Received: 22 May 2019 | Revised: 1 July 2020 | Accepted: 3 July 2020

DOI: 10.1002/jcla.23488

RESEARCH ARTICLE

WILEY

The value of circulating long non-coding RNA maternally expressed gene 3 as a predictor of higher acute respiratory distress syndrome risk and 28-day mortality in sepsis patients

Xiaoling Wu | **Dan Chen** | **Li Yu**

Department of Intensive Care Unit, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Correspondence
Li Yu, Department of Intensive Care Unit, the Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, 26 Shengli Street, Wuhan 430014, China.
Email: yuli_2020@126.com

Funding information
Natural Science Foundation of Hubei Province, Grant/Award Number: 2017CFC851

Abstract

Objective: This study was to evaluate the potential of long non-coding RNA maternally expressed gene 3 (lncRNA MEG3) in predicting acute respiratory distress syndrome (ARDS) risk and its correlation with prognosis in sepsis patients.

Methods: The plasma samples were obtained from 112 sepsis patients within 24 hours after admission and 100 healthy controls (HCs) at enrollment. The lncRNA MEG3 expression in plasma samples was determined by RT-qPCR. In sepsis patients, ARDS occurrence was assessed based on Berlin definition of ARDS and 28-day mortality risk was evaluated.

Results: LncRNA MEG3 expression was increased in sepsis patients compared with HCs. During 28-day duration, 30 sepsis patients occurred ARDS and 82 sepsis patients did not occur ARDS. LncRNA MEG3 expression was elevated in ARDS sepsis patients compared with non-ARDS sepsis patients, then the following receiver-operating characteristic (ROC) curve analysis disclosed that lncRNA MEG3 predicted ARDS risk (area under the curve (AUC) = 0.775), which was further validated as an independent risk factor by multivariate logistic regression. Furthermore, lncRNA MEG3 was positively correlated with chronic obstructive pulmonary disease, respiratory infection, acute physiology and chronic health evaluation II score, sequential organ failure assessment score, white blood cell, and C-reactive protein, while negatively correlated with albumin in sepsis patients. Additionally, lncRNA MEG3 was elevated in 28-day deaths compared with 28-day survivors, and it predicted 28-day mortality risk in sepsis patients (AUC = 0.708) by ROC curve analysis.

Conclusion: LncRNA MEG3 might represent as a valuable biomarker for individualizing prevention strategies against ARDS and improving prognosis in sepsis.

KEYWORDS

ARDS, disease severity, LncRNA MEG3, prognosis, sepsis

Xiaoling Wu and Dan Chen contributed equally to this work.

1 | INTRODUCTION

Sepsis is a major global healthy issue characterized by dysregulated immune and inflammatory responses to infecting pathogens as well as life-threatening multiple organ dysfunction.[1] As a common complication of sepsis, acute respiratory distress syndrome (ARDS) clinically presents as acute pulmonary edema, reduced lung compliance, refractory hypoxemia, and ultimately respiratory failure with a high mortality rate of approximately 40%.[2,3] In addition, ARDS also occurs in the setting of pneumonia, aspiration of gastric/oral/esophageal contents or trauma.[3] Despite the substantial progress in the optimization of supportive care for ARDS (including lung-protective mechanical ventilation and fluid-conservative therapy) after decades of dedicated effort in experimental and clinical investigations, the search for specific pharmacological therapy that effectively treat ARDS has been fruitless due to clinical/biological heterogeneity of the syndrome and a lack of specific/effective biomarkers for risk-stratifying ARDS patients.[3-5] In an attempt to overcome this challenge, the search of promising biomarkers for facilitating the recognition of ARDS and the improvement of clinical outcomes is necessary.

Long non-coding maternally expressed gene 3 (LncRNA MEG3), located within the imprinted DLK1-DIO3 gene cluster at chromosome 14q32.3, is initially identified as a lncRNA with the function of tumor suppressor.[6,7] Recent researches reveal that lncRNA MEG3 is involved in the regulation of lung injury through regulating multiple inflammation-relative signaling pathways and apoptosis-related pathways such as caspase-1 signaling and Janus kinases/signal transducer and activator of transcription proteins.[8-11] Meanwhile, lncRNA MEG3 exhibited the potential as a biomarker for identifying disease risk and progression of respiratory disease asthma.[12] As for sepsis, lncRNA MEG3 modulates the pulmonary inflammatory responses to affect the initiation and progression of sepsis, and it displays the clinical implication in predicting prognosis in sepsis patients.[13-15] In view of above-mentioned facts, it was speculated that lncRNA MEG3 might exhibit the clinical value for identifying ARDS risk in sepsis patients, while relevant report is lack. Therefore, the present study was to evaluate the potential of lncRNA MEG3 in predicting ARDS risk and its correlation with prognosis in sepsis patients.

2 | MATERIALS AND METHODS

2.1 | Participants

This study consecutively enrolled 112 sepsis patients from our hospital between January 2018 and September 2019. The screening criteria were as follows: (a) diagnosed as sepsis patients according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[16]; (b) age ≥ 18 years old; (c) admitted to intensive care unit (ICU) within the previous 24 hours; (d) not complicated with other fatal diseases (eg, hematologic malignancies, solid tumors, or acquired immune deficiency syndrome); (e) without immunosuppressive therapy within 3 months before enrollment; (f) not in pregnancy or lactation. In addition, 100 healthy subjects underwent health examination in our hospital during October 2019 and December 2019 were recruited as healthy controls (HCs). All HCs had age and gender matched with the sepsis patients, no obvious abnormalities in biochemical indexes, and no history of hematological malignancies, solid tumors, sepsis, or other severe infections. This study was approved by the Institutional Review Board of our hospital. All participants or their family members provided the written informed consent before enrollment.

