Long non-coding RNAs MALAT1, NEAT1 and DSCR4 can be serum biomarkers in predicting urosepsis occurrence and reflect disease severity

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Abstract. Sepsis commonly occurs in patients with serious infections. It severely threatens the health of patients and has very high mortality rates. Urosepsis is a type of sepsis in which the serious infection originates from the urinary system. Early diagnosis of the occurrence and severity of urogenital sepsis is crucial for improving patient prognosis. Long noncoding RNAs (LncRNAs) play important roles in the occurrence of a number of diseases, including sepsis, and can be potential biomarkers that predict disease development. The present study aimed to discover potential LncRNAs that can predict the occurrence of urosepsis. RNA-sequence data from patients with sepsis from the GEO database was analyzed and LncRNAs associated with sepsis were identified. The expression of LncRNAs associated with sepsis was tested in clinical urosepsis samples. Finally, the value of these LncRNAs in predicting urosepsis was verified using clinical samples. From the GEO database a total of nine LncRNAs (MALAT1, NEAT1, RMRP, LncIRX5, LINC01742, DSCR4, C22ORF34, LINC00381, and LINC01102) were identified that had expression changes corresponding with the occurrence of sepsis. Specifically, MALAT1, NEAT1 and DSCR4 revealed differential expression in patients with urosepsis. Moreover, MALAT1, and DSCR4 were shown to be significant risk indicators for urosepsis, and NEAT1 was shown to reflect disease severity. Therefore, the present study indicated that the LncRNAs, MALAT1, NEAT1 and DSCR4 can reflect the occurrence and severity of urosepsis and may act as potential biomarkers.

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Introduction

Sepsis is a complex disease with high mortality rates that develops as a dysregulated body response to infection (1). A previous study reported that the mortality of severe sepsis and sepsis shock ranged from 28.3-41.1% (2). Urosepsis is a type of sepsis whereby the severe infection originates in the urinary system (3). A total of ~25% of all sepsis cases occurs in adults (2). The mortality of urosepsis is high, with a study in 2018 reporting that it caused the death of >1.6 million patients in America and Europe (4). One of the primary causes of urosepsis is urinary obstruction with urinary lithiasis as a major driver (5,6). Rapid treatment is vital to improve the survival rates and outcomes of patients, as indicated by The Third International Consensus of Sepsis and Sepsis shock, which emphasized the importance of early identification of sepsis onset (7). Therefore, finding potential biomarkers to diagnose urosepsis in a timely manner is urgent.

Long noncoding RNAs (LncRNAs) are a type of RNA with >200 nucleotides that lack the potential to code proteins (8,9). Instead, LncRNAs regulate a number of important biofunctions, such as epigenetics, the cell cycle and cell differentiation regulation (8-10). Some studies have indicated that LncRNAs may be associated with the occurrence of sepsis (11-14) and can predict sepsis severity (15). However, to the best of our knowledge, no study has assessed the efficacy of LncRNAs as biomarkers to predict the occurrence of urosepsis. Therefore, the present study aimed to discover potential LncRNAs that can act as biomarkers to predict the occurrence of urosepsis.

Bioinformatic analysis has been previously used to discover potential genetic alterations in disease occurrence (16) and has proved to be an effective method for discovering the gene markers of particular diseases such as membranous nephropathy and dermatomyositis (17,18). In the present study, we the GEO database was used to find potential LncRNAs that show altered expression in sepsis. A total of 9 potential

LncRNAs were associated with sepsis. The expression of these LncRNAs was verified in clinical samples. It was found that 3 LncRNAs, MALAT1, NEAT1 and DSCR4, showed altered expression with urosepsis. Furthermore, it was indicated that their expression can predict the occurrence of urosepsis and reflect disease severity. Finally, it was found that expression of these LncRNAs normalized to control levels when patients had recovered from urosepsis. The results of the present study indicated that the LncRNAs, MALAT1, NEAT1 and DSCR4 act as potential biomarkers that can predict the risk of urosepsis occurrence and reflect disease severity.

Materials and methods

Data sourcing. The GEO database [http://www.ncbi.nlm.nih.gov/geo/; National Center for Biotechnology Information (NCBI)] is a public database that provides a genomics data repository of gene expression, chip and microarray data (19). In the present study, gene datasets within this database that included LncRNA expression in patients with sepsis and healthy controls were searched. Furthermore, the datasets must have included complete clinical data to be used in the present analysis. A total of two gene datasets, GSE145227 and GSE89376 (20,21) were included in the present study. GSE145227 included data from 12 healthy individuals and 10 patients with sepsis, and GSE89376 included sequence information from 12 healthy individuals and 12 patients with sepsis. The GSE145227 dataset includes pediatric patients with sepsis, aged <40 months, and the GSE89376 dataset includes data from patients >20 years old.

Identification of potential LncRNAs associated with urosepsis. The raw microarray expression data of LncRNAs from GSE145227 and GSE89376 were downloaded as Series Matrix files from the GEO database and mapped to the corresponding genes according to the SOFT formatted family files from the GEO database. Primary data were normalized using the Limma package of R (22,23). Genes with an adjusted $P<0.05$ and $|\log_2$ fold change$|>1$ were considered potential LncRNAs (24). After which, the online web tool, bioinformatics (http://www.bioinformatics.com.cn/), was used to construct a Venn diagram and find potential LncRNAs, with upregulated and downregulated LncRNAs recorded for further analysis.

Clinical sample collection. Samples from patients with urosepsis were collected from February 2022 to July 2023, with only patients with urosepsis caused by urinary stones included in the present study. The patients included in the study must older than 18 and did not have immunological deficiency. Patients with sepsis not caused by urinary system infection, with immunodeficiency disease or who were taking immunosuppressive drugs and who were not willing to participate in this study were excluded. Computed tomography (CT) scans were used to confirm that patients had urinary stones (Fig. S1). In total, 40 patients (14 male patients and 26 female patients; age range, 49-86 years) and 40 healthy (20 males and 20 females; age range, 48-88 years) individuals were included in the study. Blood samples were collected within 24 h of diagnosis of patients with urosepsis. Patients with a Sequential Organ Failure Assessment (SOFA) score ≥2 were defined as having urosepsis (25). Next, according to the definition of septic shock, the patients were further classified into the urosepsis group and the septic shock group. Blood samples were collected again after the patients had recovered. Simultaneously, blood samples were collected from healthy control individuals who had undergone a health examination at Tinglin Hospital of Jinshan District (Shanghai, China). The present study was approved by The Ethics Committee of Tinglin Hospital of Jinshan District (Shanghai, China; approval no. 2022-06). The study was explained to all participants, who provided written informed consent for blood sample collection.

