Circulating Long Noncoding RNAs Positively Correlate with the Increased Risk, Elevated Severity and Unfavorable Prognosis in the Sepsis Patients

Jun Yuan

First Affiliated Hospital of Anhui Medical University

Limian Cao

First Affiliated Hospital of Anhui Medical University

Junjie Bao

First Affiliated Hospital of Anhui Medical University

Yutao Zha

First Affiliated Hospital of Anhui Medical University

Shi Chen

First Affiliated Hospital of Anhui Medical University

Wenjing Fan

First Affiliated Hospital of Anhui Medical University

Ming Fang

First Affiliated Hospital of Anhui Medical University

Yonghui Gui

First Affiliated Hospital of Anhui Medical University

Nian Liu

First Affiliated Hospital of Anhui Medical University

Min Shao (✉ shaominmail@yahoo.com)

First Affiliated Hospital of Anhui Medical University <https://orcid.org/0000-0001-8886-5620>

Research

Keywords: Circulating lncRNAs, sepsis, septic shock, diagnosis, prognosis, serum

Posted Date: January 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1131221/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Objective

This study aimed to evaluate the correlation of circulating long noncoding RNAs (lncRNAs) expression with disease risk, severity, inflammatory cytokines levels and prognosis in patients with sepsis.

Methods

Differential expression profiles of lncRNA in the serum of sepsis rats were screened by high-throughput transcriptome sequencing. Homologous lncRNAs in the upregulation group were identified by homology analysis in rats and humans. The expression differences of these homologous lncRNAs in the serum of 176 sepsis patients and 176 healthy controls (HCs) were detected using reverse transcription quantitative polymerase chain reaction (RT-qPCR). And inflammatory cytokines levels were detected by enzyme-linked immunosorbent assay (ELISA). A receiver operating characteristic (ROC) curve was used to verify the diagnostic and prognosis values. Spearman correlation coefficient was used to analyze the correlation between the variables. Follow-up was performed to observe the 28-day mortality.

Results

Among the screened differentially up-regulated lncRNAs, only two lncRNAs were homologous in rats and humans, which in human named PKN2-antisense RNA 1 (PKN2-AS1) and AC068888.1, respectively. Those two lncRNAs were significantly increased in patients with sepsis compared with those in HCs ($P < 0.001$), in patients with septic shock compared with those no septic shock ($P < 0.001$), and in non-survivors compared with survivors ($P < 0.001$). And those two lncRNAs were positively correlated with sepsis-related organ failure assessment (SOFA) score, acute physiology and chronic health evaluation (APACHE) II score, lactate (Lac), c-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in sepsis patients. Likelihood ratio forward stepwise multivariate logistic regression analysis revealed that high lncRNA AC068888.1 expression was an independent risk factor for septic shock ($P < 0.001$) and unfavorable prognosis ($P = 0.006$), but high lncRNA PKN2-AS1 expression was only for unfavorable prognosis ($P = 0.019$). The ROC curve exhibited a significant predictive value for sepsis risk with area under the curve (AUC) values of 0.879 and 0.842, respectively. For predicting septic shock risk, combining lncRNA AC068888.1 with SOFA score and Lac level, the ROC curve analysis significantly improved the predictability (AUC = 0.882). For predicting 28-day death risk, combining those two lncRNAs with SOFA and APACHE II scores, the ROC curve analysis also significantly improved the predictability (AUC = 0.860). The Kaplan–Meier curves indicated that the survival probability was much worse with those two lncRNAs high expression compared to low expression in patients with sepsis ($P < 0.001$).

Conclusion

The circulating absolute expression levels of lncRNA PKN2-AS1 and AC068888.1 in the serum may be used for the early diagnosis, clinical severity evaluation and prognosis of sepsis.

Introduction

Sepsis is a life-threatening organ dysfunction resulting from a dysregulation of the host's response to infection, meanwhile, septic shock should be defined as a subset of sepsis [1–3]. According to clinical epidemiology statistics, globally, there are an estimated 31.5 million cases of sepsis and 19.4 million cases of septic shock each year, with 5.3 million deaths [4]. Despite the significant advances in clinical management and use of devices, sepsis remains a significant public health problem worldwide due to high morbidity, mortality, and rising costs associated with the complex care of patients [5]. In addition, the new data from the current emerging COVID-19 pandemic indicate a close relationship between severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) and sepsis; characterized by organ damage and systemic inflammation due to viral invasion [6–8]. Thus, it is necessary to search for new diagnostic and therapeutic approaches to improve clinical prognosis by elucidating the pathogenesis of sepsis comprehensively.

Long non-coding RNAs (lncRNAs) are a large class of 200-nucleotide long noncoding transcripts that lack the ability to encode proteins [9, 10]. Previously, they were considered to be the "noise" produced by genome transcription and have no biological functions [10]. However, now more and more studies have found that lncRNAs can participate in a variety of biological processes, including inflammation, transcriptional activation, transcriptional interference, histone modification, chromatin remodeling, cell cycle regulation, epigenetics, and RNA splicing [11–13]. With the advancement of sequencing technology and chip detection technologies, a growing number of lncRNA expression profiles have been observed recently [14]. The lncRNAs have a regulatory function in human diseases, especially cancer, which have become diagnostic markers and therapeutic targets for related tumors [15, 16]. The lncRNAs were detected not only in cancer tissue, but also in various body fluids, including urine, blood and saliva [17]. Through detection of lncRNAs in body fluids and further molecular mechanism studies, it was found that these lncRNAs play an important regulatory role in cardiovascular diseases [18] and inflammatory diseases [19]. But beyond that the corresponding regulatory function of lncRNAs in multiple organ failure caused by sepsis has been gradually reported in recent years [20]. Therefore, these reported studies on lncRNAs provide new possibilities for early rapid identification and clinical treatment of sepsis.

However, compared to the large lncRNAs families, current studies related to sepsis are very limited [21]. Therefore, through high-throughput transcriptome RNA sequencing, we found some up-regulated lncRNAs in peripheral blood of septic rat model, and two lncRNAs were identified by homology analysis of human and rat, respectively PKN2-AS1 and AC068888.1. In this paper, we further assessed their ability to distinguish between sepsis and healthy controls, as well as their association with disease severity, inflammation, and survival in patients with sepsis.

Material And Methods

Animals and LPS model of sepsis

Nine Specific Pathogen Free (SPF) male Sprague–Dawley rats (7-8 weeks old, weighing 250g to 300g) were used as an in vivo model species, which were provided by the Animal Experiment Center of Anhui Medical University (Anhui, China). The rats were placed at 3 rats per cage and labeled cage 1, 2, and 3, respectively. The food and water were freely provided, and the room temperature (22 ± 2 °C), humidity (60%-80%), and 12-hours light/dark cycle were maintained. Before onset of the experiment, the rats were allowed to adapt to the environment for a week. All animal procedures were approved by the Ethics Committee of Anhui Medical University (Ethics Approval Number LLSC20190721). The lipopolysaccharide (LPS) (Sigma, St. Louis, MO, USA) was dissolved in sterile normal saline (0.9% NaCl) at a concentration of 1 mg/mL and injected intraperitoneally at a dose of 10 mg/kg. The rats of cage 2 and 3 were treated for 6 hours and 24 hours respectively. The control group (cage 1) was injected with the same amount of saline in the same way [22].