2.2 | Date collection

Sepsis patients' clinical characteristics were recorded after admission, which included demographic characteristics, complications, primary infection site, primary organism, biochemical indexes, and disease severity. The severity of sepsis was assessed within 24 hours after admission using the acute pathologic and chronic health evaluation (APACHE) II score and sequential organ failure assessment (SOFA) score.

2.3 | Sample collection

The peripheral blood (PB) samples of sepsis patients were collected within 24 hours after admission, and the PB samples of HCs were obtained at enrollment. After collection, the PB samples were centrifuged at 3000 *g* for 15 minutes under 4°C to separate plasma. Then, the plasma samples were preserved at −80°C for next detection. The expression of lncRNA MEG3 in plasma samples was detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

2.4 | RT-qPCR

The procedures of RT-qPCR were carried out in consistent with the method described in our previous study.[17] The primers used in RT-qPCR were designed referring to the study published previously,[18] and the details were as follows: lncRNA MEG3, forward primer: GCCCTGACCTTTGCTATGCT, reverse primer: TCGACAAAGACTGACACCCC; GAPDH, forward primer: TGACCACAGTCCATGCCATCAC, reverse primer: GCCTGCTTCAC CACCTTCTTGA. Besides, the reproducibility of GAPDH and lncRNA MEG3 results was evaluated. In sepsis patients, the median Ct value of lncRNA MEG3 was 21.9 and the inter-assay CV in all samples was 2.0%; the median Ct value of GAPDH was 19.1 and the inter-assay CV in all samples was 1.2% (Table S1). In HCs, the median Ct value of lncRNA MEG3 was 22.5 and the inter-assay CV in all samples was 1.1%; the median Ct value of GAPDH was 18.4 and the inter-assay

CV in all samples was 1.7%. These findings indicated that the reproducibility was relatively good.

2.5 | Acute respiratory distress syndrome (ARDS) assessment

During hospitalization, intensive surveillance was given to the sepsis patients, and sepsis-related ADRS was monitored in time. The sepsis-related ADRS was assessed from three items according to Berlin definition of ARDS,[19] which included (a) timing, within 1 week of a known clinical insult or new or worsening respiratory symptoms; (b) chest imaging, bilateral opacities (not fully explained by effusions, lobar/lung collapse, or nodules); (c) origin of edema, respiratory failure (not fully explained by cardiac failure or fluid overload). All sepsis patients were followed up to death or 28 days after admission. During follow-up, survival status of the sepsis patients was recorded, and all sepsis patients were classified as 28-day survivors and 28-day deaths. Meanwhile, accumulating mortality was calculated from the date of admission to the date of death or completion of the 28-day follow-up.

2.6 | Statistical analysis

SPSS 24.0 software (IBM) was used for statistical analyses, and GraphPad Prism 7.01 software (GraphPad software Inc.) was used to plot figures. Continuous data were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical data were described as number (percentage). Student's *t* test, chi-square test, or Wilcoxon rank-sum test was used to compare the difference of variables between two groups. Spearman's rank correlation test was used to analyze the correlation between two variables. Receiver-operating characteristic (ROC) curve was plotted, and the area under the curve (AUC), the sensitivity and specificity at the best cut-off point were used to assess the ability of variables in distinguishing different subjects. Kaplan-Meier method was used to describe accumulating mortality, and the difference of accumulating mortality between two groups was determined by the log-rank test. Univariate logistic regression model was used to analyze the risk factors of ARDS in sepsis patients, and forward stepwise multivariate logistic regression model was used to predict the independent risk factors of ARDS in sepsis patients. *P* value < .05 was considered statistically significant. Notably, we initially performed different ways of multivariate logistic regression analyses (including enter, backward and forward stepwise multivariate logistic regression analyses). By multivariate logistic regression analysis with "Enter" method, it did not work due to the relatively small sample size and the relatively large number of covariates. Then, we performed forward stepwise multivariate logistic regression analysis and backward stepwise multivariate logistic regression analysis, respectively, which found that the results were similar. Hence, we consulted a biostatistician and chosen forward stepwise multivariate logistic regression analysis.

TABLE 1 Patient's characteristics

Items	Sepsis patients (N = 112)
Age (y), mean ± SD	54.6 ± 11.0
Gender, No. (%)	
Female	46 (41.1)
Male	66 (58.9)
BMI (kg/m^2), mean ± SD	22.6 ± 3.6
Smoke, No. (%)	39 (34.8)
COPD, No. (%)	22 (19.6)
Cardiomyopathy, No. (%)	49 (43.8)
Chronic kidney failure, No. (%)	18 (16.1)
Cirrhosis, No. (%)	22 (19.6)
Primary infection site, No. (%)	
Abdominal infection	40 (35.7)
Respiratory infection	25 (22.3)
Skin and soft tissue infection	22 (19.6)
Blood stream infection	12 (10.7)
CNS infection	6 (5.4)
Other infections	7 (6.3)
Primary organism, No. (%)	
G− bacteria	61 (54.5)
G+ bacteria	25 (22.3)
Anaerobes	12 (10.7)
Fungus	7 (6.3)
Mycoplasmas	5 (4.5)
Total culture negative	22 (19.6)
Biochemical indexes, median (IQR)	
Scr (mg/dL)	1.8 (1.2-2.6)
Albumin (g/L)	27.1 (23.2-37.0)
WBC (10^9/L)	18.3 (11.6-27.3)
CRP (mg/L)	97.6 (44.5-137.2)
Disease severity, median (IQR)	
APACHE II score	13.0 (8.2-17.0)
SOFA score	6.0 (4.0-8.0)

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; G+, Gram-positive; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