Clinical data acquisition. The baseline data from patients and healthy controls were collected following informed consent for study participation. Imaging examination (CT) was used to confirm that the urosepsis was caused by urinary stones. Other information, such as comorbidities and inflammatory indicators were also collected. Further, when patients were confirmed with urosepsis caused by urinary stones, their blood samples were collected within 24 h. Routine blood tests (level of hemoglobin, white blood cells and platelets) in addition to tests for liver function, kidney function and the level of immune indicators (TNF-α, IL-1, IL-6, IL-8) were collected and analyzed by unpaired t-test in the present study. In addition, the comorbidities of patients were also collected. All these indicators were analyzed by clinical laboratory of Tinglin Hospital.

RNA extraction and RT-qPCR. Total blood RNA was isolated using TRIzol® reagent in accordance with the manufacturer's instructions (Sigma-Aldrich; Merck KGaA). Briefly, RNA was reverse transcribed to cDNA using a RT kit (37˚C for 15 min; 85˚C for 15 sec) (Advantage® RT-for-PCR Kit; Takara Bio Inc.). According to the manufacturer's instructions, RT-qPCR was performed using the Applied Biosystems 7500 Sequence Detection system (Thermo Fisher Scientific, Inc.). The volume of cDNA, RT-qPCR reagents and experimental process (TB Green® Premix Ex Taq™ II; Takara Bio Inc.) was according to the manufacturer's instructions (denaturation at 95˚C for 30 sec; 40 cycles of 95˚C for 3 sec and 60˚C for 30 sec; 4˚C used annealing). GAPDH was used as the normal control. The primer was constructed by Sangon Biotech Co., Ltd. First, the gene ID of each LncRNA and GAPDH was taken from NCBI (https://www.ncbi.nlm.nih.gov/). After which, the gene ID was input into the primer bank (https://pga.mgh.harvard.edu/primerbank/) to obtain the potential primer sequence. The primer sequence with the minimal temperature (Tm) was selected for the present study. If the primer sequence could not be obtained from the primer bank, the whole gene sequence was sent to Sangon Biotech Co., Ltd., who then returned the primer sequence. The expression of RNA was calculated according to the $2^{-\Delta\Delta Cq}$ method (26). The primers used are shown in Table I.

Construction of risk prediction model and diagnostic model. The predictive power and risk value of the identified LncRNAs for urosepsis were assessed. A receiver operating characteristic (ROC) curve was constructed and a logistic regression model was performed by 'pROC' and 'rms' package in R software, respectively (27,28). Moreover, a forest map was utilized to show the hazard ratios more intuitively. Nomograms were

Table I. Primers used in RT-qPCR.

Gene name	Primer sequence
MALAT1	F: 5'-AAAGCAAGGTCTCCCCACAAG-3'
	R: 5'-GGTCTGTGCTAGATCAAAAGGCA-3
NEAT1	F: 5'-GCACTGGTACTGGGAGGGATG-3'
	R: 5'-CAACTTCTCACTTCCAAGCAACAAC-3'
DSCR4	F: 5'-GATGAACCCCGGATATTTACCC-3'
	R: 5'-CAGGAAACGATGTTGCAGACT-3'
RMRP	F: 5'-GTTCCTCCCCTTTCCGCCTAG-3'
	R: 5'-AGAATGAGCCCCGTGTGGTTG-3'
LncIRX5	F: 5'-TCTTGGCAGGACCTTTGCAA-3'
	R: 5'-CACCTGGCTTCTGGCTGC-3'
LINC01742	F: 5'-CTGCTGTCACTTAGAACTCATCCTG-3'
	R: 5'-TTGTCACTCACCTCTACCTTCCAG-3'
C22ORF34	F: 5'-GGCTCTGTGGCTGTCATCAATC-3'
	R: 5'-ATCTGTGGCATCCTCCTGGTG-3'
LINC00381	F: 5'-GTTCCTCAAGTGCCGCCAAAG-3'
	R: 5'-TCTCCTGTTGTTAGTGGTCAATGTG-3'
LINC01102	F: 5'-TGGAGAAGAAGCGTTTACTGAAAGG-3'
	R: 5'-AGGACTGCCGTGAACAGGAAG-3'
GAPDH	F: 5'-ACAACTTTGGTATCGTGGAAGG-3'
	R: 5'-GCCATCACGCCACAGTTTC-3'

F, forward; R, reverse.

built to predict the risk value of these factors by 'rms' package in R. At the same time, the correction curves of nomograms were created.

Correlation analysis. The correlation between the expression of LncRNAs and clinical data was analyzed by Spearman's rank correlation test and the results were reflected by scatter diagrams. $P<0.05$ was considered to indicate statistically significant correlation among these factors.

Statistical analysis. All experiments were repeated at least three times and data are presented as mean ± standard deviation. Data were analyzed using unpaired Student's t-test for two groups and in addition, the sex between different groups was analyzed by χ^2 test. The chip data from the GEO database were analyzed using different packages within R (R Version 4.0.3; http://www.r-project.org). The nomogram and ROC curve were created using R software 'autoReg' package. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of potential LncRNAs associated with sepsis. A total of two GSE datasets, GSE145227 and GSE89376, included LncRNA sequencing data from patients with sepsis and healthy controls. All RNA-sequencing data were downloaded and a volcano map was generated to reflect the LncRNAs within these datasets (Fig. 1A and B). A total of 857 upregulated LncRNAs and 431 downregulated LncRNAs were identified from GSE145227 (Fig. 1A). In addition, 5 overexpression and 12 low expression LncRNAs were identified from GSE89376 (Fig. 1B). After which, LncRNAs that were up- and down-regulated within both datasets were analyzed. Finally, five LncRNAs (MALAT1, NEAT1, RMRP, LncIRX5 and LINC01742) and four LncRNAs (DSCR4, C22ORF34, LINC00381 and LINC01102) that were up- and down-regulated, respectively, were associated with sepsis occurrence (Fig. 1C and D). In addition, the specific expression of the nine LncRNAs in these two datasets was reflected in Table SI.

Identification of potential LncRNAs associated with urosepsis. Following the identification of the potential LncRNAs associated with sepsis, whether the expression changes of these LncRNAs was correlated with urosepsis occurrence was investigated. Blood samples from 6 patients with urosepsis due to a urinary stone and 6 healthy controls were collected. The clinical information of the 6 patients is shown in Table SII. The expression of the 9 identified LncRNAs between groups was analyzed. A total of two LncRNAs, MALAT1 and NEAT1, were upregulated in patients with urosepsis, and one LncRNA, DSCR4, was downregulated in patients with urosepsis (Fig. 2). Other previously aforementioned LncRNAs did not have significantly different expression patterns between the two groups.

