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RESEARCH ARTICLE

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Association of circulating long non-coding RNA HULC expression with disease risk, inflammatory cytokines, biochemical index levels, severity-assessed scores, and mortality of sepsis

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Abstract

Background: The present study aimed to explore the correlation of long non-coding RNA highly up-regulating in liver cancer (lncRNA HULC) with disease risk, inflammatory cytokines, biochemical indexes, disease severity, infective features, and 28-day mortality of sepsis.

Methods: Totally 174 sepsis patients and 100 controls were enrolled. Peripheral blood samples were collected from sepsis patients after diagnosis and from controls at enrollment, respectively, and further for separation of peripheral blood mononuclear cell (PBMC) and serum samples. PBMC samples were for lncRNA HULC detection, and serum samples were for inflammatory cytokine detection.

Results: LncRNA HULC expression was increased in sepsis patients compared with controls. Moreover, lncRNA HULC was positively associated with TNF-α, IL-6, IL-17, intercellular adhesion molecule 1, vascular cell adhesion molecule 1, serum creatinine, white blood cell, and C-reactive protein in sepsis patients, but not in controls. Furthermore, in sepsis patients, lncRNA HULC expression was positively correlated with acute physiology and chronic health evaluation II score and sequential organ failure assessment score, but not correlated with primary infection sites or primary infection organisms; meanwhile, lncRNA HULC expression was increased in deaths compared with survivors; subsequent receiver operating characteristic curve indicated that lncRNA HULC presented good value in predicting increased 28-day mortality (AUC: 0.785, 95% CI: 0.713–0.857), and its independent predictive value for mortality was also verified by multivariate analysis.

Conclusion: LncRNA HULC is correlated with higher disease risk, severity, and inflammation and serves as an independent factor for predicting increased mortality, suggesting its potential in promoting accuracy of prognostic prediction for sepsis management.

Haiyan Wang and Qiang Feng contributed equally to this work.

KEYWORDS
disease severity, inflammatory cytokines, long non-coding RNA HULC, mortality, sepsis

1 | INTRODUCTION

Sepsis is defined as a life-threatening organ dysfunction caused by dysregulated host responses to infection.[1] Regarding the pathophysiology of sepsis, it involves the activation of phagocytic cells, formation of pro-inflammatory mediators, and the recruitment of inflammatory cells, which leads to systematic inflammation and tissue damage, and further progresses to multiple organ dysfunction, such as liver, kidney, and heart.[2,3] Current effective sepsis therapy approaches mainly rely on the timely diagnosis, removal infection source, and individualized treatment based on prognosis prediction; however, due to delay of diagnosis and absence of directed therapies to sepsis, patients with severe sepsis in intensive care units (ICU) still suffer from high mortality ranging from 30% to 50%.[4,5] These evidences highlight the importance of early diagnosis and infection recognition, and it is therefore necessary to explore novel biomarkers which help to identify the sepsis risk timely and monitor prognosis, further optimizing the treatment outcome in sepsis patients.

Long non-coding RNA (lncRNA) highly up-regulating in liver cancer (HULC) has approximately 500 nucleotides in length and contains two exons located on chromosome 6p24.3.[6,7] Existing evidences demonstrate that dysregulation of lncRNA HULC is associated with infection, inflammation response, and several organ injuries.[8-13] For instance, hepatitis B patients with high lncRNA HULC were more susceptible to infection of hepatitis B virus, and gastric cancer patients with high lncRNA HULC presented higher possibility of *H pylori* infection.[12,13] Furthermore, as for the correlation of lncRNA with inflammation, lncRNA HULC is upregulated in septic cell model of lipopolysaccharide (LPS)-induced human umbilical vein endothelial cells (HUVECs), and its high expression promotes lipopolysaccharide (LPS)-stimulated cell apoptosis, inflammatory response, and oxidative stress in HUVECs.[14] Regarding the role of lncRNA HULC in organ injury, aberrant lncRNA HULC expression mediates the apoptosis and inflammatory injury of hepatocytes and cardiomyocytes, further regulating liver injury and myocardial tissue necrosis.[10,11] According to the aforementioned evidence that lncRNA HULC was correlated with infection, inflammation, and organ injuries, we hypothesized that lncRNA HULC might have association with disease risk and prognosis of sepsis; however, there is no related research until now. We therefore performed the present study to explore the correlation of lncRNA HULC with inflammatory cytokines, biochemical indexes, disease severity, infective features, and 28-day mortality in sepsis patients.

2 | METHODS

2.1 | Participants

All procedures included in this study were approved by the Institutional Review Board of our hospital. Written informed consents were obtained from the participant or their legal representatives. In this study, 174 sepsis patients were consecutively enrolled from our hospital between January 2017 and December 2019. The inclusion criteria of sepsis patients were as follows: (i) diagnosed as sepsis referring to the Third International Consensus Definitions for Sepsis and Septic Shock[1]; (ii) aged 18–80 years old; and (iii) admitted into our department within 12 h after onset of sepsis symptom. The patients who infected with human immunodeficiency virus, complicated with hematologic malignancies, or received immunosuppressive therapy within the last 1 month were excluded. The pregnant or lactational patients were excluded as well. In addition, 100 controls were enrolled from Health Examination Center between November 2019 and January 2020. The inclusion criteria of healthy controls were as follows: (a) age and gender matched with sepsis patients; (b) had no obvious abnormality in biochemical indexes, which was confirmed in the health examination. The exclusion criteria of healthy controls were as follows: (a) history of sepsis or other severe infections; (b) history of hematological malignancies or other solid tumors; and (c) complicated with inflammatory disease.