3 | RESULTS

3.1 | Clinical characteristics of sepsis patients

The mean age was 54.6 ± 11.0 years in sepsis patients, and there were 46 (41.1%) females and 66 (58.9%) males (Table 1). For chronic complications, 22 (19.6%), 49 (43.8%), 18 (16.1%), and 22 (19.6%) sepsis patients had chronic obstructive pulmonary disease (COPD),

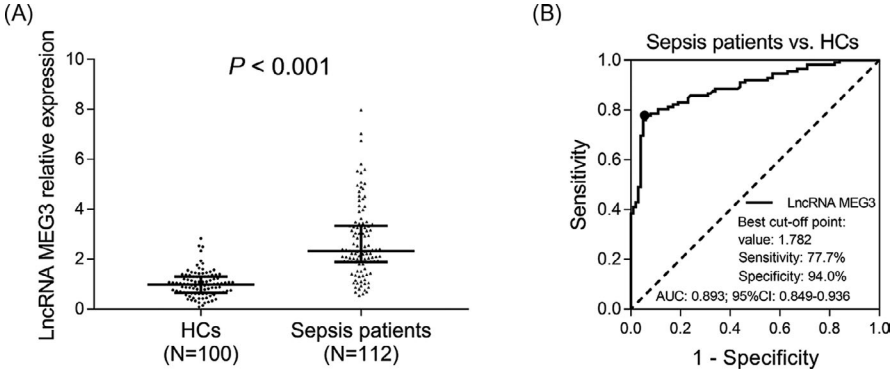

FIGURE 1 LncRNA MEG3 distinguished sepsis patients from HCs. Comparison of lncRNA MEG3 relative expressions between HCs and sepsis patients (A). ROC curve analysis for the performance of lncRNA MEG3 in discriminating sepsis patients from HCs (B). LncRNA MEG3, long non-coding RNA maternally expressed gene 3; HCs, healthy controls; ROC, receiver operating characteristic

TABLE 2 Comparison of characteristics between non-ARDS sepsis patients and ARDS sepsis patients

Items	Non-ARDS sepsis patients (n = 82)	ARDS sepsis patients (n = 30)	*P* value
Age (y), mean ± SD	53.3 ± 11.5	58.1 ± 8.5	.040
Gender, No. (%)			.150
Female	37 (45.1)	9 (30.0)	
Male	45 (54.9)	21 (70.0)	
BMI, (kg/m^2), mean ± SD	22.5 ± 3.6	23.0 ± 3.4	.500
Smoke, No. (%)	23 (28.0)	16 (53.3)	.013
COPD, No. (%)	11 (13.4)	11 (36.7)	.006
Cardiomyopathy, No. (%)	35 (42.7)	14 (46.7)	.707
Chronic kidney failure, No. (%)	11 (13.4)	7 (23.3)	.206
Cirrhosis, No. (%)	17 (20.7)	5 (16.7)	.632
Primary infection site, No. (%)			
Abdominal infection	33 (40.2)	7 (23.3)	.098
Respiratory infection	12 (14.6)	13 (43.3)	.001
Skin and soft tissue infection	16 (19.5)	6 (20.0)	.954
Blood stream infection	10 (12.2)	2 (6.7)	.402
CNS infection	4 (4.9)	2 (6.7)	.710
Other infections	7 (8.5)	0 (0.0)	.098
Primary organism, No. (%)			
G− bacteria	44 (53.7)	17 (56.7)	.777
G+ bacteria	17 (20.7)	8 (26.7)	.504
Anaerobes	8 (9.8)	4 (13.3)	.588
Fungus	5 (6.1)	2 (6.7)	.912
Mycoplasmas	4 (4.9)	1 (3.3)	.726
Total culture negative	17 (20.7)	5 (16.7)	.632
Biochemical indexes, median (IQR)			
Scr (mg/dL)	1.9 (1.2-2.7)	1.6 (1.2-2.7)	.606
Albumin (g/L)	27.2 (23.0-37.3)	26.6 (23.1-35.5)	.559
WBC (10^9/L)	15.8 (11.3-27.1)	22.6 (13.0-28.5)	.114
CRP (mg/L)	72.5 (42.9-127.4)	132.3 (68.8-226.1)	.003
Disease severity, median (IQR)			
APACHE II score	12.0 (7.0-16.3)	14.0 (10.8-18.3)	.030
SOFA score	5.0 (4.0-8.0)	7.0 (5.0-10.0)	.008

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; G+, Gram-positive; IQR, interquartile range; Scr, serum creatinine; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

cardiomyopathy, chronic kidney failure, and cirrhosis, respectively. In addition, 40 (35.7%), 25 (22.3%), 22 (19.6%), 12 (10.7%), 6 (5.4%), and 7 (6.3%) sepsis patients exhibited abdominal infection, respiratory infection, skin and soft tissue infection, blood stream infection, central nervous system infection, and other infections, respectively. Meanwhile, 61 (54.5%), 25 (22.3%), 12 (10.7%), 7 (6.3%), 5 (4.5%), and 22 (19.6%) sepsis patients were with G− bacteria, G+ bacteria, anaerobes, fungus, mycoplasmas, and total culture negative as primary organism, respectively. Besides, the median APACHE II score was 13.0 (8.2-17.0) and the median SOFA score was 6.0 (4.0-8.0) in sepsis patients. The detailed information regarding other characteristics such as body mass index (BMI) and biochemical indexes was disclosed in Table 1.

3.2 | The value of lncRNA MEG3 for distinguishing sepsis patients from HCs

The lncRNA MEG3 relative expression was 2.317 (1.889-3.336) in sepsis patients and 0.990 (0.655-1.306) in HCs, and further comparison analysis showed that lncRNA MEG3 relative expression was increased in sepsis patients compared with HCs ($P < .001$) (Figure 1A). The following ROC curve exhibited that lncRNA MEG3 could differentiate sepsis patients from HCs with an AUC of 0.893 (95% CI: 0.849-0.936). At the best cut-off point (lncRNA MEG3 = 1.782; the point at which the sum of sensitivity and specificity was the largest), the sensitivity was 77.7% and the specificity was 94.0% (Figure 1B).