LncRNA MALAT1, NEAT1 and DSCR4 are associated with the occurrence and disease severity of urosepsis. Following the discovery of the three LncRNAs linked to urosepsis, it was investigated whether these LncRNAs could reflect disease progression. Therefore, blood samples from 40 patients with

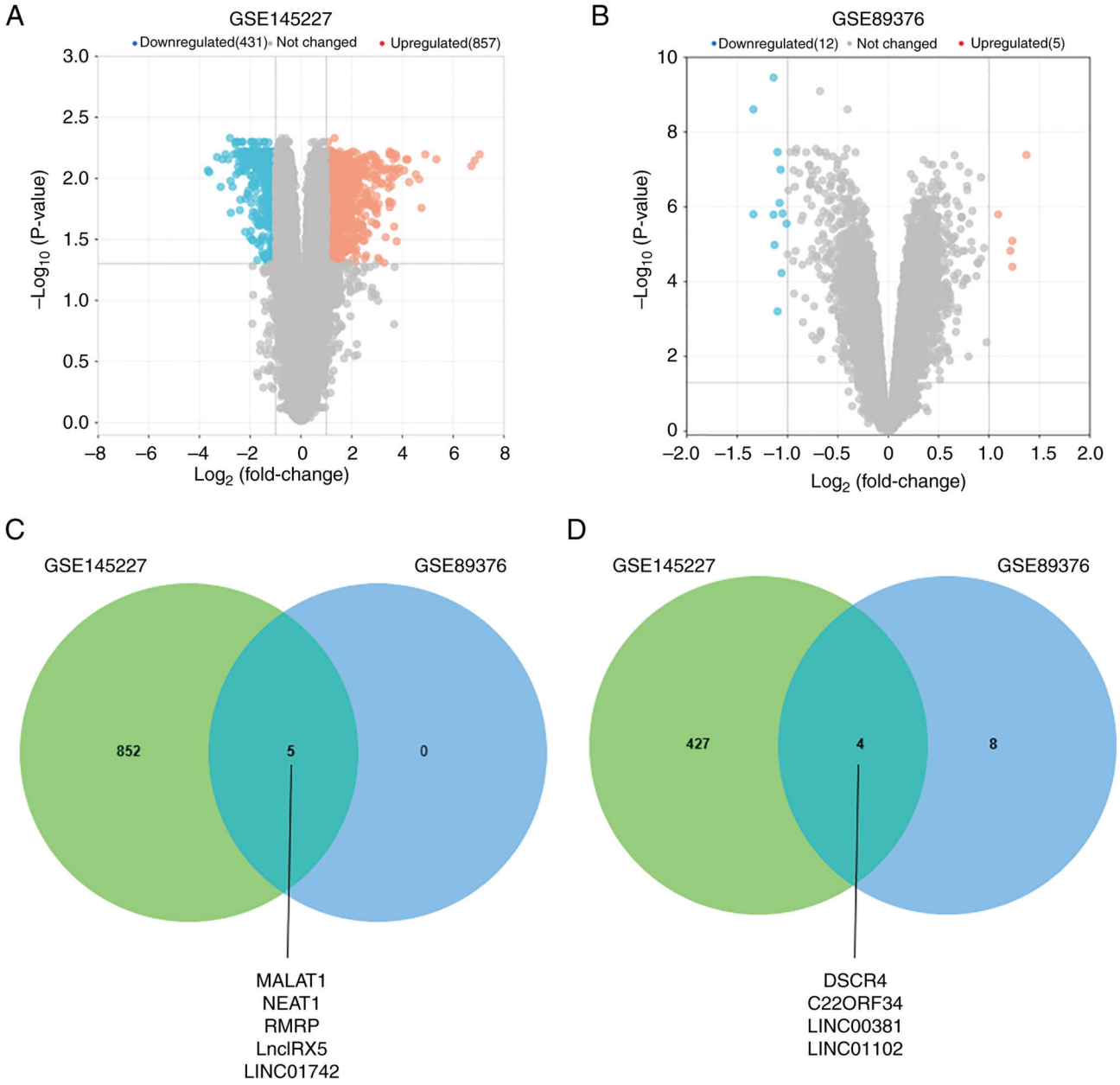

Figure 1. Potential LncRNAs correlated with sepsis detected from GSE145227 and GSE89376 datasets. (A) Volcano map reflects DELs from GSE145227. (B) Volcano map reflects DELs from GSE89376. (C) Venn map reflects the upregulated DELs between GSE145227 and GSE89376. (D) Venn map reflects the downregulated DELs between GSE145227 and GSE89376. LncRNAs, long noncoding RNAs; DELs, differently expressed LncRNAs.

urosepsis and 40 healthy controls were collected. The baseline characteristics of participants are shown in Table II. Next, the 40 patients were divided into urosepsis and septic shock groups according to the definition of septic shock from Sepsis-3 (7). Detailed information regarding these patient groups is shown in Table III. No differences among other indices, except SOFA score (P=0.004), were found. This suggested that SOFA score can reflect the severity of urosepsis in patients. Next, the expression of the three LncRNAs was detected in a large number of patients with urosepsis (40 patients). It was found MALAT1 and NEAT1 significantly increased and DSCR4 decreased in patients with urosepsis (Fig. 3A-C). In addition, MALAT1, NEAT1 and DSCR4 also reflected sepsis severity, as there was a greater change in the expression of MALAT1, NEAT1 and DSCR4 in patients with septic shock (Fig. 3D-F). These results indicated that MALAT1, NEAT1 and DSCR4 are potential biomarkers of the occurrence and severity of urosepsis.

Value of MALAT1, NEAT1 and DSCR4 in diagnosing urosepsis. Next, whether these LncRNAs could be diagnostic biomarkers of urosepsis was investigated using ROC curves. It was found that the aforementioned LncRNAs would not be effective biomarkers for predicting urosepsis occurrence. MALAT1 had an area under the curve (AUC) value of 0.632 (95% CI, 0.571-0.693), NEAT1 had an AUC value of 0.638 (95% CI, 0.584-0.692) and DSCR4 had an AUC value of 0.618 (95% CI, 0.584-0.688; Fig. 4A-C). However, combining the three LncRNAs together indicated a good diagnostic capability with an AUC value of 0.872 (Fig. 4D). The ability of the LncRNAs to predict sepsis severity was also assessed.

Table II. Baseline characteristics of patients with urosepsis by calculus.

Characteristics	Patients with urosepsis (n=40)	Healthy controls (n=40)
Sex		
Male	14	20
Female	26	20
Age, years	69.5±12.19	65.4±8.94
BMI, kg/m^2	24.9±2.47	23.71±3.11
WBC, 10^9/l	14.86±9.37	8.76±3.12
PLT, 10^9/l	124.97±73.1	182.11±94.73
Total bilirubin, μmol/l	23.85±21.44	16.77±11.32
Scr, μmol/l	165.62±134.92	74.87±45.89
Albumin, g/l	38.73±7.13	46.74±10.52
Lactate, mmol/l	2.64±1.49	NA
SOFA score	6.25±3.87	NA
CRP, mg/l	137.1±89.54	NA
PCT, ng/ml	31.84±21.42	NA
TNF-α, pg/ml	96.68±84.66	NA
IL-1, pg/ml	6.32±2.21	NA
IL-6, pg/ml	412.14±296.54	NA
IL-8, pg/ml	142.76±122.99	NA
Comorbidity (%)		
Diabetes	18 (45)	NA
Hypertension	10 (25)	NA
Vascular diseases	2 (5)	NA
Malignancy tumor	3 (7.5)	NA
Other diseases	6 (15)	NA
Stone position (%)		
Kidney	11 (27.5)	NA
Ureter	29 (72.5)	NA

BMI, body mass index; WBC, white blood cell; PLT, platelet; Scr, Serum creatinine; SOFA, sequential organ failure assessment; CRP, C reactive protein; PCT, procalcitonin; NA, not applicable.

