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Original Article

High expression of long non-coding RNA MALAT1 correlates with raised acute respiratory distress syndrome risk, disease severity, and increased mortality in sepstic patients

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Abstract: This study aimed to explore the correlation of long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1) expression with acute respiratory distress syndrome (ARDS) risk, disease severity, inflammation level, and mortality in septic patients. 152 septic patients were consecutively included and surveillance was conducted daily to identify ARDS occurrence. Severity and organ failure of sepsis were assessed by APACHE II score and SOFA score respectively, and the in-hospital mortality was calculated. Patients' blood samples were extracted. Lnc-MALAT1 expression and inflammatory cytokines levels were detected using real-time qPCR and ELISA assay respectively. The incidence of ARDS was 27.0%. Lnc-MALAT1 expression was increased in ARDS patients compared to non-ARDS patients, and it could distinguish ARDS from non-ARDS by ROC with AUC of 0.674 (95% CI: 0.581-0.766). Multivariate logistic regression analysis displayed that lnc-MALAT1 high expression, increased age, higher proportion of smoking and COPD were independent factors for predicting elevated ARDS risk. Lnc-MALAT1 expression was positively correlated with APACHE II score, SOFA score, and inflammatory factors levels including C-reactive protein, procalcitonin, TNF-α, IL-1β, IL-6 and IL-17. Furthermore, the mortality was 30.9%, and lnc-MALAT1 expression was elevated in non-survivors compared to survivors, presenting a good predictive value for high mortality by ROC with AUC of 0.651 (95% CI: 0.555-0.747). Lnc-MALAT1 high expression predicts increased ARDS risk, and correlates with severe disease condition and raised mortality in sepsis patients.

Keywords: lncRNA-MALAT1, sepsis, ARDS, disease severity, inflammation, mortality

Introduction

Sepsis is characterized by a dysregulated inflammatory response to infectious pathogens and leads to life-threatening organ dysfunction [1, 2]. Regarding sepsis-induced organ dysfunction, it is considered to result from interaction among pathogens, blood and endothelial surface at the level of microcirculation, which usually leads to a lack of microvascular endothelial barrier integrity and impairment of the blood-endothelial interface [3-5]. As one of the most common sepsis-induced organ dysfunctions, acute respiratory distress syndrome (ARDS) occurs in nearly 200,000 patients yearly and is one of the leading causes of death in the critically ill sepsis patients in intensive care units (ICU) around the world [6-8]. In recent years, a growing number of efforts in disease management such as lung protective ventilation, prone positioning, use of neuromuscular blockade, and extracorporeal membrane oxygenation have been applied for these patients, while the mortality rate still remains high, with mortality of 20%-30% in sepsis patients and mortality more than 40% in ARDS patients, which poses a challenge for the clinician [7, 9-12]. Emerging studies have revealed that timely treatment of sepsis and early identification of sepsis patients at risk of ARDS may contribute to decreasing the disease development and improving prognosis. Application of specific biomarkers has been recognized as an efficient strategy that predicts disease risk or progression [9, 13].

Therefore, exploration of novel and sensitive biomarkers is urgently needed to monitor ARDS risk and disease progression in sepsis.

Long non-coding RNA (lncRNA), which comprises more than 200 nucleotides with limited protein-coding ability, has been reported to play a role in genomic regulation by diverse mechanisms (such as epigenetic regulation, transcription modulation and post-transcription modulation) [14]. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (lnc-MALAT1), located on human chromosome 11q13.1, was originally known as a lncRNA that typically exists in non-small cell lung cancer patients who have elevated metastasis risk [2, 15]. In recent years, lnc-MALAT1 was revealed in some *in vivo* or *in vitro* experiments to exacerbate a pro-inflammatory effect in some diseases such as diabetes mellitus and renal ischemia/reperfusion-injury, and it wasd found upregulated in heart tissue of a sepsis model, indicating that lnc-MALAT1 might have influence in inflammation-related diseases, including sepsis [2, 16-18]. Also, lnc-MALAT1 has become attractive in the investigation of pathology in lung diseases, such as the effect of lnc-MALAT1 knockdown on ameliorating histopathologic changes in lung injury and repressing proliferation of human pulmonary artery smooth muscle cells in hypoxia-induced pulmonary hypertension [19, 20]. Furthermore, our preliminary investigations with small sample size found that lnc-MALAT1 high expression was associated with worse disease severity and inflammation in sepsis patients. Considering the likely pro-inflammatory effect of lnc-MALAT1 in sepsis and its participation in the pathology of lung injury, we hypothesized that lnc-MALAT1 might play a role in the mechanism of ARDS in sepsis and have the potential to serve as a diagnostic biomarker for ARDS, while related evidence was rarely reported. Based on the above, we conducted this study to explore the correlation of lnc-MALAT1 expression with ARDS risk, disease severity, inflammation level, and mortality in septic patients.