2.2 | Data collection

For all participants, the demographics, medical history, and biochemical index were recorded after enrollment, which included age, gender, body mass index (BMI), smoke, drink, history of hypertension, history of hyperlipidemia, history of diabetes, history of chronic kidney disease (CKD), history of cardio-cerebrovascular diseases (CCVDs), serum creatinine (Scr) level, albumin level, white blood cell (WBC) level, and C-reactive protein (CRP) level. Apart from those characteristics, the primary infection sites, the primary infection organism species, and disease severity of sepsis patients were recorded as well. The disease severity of sepsis patients was assessed within 24 h after admission using acute physiology and chronic health evaluation II (APACHE II) score and sequential organ failure assessment (SOFA) score. All sepsis patients were daily follow-up to death or 28 days after admission.

2.3 | Sample collection and processing

After admission, patients' initial peripheral blood samples (2 ml) were drawn as soon as the diagnosis of sepsis was made clinically. After collection, the peripheral blood samples were divided into two parts. One part was immediately processed with gradient density centrifugation to isolate peripheral blood mononuclear cell (PBMC), and another part was centrifuged to separate serum samples. The PBMC samples and serum samples of controls were separated from

peripheral blood samples using the same method described above. Then, the PBMC samples and serum samples were preserved at −80°C.

2.4 | LncRNA HULC detection

The level of lncRNA HULC in PBMC samples was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). In brief, total RNA was extracted from PBMC samples using RNeasy Protect Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German) and then reversely transcribed using PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara, Dalian, Liaoning, China). Following that, qPCR was performed using THUNDERBIRD® SYBR® qPCR Mix (Toyobo, Osaka, Kansai, Japan) to quantify lncRNA HULC expression. In addition, the expression level of lncRNA HULC was calculated using $2^{-\triangle\triangle Ct}$ method with GAPDH as an internal reference. Primers used for amplification were designed referring to a pervious study.[14] LncRNA HULC forward primer: 5′-TCATGATGGAATTGGAGCCTT-3′, reverse primer: 5′-CTCTTCCTGGCTTGCAGATTG-3′; GAPDH forward primer: 5′-GCCAAAAGGGTCATCATCTC-3′; reverse primer: 5′-GGCCATCCACAGTCTTCT-3′.

2.5 | Inflammatory cytokine detection

The level of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-17, intercellular adhesion molecule 1 (ICAM1), and vascular cell adhesion molecule 1 (VCAM1) in serum samples was detected by enzyme-linked immunosorbent assay (ELISA) with the use of commercial human ELISA kits. All human ELISA kits were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA), and the procedures were performed according to the operation manuals of the ELISA kits as follows: In brief, firstly, samples or standards were added to the 96-well plate, followed by the antibody mix. After incubation, the wells were washed to remove unbound material. TMB substrate (tetramethylbenzidine, TMB) was added, generating blue coloration. This reaction was then stopped by addition of stop solution completing any color change from blue to yellow. Signal was generated proportionally to the amount of bound analyte (BioTek, Winooski, Vermont, USA), and the intensity was measured at 450 nm.

2.6 | Treatment and follow-up

Standard treatments and resuscitation were administered to patients after the diagnosis was established, which were performed as recommended by the International Guidelines for Management of Sepsis and Septic Shock.[15] All patients were followed up for 28 days, and the patients who died during follow-up were recorded for evaluation of 28-day mortality.

2.7 | Statistical analyses

Statistical analyses were performed using SPSS 22.0 software (IBM, Chicago, IL, USA). Figures were plotted with the use of GraphPad Prism 7.01 software (GraphPad Software Inc., San Diego, California, USA). Quantitative data were expressed as mean ± standard deviation (*SD*) or median with interquartile range (IQR) according to their normality. Qualitative data were described as number and percentage. The difference in clinical characteristics and lncRNA HULC between sepsis patients and controls was compared by Student's *t* test, chi-square test, or Wilcoxon rank-sum test. The correlation of lncRNA HULC with disease severity, inflammatory cytokine level, and biochemical index level of sepsis patients or the correlation of lncRNA HULC with inflammatory cytokine level and biochemical index level of controls was determined by Spearman's rank correlation test. The correlation of lncRNA HULC with primary infection sites and primary infection organism of sepsis patients was determined by Kruskal-Wallis H rank-sum test. For further analysis, all sepsis patients were divided into survivors and deaths based on their survival status in the 28-day follow-up. The difference in lncRNA HULC between survivors and deaths was compared by Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curve was plotted, and the area under the curve (AUC) with 95% confidence interval (CI) was used to assess the ability of variables in distinguishing sepsis patients from controls or in distinguishing survivors from deaths. Factors independently related to 28-day mortality were analyzed by forward stepwise multivariate logistic regression model. p value <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical characteristics in sepsis patients and controls

The mean age was 55.2 ± 12.5 years and 53.9 ± 11.9 years in sepsis patients and controls, respectively (Table 1). As for gender, the number of females and males were 62 (35.6%) and 112 (64.4%) in sepsis patients, 36 (36.0%) and 64 (64.0%) in controls, respectively. No difference in demographics (including age, gender, BMI, smoke, and drink) or medical history (including hypertension, hyperlipidemia, diabetes, CKD, and CCVDs) was observed between sepsis patients and controls (all $p > 0.05$). However, biochemical indexes (such as Scr, WBC, CRP), and inflammatory cytokines (such as TNF-α, IL-6, IL-17, ICAM1, VCAM1) were increased, but albumin was decreased in sepsis patients compared with controls (all $p < 0.001$). Furthermore, there were 55 (31.6%), 50 (28.7%), 35 (20.1%), 22 (12.6%), and 12 (6.9%) patients with primary infection site at abdominal, respiratory, skin and soft tissue, bloodstream, and other infection, respectively. As for primary organism, there were 101 (58.0%), 60 (34.5%), 21 (12.1%),

