



Research article

The importance of serum LMAN2 level in septic shock and prognosis prediction in sepsis patients

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ABSTRACT

Objectives: To study the importance of LMAN2 in septic shock and prognosis prediction in sepsis patients.**Methods:** Serum LMAN2 was measured by ELISA in 109 sepsis patients within 24 h after their admission to ICU. We also collected clinical and laboratory variables.**Results:** Compared with sepsis group (1.21 (1.05) ng/ml), serum LMAN2 level was significantly higher in patients with septic shock (1.75 (2.04) ng/ml) on the day of admission to the ICU ($P < 0.001$), and serum LMAN2 level were significantly higher in the sepsis non-survival group (1.91 (1.66) ng/ml) than in the survival group (1.15 (1.17) ng/ml). COX regression analysis showed that high serum LMAN2 level (>1.28 ng/ml) was a predictor of 28-day mortality in sepsis patients.**Conclusions:** This study shows that high serum LMAN2 level may indicate septic shock and is associated with an unfavorable prognosis for sepsis patients.

1. Introduction

Sepsis is defined as life-threatening organ dysfunction caused by host response disorder caused by infection [1]. It is a common cause of patients entering and dying in Intensive Care Units (ICU) [2, 3]. Although the international guidelines for severe sepsis and septic shock of the Surviving Sepsis Campaign in 2013 proposed bundle treatment for 3 h–6 h, and bundle treatment for 1 h in 2018 [4]. However, the mortality rate of sepsis remains high. A recent study showed that the hospital mortality rate of sepsis is as high as 40% [5]. The main reason is that the mechanism of sepsis is complex, including the imbalance of inflammatory reaction [6, 7], immune dysfunction [8], disorder of coagulation mechanism [9], and some other factors, such as mitochondrial damage, endoplasmic reticulum stress, gene polymorphism, etc. [10, 11]. Some patients with septic shock will show persistent hypotension after full volume resuscitation and high dose of NE (Norepinephrine) in the final

stage before death. In-hospital mortality and ICU mortality in patients with sepsis can be effectively reduced by early identification and intervention in such patients. We therefore hoped to discover a biomarker that would be able to identify septic shock early.

LMAN2 (Lectin Mannose-Binding 2) is an intracellular lectin of the secretory pathway, which is rich in glycosphingolipids, widely distributed on the plasma membrane, involved in protein transport between endoplasmic reticulum, golgi apparatus [12]. It has been shown that LPS (Lipopolysaccharide) exfoliates LMAN2 from the cell surface and that the amount of LMAN2 precisely regulates the phagocytosis of macrophages [13], so it is speculated that serum LMAN2 level correlates with the severity of sepsis, and it is feasible to screen LMAN2 from serum.

The objective of this study is to investigate whether the serum LMAN2 level correlates with the severity of sepsis and its significance on the prognosis of patients with sepsis.

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2. Materials and methods

2.1. Subjects

From September 2019 to December 2020, 109 patients with sepsis were admitted to the Department of Critical Care Medicine of the First Affiliated Hospital of Anhui Medical University, were divided to two groups: sepsis group (n = 51) and septic shock group (n = 58). Inclusion criteria: (1) Age ≥ 18 years old; (2) Length of stay in ICU > 2 days; (3) The diagnosis of sepsis and septic shock conforms to the international consensus on the definition of Sepsis 3.0 published in April 2016, septic shock is defined as hypotension after full fluid resuscitation (mean blood pressure ≥ 65 mmHg under pressor maintenance) and serum lactic acid > 2 mmol/L [1]; (4) sign the informed consent form. In addition, patients who are pregnant, have recently had an acute heart attack [14], have malignant tumors were all excluded (Figure 1). This study was approved by the Ethics Committee for Clinical Medical Research of the First Affiliated Hospital of Anhui Medical University (approval number PJ2019-14-12).