However, the results indicated that the LncRNAs, individually, could not predict the occurrence of uroseptic shock effectively. MALAT1 had an AUC value of 0.625 (95% CI, 0.566-0.684), NEAT1 had an AUC value of 0.588 (95% CI, 0.517-0.658) and DSCR4 had an AUC value of 0.640 (95% CI, 0.503-0.677) in diagnosing septic shock (Fig. 4E-G). However, the results showed that the combination of these LncRNAs does appear to be effective at predicting the occurrence of septic shock with an AUC value of 0.856 (Fig. 4H). In summary, the combination of MALAT1, NEAT1 and DSCR4 LncRNAs together can predict the occurrence of urosepsis and uroseptic shock.

Correlation of MALAT1, NEAT1 and DSCR4 with SOFA score and immune factors. As SOFA score can predict sepsis severity, the correlation between SOFA score and the three LncRNAs was analyzed. NEAT1 was the only LncRNA that significantly correlated with SOFA score (R=0.35; P=0.028; Fig. 5A-C). This indicated that NEAT1 may be an effective biomarker of the severity of sepsis. The correlation of SOFA score and NEAT1 in urosepsis and septic shock groups was also analyzed and it was found that in these different groups, NEAT1 also correlated with SOFA score. Specifically, there was positive correlation between SOFA score and NEAT1 in the urosepsis group (R=0.42; P=0.022) and septic shock group (R=0.32; P=0.041; Fig. S2 and B). This suggested that NEAT1 is associated with disease severity. Sepsis is caused by an immune and inflammatory factor storm (29,30). Therefore, it was hypothesized that there is a correlation between the aforementioned LncRNAs and immune factors. To confirm this, the correlation between MALAT1, NEAT1 and DSCR4, and TNF-α, IL-1, IL-6 and IL-8 was assessed. However, no correlation between LncRNAs and immune factors was identified (Fig. 5D-O). This suggested that the change in expression of these LncRNAs was not immune-dependent.

Risk prediction of MALAT1, NEAT1 and DSCR4 in urosepsis development. The risk prediction model was constructed to assess whether MALAT1, NEAT1 and DSCR4 were risk factors for urosepsis, with both single and multiple factor logistic regression analyses performed. First, a forest map was created to visualize the predictive power of the LncRNAs. Sex, MALAT1 and DSCR4 were identified as significant risk factors for urosepsis in the single factor logistic model (Fig. 6A), but no individual risk factor was identified in the multiple factor logistic model (Fig. 6B). Following this, a nomogram and calibration curve of urosepsis was created to include information from these factors including DSCR4 and MALAT1 that may predict urosepsis. The nomogram indicated that DSCR4 carries the highest risk for urosepsis. In addition, it showed that elderly individuals and female patients were more likely to develop urosepsis (Fig. 6C and D). These results suggest that expression changes of DSCR4 and MALAT1 may indicate the occurrence of urosepsis, and age and sex are important factors for urosepsis occurrence.

Specificity of MALAT1, NEAT1 and DSCR4 in predicting urosepsis. Finally, whether the expression of MALAT1, NEAT1 and DSCR4 was urosepsis-dependent was investigated by comparing LncRNA expression in the same 40 patients after recovery from urosepsis with health individuals. When the same patients recovered from urosepsis, the expression of MALAT1, NEAT1 and DSCR4 was not significantly different from healthy individuals (Fig. 7A-C). These results confirmed that the change in expression of MALAT1, NEAT1 and DSCR4 was urosepsis-dependent. Taken together, these data showed that MALAT1, NEAT1 and DSCR4 may be indicators for predicting urosepsis occurrence.

Discussion

Sepsis is a serious disease caused by a dysregulated host response to infection (31). It triggers acute organ dysfunction and carries a high risk of death (32). Its high incidence and mortality rates mean that it is an important public health issue that can lead to serious economic burdens worldwide (33,34). Urosepsis is a types of sepsis that occurs when the infection originates from the urinary system, and also carries high incidence and mortality