TABLE 1 Clinical characteristics

Items	Controls ($N = 100$)	Sepsis patients ($N = 174$)	p value
Demographics			
Age (years), mean ± *SD*	53.9 ± 11.9	55.2 ± 12.5	0.416
Gender, no. (%)			0.951
Female	36 (36.0)	62 (35.6)	
Male	64 (64.0)	112 (64.4)	
BMI, (kg/m^2), mean ± *SD*	22.7 ± 3.0	22.8 ± 2.6	0.799
Smoke, no. (%)	28 (28.0)	60 (34.5)	0.269
Drink, no. (%)	45 (45.0)	71 (40.8)	0.499
Medical history			
Hypertension, no. (%)	32 (32.0)	62 (35.6)	0.542
Hyperlipidemia, no. (%)	14 (14.0)	31 (17.8)	0.412
Diabetes, no. (%)	10 (10.0)	23 (13.2)	0.431
CKD, no. (%)	8 (8.0)	16 (9.2)	0.736
CCVDs, no. (%)	14 (14.0)	33 (19.0)	0.294
Biochemical index			
Scr (mg/dl), median (IQR)	0.8 (0.7–1.0)	1.4 (1.0–2.2)	<0.001
Albumin (g/L), median (IQR)	42.8 (39.1–47.4)	27.6 (21.6–39.9)	<0.001
WBC ($*10^9$/L), median (IQR)	6.4 (5.4–7.5)	15.1 (11.4–26.1)	<0.001
CRP (mg/L), median (IQR)	3.7 (2.5–6.4)	96.2 (46.9–133.1)	<0.001
Inflammatory cytokine			
TNF-α (pg/ml), median (IQR)	33.0 (28.2–38.2)	145.1 (112.4–226.7)	<0.001
IL-6 (pg/ml), median (IQR)	15.3 (11.2–22.3)	55.5 (38.7–78.2)	<0.001
IL-17 (pg/ml), median (IQR)	31.9 (22.3–44.2)	141.2 (84.1–230.1)	<0.001
ICAM1 (pg/ml), median (IQR)	205.4 (138.7–294.3)	557.7 (448.5–727.3)	<0.001
VCAM1 (pg/ml), median (IQR)	749.0 (468.9–1006.4)	1868.8 (1373.6–2478.7)	<0.001
Primary infection site			
Abdominal infection, no. (%)	–	55 (31.6)	–
Respiratory infection, no. (%)	–	50 (28.7)	–
Skin and soft tissue infection, no. (%)	–	35 (20.1)	–
Blood stream infection, no. (%)	–	22 (12.6)	–
Other infections, no. (%)	–	12 (6.9)	–
Primary organism			
G− bacteria, no. (%)	–	101 (58.0)	–
G+ bacteria, no. (%)	–	60 (34.5)	–
Fungus, no. (%)	–	21 (12.1)	–
Others, no. (%)	–	42 (24.1)	–
Disease severity			
APACHE II score, median (IQR)	–	12.0 (7.0–16.0)	–
SOFA score, median (IQR)	–	5.0 (4.0–7.3)	–

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; BMI, body mass index; CCVDs, cardio-cerebrovascular diseases; CKD, chronic kidney disease; CRP, C-reactive protein; G−, gram-negative; G+, gram-positive; ICAM1, intercellular adhesion molecule 1; IL, interleukin; IQR, interquartile range; Scr, serum creatinine; *SD*, standard deviation; SOFA, sequential organ failure assessment; TNF, tumor necrosis factor; VCAM1, vascular cell adhesion molecule 1; WBC, white blood cell.

and 42 (24.1%) patients infected with G− bacteria, G+ bacteria, fungus, and others, respectively. Regarding disease severity, the APACHE II score and SOFA score were 12.0 (7.0–16.0) and 5.0 (4.0–7.3), respectively. More detailed information of clinical characteristics in sepsis patients and controls was shown in Table 1.

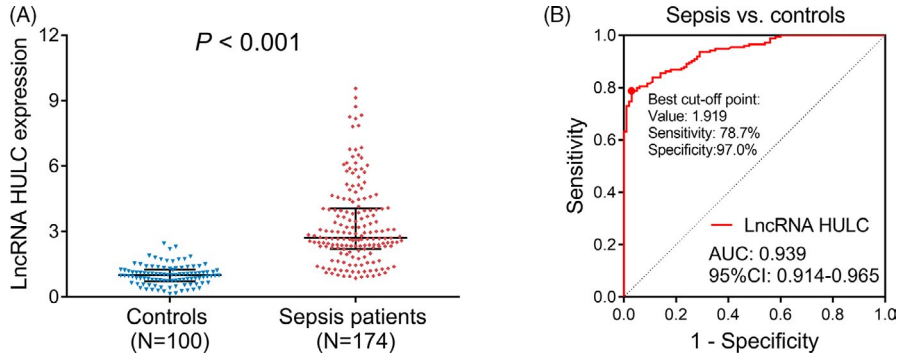


FIGURE 1 Correlation of lncRNA HULC with sepsis susceptibility. LncRNA HULC expression in sepsis patients and controls (A). The ability of lncRNA HULC in differentiating sepsis patients from controls (B). The difference in lncRNA HULC between sepsis patients and controls was compared by Wilcoxon rank-sum test. ROC curve was used to assess the ability of variables in distinguishing sepsis patients from controls. AUC, area under the curve; CI, confidence interval; lncRNA HULC, long non-coding RNA highly up-regulating in liver cancer; ROC, receiver operating characteristic

3.2 | LncRNA HULC expression in sepsis patients and controls

LncRNA HULC expression was increased in sepsis patients (2.720 (2.196–4.055)) compared with controls (0.996 (0.728–1.256)) ($p < 0.001$) (Figure 1A). Furthermore, ROC curve indicated that lncRNA HULC was of excellent value in differentiating sepsis patients from controls (AUC: 0.939, 95% CI: 0.914–0.965), and the sensitivity and specificity at the best cutoff point (the point where the largest sum of sensitivity and specificity occurred) were 78.7% and 97.0%, respectively, and the cutoff value of lncRNA HULC relative expression was 1.919 (Figure 1B).