Methods

Participants

One hundred and fifty-two septic patients who were admitted to ICUs of The Central Hospital of Wuhan between January 2016 and June 2018 were consecutively included in this study. All enrolled patients were diagnosed as septic according to the criteria of 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [21], and patients were excluded from study if they were younger than 18 years, transferred from other hospitals, suffering from cancers or hematologic malignancies, pregnant or lactating. This study was approved by the Ethics Committee of the Hospital. All patients or their guardians signed informed consents before enrollment.

Clinical data collection

Clinical data of patients were recorded after enrollment, which consisted of (1) basic characteristics: age, gender, body mass index (BMI) and smoking; (2) chronic comorbidities: chronic obstructive pulmonary disease (COPD), cardiomyopathy, chronic kidney failure and cirrhosis); (3) laboratory testing: serum creatinine (Scr), albumin, white blood cell (WBC), C-reactive protein (CRP) and procalcitonin (PCT).

Disease severity assessment

Severity of sepsis was assessed by use of acute physiology and chronic health evaluation (APACHE) II score, which was based on the initial score of 12 components, and the evaluation consisted of three parts, including physiologic scores ranging from 0 to 60 points, age scores ranging from 0 to 6 points and comorbid disease scores ranging from 0 to 5 points, with a total score ranging from 0 to 71 points. A higher score indicated more serious disease [22]. Besides, organ failure of septic patients was evaluated using the sequential organ failure assessment (SOFA) score, which scored 1 to 4 points to each of the levels of dysfunction of six organs including respiratory, circulatory, renal, hematological, hepatic and central nervous systems. Total score ranged from 0 to 24 points, and a higher score was associated with higher severity [23]. Both APACHE II score and SOFA score were evaluated within 24 hours after ICU admission.

Measurements

Septic patients' blood samples were extracted within 24 hours after ICU admission. Subsequently, the plasma was isolated by centrifugation at 4°C, 2500 g for 15 minutes and then stored at -70°C until detection. Lnc-MALAT1 relative expression in plasma was determined

using real-time quantitative polymerase chain reaction (RT-qPCR); the levels of inflammatory cytokines (tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), IL-6 and IL-17) in plasma were measured by enzyme-linked immunosorbent assay (ELISA), which was performed by use of human TNF-α, IL-1β, IL-6 and IL-17 ELISA kits (Abcam, Massachusetts, USA) according to kit instructions.

RT-qPCR

Total RNA was extracted from plasma with TRIzol reagent (Invitrogen, USA). Then, transcription to cDNA was performed using PrimeScript™ RT Master Mix reagent (Takara, China). Subsequently, SYBR® Green Realtime PCR Master Mix (Toyobo, Japan) was applied for qPCR, and the qPCR amplification was performed. The lnc-MALAT1 expression was calculated by the $2^{-\Delta\Delta CT}$ method and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference. Primers used in the present study were as follows: lnc-MALAT1, forward: TCCTAAGGTCAAGAGAAGTGTCAG, reverse: GTGGCGATGTGGCAGAGAA; GAPDH, forward: GAGTCCACTGGCGTCTTCAC, reverse: ATCTTGAGGCTGTTGTCATACTTCT.

Follow up

Surveillance was conducted daily for enrolled patients after admission, aimed at screening for ARDS development according to the Berlin definition of ARDS [24]. All enrolled patients were followed up until death in the hospital or discharge, and numbers of survivors and non-survivors were calculated.

Statistical analysis

Statistical analysis was performed by use of SPSS 22.0 software (SPSS Inc, Chicago, USA). Figures were made using GraphPad Prism 7.01 software (GraphPad Software, San Diego, USA). Quantitative data were described as mean ± standard deviation if normally distributed or median (25^{th}-75^{th} quantiles) if not normally distributed, and comparison between two groups was determined by *t* test or Wilcoxon rank sum test. Qualitative data were expressed as count or count (percentage), and comparison between two groups was determined by Chi-square test. Spearman's rank correlation test was performed for correlation analysis. Receiver-operating characteristic (ROC) curves and the area under the curve (AUC) were used to assess the value of lnc-MALAT1 relative expression in distinguishing ARDS from non-ARDS as well as survivors from non-survivors. Univariate and multivariate logistic regression model analyses were performed to assess factors predicting ARDS and mortality. Reported statistical significance levels were all two-sided. *P* value < 0.05 was considered significant.

Results

Baseline characteristics in sepsis patients

Totally 152 sepsis patients were enrolled in our study (including 112 males and 40 females), with mean age of 59.7 ± 11.2 years (**Table 1**). There were 41 patients who developed ARDS and 111 patients did not, and the incidence of ARDS was 27.0%. Higher values of age (*P* = 0.017), APACHE II score (*P* < 0.001), SOFA score (*P* = 0.015), Scr (*P* = 0.033), CRP (*P* = 0.002) and PCT (*P* = 0.023), as well as increased proportion of smoking (*P* = 0.001) and COPD (*P* = 0.022) were observed in ARDS group compared to non-ARDS group. The other detailed information is listed in **Table 1**.