Treatment of model specimens and high-throughput transcriptome sequencing analysis

The rats of cage 2 and 3 were given pentobarbital sodium (Sigma, St. Louis, MO, USA) (100 mg/kg) intraperitoneally 6 h and 24 h after LPS treatment, and the control group (cage 1) was anesthetized using the same method. After satisfactory anesthesia, 7 mL of whole heart blood was collected under direct vision, and the serum was separated using a low temperature and high-speed centrifugation method (Step 1: 4 °C, 3000 rpm, 10 min, Step 2: 4 °C, 12000 rpm, 10 min). High-throughput transcriptome sequencing was performed on the collected sera to detect the information of abnormally expressed lncRNAs by BGI Company (Shenzhen, China). The sequencing item number was F19FTSCCKF1548_ou201909271103114221RA0305. Significantly DElncRNAs were studied using DESeq R software package (3.5.1). A threshold value of $|\log_2(\text{fold change})| > 1$ with a P value < 0.05 was determined. In order to obtain an overview of the expression profiles of lncRNAs, 'pheatmap' and 'ggplot2' R packages were used to draw the heat map and volcano plot, respectively. Through genomic analysis, human-rat homologous lncRNAs were screened out from all the highly expressed lncRNAs, and representative highly expressed lncRNAs were selected for clinical verification.

Study subjects and general data

This study consecutively enrolled 176 sepsis patients from intensive care unit (ICU) of the first affiliated Hospital of Anhui Medical University between September 2019 and March 2021, including 114 males and 62 females, aged 18-75 years, with a mean age of 57.5 (22) years. Meanwhile, we continuously recruited 176 healthy controls (HCs) who underwent a regular physical examination at our Health Examination Center and compared them with sepsis patients in the same period, including 94 males and 82 females, aged 21-73 years, with a mean age of 56.7 (23) years. All HCs showed no significant abnormalities in the biochemical indexes, and no history of malignant blood diseases, solid tumors, sepsis or other serious infections. This study was approved by the Human Ethics Committee of the First Affiliated Hospital of

Anhui Medical University (Ethics Approval Number PJ2019-14-12). All participants or their family members provided written informed consent before the registration for this study.

Inclusion and exclusion criteria

The screening criteria were as follows: (a) patients diagnosed with sepsis according to the third International Consensus Definitions for Sepsis and Septic Shock (1), (b) patients with the age of ≥ 18 years old and ≤ 75 years old, (c) patients who admitted to the intensive care unit (ICU) within the previous 24 hours, (d) patients who did not have other fatal diseases (e.g., hematologic malignancies, solid tumors, or acquired immune deficiency syndrome), (e) patients without immunosuppressive therapy within 3 months before the enrollment, (f) patients who were not in pregnancy or lactation.

Date collection

Sepsis patients' clinical characteristics were recorded after admission, which included demographic characteristics, complications, primary infection sites, organ dysfunction, glucocorticoid drugs intakes, biochemical indexes, and disease severity. The severity of the sepsis was assessed within 24 hours after admission using the APACHE II score and SOFA score. All patients were treated in accordance with the current guidelines for treatment of sepsis and followed up for 28 days (28-day survival was recorded as well).

Sample collection

The peripheral blood (PB) samples of the sepsis patients were collected within 24 hours after admission, and the PB samples of the HCs were obtained at the time of enrollment. After collection, the PB samples were centrifuged at 3000 rpm for 10 minutes at 4°C to separate plasma. Then, the plasma samples were centrifuged at 12000 rpm for 10 minutes at 4°C to separate serum, which was preserved at -80°C for the subsequent analysis. The absolute expression levels of lncRNAs in the serum samples were detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Real-time quantitative polymerase chain reaction (RT-qPCR)

The serum was separated from the blood samples by centrifugation, and the total RNA in serum was extracted using Bioo cRNA Easy Kit (Baidai Biotechnology Co., LTD, Changzhou, Zhejiang, China) following the manufacturer's instructions. The RNA concentrations were determined using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The complementary DNA (cDNA) synthesis with extracted the total RNA was performed using a PrimeScript™ RT Reagent Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All quantitative RNA detections were carried out in triplicates with 2X Universal SYBR Green Fast qPCR Mix (Abclonal Biotech. Co., Ltd., Wuhan, Hubei, China) using a Bio-Rad CFX96 Touch Real-time Thermocycler (Bio-Rad Laboratories GmbH, Munich, Germany). The reaction was incubated for at 95°C for 3 minutes, followed by 40 cycles of 95°C for 5 seconds and 60°C for 32 seconds. Calculation of absolute expression of lncRNA by standard curve method. The primers were as follows: lncRNA PKN2-AS1 forward: GACCAGTGAGAGACCAGCAT,

reverse: GTCCGTGAAATGCAGCAGAT, lncRNAAC068888.1 forward: CCATTGGAGAGGAGCTAGGG, reverse: TCATCCCTTCTGCAAGGTGT (General Biosystems, Anhui, China). The exact gene copy numbers of lncRNA PKN2-AS1 and AC068888.1 transcripts per milliliter of serum were quantified by using RT-qPCR assay. In this assay, serially diluted RT-PCR products of lncRNAPKN2-AS1 and AC068888.1 were used as templates to formulate standard curves, and then, the exact copies of lncRNAPKN2-AS1 and AC068888.1 per milliliter of serum were calculated accordingly. Using the data of three independent tests, the absolute expression of the target gene was calculated by the standard curve method [23].

Detections of inflammatory cytokines in the sepsis patients

Serum inflammatory cytokines, IL-6 and TNF- α , were detected by ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) in patients with sepsis.

Statistical analysis

SPSS26.0 software (IBM Corporation, Armonk, NY, USA) and R language version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis and generating graphs, respectively. Continuous data were represented as the mean $\pm$ standard deviation (SD) or the median (IQR) of interquartile intervals. The categorical data were expressed in numbers (percentages). The T-test, Chi-square test or Wilcoxon rank-sum test were used to compare the differences of variables between the two groups. The Spearman rank correlation test was used to analyze the correlation between the two variables. The ROC curve was plotted to evaluate the abilities of different subjects using the AUC, sensitivity, and specificity of the optimal cut-off point. The survival probability was described by the Kaplan-Meier method, and the difference of survival probability between the two groups was described by logarithmic rank test. The univariate and likelihood ratio forward stepwise multivariate logistic regression model were used to predict the independent risk factors in patients with sepsis. $P < 0.05$ was considered statistically significant.

Results

High-throughput transcriptome sequencing RNA gene analysis

Unsupervised hierarchical cluster analysis was used to analyze the expression pattern of lncRNAs in the serum from the sepsis rats at different periods (0- 6- 24 hour). The results showed that there was a significant difference in the lncRNA expression profiles between volcano map (Fig. 1a) and heat map (Fig. 1b). In this study, 63 lncRNAs (29 up-regulated and 34 down-regulated) were identified as significantly differentially expressed between the normal control group (NC) and sepsis group (Supplementary Table S1 online). Here we select the 29 up-regulated ones for confirmatory analysis. Among those up-regulated lncRNAs, we identified the human-rat orthologous sequences by UCSC (University of California, Santa Cruz) Genome Browser and found only lncRNA PKN2-AS1 (LOC108350126, ENST00000645056.1) and AC068888.1 (LOC108351556, ENST00000663863.1) had homologous conserved gene sequences. Other DElncRNAs were mRNA (messenger RNA) sequences or

not having homologous gene sequences in the human genome (Supplementary Table S2 online). Neither lncRNA PKN2-AS1 nor AC068888.1 has ever reported in sepsis before. Hence, those two lncRNAs were screened out for clinical verification.