3.3 | Correlation of lncRNA HULC with inflammatory cytokines in sepsis patients and controls

In sepsis patients, lncRNA HULC expression was positively correlated with TNF-α ($r = 0.237$, $p = 0.002$) (Figure 2A), IL-6 ($r = 0.232$, $p = 0.002$) (Figure 2B), IL-17 ($r = 0.276$, $p < 0.001$) (Figure 2C), ICAM1 ($r = 0.227$, $p = 0.003$) (Figure 2D), and VCAM1 ($r = 0.275$, $p < 0.001$) (Figure 2E), whereas in controls lncRNA HULC expression was not correlated with TNF-α ($r = 0.144$, $p = 0.153$) (Figure 2F), IL-6 ($r = 0.094$, $p = 0.352$) (Figure 2G), IL-17 ($r = 0.162$, $p = 0.106$) (Figure 2H), or ICAM1 ($r = 0.143$, $p = 0.157$) (Figure 2I), while it presented a weak positive correlation with VCAM1 ($r = 0.204$, $p = 0.042$) (Figure 2J).

3.4 | Correlation of lncRNA HULC with biochemical indexes in sepsis patients and controls

In sepsis patients, lncRNA HULC was positively correlated with Scr ($r = 0.339$, $p < 0.001$), WBC ($r = 0.245$, $p = 0.001$), and CRP ($r = 0.386$, $p < 0.001$) but negatively correlated with albumin ($r = -0.433$, $p < 0.001$) (Table 2). However, in controls, there is no correlation of lncRNA HULC with these biochemical indexes including Scr ($r = -0.080$, $p = 0.427$), albumin ($r = -0.092$, $p = 0.364$), WBC ($r = 0.057$, $p = 0.574$), and CRP ($r = 0.036$, $p = 0.725$).

3.5 | Correlation of lncRNA HULC with disease severity and infective features in sepsis patients

In sepsis patients, lncRNA HULC expression was positively correlated with APACHE II ($r = 0.414$, $p < 0.001$) (Figure 3A) and SOFA score ($r = 0.447$, $p < 0.001$) (Figure 3B). In addition, lncRNA HULC expression was not correlated with primary infection sites (including abdominal site, respiratory site, skin and soft tissue, blood stream, and others) ($p = 0.436$) (Figure 4A). Furthermore, lncRNA HULC expression was not associated with primary infection organism (including G− bacteria, G+ bacteria, fungus, and others) ($p = 0.875$) (Figure 4B).

3.6 | Correlation of lncRNA HULC with 28-day mortality in sepsis patients

In sepsis patients, lncRNA HULC expression was increased in deaths (3.957 (2.990–6.388)) compared with survivors (2.570 (1.731–3.758)) ($p < 0.001$) (Figure 5A). Furthermore, ROC curve indicated that lncRNA HULC was of good value in predicting 28-day mortality (AUC: 0.785, 95% CI: 0.713–0.857) with the sensitivity and specificity at the best cutoff point 58.7% and 91.7%, respectively, and the best cutoff value of lncRNA HULC relative expression 2.686 (Figure 5B). (Figure 5B) Furthermore, the 28-day mortality predictive value of lncRNA HULC was numerically superior to some inflammatory cytokines including TNF-α (Figure 6C),