Correlation of lnc-MALAT1 expression with ARDS risk in sepsis patients

Lnc-MALAT1 expression in ARDS group was remarkably increased than that in non-ARDS group (*P* = 0.001) (**Figure 1A**). ROC curve showed that lnc-MALAT1 presented a good predictive value for ARDS risk in sepsis patients with AUC of 0.674 (95% CI: 0.581-0.766). At the best cut off point where the sum of sensitivity and specificity reached the maximum value, lnc-MALAT1 expression was 1.808, and the sensitivity as well as specificity were 65.9% and 68.5% respectively (**Figure 1B**).

Analysis of factors predicting ARDS risk in sepsis patients

Univariate logistic regression analysis displayed that lnc-MALAT1 expression (P = 0.007) was positively correlated with ARDS risk; meanwhile, age (≥ 60 years) (*P* = 0.009), smoking (yes vs. no) (*P* = 0.026), COPD (yes vs. no) (*P* = 0.001), CRP (≥ 90.3 mg/L) (*P* = 0.047) and APACHE II score (≥ 16 vs. < 16) (*P* = 0.011) were associated with higher ARDS risk in sepsis

Table 1. Characteristics of septic patients

Parameter	Total sepsis patients (N = 152)	ARDS patients (N = 41)	Non-ARDS patients (N = 111)	*P* value*
Characteristics				
Age (years)	59.7 ± 11.2	63.2 ± 11.0	58.4 ± 11.1	0.017
Gender (male/female)	112/40	32/9	80/31	0.458
BMI (kg/m^2)	22.9 ± 4.8	23.4 ± 4.7	22.7 ± 4.8	0.482
Smoking (n/%)	49 (32.2)	22 (53.7)	27 (24.3)	0.001
Chronic comorbidities				
COPD (n/%)	21 (13.8)	10 (24.4)	11 (9.9)	0.022
Cardiomyopathy	54 (35.5)	15 (36.6)	39 (35.1)	0.868
Chronic kidney failure	14 (9.2)	4 (9.8)	10 (9.0)	0.888
Cirrhosis	28 (18.4)	6 (14.6)	22 (19.8)	0.464
Disease severity				
APACHE II score	16.3 ± 6.0	19.6 ± 5.5	15.1 ± 5.8	< 0.001
SOFA score	8.8 ± 4.0	10.1 ± 4.2	8.3 ± 3.9	0.015
Laboratory testing				
Scr (mg/dL)	1.7 (1.2-2.3)	1.8 (1.3-3.5)	1.6 (1.1-2.3)	0.033
Albumin (g/L)	27.1 (21.5-36.7)	27.8 (20.6-38.3)	27.1 (21.6-36.7)	0.680
WBC (× 10^9/L)	15.2 (3.0-28.6)	19.1 (3.3-31.8)	12.6 (2.9-28.3)	0.287
CRP (mg/L)	90.3 (50.2-121.6)	117.5 (67.7-197.1)	81.1 (48.6-109.6)	0.002
PCT (ng/mL)	13.1 (7.7-21.7)	18.5 (8.1-31.7)	10.8 (7.3-19.2)	0.023
Inflammatory cytokines				
TNF-α (pg/mL)	173.0 (109.9-247.4)	211.7 (109.6-269.5)	158.3 (109.0-230.6)	0.157
IL-1β (pg/mL)	11.3 (4.9-21.4)	13.0 (5.3-33.0)	11.0 (4.6-18.9)	0.073
IL-6 (pg/mL)	63.7 (37.2-119.2)	72.2 (45.1-163.5)	61.5 (34.7-112.2)	0.099
IL-17 (pg/mL)	144.6 (68.5-218.0)	164.4 (85.6-208.9)	136.9 (64.7-229.4)	0.629

Data are presented as mean value ± standard deviation, count (percentage) or median (25th-75th quantiles). *All comparisons between ARDS patients and non-ARDS patients were determined by *t* test, Chi-Square test, or Wilcoxon rank sum test. *P* value < 0.05 was considered significant. BMI: body mass index; COPD: chronic obstructive pulmonary disease; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; Scr: serum creatinine; WBC: white blood cells; CRP: C-reactive protein; PCT: procalcitonin; TNF-α: tumor necrosis factor-α; IL: interleukin.