Baseline characteristics

The average age was 57.5(22.0) years in patients with sepsis, and there were 62 (35.2%) females and 114 (64.8%) males. Specific to chronic complications, 12 (6.8%), 32 (18.2%), 9 (5.1%), 1 (0.6%), 31 (17.6%) and 62 (35.2%) sepsis patients had chronic obstructive pulmonary disease (COPD), cardiomyopathy, chronic kidney failure, cirrhosis, diabetes and hypertension disease, respectively. Then, 75 (42.6%), 19 (10.8%), 12 (6.8%), 13 (7.4%), 35 (19.9%), 10 (5.7%) and 12 (6.8%) sepsis patients had a respiratory infection, abdominal infection, skin and soft tissue infection, urinary tract infection, blood stream infection, central nervous system (CNS) infection, and other infections, respectively. In addition, the sepsis patients suffered from multiple organs failure, and 88 (50%), 51 (29%), 77 (43.8%), 40 (22.7%), 63 (35.8%), 163 (92.6%), 151 (85.8%), 68 (38.6%) had heart failure, acute liver failure, acute renal failure, gastrointestinal failure, CNS failure, respiratory failure, circulatory failure, clotting dysfunction, respectively. And the average number of organ failures was 4.0 (2.0). Some sepsis patients were treated with glucocorticoids, such as dexamethasone, methylprednisolone, and hydrocortisone. Here, we converted multiple glucocorticoids into hydrocortisone equivalents, and the final statistical median use of glucocorticoids was 80.0 (1000.0). Besides, the median APACHE II score was 20.0 (8.0) and the median SOFA score was 10.0 (4.0) in sepsis patients. The detailed information regarding other characteristics such as body mass index (BMI) and biochemical indexes were shown in Table 1.

Absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 and their diagnostic value in the sepsis

The absolute expression of serum lncRNA PKN2-AS1 was 329.3 (77.4) gc/mL in patients with sepsis and 203.1 (96.1) gc/mL in the HCs. Meanwhile, the absolute expression of lncRNA AC068888.1 was 312.1 (91.5) gc/mL in patients with sepsis and 175.0 (93.6) gc/mL in the HCs, and further comparison analysis showed that lncRNA PKN2-AS1 and AC068888.1 expression were increased in in patients with sepsis compared with the HCs ($P < 0.001$) (Fig. 2a, 2b). From the ROC curve, the lncRNA PKN2-AS1 and AC068888.1 were differentiated between the sepsis patients and the HCs with an AUC of 0.879 (95% CI: 0.843-0.915) and 0.842 (95% CI: 0.801-0.884) (Fig. 2c, 2d). The optimal cut-off point for the level of lncRNA PKN2-AS1 that distinguished patients with sepsis from HCs was >238.0, and the specificity and sensitivity were 93.8% and 67.0%, respectively. Correspondingly, the optimal cut-off point for lncRNA AC068888.1 level that distinguished patients with sepsis from HCs was >210.6, and the specificity and sensitivity were 88.6% and 71.0%, respectively. These data suggested that both lncRNA PKN2-AS1 and AC068888.1 might be biomarkers for the sepsis risk. However, the predictive effect of lncRNA PKN2-AS1 was more prominent in comparison (Table 2).

Correlation of the lncRNA PKN2-AS1 and AC068888.1 absolute expression with the conventional evaluation indicators of sepsis, and inflammatory cytokines

Spearman's correlation analysis revealed that the serum absolute expression level of lncRNA PKN2-AS1 exhibited positively correlations with SOFA score ($r = 0.427$, $P < 0.001$), APACHE II score ($r = 0.303$, $P < 0.001$), Lac level ($r = 0.347$, $P < 0.001$), PCT level ($r = 0.245$, $P = 0.001$), and CRP level ($r = 0.156$, $P = 0.039$) (Fig. 3a-e). Meanwhile, the serum absolute expression level of lncRNA AC068888.1 also exhibited positively correlations with SOFA score ($r = 0.492$, $P < 0.001$), APACHE II score ($r = 0.317$, $P < 0.001$), Lac level ($r = 0.480$, $P < 0.001$), PCT level ($r = 0.261$, $P < 0.001$), and CRP level ($r = 0.149$, $P = 0.048$) (Fig. 3h-l). As to the inflammatory cytokines, lncRNA PKN2-AS1 absolute expression was positively correlated with IL-6 ($r = 0.422$, $P < 0.001$) and TNF- α ($r = 0.401$, $P < 0.001$) (Fig. 3f-g), and the lncRNA AC068888.1 absolute expression level also showed homogeneously correlations with IL-6 ($r = 0.476$, $P < 0.001$) and TNF- α ($r = 0.516$, $P < 0.001$) (Fig. 3m-n). These data indicated that both lncRNA PKN2-AS1 and AC068888.1 might be biomarkers for disease severity and inflammation in patients with sepsis, however, neither lncRNA PKN2-AS1 nor lncRNA AC068888.1 showed absolute advantages.

Clinical characteristics of septic shock and no septic shock sepsis patients and risk factors for septic shock

During the 28-day follow-up period, 100 (56.8%) sepsis patients occurred septic shock, and they were grouped as septic shock patients, 76 (43.2%) patients did not occur septic shock, and they were grouped as no septic shock patients. Univariate analysis revealed that the number of organ dysfunction, platelet (PLT), serum creatinine (Scr), PCT, Lac, prothrombin time (PT), activated partial thromboplastin time (APTT), APACHE II score, SOFA score ($P < 0.001$), other infections ($P = 0.021$), CRP ($P = 0.022$), fibrinogen (FIB) ($P = 0.016$), the absolute expression of lncRNA PKN2-AS1 ($P < 0.001$, Fig. 4a) and lncRNA AC068888.1 ($P < 0.001$, Fig. 4b) were significantly higher in patients with septic shock compared with in those without septic shock. The others were not significantly associated with the occurrence of septic shock (Table 3). In addition, likelihood ratio forward stepwise multivariate logistic regression analysis demonstrated that the highly absolute expression of lncRNA AC068888.1 ($P < 0.001$), Lac level ($P = 0.045$), SOFA score ($P = 0.031$), and PT ($P = 0.026$) were independent risk factors for septic shock, whereas the absolute expression of lncRNA PKN2-AS1 and others exhibited no association with the severity of septic shock (Table 4).

Diagnostic value of the lncRNA PKN2-AS1 and AC068888.1 level for septic shock

The ROC curve analysis exhibited significant predictive value for lncRNA PKN2-AS1 (AUC = 0.704) and AC068888.1 (AUC = 0.812) in distinguishing patients with septic shock from those without septic shock (Fig. 4c). The predictive value of lncRNA AC068888.1 was parallel to Lac (AUC = 0.816), but apparently higher compared with those for APACHE II score (AUC = 0.668), SOFA score (AUC = 0.791) and lncRNA PKN2-AS1. At the optimal cut-off point of >302.3 for the serum absolute expression level of lncRNA AC068888.1, the specificity and sensitivity were 78.0% and 77.6%, respectively. At the optimal cut-off point of >297.6 for the serum absolute expression level of lncRNA PKN2-AS1, the specificity and sensitivity were 85.0% and 51.3%, respectively. At the optimal cut-off point of >3.6 for Lac levels, the specificity and sensitivity were 81.0% and 76.3%, respectively. At the optimal cut-off point of >9.5 for

SOFA score, the specificity and sensitivity were 81.0% and 64.5%, respectively. At the optimal cut-off point of >20.5 for APACHE II score, the specificity and sensitivity were 56.0% and 72.4%, respectively. Those independent risk factors of septic shock were used to construct the predictive model for septic shock risk in sepsis patients (including lncRNA AC068888.1 score, SOFA score, Lac level), then the following ROC curve analysis manifested that the predictive model exhibited a good value for identifying septic shock risk in sepsis patients (AUC = 0.882) (Fig. 4c and Table 5).