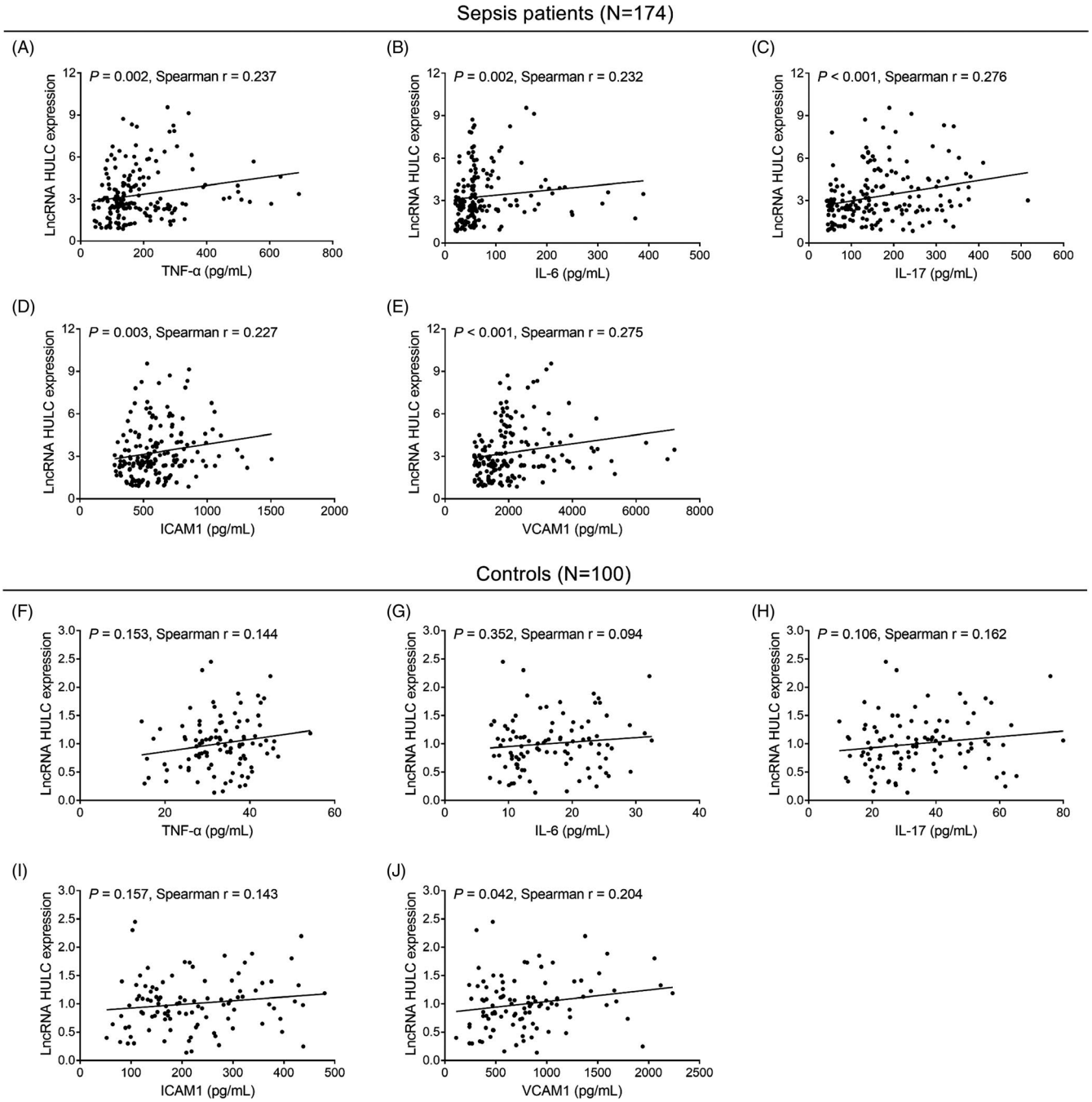


FIGURE 2 Correlation of lncRNA HULC with inflammatory cytokines. Correlation of lncRNA HULC with TNF-α (A), IL-6 (B), IL-17 (C), ICAM1 (D), VCAM1 (E) in sepsis patients. Correlation of lncRNA HULC with TNF-α (F), IL-6 (G), IL-17 (H), ICAM1 (I), VCAM1 (J) in controls. The correlation of lncRNA HULC with inflammatory cytokine level was determined by Spearman's rank correlation test. ICAM1, intercellular adhesion molecule 1; IL, interleukin; LncRNA HULC, long non-coding RNA highly up-regulating in liver cancer; TNF, tumor necrosis factor; VCAM1, vascular cell adhesion molecule 1

IL-6 (Figure 6D), ICAM1 (Figure 6F), VCAM1 (Figure 6G), and biochemical indexes including Scr (Figure 6H), albumin (Figure 6I), and WBC (Figure 6J), meanwhile was similar to IL-17 (Figure 6E), but was inferior to comprehensive disease severity-assessed scores such as APACHE II score (Figure 6A), SOFA score (Figure 6B) and biochemical indexes CRP (Figure 6K). The detailed information about the ability of each clinical indicator in predicting 28-day mortality was shown in Figure 6.

3.7 | Independent factors affecting 28-day mortality in sepsis patients

Multivariate logistic regression analysis indicated that lncRNA HULC expression (OR = 1.494, $p = 0.016$), age (OR = 1.084, $p = 0.004$), fungus infection (OR = 5.399, $p = 0.015$), CRP (OR = 1.010, $p = 0.010$), and APACHE II score (OR = 1.210, $p < 0.001$) were independent factors for predicting increased 28-day mortality (Table 3).

TABLE 2 Correlation of lncRNA HULC with biochemical indexes

Items	LncRNA HULC	
	Spearman *r*	*p* value
Sepsis patients		
Scr	0.339	<0.001
Albumin	−0.433	<0.001
WBC	0.245	0.001
CRP	0.386	<0.001
Controls		
Scr	−0.080	0.427
Albumin	−0.092	0.364
WBC	0.057	0.574
CRP	0.036	0.725

Abbreviations: CRP, C-reactive protein; LncRNA HULC, long non-coding RNA highly upregulated in liver cancer; Scr, serum creatinine; WBC, white blood cell.

example, lncRNA HULC is upregulated in hepatitis B patients and interacts with hepatitis B X-interacting protein in hepatitis B-related diseases.[12] Furthermore, mounting recent studies reveal that lncRNA HULC high expression is correlated with secretion of inflammation factors, the regulation of oxidative stress response in inflammatory process of various diseases.[9,14,17] In addition, activation of systemic inflammation by toxic effects of LPS is a way to create septic model, and a recent study observes that lncRNA HULC is increased for pro-inflammatory response in LPS-induced cell model of sepsis.[14,18] In addition, as for the lncRNA HULC with organ injury, one study indicates that reduced lncRNA HULC expression suppresses oxidative stress and inflammatory injury in rates with cirrhosis.[10] Another study reveals that lncRNA HULC is dysregulated in ischemia/reperfusion-injured injury myocardial tissue, and regulates the apoptosis in the hypoxia/reoxygenation-induced cardiomyocytes via inflammation-related (NLRP3/Caspase-1/IL-1β) signaling pathway.[11] However, the clinical value of lncRNA HULC in sepsis was still unknown, which was investigated in the present study.