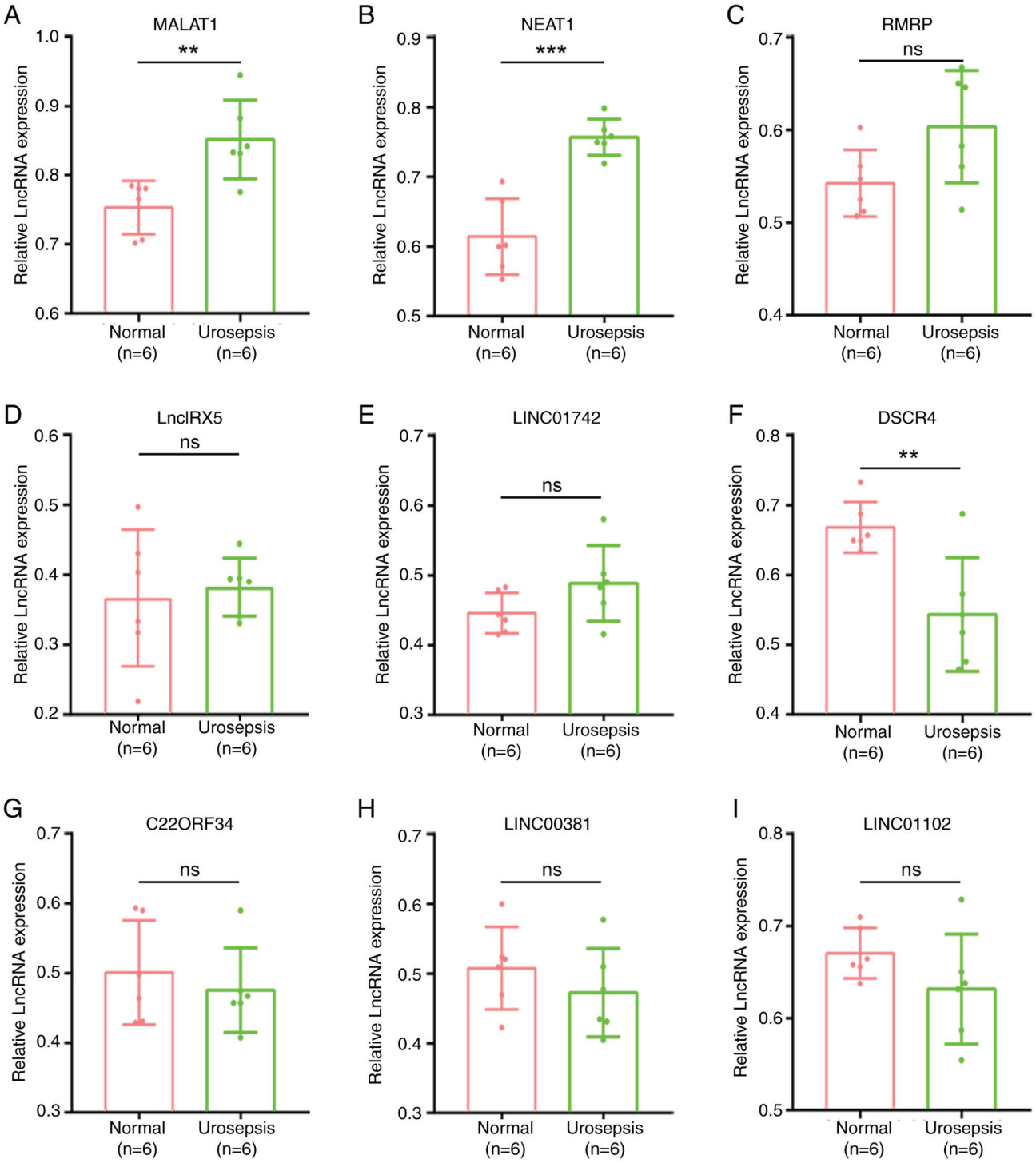

Figure 2. Expression of nine LncRNAs in patients with urosepsis and healthy individuals. (A) Expression of MALAT1, (B) NEAT1, (C) RMRP, (D) LncIRX5, (E) LINC01742, (F) DSCR4, (G) C22ORF4, (H) LINC00381 and (I) LINC01102. The RT-qPCR experiments used GAPDH as the inner control. The results were presented as mean ± SD. **P<0.01 and ***P<0.001. ns, not significant; LncRNAs, long noncoding RNAs.

rates (2,5). Timely diagnosis and treatment of urosepsis is very important for improving the outcomes of patients (35,36). The most common cause for urosepsis is obstructive diseases, and a number of factors, such as ureteral stones, anomalies, stenosis or tumors can lead to urinary tract obstruction (37,38). However, ureteral stones are the most common cause of urinary tract obstruction and urosepsis (39). Therefore, finding effective biomarkers to predict the occurrence of urosepsis is important to improve the prognosis of patients.

Numerous biomarkers are used for the diagnosis of sepsis, such as C-reactive protein (CRP) and procalcitonin (PCT) (40). CRP levels can arise rapidly in acute injury, acute inflammation and sepsis, and additionally can reflect infection severity. However, a number of other factors can lead to increased CRP, such as injury and hepatic disease (40,41). Additionally, PCT levels can rise with the occurrence of serious infection (40) and can also reflect the severity of the infection (42). However, a number of other factors can also lead to the rise of PCT

Table III. Characteristics of patients with urosepsis, with or without septic shock.

Characteristics	Patients without septic shock, n=25	Patients with septic shock, n=15	P-value
Sex			0.86
Male	9	5	
Female	16	10	
Age, years	71.5±10.87	66±14.7	0.19
BMI, kg/m^2	24.6±2.16	25.5±3.31	0.43
Temperature, ˚C	38.4±1.21	38.44±1.97	0.92
WBC, 10^9/l	13.52±8.82	17.36±11.59	0.23
SOFA score	4.85±3.41	8.76±3.53	0.004
CRP, mg/l	119.74±84.88	169.95±91	0.08
PCT, ng/ml	30.32±24.17	35.37±27.14	0.77
TNF-α, pg/ml	94.92±53.32	98.67±69.82	0.59
IL-1, pg/ml	5.96±1.85	6.71±4.21	0.51
IL-6, pg/ml	400.57±268.01	413.36±335.49	0.62
IL-8, pg/ml	122.26±118.41	196.84±135.04	0.77

WBC, white blood cell; PLT, platelet; Scr, Serum creatinine; SOFA, sequential organ failure assessment; CRP, C reactive protein; PCT, procalcitonin.