Clinical characteristics of survival sepsis and non-survival sepsis patients, and risk factors for the unfavored prognosis of sepsis

96 (54.6%) sepsis patients survived during the 28-day follow-up period, and they were grouped as survival patients, 80 (45.4%) sepsis patients died, and they were grouped as non-survival patients. As presented in Table 6, univariate analysis revealed that number of organ dysfunction, the levels of PCT and Lac, APACHE II score, SOFA score ($P < 0.001$), glucocorticoid drugs ($P = 0.007$), septic shock ($P = 0.021$), PLT ($P = 0.010$), albumin ($P = 0.030$), Scr ($P = 0.004$), CRP ($P = 0.013$), PT ($P = 0.011$) and FIB ($P = 0.008$) were significantly different between survivors and non-survivors in patients with sepsis. Meanwhile, lncRNA PKN2-AS1 level ($P < 0.001$, Fig. 5a) and lncRNA AC068888.1 level ($P < 0.001$, Fig. 5b) were significantly increased in non-survivors compared with survivors. The other variables were not significantly different between survivors and non-survivors. In addition, the likelihood ratio forward stepwise multivariate logistic regression analysis demonstrated that APACHE II score ($P < 0.001$), high PCT level ($P = 0.032$), number of organ dysfunction ($P = 0.001$), septic shock ($P = 0.008$), the highly absolute expression of lncRNA PKN2-AS1 ($P = 0.019$) and lncRNA AC068888.1 levels ($P = 0.006$) were independent risk factors for the poor prognosis of sepsis, whereas other variables exhibited no association with prognosis in patients with sepsis (Table 7).

Prognostic value of the lncRNA PKN2-AS1 and AC068888.1 levels for sepsis

The ROC curve analysis exhibited significant predictive value for lncRNA PKN2-AS1 (AUC = 0.747) and AC068888.1 (AUC = 0.717) in distinguishing non-survivors from survivors (Fig. 5c), which were higher compared with its for Lac level (AUC = 0.694), but lower compared with those for APACHE II score (AUC = 0.806) and SOFA score (AUC = 0.778). At the optimal cut-off point of >342.13 for the serum absolute expression of lncRNA PKN2-AS1, the specificity and sensitivity were 60.0% and 81.2%, respectively. At the optimal cut-off point of >330.1 for the serum absolute expression of lncRNA AC068888.1, the specificity and sensitivity were 52.5% and 83.3%, respectively. At the optimal cut-off point of >5.7 for Lac level, the specificity and sensitivity were 45.5% and 88.5%, respectively. At the optimal cut-off point of >12.5 for SOFA score, the specificity and sensitivity were 46.3% and 94.8%, respectively. At the optimal cut-off point of >19.5 for APACHE II score, the specificity and sensitivity were 78.8% and 67.7%, respectively. The addition of either SOFA score alone (AUC = 0.778) or APACHE II score alone (AUC = 0.806) or both (AUC = 0.825) did not significantly improve the predictive ability to predict prognosis of septic patients. However, if SOFA score and APACHE II score were combined with lncRNA PKN2-AS1 and AC068888.1, the prognosis of sepsis patients was improved to a certain extent (AUC = 0.860) (Fig. 5c and Table 8).

Kaplan–Meier survival analysis

Kaplan–Meier survival analysis was performed to evaluate the clinical outcomes between two subgroups of sepsis patients divided by the absolute expression of lncRNA PKN2-AS1 or AC068888.1. We found that the 28-day survival exhibited much worse in sepsis patients with lncRNA PKN2-AS1 high expression than in sepsis patients with lncRNA PKN2-AS1 low expression (hazard ratio (HR) = 4.089, 95% CI: 2.520 - 6.634, $\chi^2 = 32.529$, $P < 0.001$, Fig. 6a). Likewise, analogous consequence was observed between two subgroups of sepsis patients which was distributed by lncRNA AC068888.1 expression (HR = 3.156, 95% CI: 1.914 - 5.204, $\chi^2 = 30.794$, $P < 0.001$, Fig. 6b).

Discussion

Sepsis is a medical emergency of serious organs dysfunction caused by the host's imbalanced and extreme inflammatory and immune response to an infection, which can lead to poor prognosis [1, 24]. Common clinical symptoms are fever, leukocytosis, edema, and accumulation of inflammatory cells (neutrophils, macrophages, and monocytes) in various tissues and organs [25]. Septic shock, a more severe form of sepsis, is a subset of sepsis in which severe circulatory, cellular, and metabolic abnormalities are found with a greater risk of death than sepsis alone [26]. The clinical definition of septic shock is that, on the basis of sepsis, vasopressors are required to maintain a mean arterial pressure of 65 mmHg or greater and serum lactic acid level greater than 2 mmol/L, despite adequate fluid resuscitation [1–3]. Sepsis and its consequence of multiple organ dysfunction are still one of the dominating causes of morbidity and mortality in critically ill patients in ICU [27]. The latest guidelines for sepsis management indicated that early identification and diagnosis of sepsis is critical for early target-oriented treatment of sepsis and septic shock, helping to reduce mortality in patients with sepsis [28]. Therefore, the search for reliable biomarkers of sepsis is of great significance for the early diagnosis and prognosis of sepsis.

In this context, high-throughput transcriptome RNA sequencing has emerged, providing unprecedented insights into the study of the human genome. Sepsis is a multi-system affected condition, involving not only the early activation of inflammatory responses [29], but also major changes in non-immune pathways, such as cardiovascular, renal, autonomic nervous, hepatic, metabolic, and coagulation pathways, all of which are regulated by lncRNAs [1]. Studies showed that lncRNAs were involved in the pathological process of sepsis and sepsis-induced organ dysfunction [30], and identified them being as potential biomarkers and therapeutic targets. At present stage, numerous reports have proved that lncRNAs were involved in the occurrence and development of sepsis with crucial roles in modulating gene expression and signaling pathways in the serum of sepsis patients [31–33]. Meanwhile, Zheng et al. showed that lncRNAs regulated the inflammatory immune-related genes and may serve as potential diagnostic biomarkers for sepsis [34]. Furthermore, the study of Chen et al. demonstrated that the lncRNA MALAT1 could be used as a diagnostic marker and therapeutic target for sepsis by influencing the p38 MAPK/NF κ B signaling pathway through miR-125b related effects, and thereby exacerbating cardiac inflammation and dysfunction during sepsis [35].

In this study, human-rat homologous lncRNA PKN2-AS1 and AC068888.1 were selected as biomarker candidates of sepsis, which were screened from the rat sepsis model induced by LPS and verified clinically. Before this trial, lncRNA PKN2-AS1 and AC068888.1 were only associated with the prognosis of bladder cancer [36] and postoperative survival of patients with glioblastoma multiforme [37], respectively. Nevertheless, there were no reports related to other diseases, including sepsis. In *homo sapiens*, lncRNA PKN2-AS1 is located on chromosome 1 and its length was 3160-bp with the NONCODE transcript ID ENST00000645056.1. Likewise, lncRNA AC068888.1 is located in chromosome 12, and its length was 1849-bp with the NONCODE transcript ID ENST00000663863.1. To verify the sequencing results, the RT-qPCR was therefore performed in 176 expanded pair samples. Gratifyingly, the absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 in patients with sepsis were higher than those in the healthy controls, reflecting the involvement of those lncRNAs in sepsis. We speculate that the above-mentioned two lncRNAs may activate multiple inflammatory signaling pathways to regulate the expression of inflammation-related proteins, thus inducing systemic inflammatory response in patients with sepsis, resulting in higher expression in patients with sepsis. The ROC curve showed that the AUC of lncRNA PKN2-AS1 and AC068888.1 were 0.881 and 0.842, respectively. Compared with the conventional evaluation of sepsis score index or laboratory indicators, including PCT, CRP and Lac levels, as well as SOFA and APACHE II scores, the two lncRNAs have apparent advantages, which may be favorable candidates for early diagnosis of sepsis.