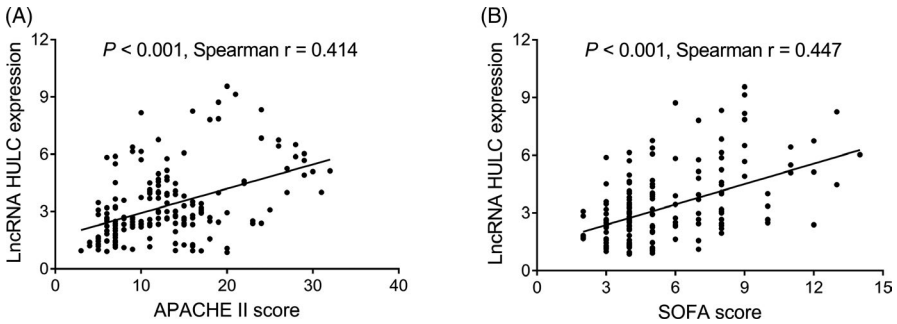
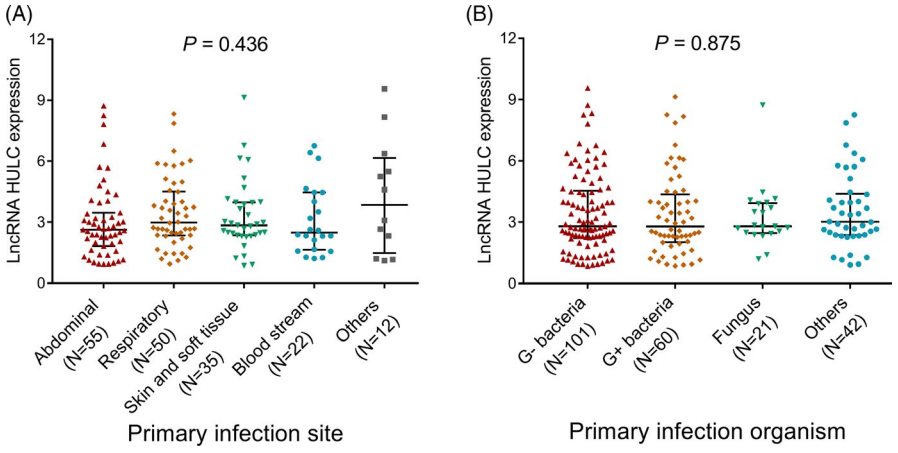


FIGURE 3 Correlation of lncRNA HULC with disease severity. The correlation of lncRNA HULC with APACHE II score (A) and SOFA score (B) in sepsis patients. The correlation of lncRNA HULC with disease severity was determined by Spearman's rank correlation test. APACHE II, acute physiology and chronic health evaluation II; LncRNA HULC, long non-coding RNA highly up-regulating in liver cancer; SOFA, sequential organ failure assessment

FIGURE 4 LncRNA HULC expression in different primary infection sites and primary infection organisms. Correlation of lncRNA HULC with primary infection sites (A) and primary infection organism (B) in sepsis patients. The correlation of lncRNA HULC with primary infection sites and primary infection organism of sepsis patients was determined by Kruskal-Wallis *H* rank-sum test. G−, gram-negative; G+, gram-positive; LncRNA HULC, long non-coding RNA highly up-regulating in liver cancer



4 | DISCUSSION

Existing evidence has demonstrated that highly expressed lncRNA HULC is positively correlated with several infections.[12,13,16] For

In the present study, we consecutively enrolled 174 sepsis patients and another 100 age- and gender-matched controls, and collected their peripheral blood samples for lncRNA HULC and inflammatory cytokine detection, which observed that lncRNA

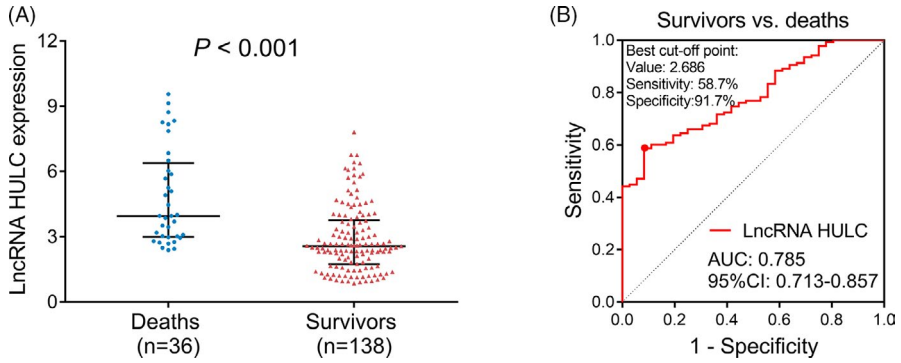


FIGURE 5 Correlation of LncRNA HULC expression with mortality risk. LncRNA HULC expression in deaths and survivors of sepsis patients (A). The ability of lncRNA HULC in differentiating deaths from survivors (B). The difference in lncRNA HULC between survivors and deaths was compared by Wilcoxon rank-sum test. ROC curve was used to assess the ability of variables in distinguishing survivors from deaths. AUC, area under the curve; CI, confidence interval; lncRNA HULC, long non-coding RNA highly up-regulating in liver cancer; ROC, receiver operating characteristic