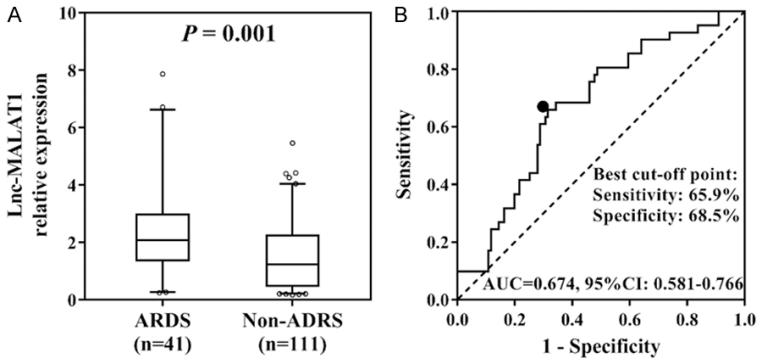

Figure 1. Lnc-MALAT1 expression in ARDS and non-ARDS patients and ROC curve. Lnc-MALAT1 expression was elevated in the ARDS group compared to non-ARDS group (A). ROC curve showed that lnc-MALAT1 had a good predictive value for ARDS (B). Comparison of lnc-MALAT1 expression was determined by Wilcoxon rank sum test. ROC curve was drawn to evaluate the predictive value of ARDS risk. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; ARDS, acute respiratory distress syndrome; ROC curve, receiver-operating characteristic curve. P < 0.05 was considered significant.

patients (**Table 2**). Furthermore, multivariate logistic regression analysis revealed that lnc-MALAT1 high expression (*P* = 0.002) was an independent factor for predicting elevated ARDS risk in sepsis patients, as well as age (≥ 60 years) (*P* = 0.021), smoking (yes vs. no) (*P* = 0.001) and COPD (yes vs. no) (*P* = 0.002).

Correlation of lnc-MALAT1 expression with disease severity in sepsis patients

Correlation of lnc-MALAT1 expression with disease severity including APACHE II score (**Figure 2A**) and SOFA score

Table 2. Univariate and multivariate logistic regression model analysis of factors predicting ARDS

Items	Univariate logistic regression				Multivariate logistic regression			
	P value	OR	95% CI		*P* value	OR	95% CI	
			Lower	Higher			Lower	Higher
Lnc-MALAT1 (high vs. low)	0.007	2.827	1.325	6.029	0.002	7.923	2.107	29.798
Age (≥ 60 years vs. < 60 years)	0.009	2.725	1.278	5.810	0.021	3.428	1.199	9.799
Gender (male vs. female)	0.360	0.692	0.315	1.522	0.788	0.851	0.261	2.772
BMI (≥ 22.4 kg/m^2 vs. < 22.4 kg/m^2)	0.203	1.602	0.776	3.306	0.578	1.340	0.478	3.754
Smoking (yes vs. no)	0.026	2.332	1.109	4.903	0.001	7.859	2.337	26.432
COPD (yes vs. no)	0.001	5.262	2.041	13.568	0.002	10.655	2.386	47.587
Cardiomyopathy (yes vs. no)	0.868	1.065	0.505	2.245	0.378	0.613	0.207	1.820
Chronic kidney failure (yes vs. no)	0.888	1.092	0.323	3.695	0.714	1.346	0.275	6.594
Cirrhosis (yes vs. no)	0.466	0.694	0.259	1.855	0.374	2.205	0.386	12.593
Scr (≥ 1.7 mg/dL vs. < 1.7 mg/dL)	0.150	1.710	0.824	3.547	0.564	1.489	0.385	5.755
Albumin (≥ 27.1 g/L vs. < 27.1 g/L)	0.855	1.069	0.522	2.189	0.383	2.066	0.404	10.563
WBC (≥ 15.2*10^9/L vs. < 15.2*10^9/L)	0.362	1.398	0.680	2.874	0.490	1.870	0.316	11.062
CRP (≥ 90.3 mg/L vs. < 90.3 mg/L)	0.047	2.115	1.012	4.420	0.737	1.287	0.295	5.606
PCT (≥ 13.2 ng/mL vs. < 13.2 ng/mL)	0.083	1.906	0.918	3.957	0.156	2.506	0.705	8.913
APACHE II score (≥ 16 vs. < 16)	0.011	2.777	1.267	6.086	0.078	4.026	0.856	18.941
SOFA score (≥ 6 vs. < 6)	0.170	1.968	0.749	5.171	0.574	0.595	0.097	3.645
TNF-α (≥ 173.0 pg/mL vs. < 173.0 pg/mL)	0.203	1.602	0.776	3.306	0.064	3.999	0.921	17.375
IL-1β (≥ 11.3 pg/mL vs. < 11.3 pg/mL)	0.855	1.069	0.522	2.189	0.589	0.666	0.152	2.910
IL-6 (≥ 63.7 pg/mL vs. < 63.7 pg/mL)	0.584	1.222	0.596	2.506	0.269	0.512	0.156	1.678
IL-17 (≥ 144.55 pg/mL vs. < 144.55 pg/mL)	0.584	1.222	0.596	2.506	0.513	0.644	0.173	2.405

Factors predicting ARDS were determined by univariate and multivariate logistic regression analyses. *P* value < 0.05 was considered significant. All continuous variables were classified by the median values. ARDS: acute respiratory distress syndrome; BMI: body mass index; COPD: chronic obstructive pulmonary disease; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; Scr: serum creatinine; WBC: white blood cells; CRP: C-reactive protein; PCT: procalcitonin; TNF-α: tumor necrosis factor-α; IL: interleukin.