The CRP and PCT have been reported to be closely related to the diagnosis of infection and the severity of sepsis, but due to the low specificity, they can no longer be used as the only criteria for septic infection [38]. A multitude of inflammatory cytokines, including IL-6 and TNF- α , were closely related to the degree of tissue and organ damage during the occurrence and development of sepsis [39, 40]. Current tools used to stratify the inflammation in sepsis included clinical severity scores, such as SOFA and APACHE II scores, as well as Lac level [41]. To verify the role of lncRNA PKN2-AS1 and AC068888.1 in the assessment of disease severity and inflammation, our clinical study found that those two lncRNAs were significantly correlated with SOFA, APACHE II scores and Lac level, but weakly correlated with PCT and CRP levels. The possible explanation is that, like other lncRNAs (such as MALAT1 and NEAT1), these two lncRNAs may promote the production of inflammatory factors through the NF- κ B or TLRs pathway [42, 43], aggravating the inflammation and organ dysfunction. In the diagnosis of sepsis, lncRNA PKN2-AS1 (AUC = 0.704) and AC068888.1 (AUC = 0.812) clearly distinguished patients with septic shock from those without septic shock. Furthermore, the lncRNA AC068888.1 showed a certain degree of superiority. Multivariate logistic regression analysis showed that high expression of AC068888.1 was an independent risk factor for shock in patients with sepsis as well as SOFA score and high Lac level, but the PKN2-AS1 was not associated with them. In the septic shock prediction model, the combination of the above independent risk factors well predicted the occurrence of septic shock (AUC = 0.882).

We further evaluated the prognostic values of lncRNA PKN2-AS1 and AC068888.1 in patients with sepsis, and found that the absolute expression levels of lncRNA PKN2-AS1 and AC068888.1 in non-survivors at 28 days were higher than those in survivors at 28 days. Multivariate logistic regression analysis showed that lncRNA PKN2-AS1 and AC068888.1 were independent risk factors for the prognosis of sepsis

patients, and the patients with high expression of PKN2-AS1 and AC068888.1 had worse prognosis. In addition, lncRNA PKN2-AS1 and AC068888.1 well predicted the 28-day death risk of sepsis patients by the ROC curve. In the prognostic model, the combination of those two lncRNAs with the SOFA and APACHE II scores was able to better predict the poor prognosis of the patients with sepsis (AUC = 0.860). To verify the validity of the prognostic model, we used the K-M survival curve to represent the survival time of survival group and non-survival group. The P-values of K-M survival curves of those two lncRNAs were less than 0.001, indicating that our predicted model was strongly correlated with the survival outcome of patients with sepsis.

There were still some limitations in this study. First of all, the sample size was small, and it was a single-center study, so the sample size needs to be expanded. Secondly, the expression of those two lncRNAs was detected only once in patients with sepsis (within 24 hours after admission), so it is necessary to further elucidate the changes of those two lncRNAs in the course of disease and treatment. Third, only 28-day mortality in patients with sepsis was followed up, so it is necessary to extend the follow-up time and evaluate the long-term predictive values of those two lncRNAs. Finally, the specific regulatory mechanism of those two lncRNAs in sepsis is not clear.

Conclusion

To our knowledge, this is the first study to report an association between lncRNA PKN2-AS1 and AC068888.1 and sepsis. Our study strongly suggests that the serum lncRNA PKN2-AS1 and AC068888.1 can be used for the early diagnosis, disease severity assessment and adverse prognosis of sepsis.

Abbreviations

APACHE II	Acute physiology and chronic evaluation II
APTT	Activated partial thromboplastin time
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
DE	Differentially expressed
ELISA	Enzyme-linked immunosorbent assay
FIB	Fibrinogen
gc/mL	gene copies per milliliter serum
HCS	Healthy controls
HR	Hazard ratio
ICU	Intensive care unit
IL-6	Interleukin-6
IQR	Interquartile ranges
K-M	Kaplan–Meier
LncRNA	Long noncoding RNA
LPS	Lipopolysaccharide
Lac	Lactate
mRNA	messenger RNA
NF- κ B	Nuclear factor kappa-B
OR	Odds ratio
PB	Peripheral blood
PCT	Procalcitonin
PKN2-AS1	PKN2 antisense RNA1
PLT	Platelet
PT	Prothrombin time

ROC	Receiver operating characteristic
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SARS-COV-2	Severe acute respiratory syndrome coronavirus-2
Scr	Serum creatinine
SD	Standard deviation
SPF	Specific pathogen Free
SOFA	Sequential organ failure assessment
TLRs	Toll-like receptors
TNF- α	Tumor necrosis factor- α
WBC	White blood cell

Declarations

Authors' contributions

The authors contributed in the following way: Min Shao, Nian Liu and Limian Cao designed and coordinated the study. Jun Yuan, Junjie Bao, Yutao Zha, Shi Chen, Wenjing Fan, Ming Fang and Yonghui Gui collected the samples and data. Jun Yuan and Junjie Bao performed the statistical analyses and produced graphs. Jun Yuan and Limian Cao drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

None.

Competing interests

The authors declared no conflict of interest.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The procedures for care and use of animals were approved by the Ethics Committee of Anhui Medical University and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of the First Affiliated Hospital of Anhui Medical University. Written informed consent was obtained from individual or guardian participants.

Funding

This work was supported by grants from the Natural Science Foundation of Anhui Province (2008085QC113 and 1808085MH228), the fund from The First Affiliated Hospital of Anhui Medical University for Youth Cultivation Program (2020kj25), the fund from The First Affiliated Hospital of Anhui Medical University for Doctoral Talent Program (BSKY2019032), the fund from The First Affiliated Hospital of Anhui Medical University for Clinical Medicine Discipline Construction Program (2021lcxk019).

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM, et al: The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016, 315:801-810.
2. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M: Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016, 315:775-787.
3. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, Rubenfeld G, Kahn JM, Shankar-Hari M, Singer M, et al: Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016, 315:762-774.
4. Fleischmann C, Scherag A, Adhikari NKJ, Hartog CS, Tsaganos T, Schlattmann P, Angus DC, Reinhart K: Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American journal of respiratory and critical care medicine* 2016, 193:259-272.
5. Wang W, Yang N, Wen R, Liu C-F, Zhang T-N: Long Noncoding RNA: Regulatory Mechanisms and Therapeutic Potential in Sepsis. *Frontiers in cellular and infection microbiology* 2021, 11:563126.
6. Arentz M, Yim E, Klaff L, Lokhandwala S, Riedo FX, Chong M, Lee M: Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA* 2020, 323:1612-1614.
7. Oude Munnink BB, Worp N, Nieuwenhuijse DF, Sikkema RS, Haagmans B, Fouchier RAM, Koopmans M: The next phase of SARS-CoV-2 surveillance: real-time molecular epidemiology. *Nature medicine* 2021, 27:1518-1524.