2.2. Data collection

Vital signs (blood pressure, heart rate, respiratory rate, and body temperature), routine laboratory test results (creatinine, bilirubin, platelet count, C-reactive protein (CRP), Procalcitonin (PCT), hemoglobin, hematocrit, sodium, potassium, white blood cell count and blood culture), blood gas analysis (pH, lactate (Lac), PaO₂, PaCO₂, bicarbonate (HCO₃), and base excess (BE)), Sequential Organ Failure Assessment (SOFA) score [15], Acute Physiology and Chronic Health Evaluation (APACHE) II score [16] and personal information (age, sex) were collected.

2.3. Blood sample collection and ELISA detection of subjects

Blood samples were collected within 24 h after admission, all samples were immediately centrifuged (3000 rpm, 4 °C, 10 min, then 12,000 rpm, 4 °C, 10 min), and serum was extracted and stored in a refrigerator at -80 °C. Serum LMAN2 level was determined by human LMAN2 ELISA kit (Shanghai Enzyme-linked Biotechnology Co., Ltd. Cat. #m1036944-J). Each sample was tested only once.

2.4. Statistical analysis

Kolmogorov-Smirnov test was used to evaluate the normality of data, Mean ± Standard Deviation ($\bar{x} \pm SD$) was used to describe the variables of normal distribution, students' t test was used for comparison between two groups; non-normal distribution data were described by median and quartile spacing, and Mann-Whitney U test was used for comparison between two groups; qualitative data were described by n (%), Chi-square test or Fisher precision test was used for comparison between groups; ROC curve analysis was used for sensitivity and specificity analysis. COX regression analysis was used to analyze the survival data. All data were analyzed by IBM SPSS Statistics 26.0 and GraphPad prism 9.0. The area under ROC curve (AUC) of different indexes was compared by Medcale20 software. The threshold P-value for statistical significance was 0.05.

3. Result analysis

3.1. Comparison of clinical data characteristics and baseline data between sepsis group and septic shock

The clinical characteristics and baseline data of the sepsis, septic shock were compared in Table 1. There were 51 cases in the sepsis group,