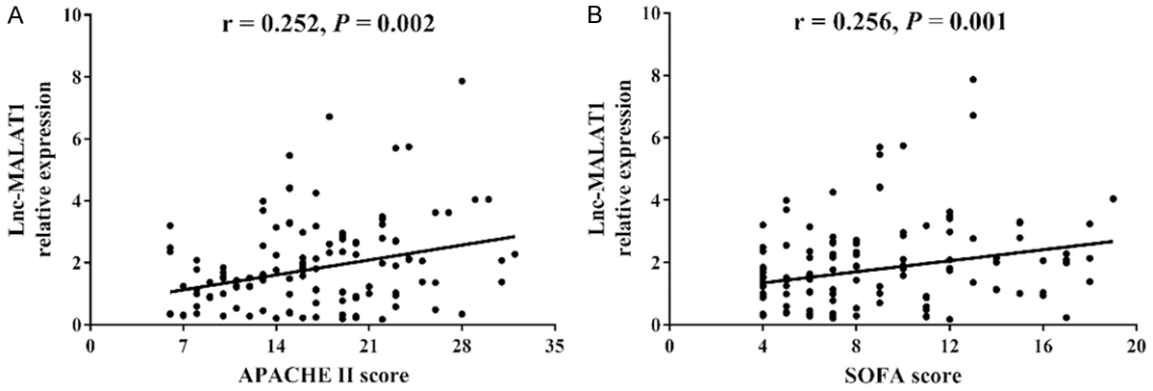

Figure 2. lnc-MALAT1 expression is correlated with disease severity. Lnc-MALAT1 expression is positively correlated with APACHE II score (A). Increased lnc-MALAT1 expression was correlated with higher SOFA score (B). Correlation of lnc-MALAT1 relative expression with severity of the patients were determined by Spearman rank correlation test. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; APACHE II score, acute physiology and chronic health evaluation II score; SOFA score, sequential organ failure assessment score. P < 0.05 was considered significant.

(**Figure 2B**) was assessed by Spearman rank correlation test, which disclosed that lnc-MALAT1 expression was positively correlated with APACHE II score (r = 0.252, *P* = 0.002) and