8. Hu B, Guo H, Zhou P, Shi Z-L: Characteristics of SARS-CoV-2 and COVID-19. *Nature reviews Microbiology* 2021, 19:141-154.
9. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, Barrette TR, Prensner JR, Evans JR, Zhao S, et al: The landscape of long noncoding RNAs in the human transcriptome. *Nature genetics* 2015, 47:199-208.
10. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, Horlings HM, Shah N, Umbricht C, Wang P, et al: Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nature genetics* 2011, 43:621-629.
11. Zhang L, Zhou Y, Huang T, Cheng ASL, Yu J, Kang W, To KF: The Interplay of LncRNA-H19 and Its Binding Partners in Physiological Process and Gastric Carcinogenesis. *International journal of molecular sciences* 2017, 18.
12. Fang Y, Fullwood MJ: Roles, Functions, and Mechanisms of Long Non-coding RNAs in Cancer. *Genomics, proteomics & bioinformatics* 2016, 14:42-54.
13. Quinn JJ, Chang HY: Unique features of long non-coding RNA biogenesis and function. *Nature reviews Genetics* 2016, 17:47-62.
14. Jathar S, Kumar V, Srivastava J, Tripathi V: Technological Developments in lncRNA Biology. *Advances in experimental medicine and biology* 2017, 1008:283-323.
15. Chen M, Fan L, Zhang S-M, Li Y, Chen P, Peng X, Liu D-B, Ma C, Zhang W-J, Zou Z-W, Li P-D: LINC01939 inhibits the metastasis of gastric cancer by acting as a molecular sponge of miR-17-5p to regulate EGR2 expression. *Cell death & disease* 2019, 10:70.
16. Chen P, Fang X, Xia B, Zhao Y, Li Q, Wu X: Long noncoding RNA LINC00152 promotes cell proliferation through competitively binding endogenous miR-125b with MCL-1 by regulating mitochondrial apoptosis pathways in ovarian cancer. *Cancer medicine* 2018, 7:4530-4541.
17. Da M, Jiang H, Xie Y, Jin W, Han S: The Biological Roles of Exosomal Long Non-Coding RNAs in Cancers. *OncoTargets and therapy* 2021, 14:271-287.
18. Tang Y, Bao J, Hu J, Liu L, Xu D-Y: Circular RNA in cardiovascular disease: Expression, mechanisms and clinical prospects. *Journal of cellular and molecular medicine* 2021, 25:1817-1824.
19. Peng H, Ren S, Liu Y, Zhou H, Tang X, Yang J, Tian J, Xu P, Xu H, Wang S: Elevated Expression of the Long Noncoding RNA IFNG-AS1 in the Peripheral Blood from Patients with Rheumatoid Arthritis. *Journal of immunology research* 2020, 2020:6401978.
20. Wang M, Wei J, Shang F, Zang K, Ji T: Long non-coding RNA CASC2 ameliorates sepsis-induced acute kidney injury by regulating the miR-155 and NF- κ B pathway. *International journal of molecular medicine* 2020, 45:1554-1562.
21. Wu B, Wang K, Fei J, Bao Y, Wang X, Song Z, Chen F, Gao J, Zhong Z: Novel three-lncRNA signature predicts survival in patients with pancreatic cancer. *Oncology reports* 2018, 40:3427-3437.
22. Yang C, Xia W, Liu X, Lin J, Wu A: Role of TXNIP/NLRP3 in sepsis-induced myocardial dysfunction. *International journal of molecular medicine* 2019, 44:417-426.

23. Zhang P, Cao L, Fan P, Mei Y, Wu M: LncRNA-MIF, a c-Myc-activated long non-coding RNA, suppresses glycolysis by promoting Fbxw7-mediated c-Myc degradation. *EMBO reports* 2016, 17:1204-1220.
24. Oh BM, Lee S-J, Park GL, Hwang YS, Lim J, Park ES, Lee KH, Kim BY, Kwon YT, Cho HJ, Lee HG: Erastin Inhibits Septic Shock and Inflammatory Gene Expression via Suppression of the NF- κ B Pathway. *Journal of clinical medicine* 2019, 8.
25. Fattahi F, Ward PA: Understanding Immunosuppression after Sepsis. *Immunity* 2017, 47:3-5.
26. Cecconi M, Evans L, Levy M, Rhodes A: Sepsis and septic shock. *Lancet (London, England)* 2018, 392:75-87.
27. Mayr FB, Yende S, Angus DC: Epidemiology of severe sepsis. *Virulence* 2014, 5.
28. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al: Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. *Intensive care medicine* 2017, 43:304-377.
29. Adhikari A, Martel C, Marette A, Olivier M: Hepatocyte SHP-1 is a Critical Modulator of Inflammation During Endotoxemia. *Scientific reports* 2017, 7:2218.
30. Lu D, Thum T: RNA-based diagnostic and therapeutic strategies for cardiovascular disease. *Nature reviews Cardiology* 2019, 16:661-674.
31. Schmitz SU, Grote P, Herrmann BG: Mechanisms of long noncoding RNA function in development and disease. *Cellular and molecular life sciences : CMLS* 2016, 73:2491-2509.
32. Li Y, Guo W, Cai Y: NEAT1 Promotes LPS-induced Inflammatory Injury in Macrophages by Regulating MiR-17-5p/TLR4. *Open medicine (Warsaw, Poland)* 2020, 15:38-49.
33. Yu B, Cui R, Lan Y, Zhang J, Liu B: Long non-coding RNA H19 as a diagnostic marker in peripheral blood of patients with sepsis. *American journal of translational research* 2021, 13:2923-2930.
34. Zheng X, Leung K-S, Wong M-H, Cheng L: Long non-coding RNA pairs to assist in diagnosing sepsis. *BMC genomics* 2021, 22:275.
35. Chen H, Wang X, Yan X, Cheng X, He X, Zheng W: RETRACTED: LncRNA MALAT1 regulates sepsis-induced cardiac inflammation and dysfunction via interaction with miR-125b and p38 MAPK/NF κ B. *International immunopharmacology* 2018, 55:69-76.
36. Yang FL, Hong K, Zhao GJ, Liu C, Song YM, Ma LL: [Construction of prognostic model and identification of prognostic biomarkers based on the expression of long non-coding RNA in bladder cancer via bioinformatics]. *Beijing da xue xue bao Yi xue ban = Journal of Peking University Health sciences* 2019, 51:615-622.
37. Gao W-Z, Guo L-M, Xu T-Q, Yin Y-H, Jia F: Identification of a multidimensional transcriptome signature for survival prediction of postoperative glioblastoma multiforme patients. *Journal of translational medicine* 2018, 16:368.
38. Tsalik EL, Jaggars LB, Glickman SW, Langley RJ, van Velkinburgh JC, Park LP, Fowler VG, Cairns CB, Kingsmore SF, Woods CW: Discriminative value of inflammatory biomarkers for suspected sepsis. *The Journal of emergency medicine* 2012, 43.

39. Zhu W, Bao R, Fan X, Tao T, Zhu J, Wang J, Li J, Bo L, Deng X: PD-L1 blockade attenuated sepsis-induced liver injury in a mouse cecal ligation and puncture model. *Mediators of inflammation* 2013, 2013:361501.

40. Li T, Qian Y, Miao Z, Zheng P, Shi T, Jiang X, Pan L, Qian F, Yang G, An H, Zheng Y: Xuebijing Injection Alleviates Pam3CSK4-Induced Inflammatory Response and Protects Mice From Sepsis Caused by Methicillin-Resistant. *Frontiers in pharmacology* 2020, 11:104.

41. Sweeney TE, Perumal TM, Henao R, Nichols M, Howrylak JA, Choi AM, Bermejo-Martin JF, Almansa R, Tamayo E, Davenport EE, et al: A community approach to mortality prediction in sepsis via gene expression analysis. *Nature communications* 2018, 9:694.

42. Deng X, Guo J, Sun Z, Liu L, Zhao T, Li J, Tang G, Zhang H, Wang W, Cao S, et al: -Induced Downregulation of lncRNA Gm28309 Triggers Macrophages Inflammatory Response Through the miR-3068-5p/NF-κB Pathway. *Frontiers in immunology* 2020, 11:581517.

43. Kouli A, Camacho M, Allinson K, Williams-Gray CH: Neuroinflammation and protein pathology in Parkinson's disease dementia. *Acta neuropathologica communications* 2020, 8:211.