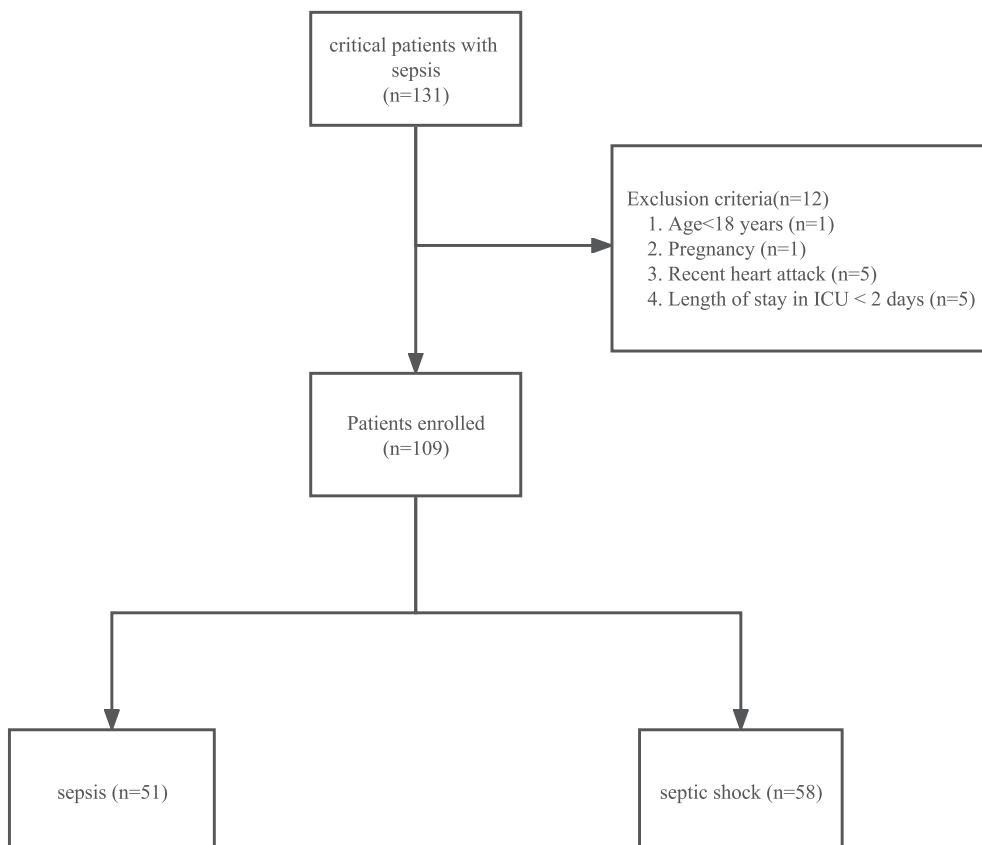


Figure 1. Flow chart for screening patients with sepsis.

Table 1. Comparison of clinical data characteristics and baseline data between sepsis group and septic shock group.

Characteristics	Sepsis (n = 51)	Septic shock (n = 58)	P value
Age, years (IQR)	63.00 (19.00)	63.00 (24.25)	0.806
Male No. (%)	34 (66.7)	37 (63.8)	0.753
MAP, mmHg (IQR)	84.00 (14.85)	78.00 (14.00)	0.002
NE (24 h, max), µg/kg. min (IQR)	0.00 (0.20)	0.36 (0.67)	< 0.001
Biochemical indexes (IQR)			
WBC, 10 ⁹ /L	12.47 (7.61)	11.90 (9.21)	0.634
PLT, 10 ⁹ /L	143.00 (117.50)	110.00 (146.50)	0.057
Lac, mmol/L	1.48 (0.76)	2.97 (2.83)	< 0.001
PCT, ng/ml	3.22 (12.33)	12.06 (41.37)	0.008
CRP, mg/L	80.30 (66.19)	94.7 (140.07)	0.207
Chronic complications, NO. (%)			
COPD	3 (5.9)	2 (3.4)	0.663
CHF	7 (13.7)	12 (20.7)	0.339
CKD	2 (3.9)	3 (5.2)	1.000
Diabetes	14 (27.5)	11 (19.0)	0.293
Cirrhosis	0 (0.0)	3 (5.2)	0.246
Hypertension	29 (56.9)	23 (39.7)	0.073
Infection site, NO. (%)			
Respiratory	43 (84.3)	47 (81.0)	0.653
Abdominal	7 (13.2)	22 (37.9)	0.004
Urinary	5 (9.8)	10 (17.2)	0.261
Other	10 (19.6)	8 (13.8)	0.288
Isolates, NO. (%)			
Gram-positive	8 (15.7)	12 (20.7)	0.501
Gram-negative	29 (56.9)	31 (53.4)	0.721
Other	9 (17.6)	19 (32.8)	0.072
Disease severity (IQR)			
SOFA score	9.00 (6.00)	11.00 (4.00)	< 0.001
APACHE II score	18.00 (7.50)	22.00 (10.00)	0.031

Data are expressed in n (%) and median (quartile spacing). IQR: Interquartile range; MAP: Mean artery pressure; NE: Norepinephrine; WBC: White blood cells; PLT: Platelet; Lac: lactic acid; PCT: Procalcitonin; CRP: C-reactive protein; COPD: Chronic obstructive pulmonary disease; CHF: Chronic heart failure; CKD: Chronic kidney disease; SOFA score: Sequential organ failure score; APACHE II score: Acute physiology and chronic health score.

aged 63.00 (19.00) years old, 58 cases in the septic shock group, aged 63.00 (24.25) years old, there was no significant difference in age ($P = 0.806$) and gender ($P = 0.753$). Furthermore, no difference in basic

Table 2. Receiver operating characteristic (ROC) analysis for LMAN2.