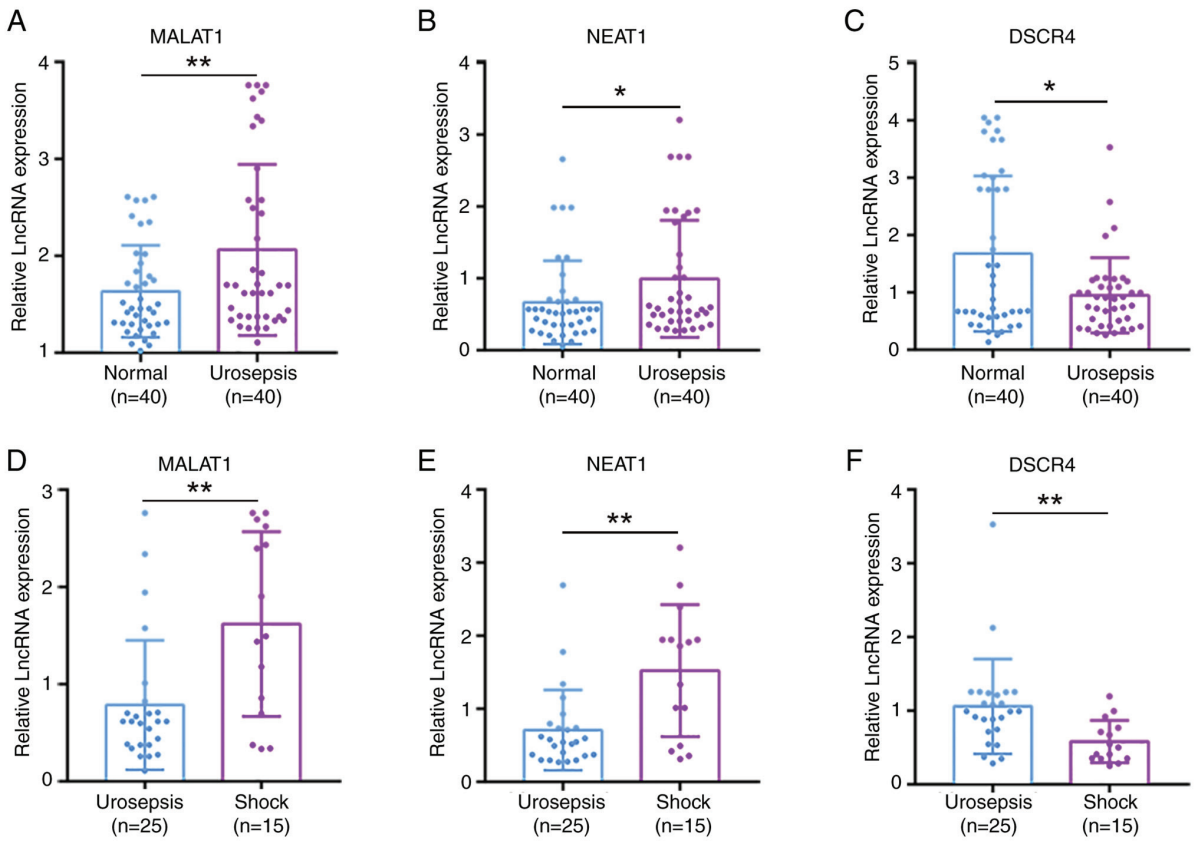

Figure 3. (A) Expression of MALAT1, (B) NEAT1 and (C) DSCR4 in healthy individuals and patients with urosepsis. (D) Expression of MALAT1, (E) NEAT1 and (F) DSCR4 in patients with urosepsis or septic shock. The RT-qPCR experiments used GAPDH as the inner control. The results were presented as mean ± SD. *P<0.05, **P<0.01. ns, not significant; LncRNAs, long noncoding RNAs.

such renal inadequacy and a previous serious infection (43). Therefore, both CRP and PCT have high sensitivity but low specificity in diagnosing sepsis. Other biomarkers, such as IL-6, heparin-binding protein and serum amyloid A, can aid the predicting of sepsis (44-46); however, these biomarkers also have shortcomings in their diagnostic ability, such as

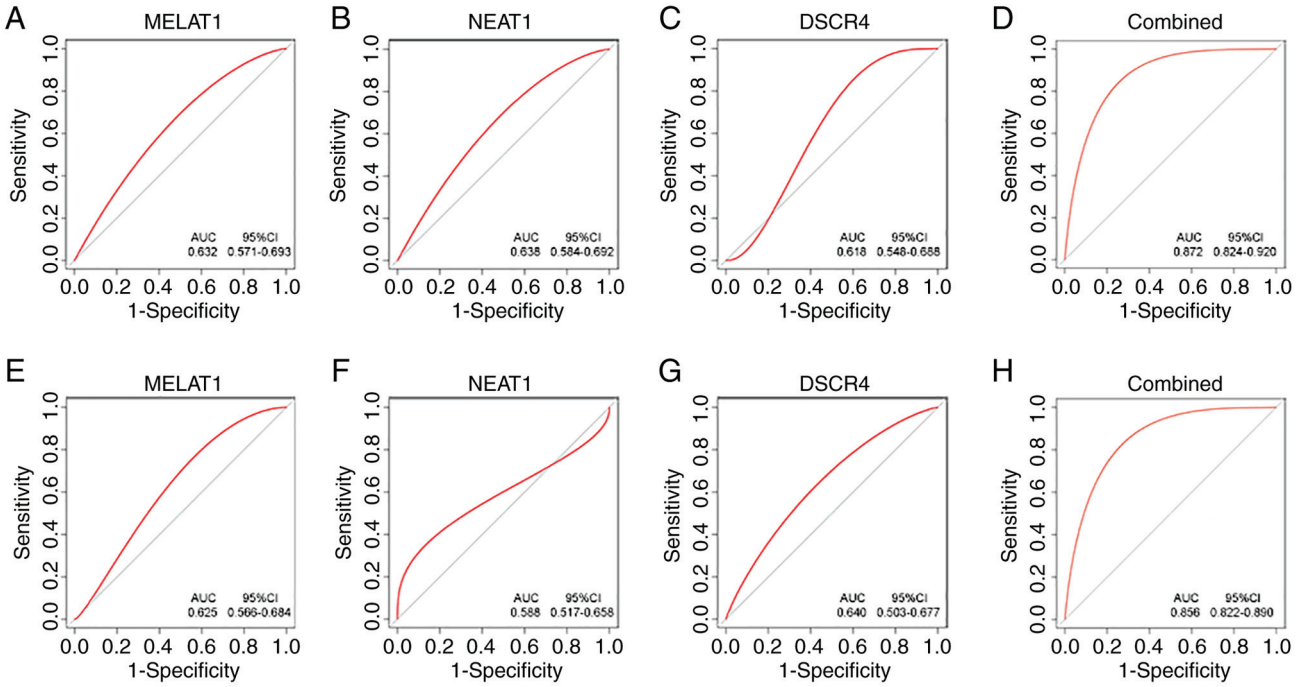

Figure 4. ROC curves for (A) MALAT1 in diagnosing urosepsis, (B) NEAT1 in diagnosing urosepsis, (C) DSCR4 in diagnosing urosepsis, (D) the combined three LncRNAs in diagnosing urosepsis, (E) MALAT1 in diagnosing septic shock, (F) NEAT1 in diagnosing septic shock, (G) DSCR4 in diagnosing septic shock and (H) the combined three LncRNAs in diagnosing septic shock. AUC, area under the curve; ROC, receiver operating characteristic; LncRNAs, long noncoding RNAs.

their insufficient specificity to diagnose sepsis. Therefore, for the aforementioned reasons, these biomarkers are not ideal for diagnosing sepsis (47,48).

LncRNAs do not code proteins, and instead regulate biological functions in various ways such as by influencing gene expression by post-transcriptional regulation and participating in cancer development, which is a key factor leading cancer progress (8-10). Previous studies have shown the importance of LncRNAs for sepsis as predictive biomarkers. Qiu *et al* (14) has reported that RMRP can prevent sepsis-associated cardiomyocyte apoptosis in mice by regulating miR-1-5p/hsp70. Furthermore, lncRNA taurine upregulated 1 can alleviate sepsis-induced lung injury by targeting miR-34b-5b/GAB1 (14). In addition, lncRNA MEG3 can predict sepsis severity and prognosis (15). Taken together, these studies have shown that LncRNAs are important for the diagnosis of sepsis and can also act as prediction biomarkers. Therefore, the present study was undertaken to find other biomarkers for predicting the occurrence of urosepsis.

Bioinformatic methods were used to find potential LncRNAs associated with sepsis. A total of nine LncRNAs hypothesized be important for the occurrence of sepsis were taken from GSE145227 and GSE89376 datasets. However, there was a large variation between the number of LncRNAs found in each of the datasets. This difference may firstly have been because the two datasets used different sequencing methods with different genetic screening approaches. Second, the GSE145227 dataset includes patients aged <40 months, however the GSE89376 dataset includes data from patients >20 years old, and it was considered that age may a have diverse reflection on sepsis. These aforementioned reasons may have caused the variation of LncRNAs found in the two datasets.

The expression of nine LncRNAs in patients with urosepsis were assessed. It was found that three LncRNAs, including MALAT1, NEAT1 and DSCR4, had altered expression in patients with urosepsis. Furthermore, these LncRNAs could predict the occurrence of urosepsis and septic shock. Notably, changes in the expression levels of MALAT1 and DSCR4 were identified as potential risk factors for urosepsis, and NEAT1 was also associated with urosepsis severity. Therefore the results of the present study suggested that these LncRNAs are potential biomarkers for urosepsis and sepsis shock. Moreover, the nomogram data revealed that age and sex were important factors leading to urosepsis. Specifically, elderly female patients were at a higher risk of developing urosepsis, which is consistent with previous studies (49,50), and thus these patients require more proactive treatments.