Tables

Table 1. Baseline characteristics of patients with sepsis

Characteristics	Sepsis (n=176)
Demographic characteristics	
Age, y	57.5 (22.0)
Male, n (%)	114 (64.8)
BMI (kg/m ²)	23.4 (4.9)
Chronic disease, n (%)	
COPD	12 (6.8)
Cardiomyopathy	32 (18.2)
Chronic kidney failure	9 (5.1)
Cirrhosis	1 (0.6)
Diabetes	31 (17.6)
Hypertension disease	62 (35.2)
Primary infection site, n (%)	
Respiratory infection	75 (42.6)
Abdominal infection	19 (10.8)
Skin and soft tissue infection	12 (6.8)
Urinary tract infection	13 (7.4)
Blood stream infection	35 (19.9)
CNS infection	10 (5.7)
Other infections	12 (6.8)
Organ failure, n (%)	
Heart failure	88 (50.0)
Acute liver failure	51 (29.0)
Acute renal failure	77 (43.8)
Gastrointestinal failure	40 (22.7)
CNS failure	63 (35.8)
Respiratory failure	163 (92.6)
Circulatory failure	151 (85.8)
Clotting dysfunction	68 (38.6)

Number of organ dysfunction	4.0 (2.0)
Glucocorticoid drugs (mg)	80.0 (1000.0)
Biochemical indexes, median (IQR)	
WBC (10 ⁹ /L)	12.5 (8.0)
PLT (10 ⁹ /L)	129.5 (142.8)
Albumin (g/L)	30.9 (8.1)
Scr (umol/L)	101.1 (99.8)
CRP (mg/L)	61.7 (70.2)
PCT (ng/mL)	14.3 (38.9)
Lac (mmol/L)	3.8 (3.1)
PT (s)	16.7 (4.4)
APTT (s)	44.6 (14.1)
FIB (g/L)	4.4 (3.0)
Disease severity, median (IQR)	
APACHE II score	20.0 (8.0)
SOFA score	10 .0 (4.0)

Table 2. ROC markers of serum lncRNA PKN2-AS1 and AC068888.1 in patients with sepsis

Diagnostic index	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
PKN2-AS1	0.879	0.843-0.915	0.018	238.0	93.8	67.0
AC068888.1	0.842	0.801-0.884	0.021	210.6	88.6	71.0

Table 3. Univariate analysis of clinical characteristics between no septic shock sepsis patients and septic shock patients

Characteristics	Septic shock (n=100)	No septic shock (n=76)	F/Z/ χ^2	P value
Demographic characteristics				
Age, y	56.0 (25.0)	58.0 (19.0)	-0.599	0.549
Male, n (%)	60 (60.0)	54 (71.1)	2.312	0.128
BMI (kg/m ²)	23.1 (4.8)	23.7 (5.3)	-1.022	0.307
Chronic disease, n (%)				
COPD	8 (8.0)	4 (5.3)	0.509	0.476
Cardiomyopathy	20 (20.0)	12 (15.8)	0.515	0.473
Chronic kidney failure	5 (5.0)	4 (5.3)	0.000	1.000
Cirrhosis	1 (1.0)	0 (0.0)	/	1.000
Diabetes	14 (14.0)	17 (22.4)	2.084	0.149
Hypertension disease	34 (34.0)	28 (36.8)	0.153	0.696
Primary infection site, n (%)				
Respiratory infection	40 (46.1)	35 (40.0)	0.647	0.421
Abdominal infection	13 (13.0)	6 (7.9)	1.169	0.280
Skin and soft tissue infection	10 (10.0)	2 (2.6)	3.690	0.055
Urinary tract infection	5 (5.0)	8 (10.5)	1.928	0.165
Blood stream infection	25 (25.0)	10 (13.2)	3.801	0.051
CNS infection	4 (4.0)	6 (7.9)	0.604	0.437
Other infections	3 (3.0)	9 (11.8)	5.314	0.021
Organ failure, n (%)				
Heart failure	61 (61.0)	27 (35.5)	11.208	0.001
Acute liver failure	41 (41.0)	10 (13.2)	16.265	< 0.001
Acute renal failure	53 (53.0)	24 (31.6)	8.052	0.005
Gastrointestinal failure	25 (25.0)	15 (19.7)	0.681	0.409
Central failure	35 (35.0)	28 (36.8)	0.064	0.801
Respiratory failure	94 (94.0)	69 (90.8)	0.651	0.420
Circulatory failure	93 (93.0)	58 (76.3)	9.863	0.002

Clotting dysfunction	51 (51.0)	17 (22.4)	14.931	< 0.001
Number of organ dysfunction	5.0 (3.0)	3.0 (2.0)	-4.804	< 0.001
Glucocorticoid drugs (mg)	166.7 (1148.3)	40.0 (500.0)	-1.593	0.111
Biochemical indexes, median (IQR)				
WBC (10 ⁹ /L)	12.6 (8.8)	12.3 (7.4)	-0.202	0.840
PLT (10 ⁹ /L)	110.0 (128.0)	171.0 (120.8)	-3.754	< 0.001
Albumin (g/L)	30.4 (8.2)	31.0 (8.1)	-0.421	0.674
Scr (umol/L)	127.1 (108.3)	77.3 (62.1)	-4.274	< 0.001
CRP (mg/L)	70.4 (71.5)	56.0 (63.0)	-2.285	0.022
PCT (ng/mL)	21.9 (55.2)	7.1 (16.9)	-5.302	< 0.001
Lac (mmol/L)	5.0 (3.8)	2.8 (1.5)	-7.173	< 0.001
PT (s)	17.8 (5.6)	15.8 (2.2)	-4.804	< 0.001
APTT (s)	47.9 (16.5)	42.5 (8.1)	-3.714	< 0.001
FIB (g/L)	3.9 (3.1)	5.1 (3.1)	-2.403	0.016
Disease severity, median (IQR)				
APACHE II score	21.0 (10.0)	18.0 (7.0)	-3.821	< 0.001
SOFA score	12.0 (4.0)	9.0 (4.0)	-6.634	< 0.001

Table 4. Likelihood ratio forward stepwise multivariate logistic regression of risk factors for septic shock patients

Variable	B	S.E	Wals	P	OR	95% CI
SOFA score	0.168	0.078	4.636	0.031	1.183	1.015-1.378
Lac	0.279	0.139	4.021	0.045	1.322	1.006-1.737
AC068888.1	0.014	0.004	16.219	<0.001	1.015	1.007-1.022
PT	0.190	0.085	4.924	0.026	1.209	1.022-1.429

Table 5. Predictive value of serum lncRNA PKN2-AS1 and AC068888.1 in patients with septic shock

Diagnostic index	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
SOFA score	0.791	0.724-0.858	0.034	9.5	81.0	64.5
APACHE II score	0.668	0.589-0.747	0.040	20.5	56.0	72.4
Lac	0.816	0.753-0.879	0.032	3.6	81.0	76.3
PKN2-AS1	0.704	0.627-0.782	0.040	297.6	85.0	51.3
AC068888.1	0.812	0.748-0.876	0.033	302.3	78.0	77.6
Combined prediction	0.882	0.831-0.932	0.026	0.54	82.0	84.2

Table 6. Univariate analysis of clinical characteristics between survivors patients and non-survivors patients