	AUC	P value	Cut-off value (ng/ml)	Youden index	Sensitivity (%)	Specificity (%)
Septic shock	0.699	<0.001	1.37	0.339	67.24	66.91

AUC: Area under concentration-time curve.

disease between patients in the sepsis and septic shock group. Compared with the sepsis group, SOFA score, APACHE II score, lactic acid level, PCT level and maximum dosage of NE within 24 h in septic shock group were higher ($P < 0.001$; $P = 0.031$; $P < 0.001$; $P = 0.008$; $P < 0.001$), MAP was lower ($P = 0.002$), and abdominal infection were more frequent in the septic shock group. In addition, there was no significant difference in WBC and CRP between sepsis group and septic shock group (Table 1).

3.2. Comparison of serum LMAN2 level between sepsis group and septic shock group

Serum LMAN2 level in septic shock group were also higher than those in sepsis group (Figure 2A), and the difference was statistically significant. The ROC curve of serum LMAN2 level in diagnosing septic shock is shown in Figure 2B. In the septic shock prediction, The AUC of serum LMAN2 level (0.699, $P < 0.001$, [95% CI]: 0.600–0.797) was higher than the AUC of APACHE II, CRP and PCT ((AUC = 0.662 ($P = 0.004$, [95% CI]: 0.560–0.764), AUC = 0.570 ($P = 0.208$, [95% CI]: 0.463–0.678), AUC = 0.647 ($P = 0.008$, [95% CI]: 0.544–0.750)), but lower than the AUC of SOFA in predicting septic shock (AUC = 0.708($P < 0.001$, [95% CI]: 0.608–0.808)).

The cut-off value, sensitivity, and specificity of LMAN2 as a predictor of septic shock are presented in Table 2.

Furthermore, correlation analysis showed that serum LMAN2 level was positively correlated with SOFA score (Figure 3A), APACHE II score (Figure 3B), and serum LMAN2 level increased with the number of organ failure (age was not statistically significant among the groups, $P = 0.942$) (Figure 3C).

3.3. Comparison of clinical data characteristics and baseline data between survival group and non-survival group of sepsis

Patients with sepsis were divided into survival group and non-survival group according to clinical outcome. The comparison of clinical data characteristics and baseline data is shown in Table 3. 58 patients in survival group were 63.00 (21.5) years old, and 51 patients in non-

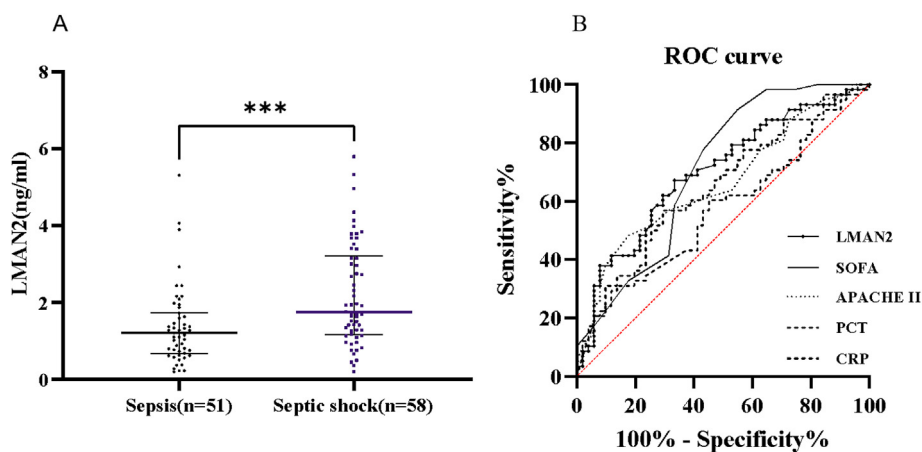


Figure 2. Prediction of septic shock by serum LMAN2 level. Serum LMAN2 levels increase when patients with sepsis are admitted to the hospital. (A) Comparison of the corrected value of serum LMAN2 level between the septic shock group and the sepsis group; (B) ROC curve of serum LMAN2, SOFA score, APACHE II score, PCT, CRP predicting septic shock.