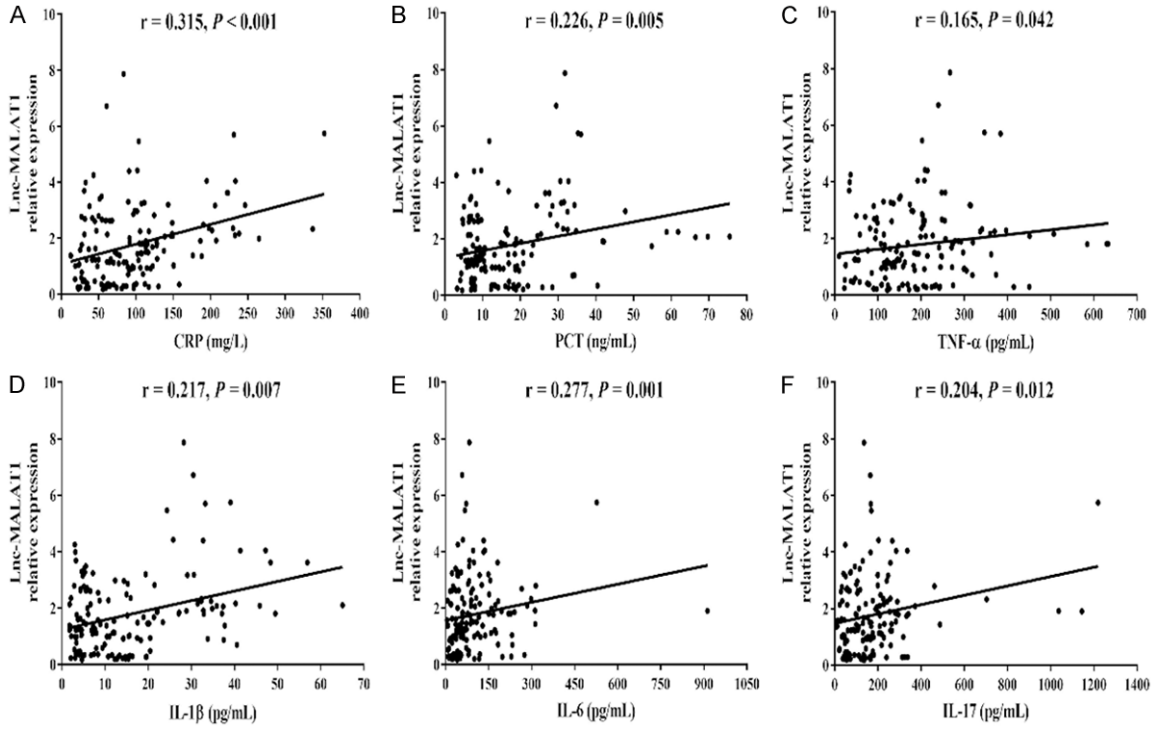

Figure 3. Increased lnc-MALAT1 expression is correlated with higher inflammation level. Lnc-MALAT1 expression is positively correlated with CRP (A), PCT (B), TNF-α (C), IL-1β (D), IL-6 (E) and IL-17 (F). Correlation of lnc-MALAT1 relative expression with inflammation levels was determined by Spearman correlation analyses. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; CRP, C-reactive protein; PCT, procalcitonin; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-17, interleukin-17. P < 0.05 was considered significant.

SOFA score (r = 0.256, *P* = 0.001) in sepsis patients.

Correlation of lnc-MALAT1 expression with inflammatory factor levels in sepsis patients

Lnc-MALAT1 expression was positively correlated with inflammatory factor levels including CRP (r = 0.315, *P* < 0.001) (**Figure 3A**), PCT (r = 0.226, *P* = 0.005) (**Figure 3B**), TNF-α (r = 0.165, *P* = 0.042) (**Figure 3C**), IL-1β (r = 0.217, *P* = 0.007) (**Figure 3D**), IL-6 (r = 0.227, *P* = 0.001) (**Figure 3E**) and IL-17 (r = 0.204, *P* = 0.012) (**Figure 3F**) in septic patients.

Prognostic value of lnc-MALAT1 for mortality in septic patients

The mortality of septic patients was 30.9% in our study, and lnc-MALAT1 expression in non-survivor group (N = 47) was elevated compared to survivor group (N = 105) (*P* = 0.003) (**Figure 4A**). Moreover, the ROC curve disclosed that lnc-MALAT1 had a good predictive value of high mortality in sepsis patients (AUC = 0.651, 95% CI: 0.555-0.747), with the sensitivity and specificity of 88.6% and 38.3% respectively at the best cut-off point (**Figure 4B**).

Analysis of factors affecting mortality in septic patients

Univariate logistic regression analysis disclosed that lnc-MALAT1 high expression was associated with raised mortality (*P* = 0.024). Moreover, Scr (≥ 1.7 mg/dL) (*P* = 0.006), CRP (≥ 90.3 mg/L) (*P* < 0.001), PCT (≥ 13.2 ng/mL) (*P* = 0.043), APACHE II score (≥ 16) (*P* = 0.007), TNF-α (≥ 173.0 pg/mL) (*P* = 0.009) and IL-1β (≥ 11.3 pg/mL) (*P* < 0.001) were correlated with elevated mortality in septic patients as well (**Table 3**). In the multivariate logistic regression analysis, it was revealed that only CRP (≥ 90.3 mg/L) (*P* = 0.031) was an independent factor predicting worse mortality in septic patients.

Discussion

In this study, we observed that: (1) The incidence of ARDS was 27.0%, and lnc-MALAT1

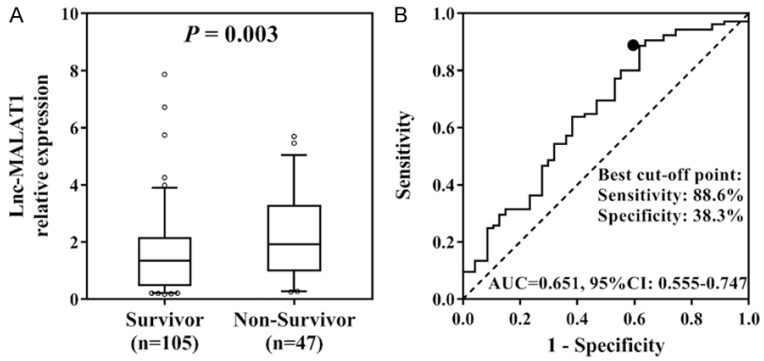

Figure 4. Increased lnc-MALAT1 expression is associated with higher mortality. Compared to the survivor group, elevated lnc-MALAT1 expression was observed in the non-survivor group (A). Lnc-MALAT1 expression presented with a good value for predicting mortality (B). Comparison between two groups was determined by Wilcoxon rank sum test; ROC curve was performed to assess the predictive value of lnc-MALAT 1 for mortality. Lnc-MALAT1, long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1; ROC curve, receiver-operating characteristic curve. $P < 0.05$ was considered significant.

was overexpressed in ARDS group compared to non-ARDS group. Moreover, its high expression was an independent predictive factor for elevated ARDS risk in sepsis patients, as well as increased age and higher proportion of smoking and COPD; (2) lnc-MALAT1 high expression was correlated with increased disease severity, elevated inflammation level, and raised mortality in sepsis patients.