In addition, it was hypothesized that a number of other comorbidities such as diabetes and ureteral stone position may also affect the occurrence of urosepsis. Numerous studies have reported that patients with diabetes exhibit impaired immune function and aggravated infectious diseases, thus these patients carry a higher risk for sepsis development (51,52). Furthermore, stone position is also an important factor which can affect the occurrence of urosepsis. Studies have found that the location and size of stones can influence urosepsis occurrence. A larger stone leads to more severe obstruction, and makes patients more likely to develop urosepsis (53,54). Although these aforementioned reasons can also affect urosepsis, the aim of the present study was to find potential LncRNAs which can predict the occurrence of urosepsis, and therefore, their function in causing urosepsis was not analyzed further.

MALAT1 has a number of biofunctions. For example, it can regulate antiviral innate immunity via TDP43 (55).

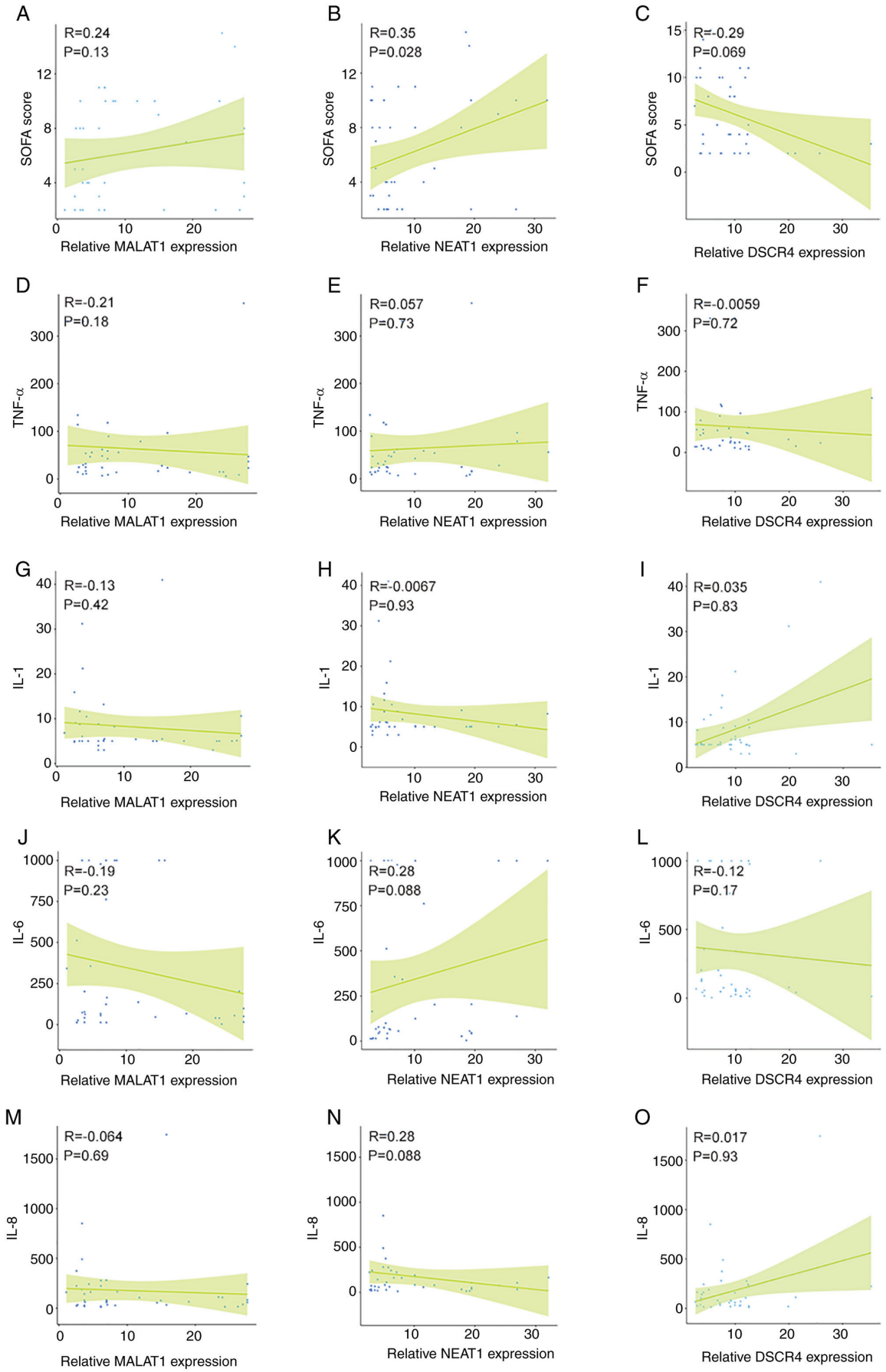

Figure 5. Correlation between LncRNAs, SOFA score and immunological factors. (A) Correlation between MALAT1, (B) NEAT1, (C) DSCR4 and SOFA score. (D) Correlation between MALAT1, (E) NEAT1, (F) DSCR4 and TNF-α. (G) Correlation between MALAT1, (H) NEAT1, (I) DSCR4 and IL-1. (J) Correlation between MALAT1, (K) NEAT1, (L) DSCR4 and IL-6. (M) Correlation between MALAT1, (N) NEAT1, (O) DSCR4 and IL-8. LncRNAs, long noncoding RNAs; SOFA, Sequential Organ Failure Assessment.