3.3 | Clinical characteristics of non-ARDS sepsis patients and ARDS sepsis patients

During 28-day follow-up period, 30 (26.8%) sepsis patients occurred ARDS, and they were grouped as ARDS sepsis patients; 82 (73.2%) patients did not occur ARDS, and they were grouped as non-ARDS sepsis patients. The median time of occurrence of ARDS after onset sepsis was 2.5 (2.0-4.0) days. The following comparisons analyses displayed that ARDS sepsis patients presented older age ($P = .040$), elevated percentage of smoking cases ($P = .013$), percentage of COPD cases ($P = .006$), percentage of respiratory infection cases ($P = .001$), increased C-reactive protein (CRP) ($P = .003$), APACHE II score ($P = .030$), and SOFA score ($P = .008$) compared with non-ARDS sepsis patients (Table 2).

3.4 | The value of lncRNA MEG3 for predicting ARDS risk in sepsis patients

LncRNA MEG3 relative expression was higher in ARDS patients than that in non-ARDS patients ($P < .001$) (Figure 2A). Subsequent ROC curve showed that lncRNA MEG3 could predict ARDS risk in sepsis patients with an AUC of 0.775 (95% CI: 0.678-0.872) (Figure 2B). At the best cut-off point (lncRNA MEG3 = 2.259; The point at which the sum of sensitivity and specificity was the largest), the sensitivity was 86.7% and the specificity was 58.5%.

3.5 | Risk factors for ARDS in sepsis patients

Univariate logistic regression analysis displayed that lncRNA MEG3 ($P < .001$, OR = 2.058), age ($P = .043$, OR = 1.043), smoke ($P = .015$, OR = 2.932), COPD ($P = .008$, OR = 3.737), primary infection site (respiratory *vs* others) ($P = .002$, OR = 4.461), CRP ($P = .001$, OR = 1.011), and SOFA score ($P = .005$, OR = 1.235) were risk factors of ARDS in sepsis patients (Table 3), and further forward stepwise multivariate logistic regression analysis showed that lncRNA MEG3 ($P = .004$, OR = 1.869), age ($P = .044$, OR = 1.063), smoke ($P = .007$, OR = 5.114), and CRP ($P = .003$, OR = 1.012) were independent risk factors for ARDS in sepsis patients. These Independent risk factors were used to construct the predictive model for ARDS risk in sepsis patients (including lncRNA MEG3, age, smoke, and CRP), then the following ROC curve analysis manifested that the predictive model exhibited a good value for identifying ARDS risk in sepsis patients (AUC: 0.851, 95% CI: 0.776-0.926) (Figure 3).

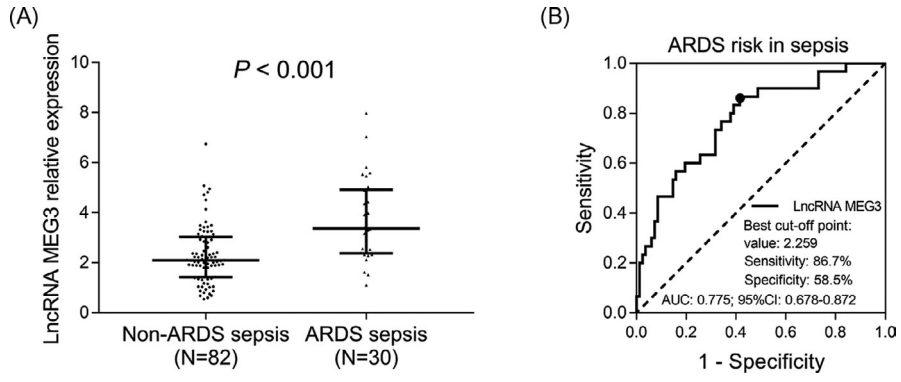

FIGURE 2 LncRNA MEG3 predicted ARDS risk in sepsis patients. Comparison of lncRNA MEG3 relative expressions between ARDS sepsis patients and non-ARDS sepsis patients (A). ROC curve analysis for the performance of lncRNA MEG3 for predicting ARDS risk in sepsis patients (B). LncRNA MEG3, long non-coding RNA maternally expressed gene 3; HCs, healthy controls. ARDS, acute respiratory distress syndrome; ROC, receiver operating characteristic

TABLE 3 Analysis of risk factors of ARDS in sepsis patients

	Logistic regression model			
			95% CI	
Items	*P* value	OR	Lower	Higher
Univariate logistic regression				
LncRNA MEG3	<.001	2.058*	1.448	2.926
Age	.043	1.043	1.001	1.086
Male	.153	1.919	0.785	4.690
BMI	.497	1.042	0.926	1.171
Smoke	.015	2.932	1.236	6.956
COPD	.008	3.737	1.407	9.928
Cardiomyopathy	.707	1.175	0.507	2.722
Chronic kidney failure	.211	1.964	0.682	5.658
Cirrhosis	.632	0.765	0.255	2.294
Primary infection site (Respiratory *vs* others)	.002	4.461	1.731	11.498
Primary infection organism				
G- vs. others	.777	1.129	0.486	2.623
G + vs. others	.505	1.390	0.527	3.666
Anaerobes/fungus/mycoplasmas vs. others	.658	1.255	0.459	3.437
Scr	.465	0.888	0.647	1.220
Albumin	.409	0.980	0.936	1.027
WBC	.183	1.027	0.987	1.069
CRP	.001	1.011	1.005	1.017
APACHE II	.106	1.058	0.988	1.133
SOFA score	.005	1.235	1.067	1.430
Forward stepwise multivariate logistic regression				
LncRNA MEG3	.004	1.869	1.222	2.860
Age	.044	1.063	1.002	1.127
Smoke	.007	5.114	1.567	16.687
CRP	.003	1.012	1.004	1.020

Note: Risk factors of ARDS were analyzed by univariate logistic regression model, and the independent risk factors were analyzed by 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; G+, Gram-positive; lncRNA MEG3, long non-coding RNA maternally expressed gene 3; OR, odds ratio; Scr, serum creatinine; SOFA, sequential organ failure assessment; WBC, white blood cell.