LncRNAs have been revealed to participate in a number of biologicaprocesses, and their major mechanisms include the following: (1) influence gene transcription; (2) organize RNA-protein complex with protein and affect mRNA cleavage; (3) manage protein activity via binding to proteins; (4) regulate epigenetic changes; (5) change the cellular localization of functional proteins; (6) serve as ceRNA [15]. As to lnc-MALAT1, it has been found dysregulated and influences biologic or pathologic processes in several inflammation-related diseases [2, 17, 19, 20]. For instance, a study shows that lnc-MALAT1 is overexpressed in septic mice, and it aggravates cardiac inflammation as well as dysfunction by interacting with miR-125b and p38 mitogen-activated protein kinase (MAPK)/nuclear factor κB (NFκB) [17]. Another study discloses that lnc-MALAT1 is overexpressed in the LPS-induced cardiac microvascular endothelial cells and the heart of sepsis rats [2]. In addition, lnc-MALAT1 also has been reported to participate in the mechanisms of some lung diseases [19, 20]. An interesting study displays that lnc-MALAT1 knockdown suppresses the inflammatory responses by up-regulating miR-146a in LPS-induced acute lung injury rats [19]. Another study shows that depletion of lnc-MALAT1 represses migration and proliferation of human pulmonary artery smooth muscle cells and also ameliorates heart hypertrophy in mice with hypoxia-induced pulmonary hypertension, and consequently reduces the vascular remodeling and right heart failure in pulmonary hypertension [20]. Therefore, these previous data indicate that lnc-MALAT1 plays a pro-inflammatory role and is involved in the pathology of sepsis as well as some lung diseases, which might provide support to investigations of lnc-MALAT1 in sepsis and its predictive risk for ARDS.

Regarding the explorations of lnc-MALAT1 in clinical practice, most of them are observed in the field of cancer, which disclose that lnc-MALAT1 is dysregulated and has extensive potential for application of the early detection and diagnosis of these cancer, especially lung cancer [25-28]. Apart from cancers, lnc-MALAT1 is also found to play a role in some inflammation-related diseases [18, 29]. For example, lnc-MALAT1 is overexpressed in plasma samples and kidney biopsies of human hypoxic/ischemic kidney injury. Moreover, it presents excellent diagnostic value for diabetic retinopathy [18, 29]. Nevertheless, investigation of lnc-MALAT1 expression in sepsis and its diagnostic value for ARDS risk in sepsis patients are seldomly reported. In our study, we found that lnc-MALAT1 was increased in ARDS patients compared with the non-ARDS, and lnc-MALAT1 high expression predicted ARDS risk in sepsis patients, which might be because: lnc-MALAT1 participates in the interplay among cytokines, inflammation mediators, and oxidative stress responses, and these interactions induce cytoskeletal remodeling or cell-to-cell connection alteration, and consequently lead to dysfunction of lung alveolar epithelial cells

Table 3. Univariate and multivariate logistic regression model analysis of factors affecting mortality