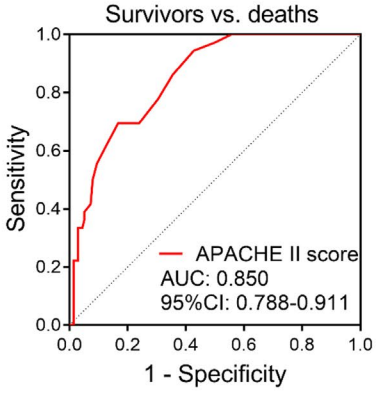
HUCL was upregulated in sepsis patients compared with matched controls. The possible reasons might be that lncRNA HULC might promote the feedback loop between oxidative stress and inflammation, leading to cytokine storm and injured tissue, eventually contributing to increased sepsis risk.[4,9] In addition, according to existing evidence, there is certain evidence indicating the involvement of TNF-α, IL-6, IL-17, ICAM1, and VCAM1 in the sepsis pathology.[19-21] For example, there is evidence displaying that ICAM-1 and VCAM-1, as members of the immunoglobulin superfamily, are increased expressed in endothelial cells by inflammatory mediators, and play fundamental roles of inflammatory processes and development of multiple organ dysfunction.[21] In our study, further analysis indicated the correlation of lncRNA HULC with increased inflammatory cytokines (TNF-α, IL-6, IL-17, ICAM1, and VCAM1) and dysregulated biochemical indexes (including Scr, albumin, WBC and CRP) in sepsis patients, but not in controls, which suggested the promoting effect of lncRNA HULC on the release of pro-inflammatory factors as well as the deterioration of organ injuries in sepsis patients. Meanwhile, the absence of correlation between lncRNA HULC with inflammatory cytokines and biochemical indexes in controls might be explained by the non-obvious increased inflammation and organ injuries.

Meanwhile, we also observed that lncRNA HULC was positive correlated with disease severity in sepsis patients. The possible reasons might include that (I) lncRNA HULC high expression might activate uncontrolled host inflammatory response via production of pro-inflammatory mediators (such as ICAM1, IL-6, and VCAM-1) and recruitment of inflammatory cells (such as WBC), which results to cellular injury and further organ dysfunction, contributing to increased severity of sepsis.[4,14] (II) In addition to activation of imbalanced inflammatory response and immune dysfunction via elevating pro-inflammatory cytokines, a complex chain of oxidative stress events caused by lncRNA HULC upregulation paralleled might exacerbate the progression of multiple organ dysfunction, and further elevate the severity of sepsis.[4,22] Interestingly, in our study, lncRNA HULC was not correlated with primary infection sites and primary infection organism in sepsis patients. The possible reason might be that lncRNA HULC was mainly correlated with inflammation level and organ injuries, but the sensitivity of lncRNA HULC on regulating infection source was poor.

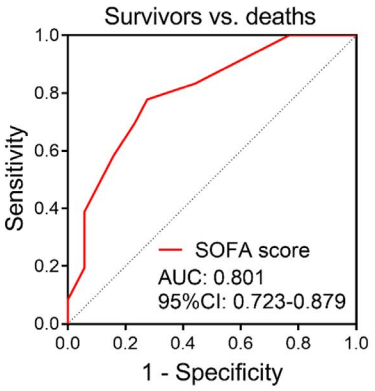
Furthermore, we also observed that lncRNA HULC could predict increased 28-day mortality independently, and notably, the predictive value of lncRNA HULC was superior to some common inflammatory cytokines and biochemical indexes (TNF-α, IL-6, IL-17, ICAM1, VCAM1, Scr, albumin, WBC) of sepsis. The possible reasons might include that (I). According to the prior results in our study, lncRNA HULC high expression might be correlated with worse overall health condition and exacerbated organ failure via affecting APACHE II and SOFA score; therefore, lncRNA HULC could predict the higher possibility of 28-day mortality in sepsis patients but presented lower predictive value compared with APACHE II and SOFA score. (II) Furthermore, lncRNA HULC might promote the amplification of inflammation response and oxidative stress, leading to increased rate of organ dysfunction, which directly contributed to higher mortality of sepsis patients. (III) Furthermore, considering the correlation of lncRNA HULC with various key prognostic predictors of sepsis (such as inflammatory cytokines, biochemical indexes, SOFA score, APACHE II score, etc),

FIGURE 6 Ability of comprehensive severity-assessed scores, inflammatory cytokines, and biochemical indexes in predicting mortality of sepsis. Ability of APACHE II score (A), SOFA score (B), TNF-α (C), IL-6 (D), IL-17 (E), ICAM1 (F), VCAM1 (G), Scr (H), albumin (I), WBC (J), and CRP (K) in differentiating survivors from deaths of sepsis. ROC curve was used to assess the ability of variables in distinguishing survivors from deaths. APACHE II, acute physiology and chronic health evaluation II; AUC, area under the curve; CI, confidence interval; ICAM1, intercellular adhesion molecule 1; IL, interleukin; lncRNA HULC, long non-coding RNA highly up-regulating in liver cancer; ROC, receiver operating characteristic; SOFA, sequential organ failure assessment; TNF, tumor necrosis factor; VCAM1, vascular cell adhesion molecule 1

(A)



(B)



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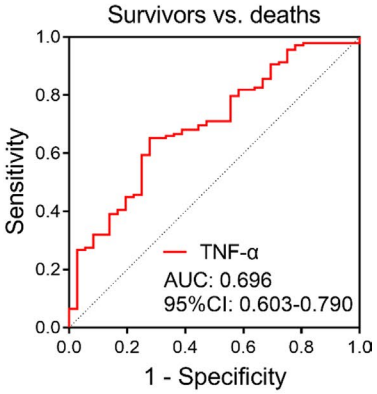
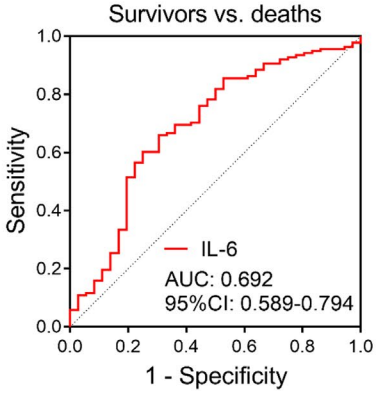


(D)



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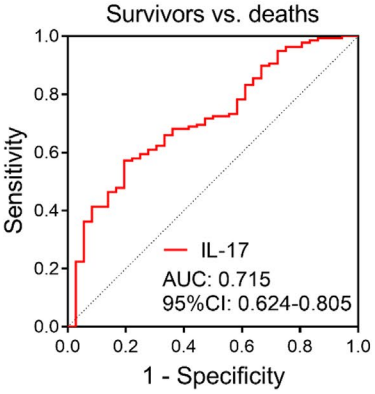