*One unit increase in the lncRNA MEG3 expression would elevate the risk of ARDS 2.058 times in sepsis patients. The model was as follows: $P = \exp[-8.633 + 0.625 * (\text{lncRNA MEG3}) + 0.061 * (\text{age}) + 1.632 * (\text{smoke}) + 0.012 * (\text{CRP})]/1 + \exp[-8.633 + 0.625 * (\text{lncRNA MEG3}) + 0.061 * (\text{age}) * 1.632 * (\text{smoke}) + 0.012 * (\text{CRP})]$. Goodness of fit: $-2\ln(R) = 84.968$, Nagelkerke $R^2 = 0.483$.

3.6 | Correlation of lncRNA MEG3 with primary infection site in sepsis patients

LncRNA MEG3 was positively correlated with respiratory infection ($P = .005$) (Figure 4B), while it was not correlated with abdominal infection ($P = .375$) (Figure 4A), skin and soft tissue infection ($P = .158$) (Figure 4C), blood stream infection ($P = .569$) (Figure 4D), or CNS infection ($P = .369$) (Figure 4E) in sepsis patients.

3.7 | Correlation of lncRNA MEG3 with complications, primary infection site, primary organism biochemical indexes, and disease severity in sepsis patients

LncRNA MEG3 was positively correlated with COPD ($P = .014$) (Table 4), white blood cell (WBC) ($P = .008$, $r = 0.248$), CRP ($P = .001$, $r = 0.300$) (Table 5), APACHE II score ($P < .001$, $r = 0.440$) (Figure 5A), and SOFA score ($P < .001$, $r = 0.366$) (Figure 5B), while negatively correlated albumin ($P < .001$, $r = -0.325$) (Table 5) in sepsis patients.

3.8 | The value of lncRNA MEG3 for predicting 28-day mortality risk in sepsis patients

During the 28-day follow-up duration, there were 83 (74.1%) 28-day survivors and 29 (25.9%) 28-day deaths, then the following comparison analysis revealed that lncRNA MEG3 expression was raised in 28-day deaths compared with 28-day survivors ($P < .001$) (Figure 6A). Furthermore, ROC curve displayed that the predictive value of lncRNA MEG3 (AUC: 0.708, 95% CI: 0.608-0.808) for 28-day mortality risk in sepsis patients was non-inferior to common biochemical indexes such as Scr (AUC: 0.694, 95% CI: 0.590-0.798), albumin (AUC: 0.629, 95% CI: 0.511-0.748), WBC (AUC: 0.637, 95% CI: 0.533-0.741), and CRP (AUC: 0.757, 95% CI: 0.611-0.853), while less than common comprehensive score such as APACHE II score (AUC: 0.799, 95% CI: 0.707-0.891) and SOFA score (AUC: 0.848, 95% CI: 0.767-0.929) (Figure 6B). In addition, the accumulating mortality was elevated in sepsis patients with lncRNA MEG3 high expression compared to those with lncRNA MEG3 low expression ($P = .005$) (Figure 7).

4 | DISCUSSION

Preceding researches identified lncRNA MEG3 as a regulator for lung injury through modulating inflammatory signaling pathways or cell apoptosis-related pathways in multiple inflammatory diseases.[8-11] As an example, Zou et al unravel that lncRNA MEG3 knockdown attenuates cell damage to subsequently attenuate hyperoxia-induced lung injury via inhibiting non-obese diabetic-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activity

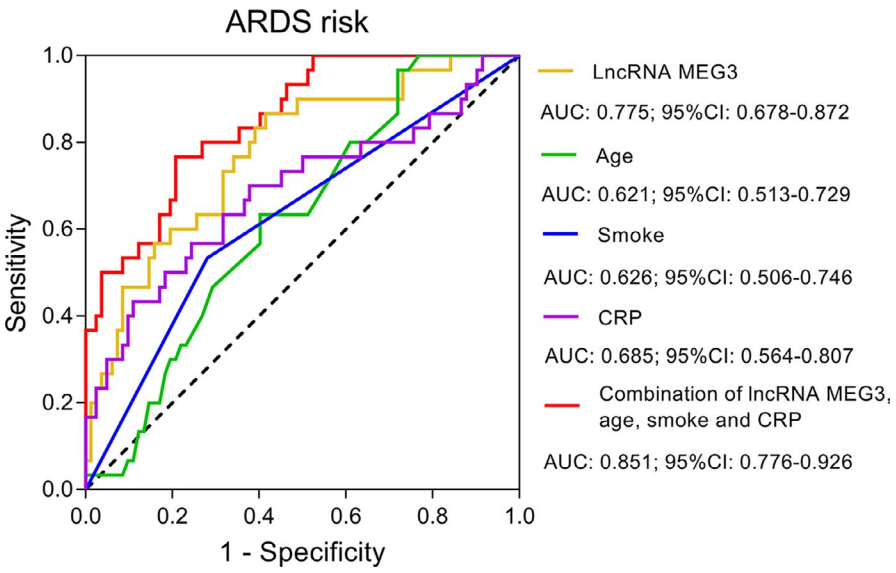

FIGURE 3 LncRNA MEG3, age, smoke, CRP, combination of lncRNA MEG3, age, smoke, and CRP predicted ARDS risk in sepsis patients. ROC curve analysis for the performances of lncRNA MEG3, age, smoke, CRP, combination of lncRNA MEG3, age, smoke, and CRP in predicting ARDS risk in sepsis patients. LncRNA MEG3, long non-coding RNA maternally expressed gene 3; CRP, C-reactive protein; ARDS, acute respiratory distress syndrome; ROC, receiver operating characteristic