Items	Univariate logistic regression				Multivariate logistic regression			
	P value	OR	95% CI		*P* value	OR	95% CI	
			Lower	Higher			Lower	Higher
Lnc-MALAT1 (high vs. low)	0.024	2.263	1.114	4.599	0.474	1.433	0.535	3.840
Age (≥ 60 years vs. < 60 years)	0.676	1.158	0.582	2.307	0.754	1.157	0.465	2.876
Gender (male vs. female)	0.586	1.249	0.561	2.777	0.436	1.501	0.541	4.164
BMI (≥ 22.4 kg/m^2 vs. < 22.4 kg/m^2)	0.861	1.064	0.534	2.116	0.484	1.387	0.555	3.464
Smoking (yes vs. no)	0.420	0.733	0.344	1.560	0.985	1.009	0.387	2.636
COPD (yes vs. no)	0.922	1.050	0.397	2.774	0.566	1.442	0.413	5.033
Cardiomyopathy (yes vs. no)	0.534	0.793	0.382	1.646	0.919	1.050	0.409	2.699
Chronic kidney failure (yes vs. no)	0.684	1.270	0.401	4.018	0.431	1.788	0.421	7.600
Cirrhosis (yes vs. no)	0.454	0.700	0.275	1.782	0.303	2.095	0.513	8.551
Scr (≥ 1.7 mg/dL vs. < 1.7 mg/dL)	0.006	2.736	1.326	5.646	0.095	2.985	0.825	10.793
Albumin (≥ 27.1 g/L vs. < 27.1 g/L)	0.381	0.734	0.368	1.465	0.711	1.315	0.308	5.612
WBC (≥ 15.2*10^9/L vs. < 15.2*10^9/L)	0.116	1.750	0.871	3.516	0.880	0.878	0.162	4.758
CRP (≥ 90.3 mg/L vs. < 90.3 mg/L)	< 0.001	4.553	2.121	9.774	0.031	3.941	1.131	13.727
PCT (≥ 13.2 ng/mL vs. < 13.2 ng/mL)	0.043	2.066	1.023	4.175	0.478	0.667	0.217	2.044
APACHE II score (≥ 16 vs. < 16)	0.007	2.769	1.315	5.833	0.118	2.596	0.785	8.583
SOFA score (≥ 6 vs. < 6)	0.063	2.485	0.952	6.488	0.997	0.997	0.237	4.203
TNF-α (≥ 173.0 pg/mL vs. < 173.0 pg/mL)	0.009	2.583	1.262	5.289	0.792	0.854	0.264	2.763
IL-1β (≥ 11.3 pg/mL vs. < 11.3 pg/mL)	< 0.001	3.923	1.855	8.296	0.074	3.178	0.896	11.277
IL-6 (≥ 63.7 pg/mL vs. < 63.7 pg/mL)	0.381	1.362	0.682	2.718	0.286	0.565	0.198	1.612
IL-17 (≥ 144.55 pg/mL vs. < 144.55 pg/mL)	0.055	1.988	0.985	4.015	0.769	1.185	0.383	3.668

Factors affecting mortality were determined by univariate and multivariate logistic regression analyses. *P* value < 0.05 was considered significant. All continuous variables were classified by the median values. BMI: body mass index; COPD: chronic obstructive pulmonary disease; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; Scr: serum creatinine; WBC: white blood cells; CRP: C-reactive protein; PCT: procalcitonin; TNF-α: tumor necrosis factor-α; IL: interleukin.

and lung capillary endothelial cells. Thus the lnc-MALAT1 high expression predicted ARDS risk in sepsis patients [30].

As to the correlation of lnc-MALAT1 with disease severity, most of studies focus on cancer patients (including osteosarcoma, esophageal squamous cell carcinoma and breast cancer). They reveal thatcancer patients with lnc-MALAT1 high expression suffer enhanced disease progression compared to patients with lnc-MALAT1 low expression [31-33]. Limited studies investigate the influence of lnc-MALAT1 in inflammation-related diseases; for instance, one study discloses that lnc-MALAT1 enhances inflammation levels in patients with nonalcoholic steatohepatitis and chronic hepatitis C [34, 35]. However, there is no evidence about the predictive value of lnc-MALAT1 for severity or inflammation in septic patients. In our present study, we observed that lnc-MALAT1 high expression was associated with worse disease severity (including higher APACHE II score and SOFA score) and raised inflammation levels (including increased CRP, PCT, TNF-α, IL-1β, IL-6 and IL-17 levels), and the reasons might be as follows: (1) lnc-MALAT1 might have interaction with p38 MAPK/NFκB, thereby promotes the release of inflammatory cytokines and aggravates the inflammation levels in sepsis patients [17]; (2) lnc-MALAT1 might sponge miRNAs including miR-125b, miR-146a, miR-320a and miR-22-3p, which are found to protect against inflammation or dysfunction of endothelial cells, thus it aggravates organ dysfunction and enhances progression in sepsis patients [17, 19, 36, 37]. Regarding the prognostic value of lnc-MALAT1, it has been identified as a biomarker predicting prognosis in diverse cancers, whereas it has not yet been reported in sepsis [31, 33, 37]. In this study, we found that lnc-MALAT1 expression was elevated in the non-

survivors compared to the survivors, and its high expression predicted increased mortality in sepsis patients. This might be partially due to the promotion on inflammatory cytokines of lnc-MALAT1, which accelerates the disease progression and further increases the mortality in sepsis patients [17, 19]. Besides, lnc-MALAT1 might facilitate resistance to therapy in sepsis, thereby reducing treatment efficacy and leading to high mortality. The evidence is limited, and further mechanisms underlying the correlation of lnc-MALAT1 high expression with increased mortality need exploration [32, 38].

Some limitations existed in our study: (1) sample size in our study (of 152 sepsis patients) was relatively low, which might have insufficient statistical efficacy; (2) this was a single-center study, which might present insufficient representation; (3) detailed mechanisms of lnc-MALAT1 in sepsis and ARDS in sepsis patients had not yet been fully elucidated, and further study is needed.

In conclusion, lnc-MALAT1 high expression predicts increased ARDS risk, and correlates with worse disease severity, elevated inflammation, and raised mortality in sepsis patients.

Disclosure of conflict of interest

None.

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