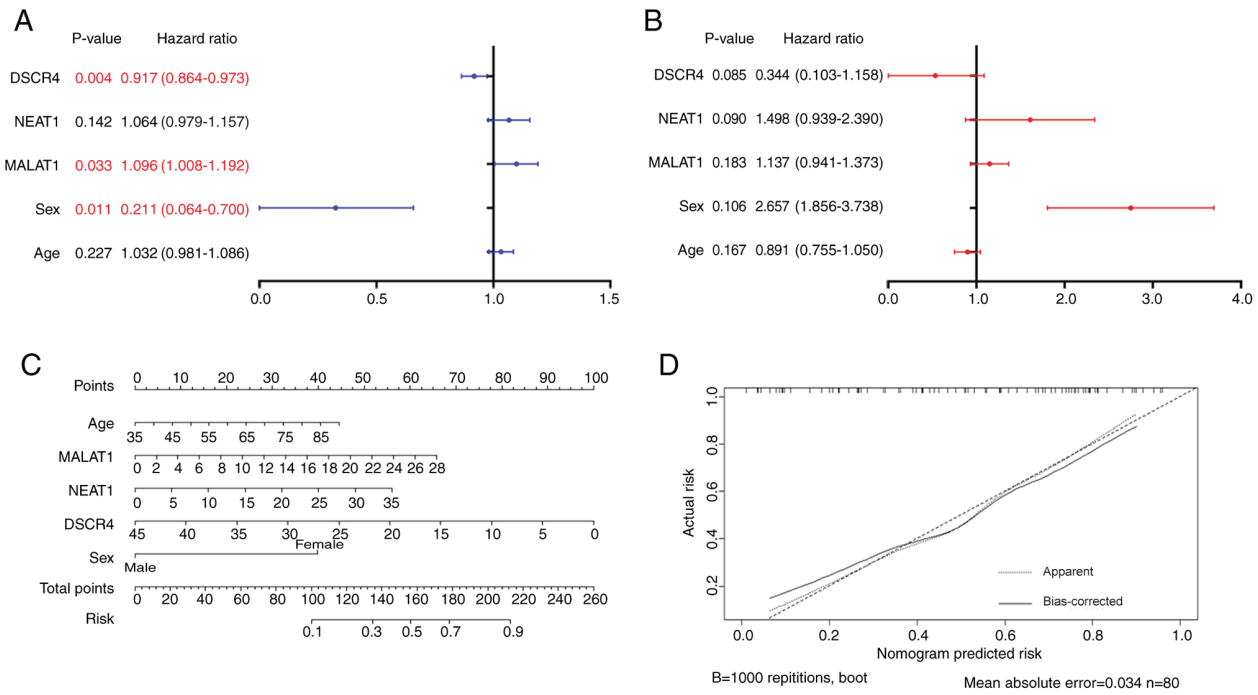

Figure 6. Forest map and nomogram reflect the risk of MALT1, NEAT1 and DSCR4 LncRNAs in developing urosepsis. (A) Single-factor logistic regression and (B) multiple-factor regression analyses reflecting the value of the LncRNAs in causing urosepsis. (C) Nomogram reflecting the risk of numerous factors (age, sex, expression of LncRNAs) in causing urosepsis. (D) Calibration plot of actual risk probability and nomogram risk of nomogram. LncRNAs, long noncoding RNAs.

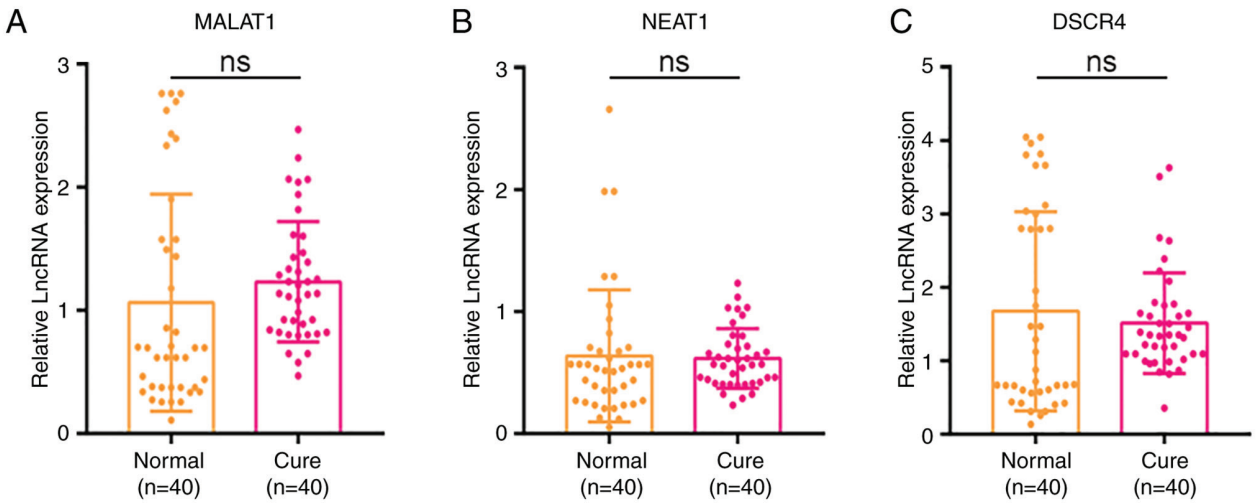

Figure 7. Expression of three LncRNAs of patients that have recovered from urosepsis compared with healthy individuals including (A) MALAT1 (B) NEAT1 (C) DSCR4. The RT-qPCR experiments used GAPDH as the inner control. The results were presented as mean ± SD. ns, not significant; LncRNAs, long noncoding RNAs.

Additionally, it is associated with the occurrence of breast and ovarian cancers (56,57). In sepsis MALAT1 mediates the proliferation of LPS-treated articular chondrocytes by targeting the miR-146a-PI3K/Akt/mTOR axis (58). In addition, MALAT1 accelerates skeletal muscle cell apoptosis and inflammatory responses in sepsis (11). NEAT1 can lead to the occurrence of numerous cancers, such as breast and gastric cancers (59,60). Additionally, it can regulate inflammatory responses including the regulation of corneal neovascularization by promoting an inflammatory response (61). Although NEAT1 reportedly promotes sepsis-relevant inflammation (62-64), one study has reported that NEAT1 can alleviate inflammatory responses (12). Furthermore, it can reflect sepsis severity and progress (65). However, no previous studies have found a correlation between DSCR4 and sepsis. In the present study it was found that NEAT1 can also predict urosepsis severity. Taken together, these results indicated that MALAT1, NEAR1 and DSCR4 LncRNAs could be biomarkers for predicting urosepsis occurrence and reflect disease severity.

The present study had several limitations. First, although the RNA sequence data used were obtained from patients with

sepsis, these data may differ from patients with urosepsis. This may mean that these LncRNAs may not only predict urosepsis but also sepsis. Therefore, the specificity of these LncRNAs in predicting urosepsis may be defective. Second, only included 40 patients were included in the present study and this small sample size may have created bias. Therefore, the experimental results need validation with a larger sample size. Moreover, further studies are also required to measure whether other factors can affect the expression of these LncRNAs. Third, the underlying mechanisms of this change in LncRNA expression in urosepsis is still unclear and needs elucidating in the future.

In conclusion, three LncRNAs, MALAT1, NEAT1, and DSCR4 were identified as potential biomarkers to predict the occurrence of urosepsis in the present study. Furthermore, it was also shown that these LncRNAs may reflect the severity of urosepsis.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JS and LP collected the blood samples and clinical data from patients and healthy individuals and carried out the RT-qPCR experiments. WC analyzed the data and performed the statistical analyses. YW collected data from public databases and wrote the manuscript. All authors read and approved the final version of the manuscript. JS and YW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study was approved by The Ethics Committee of Tinglin Hospital of Jinshan District (Shanghai, China; approval no. 2022-06) and conducted in accordance with the Declaration of Helsinki. All participants provided informed consent for blood sample collection and consented for publication.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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