logistic regression revealed that both higher lncRNA NEAT1 and higher miR-125a were correlated with ARDS in sepsis patients; however, further multivariate logistic regression analysis showed that higher miR-125a, but not higher lncRNA NEAT1, was correlated with ARDS risk independently in sepsis patients. Possible explanations for our data might be that (a) both lncRNA NEAT1 and miR-125a might modulate the injury of lung epithelial cells in sepsis[11,19]; thus, higher lncRNA NEAT1 and miR-125a were correlated with ARDS in sepsis patients; (b) lncRNA NEAT1 might interact with other ARDS independent correlated factors including miR-125a (as was shown by correlation analysis mentioned above) and CRP to affect ARDS in sepsis patients. Notably, the ROC curve showed that the combination of ARDS independent correlated factors (including miR-125a, smoking behavior, COPD, respiratory infection and CRP) could well-predict ARDS risk in sepsis patients, indicating that it might be a potential tool to recognize sepsis patients who had high ARDS risk, which may improve the management toward these patients.

Additionally, we investigated the correlation of lncRNA NEAT1 and miR-125a with major biochemical indexes and disease severity in sepsis patients. Data showed that (a) lncRNA NEAT1 was positively correlated with Scr, WBC, and CRP, while negatively correlated with albumin in sepsis patients, which could be explained, respectively, by the regulatory effect of lncRNA NEAT1 on sepsis-induced acute kidney injury,[10] inflammation,[13] and sepsis-induced liver injury[11]; (b) miR-125a was negatively associated with Scr, WBC, and CRP, but positively associated with albumin. Possible explanations might be that the down-regulation of miR-125a could promote inflammation through suppressing macrophage M2 polarization and neutrophils development,[19,20] which further exaggerated kidney and liver injury in sepsis patients[28,29]; (c) lncRNA NEAT1 was positively, while miR-125a was negatively correlated with APACHE II score and SOFA score in sepsis patient, which could be explained by that high lncRNA NEAT1 and low miR-125a expressions might promote inflammation and accelerate multiple organ injury,[10,13,19] which resulted in elevated disease severity in sepsis patients.

Furthermore, the short-term prognostic values of lncRNA NEAT1 and miR-125a were explored. Data indicated that lncRNA NEAT1 was elevated, but miR-125a was reduced in 28-day deaths compared to 28-day survivors. Meanwhile, lncRNA NEAT1 high expression and miR-125a low expression showed a certain degree of predictive value for higher 28-day mortality risk in sepsis patients, which was numerically similar to that of biochemical indexes (including Scr, albumin, WBC, and CRP), but was weaker than that of general disease severity assessments (including APACHE II score and SOFA score). Additionally, both lncRNA NEAT1 high expression and miR-125a low expression were correlated with higher accumulating mortality in sepsis patients. Possible explanations for our data might be that (a) elevated lncRNA NEAT1 and decreased miR-125a were correlated with enhanced disease severity, which indirectly promoted mortality in sepsis patients; (b) lncRNA NEAT1 high expression and miR-125a low expression might promote inflammation and exacerbate multiple organ injury,[10,13,19] and thus directly increased mortality in sepsis patients. Notably, a negative correlation between lncRNA NEAT1 and miR-125a was observed in sepsis patients, indicating that lncRNA NEAT1 might interact with miR-125a to exert potential prediction for ARDS risk, and reflection for disease severity as well as prognosis in sepsis patients.

There were several limitations in this study. First of all, the sample size of this study was relatively small, which might cause low statistical power, especially in the analyses that included ARDS sepsis patients and 28-day deaths. Therefore, further studies with larger sample sizes could be conducted. Secondly, the interaction between lncRNA NEAT1 and miR-125a in sepsis patients at molecular level was not investigated in this study, which could be explored further. Finally, lncRNA NEAT1 high and miR-125a low expressions were correlated with increased Scr and decreased albumin, implying their potential clinical role in sepsis-induced acute kidney injury and liver injury, and further studies could be performed to investigate those hypotheses.

To be conclusive, lncRNA NEAT1 sufficiency and miR-125a deficiency correlate with increased ARDS risk, enhanced disease severity, and worse short-term prognosis; meanwhile, lncRNA NEAT1 is negatively associated with miR-125a in sepsis patients.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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