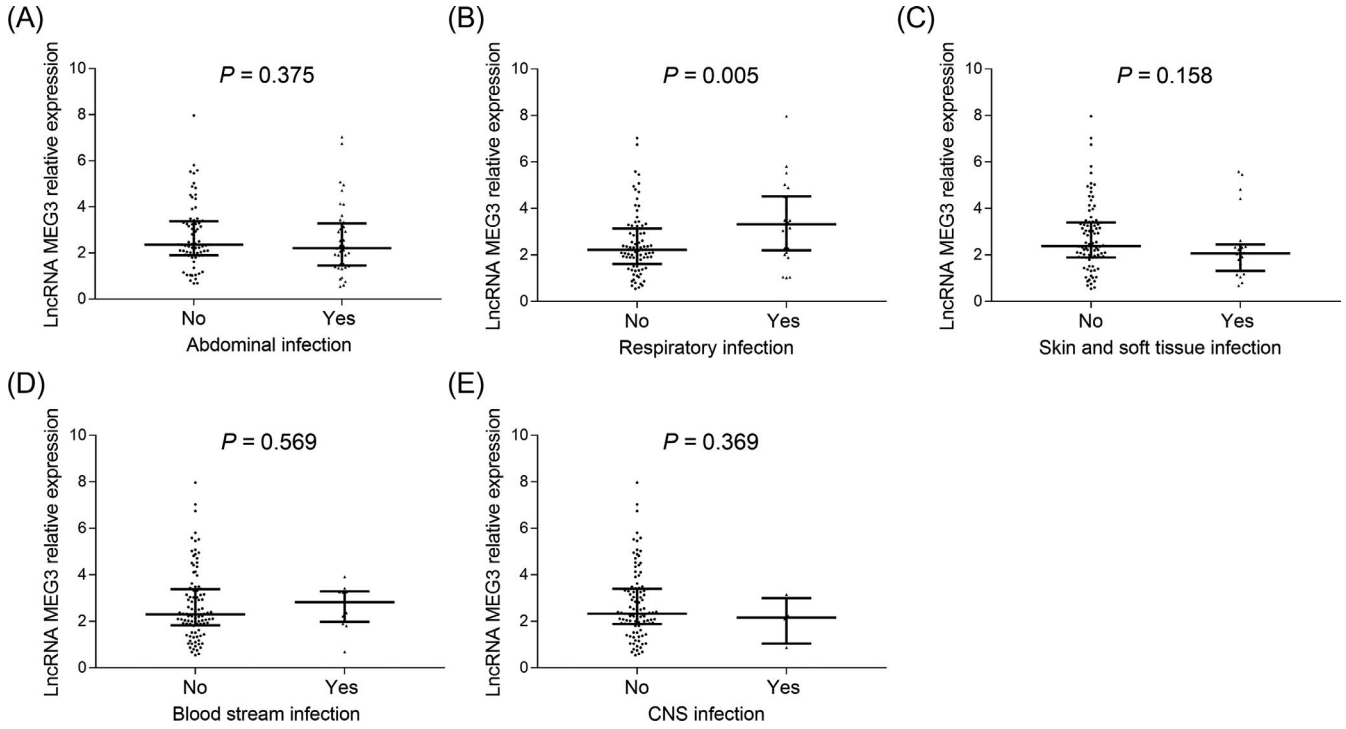

FIGURE 4 LncRNA MEG3 correlated with respiratory infection in sepsis patients. Correlation of lncRNA MEG3 with abdominal infection (A), respiratory infection (B), skin and soft tissue infection (C), blood steam infection (D), and CNS infection (E) in sepsis patients. LncRNA MEG3, long non-coding RNA maternally expressed gene 3; CNS, central nervous system

and caspase-1 signaling.[10] Another study illuminates that lncRNA MEG3 induces pulmonary microvascular endothelial cell apoptosis via the upregulation of caspase-3 activity, reduction of Bcl-2 level, and elevation of Bax in chronic obstructive pulmonary disease.[11] Meanwhile, lncRNA MEG3 has a good predictive value for disease risk of respiratory disease asthma.[12] In addition, lncRNA MEG3 upregulates IL-1β abundance and exaggerates inflammatory responses in alveolar macrophages and lung epithelial cells, which is involved in the progression of sepsis,[13] and clinically, lncRNA MEG3 is proved to be associated with higher mortality risk in sepsis patients.[14,15] While the clinical value of lncRNA MEG3 in sepsis-related ARDS is still unknown.

In the present study, lncRNA MEG3 expression was higher in sepsis patients than that in HCs, and it could distinguish sepsis patients from HCs. The findings could be explained by first, lncRNA MEG3 might promote the expression of inflammatory cytokines CRP, IL-1β, IL-6, and monocyte chemoattractant protein-1, which subsequently intensified inflammatory response in

TABLE 4 Correlation of lncRNA MEG3 with complications and primary organism

Items	LncRNA MEG3 expression	*P* value
Complications, median (IQR)		
COPD		
No	2.259 (1.804-3.164)	.014
Yes	3.214 (2.017-5.176)	
Cardiomyopathy		
No	2.373 (1.893-3.321)	.626
Yes	2.221 (1.605-4.012)	
Chronic kidney failure		
No	2.325 (1.890-3.273)	.659
Yes	2.247 (1.734-4.189)	
Cirrhosis		
No	2.317 (1.890-3.326)	.858
Yes	2.568 (1.704-3.425)	
Primary organism, median (IQR)		
G− bacteria		
No	2.289 (1.520-3.293)	.515
Yes	2.361 (1.911-3.368)	
G+ bacteria		
No	2.407 (1.896-3.395)	.125
Yes	2.073 (1.362-2.458)	
Anaerobes/fungus/ mycoplasmas		
No	2.361 (1.895-3.456)	.245
Yes	2.102 (1.436-2.941)	

Note: Comparison was determined by Wilcoxon rank-sum test.