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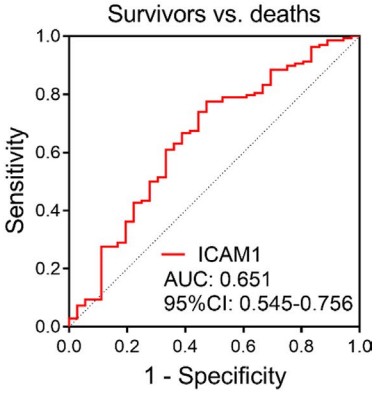
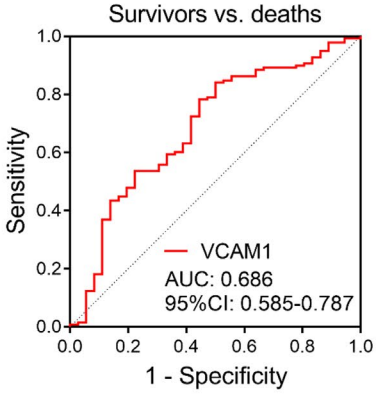


(G)



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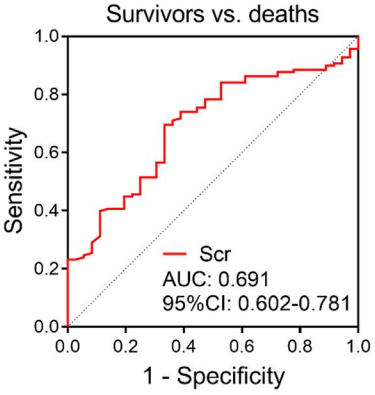
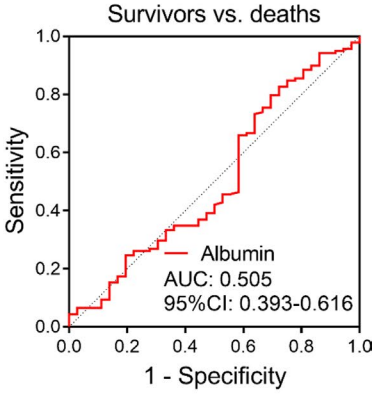
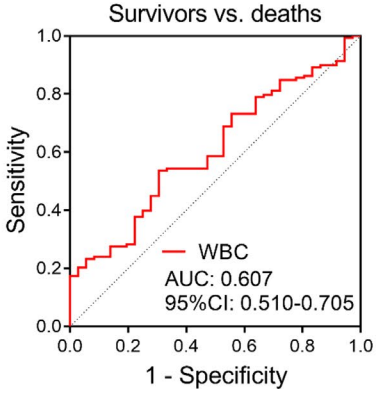


(I)



(J)



(K)

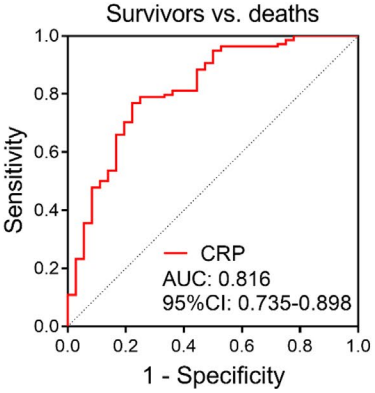


TABLE 3 Multivariate logistic regression model analysis of factors related to 28-day mortality

	Multivariate logistic regression model[a]			
			95% CI	
Items	*p* value	OR	Lower	Higher
LncRNA HULC expression	0.016	1.494	1.076	2.074
Age	0.004	1.084	1.026	1.145
Fungus infection	0.015	5.399	1.385	21.048
CRP	0.010	1.010	1.002	1.018
APACHE II score	<0.001	1.210	1.099	1.331

Abbreviations: APACHE II, acute physiology and chronic health evaluation II; CI, confidence interval; CRP, C-reactive protein; ICAM1, intercellular adhesion molecule 1; IL, interleukin; lncRNA HULC, long non-coding RNA highly upregulated in liver cancer; OR, odds ratio; TNF, tumor necrosis factor; VCAM1, vascular cell adhesion molecule-1.

[a]All clinical characteristics, lncRNA HULC expression, and inflammatory cytokines including TNF-α, IL-6, IL-17, ICAM1, and VCAM1 were included in the multivariate logistic regression model analysis, and forward stepwise method was used to screen the independent factors related to 28-day mortality.

lncRNA HULC could influence the prognosis of sepsis patients via multiple ways, which contributed to the superior predictive value of lncRNA HULC to some inflammatory cytokines and biochemical indexes in sepsis management.

However, the present study existed some limitations as follows: (I) The present study was a single-centered study with a small sample size, which might lead to regional selective bias. (II) The underlying mechanism of lncRNA HULC in mediating LPS-induced organ dysfunction should be further explored in the future. (III) The present study revealed the correlation of lncRNA HULC with inflammation and disease severity as well as the value of lncRNA HULC in predicting 28-day mortality in sepsis; however, the value of lncRNA HULC for predicting the prognosis in longer period was needed for exploration. (IV) The longitudinal change in lncRNA HULC level needed further exploration in patients with sepsis. (V) The influence of treatment on the expression of lncRNA HULC in sepsis patients, which needed further investigation.

In conclusion, lncRNA HULC is highly expressed in sepsis patients compared with controls, and correlates with dysregulated biochemical indexes, increased disease severity, inflammation, and 28-day mortality in sepsis patients, suggesting the potential of lncRNA HULC as a biomarker for sepsis management.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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