Characteristics	Survivors (n=96)	Non-survivors (n=80)	F/Z/ χ^2	P value
Demographic characteristics				
Age, y	57.0 (22.0)	58.0 (21.0)	-1.127	0.260
Male n (%)	57(59.4)	57(71.3)	2.697	0.101
BMI (kg/m ²)	23.5 (4.6)	23.0 (5.1)	-1.422	0.155
Chronic disease, n (%)				
COPD	4 (4.2)	8 (10.0)	2.337	0.126
Cardiomyopathy	13 (13.5)	19 (23.8)	3.057	0.080
Chronic kidney failure	5 (5.2)	4 (5.0)	/	1.000
Cirrhosis	1 (1.0)	0 (0.0)	/	1.000
Diabetes	19 (19.8)	12 (15.0)	0.690	0.406
Hypertension disease	35 (36.5)	27 (33.8)	0.140	0.708
Primary infection site, n (%)				
Respiratory infection	44 (45.8)	31 (38.8)	0.895	0.344
Abdominal infection	9 (9.4)	10 (12.5)	0.443	0.506
Skin and soft tissue infection	9 (9.4)	3 (3.8)	2.173	0.140
Urinary tract infection	9 (9.4)	4 (5.0)	1.221	0.269
Blood stream infection	12 (12.5)	23 (28.7)	7.233	0.007
CNS infection	4 (4.2)	6 (7.5)	0.390	0.532
Other infections	9 (9.4)	3 (3.8)	2.173	0.140
Organ failure, n (%)				
Heart failure	36 (37.5)	52 (65.0)	13.200	<0.001
Acute liver failure	18 (18.8)	33 (41.3)	10.734	0.001
Acute renal failure	29 (30.2)	48 (60.0)	15.738	<0.001
Gastrointestinal failure	19 (19.8)	21 (26.3)	1.0360	0.309
CNS failure	17 (17.7)	46 (57.5)	30.063	<0.001
Respiratory failure	86 (89.6)	77 (96.3)	2.835	0.092
Circulatory failure	74 (77.1)	77 (96.3)	13.154	<0.001

Clotting dysfunction	30 (31.3)	38 (47.5)	4.860	0.027
Number of organ dysfunction	3.0 (2.0)	5.0 (2.0)	-6.317	<0.001
Glucocorticoid drugs (mg)	0.5 (500.0)	500.0 (1265.0)	-2.694	0.007
Septic shock, n (%)	47 (49.0)	53 (66.3)	5.318	0.021
Biochemical indexes, median (IQR)				
WBC (10 ⁹ /L)	12.3 (7.2)	12.7 (8.9)	-0.394	0.694
PLT (10 ⁹ /L)	142.5 (140.8)	113.5 (143.5)	-2.564	0.010
Albumin (g/L)	29.9 (7.2)	31.9 (9.4)	-2.169	0.030
Scr (umol/L)	85.4 (6.5)	116.6 (116.6)	-2.897	0.004
CRP (mg/L)	55.9 (54.7)	86.8 (84.3)	-2.493	0.013
PCT (ng/mL)	10.8 (22.6)	21.0 (76.0)	-4.322	<0.001
Lac (mmol/L)	3.4 (2.1)	4.7 (5.0)	-4.427	<0.001
PT (s)	16.1 (3.7)	17.3 (5.2)	-2.559	0.011
APTT (s)	44.8 (11.7)	44.3 (15.7)	-0.227	0.820
FIB (g/L)	4.9 (2.9)	3.8 (3.0)	-2.572	0.010
Disease severity, median (IQR)				
APACHE II score	17.0 (7.0)	23.0 (6.0)	-6.990	<0.001
SOFA score	9.0 (5.0)	12.0 (6.0)	-6.379	<0.001

Table 7. Likelihood ratio forward stepwise multivariate logistic regression of 28-day mortality risk in patients with sepsis

Variable	B	S.E	Wals	P	OR	95% CI
Sex (Male)	0.900	0.474	3.614	0.057	2.461	0.973-6.226
APACHE II score	0.193	0.048	15.977	<0.001	1.212	1.103-1.332
PKN2-AS1	0.011	0.005	5.482	0.019	1.011	1.002-1.021
AC068888.1	0.010	0.003	7.626	0.006	1.010	1.003-1.017
PCT	0.011	0.005	4.581	0.032	1.011	1.001-1.021
Number of organ dysfunction	0.553	0.172	10.314	0.001	1.738	1.240-2.436
Septic shock (Yes)	1.503	0.570	6.955	0.008	4.496	1.471-13.739

Table 8. Prognostic value of serum lncRNA PKN2-AS1 and AC068888.1 in patients with sepsis

Diagnostic index	AUC	95% CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
SOFA score	0.778	0.711-0.846	0.034	12.5	46.3	94.8
APACHE II score	0.806	0.742-0.869	0.032	19.5	78.8	67.7
Lac	0.694	0.616-0.772	0.040	5.7	45.0	88.5
PKN2-AS1	0.747	0.675-0.820	0.037	342.1	60.0	81.2
AC068888.1	0.717	0.640-0.793	0.039	330.1	52.5	83.3
Combined prediction	0.860	0.806-0.913	0.027	0.36	85.0	72.9

Figures

Figure 1

The expression profiles of lncRNAs. (a) The volcano plot of DElncRNAs. Red and blue dots indicate upregulation and downregulation, respectively. (b) The cluster analysis (heat map) of DElncRNAs. The color scale indicates the expression of DElncRNAs: red and blue indicate upregulation and downregulation, respectively. The "S" represents the rat sepsis model samples.

Figure 2

The absolute expression of serum lncRNA PKN2-AS1 and AC068888.1, and their diagnostic values in sepsis. (a, b) Both absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 in patients with sepsis were significantly higher than those in the HCs ($P < 0.001$). (c, d) The ROC curve analysis for the performance of lncRNA PKN2-AS1 and AC068888.1 in discriminating the sepsis patients from the HCs.

Figure 3

Correlation between the absolute expression of serum lncRNAs and SOFA score, APACHE II score, Lac, PCT, CRP, IL-6 and TNF- α levels in patients with sepsis. (a-g) The absolute expression of serum lncRNA PKN2-AS1 was positively correlated with SOFA score, APACHE II score, Lac, PCT, CRP, IL-6 and TNF- α level ($r = 0.427, P < 0.001, r = 0.303, P < 0.001, r = 0.347, P < 0.001, r = 0.245, P = 0.001, r = 0.156, P = 0.039, r = 0.422, P < 0.001, r = 0.401, P < 0.001$). (h-n) The absolute expression of serum lncRNA AC068888.1 was positively correlated with SOFA score, APACHE II score, Lac, PCT, CRP, IL-6 and TNF- α levels ($r = 0.492, P < 0.001, r = 0.317, P < 0.001, r = 0.480, P < 0.001, r = 0.261, P < 0.001, r = 0.149, P = 0.048, r = 0.476, P < 0.001, r = 0.516, P < 0.001$).

Figure 4

The absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 and their diagnostic values in septic shock. (a, b) Both absolute expression of the serum lncRNA PKN2-AS1 and AC068888.1 in patients with septic shock were significantly higher than those no septic shock ($P < 0.001$). (c) The ROC curve of each potential indicator for the diagnosis of septic shock. SOFA score, APACHE II score, Lac level, lncRNA PKN2-AS1 and AC068888.1. Combining lncRNA AC068888.1 with SOFA score and Lac level, the accuracy of predicting septic shock risk in patients with sepsis through the ROC curve analysis can be significantly improved (AUC = 0.882).

Figure 5

The absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 in survival and non-survival sepsis patients, and their prognostic values in sepsis. (a, b) Both absolute expression of serum lncRNA PKN2-AS1 and AC068888.1 in the survival sepsis patients were significantly higher than those non-survival sepsis patients ($P < 0.001$). (c) The ROC of each potential indicator for predicting the risk of 28-day mortality. SOFA score, APACHE II score, Lac level, lncRNA PKN2-AS1 and AC068888.1. Combining lncRNA PKN2-AS1 and AC068888.1 with SOFA and APACHE II scores, the accuracy of predicting 28-day death risk of sepsis patients through the ROC curve analysis can be significantly improved (AUC = 0.860).

Figure 6

Kaplan–Meier survival analysis of patients with sepsis. The influence of lncRNA PKN2-AS1 (a) and AC068888.1 (b) on the 28-day survival of patients with sepsis was evaluated using the Kaplan–Meier survival analysis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.docx](#)