Abbreviations: COPD, chronic obstructive pulmonary disease; G−, Gram-negative; G+, Gram-positive; IQR, interquartile range; lncRNA MEG3, long non-coding RNA maternally expressed gene 3.

TABLE 5 Correlation of lncRNA MEG3 with biochemical indexes in sepsis patients

	LncRNA MEG3	
Items	Correlation coefficient (*r*)	*P* value
Scr	0.152	.110
Albumin	−0.325	<.001
WBC	0.248	.008
CRP	0.300	.001

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

Abbreviations: CRP, C-reactive protein; lncRNA MEG3, long non-coding RNA maternally expressed gene 3; Scr, serum creatinine; WBC, white blood cell.

sepsis, thereby, lncRNA MEG3 was elevated in sepsis patients compared with HCs.[20] Second, lncRNA MEG3 probably mediated cell apoptosis in major organs including kidney and heart, which subsequently accelerated multiple organ injury in sepsis, thereby, lncRNA MEG3 discriminated sepsis patients from HCs.[15] As for sepsis-related ARDS, lncRNA MEG3 was increased in ARDS sepsis patients compared with non-ARDS sepsis patients, and lncRNA MEG3 exhibited the value for predicting ARDS risk in sepsis patients. These findings were likely to explained by that lncRNA MEG3 might trigger the production of hyperoxia-induced inflammatory cytokines in lung tissues and facilitate pulmonary microvascular endothelial cell apoptosis/subsequent lung injury by modulating its downstream pathways such as NLRP3 inflammasome-mediated caspase-1 pathway, which might contribute to augmented ARDS risk in sepsis patients.[10,11] However, these hypothetical explanations needed further validation. Of note, the association of lncRNA MEG3 with ARDS might be an epiphenomenon as an indirect consequence of pneumonia (reflected by the correlation of lncRNA MEG3 with respiratory infection). However, it was possible that lncRNA MEG3 directly induced severe inflammation, lung injury, and ADRS. Hence, we performed forward stepwise multivariate logistic regression analysis of risk factors of ARDS in sepsis patients to eliminate the confounding effects (including respiratory infection), and we found that lncRNA MEG3 was an independent risk factor for ARDS in sepsis patients. This finding indicated that lncRNA MEG3 served as a valuable predictor for ARDS in sepsis patients.

In the present study, we further assessed the prognostic value of lncRNA MEG3 in sepsis patients, then found that lncRNA MEG3 relative expression was raised in 28-day deaths compared with 28-day survivors in sepsis patients, and lncRNA MEG3 could predict 28-day mortality risk in sepsis patients by ROC curve. The following Kaplan-Meier analysis disclosed that sepsis patients with lncRNA MEG3 high expression presented with higher accumulating mortality compared to patients with lncRNA MEG3 low expression. Our findings were in accordance with findings from previous studies including sepsis patients from Eastern China and Northern China (lncRNA MEG3 correlated with worse prognosis in sepsis patients).[14,15] Herein, several possible reasons were proposed: First, as observed in this study, lncRNA MEG3 was positively associated with COPD, respiratory infection, WBC, CRP, APACHE II score, and SOFA score, thereby, lncRNA MEG3 was associated with inclined 28-day mortality risk in sepsis patients. Second, lncRNA MEG3 probably augmented the concentration of inflammatory cytokines in multiple major organs (such as lung, heart, and liver) and deteriorated inflammation-induced cell damage in a hypoxic environment through multiple downstream pathways (including activation of c-Jun N-terminal kinase/nuclear factor regulating expression of kappa light-chain immunoglobulin pathway and the enhancement of the mitochondria-mediated apoptosis pathway), which then exacerbated the disease severity and higher 28-day mortality risk in sepsis patients.[20-22] Interestingly, the prognostic value of lncRNA MEG3 for 28-day mortality risk in sepsis patients was non-inferior to common biochemical indicators such as Scr, albumin, WBC, and CRP, while

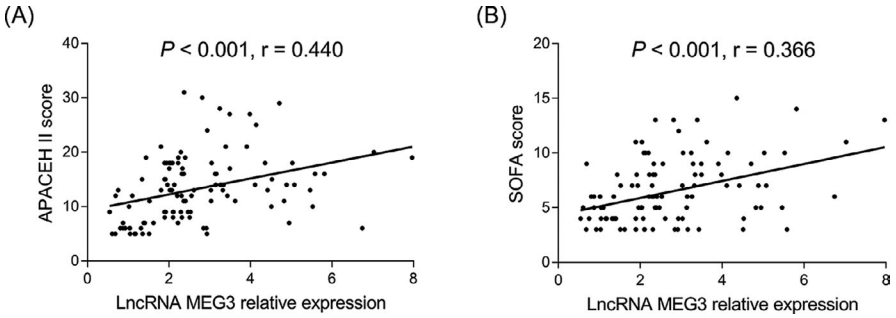

FIGURE 5 LncRNA MEG3 correlated with APACHE II score and SOFA score in sepsis patients. Correlation of lncRNA MEG3 with APACHE II score (A) and SOFA score (B) in sepsis patients. LncRNA MEG3, long non-coding RNA maternally expressed gene 3; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment

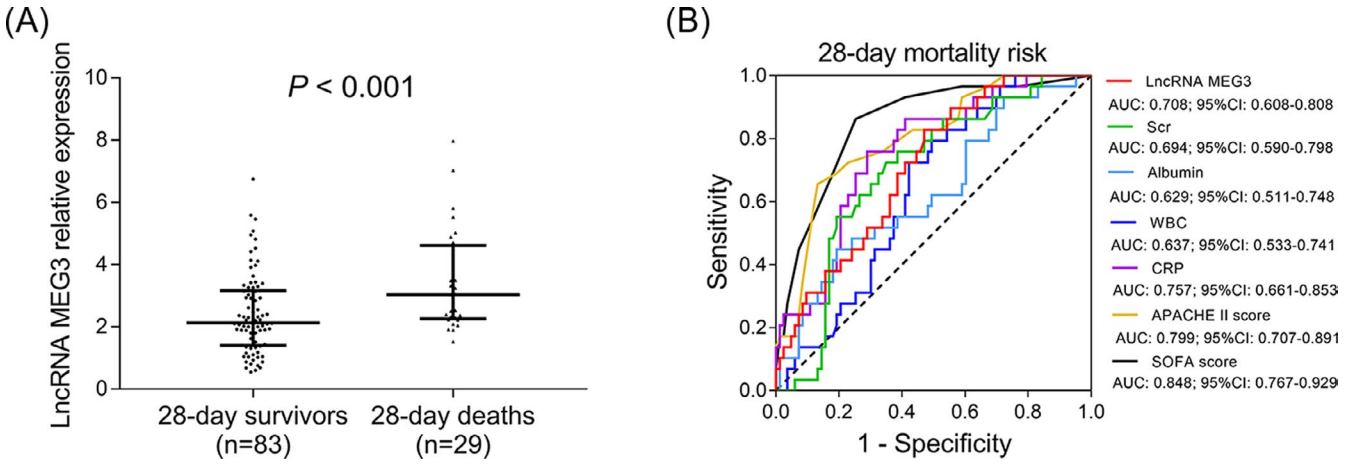

FIGURE 6 LncRNA MEG3 predicted 28-d mortality risk in sepsis patients. Comparison of lncRNA MEG3 relative expression between 28-d survivors and 28-d deaths in sepsis patients (A). ROC curve analysis of the performance of lncRNA MEG3, Scr, albumin, WBC, CRP, APACHE II score, and SOFA score in predicting 28-d mortality risk in sepsis patients (B). LncRNA MEG3, long non-coding RNA maternally expressed gene 3; ROC, receiver operating characteristic; Scr, serum creatinine; WBC, white blood cell; CRP, C-reactive protein; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment

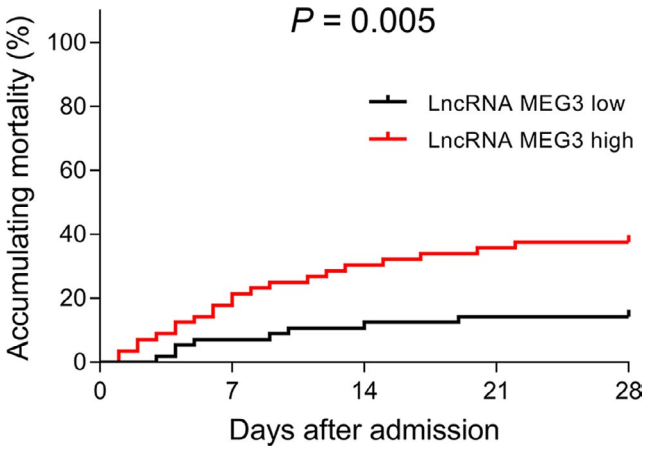

FIGURE 7 Accumulating mortality in sepsis patients with lncRNA MEG3 low expression and sepsis patients with lncRNA MEG3 high expression. Comparison of accumulating mortality in sepsis patients with lncRNA MEG3 low expression and sepsis patients with lncRNA MEG3 high expression. LncRNA MEG3, long non-coding RNA maternally expressed gene 3

less than common comprehensive score such as APACHE II score and SOFA score, which was implied that lncRNA MEG3 displayed the potential of being an additional prognostic biomarker for sepsis patients' outcome in clinical setting.

Several shortcomings should be noted when interpreting the findings of the present study. First, the lncRNA MEG3 expression was only detected at a single time (within 24 hours after admission) in sepsis patients, further study assessing the variation of lncRNA MEG3 through the course of disease and treatment was necessary. Second, the sample size of ARDS-sepsis patients included in the analysis was relatively small (n = 30), which might reduce the statistic power; thereby, further study with larger sample size was needed for validate our findings. Third, only 28-day mortality was evaluated in sepsis patients; thereby, further study with extended follow-up duration for exploring the long-term predictive value of lncRNA MEG3 for prognosis would be warranted. Last, only one inflammatory index (CRP) was assessed and included in the analysis, further studies with the detection of more inflammatory indexes (such as TNF-α, IL-6, and IL-1β) in sepsis patients were needed for indicating inflammation more comprehensively.

To conclude, circulating lncRNA MEG3 is correlated with higher ARDS risk and elevated accumulating mortality in sepsis patients, which offers a new perspective for optimizing prevention strategies against ARDS and improving prognosis in sepsis.

ACKNOWLEDGMENTS

This study was supported by Natural Science Foundation of Hubei Province (No. 2017CFC851).

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

ORCID

Li Yu https://orcid.org/0000-0003-4116-7045

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Wu X, Chen D, Yu L. The value of circulating long non-coding RNA maternally expressed gene 3 as a predictor of higher acute respiratory distress syndrome risk and 28-day mortality in sepsis patients. *J Clin Lab Anal*. 2020;34:e23488. https://doi.org/10.1002/jcla.23488