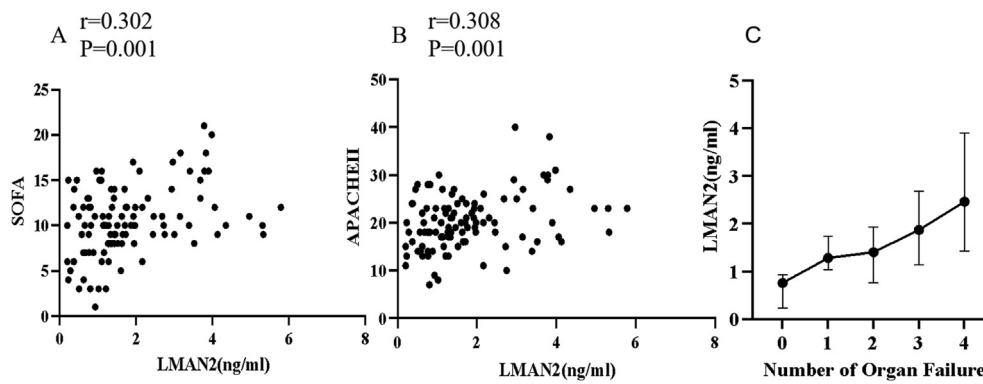


Figure 3. Correlation between serum LMAN2 level and disease severity. (A) Correlation of serum LMAN2 level with SOFA score; (B) correlation of serum LMAN2 level with APACHE II score; (C) the change in serum LMAN2 level with increasing number of organ failure.

survival group were 63.00 (20.00) years old. There was no significant difference in age ($P = 0.286$) and gender ($P = 0.473$). Compared with the survival group, the non-survival group had higher values of SOFA score,

APACHE II score and Lac, and the MAP was lower, higher proportion of patients with septic shock in the non-survival group.

Table 3. Comparison of clinical and baseline data between the survival group and the non-survival group of sepsis.

Characteristics	Survivors (n = 58)	Non-survivors (n = 51)	P value
Age, years, median (IQR)	63.00 (21.5)	63.00 (20.00)	0.286
Male No. (%)	36 (62.1)	35 (68.6)	0.473
MAP, mmHg, median (IQR)	83.00 (15.25)	78.00 (15.00)	0.010
NE (24 h, max), $\mu\text{g}/\text{kg}$, min, median (IQR)	0.13 (0.44)	0.30 (0.90)	0.054
Biochemical indexes, median (IQR)			
WBC, $10^9/\text{L}$	12.14 (8.18)	12.59 (9.74)	0.655
PLT, $10^9/\text{L}$	141.50 (146.75)	119.00 (123.00)	0.057
Lac, mmol/L	1.87 (1.57)	2.49 (1.93)	0.011
PCT, ng/ml	3.65 (20.83)	8.06 (36.48)	0.073
CRP, mg/L	85.12 (69.89)	92.37 (111.48)	0.750
Chronic complications, NO. (%)			
COPD	3 (5.2)	2 (3.9)	1.000
CHF	8 (13.8)	11 (21.6)	0.286
CKD	2 (3.4)	3 (5.9)	0.663
Diabetes	13 (22.4)	12 (23.5)	0.890
Cirrhosis	2 (3.4)	1 (2.0)	1.000
Hypertension	27 (46.6)	25 (49.0)	0.797
Infection site, NO. (%)			
Respiratory	46 (79.3)	44 (86.3)	0.339
Blood	22 (37.9)	25 (49.0)	0.243
Urinary	7 (12.1)	8 (15.7)	0.584
Other	10 (17.2)	8 (15.7)	0.827
Isolates, NO. (%)			
Gram-positive	13 (22.4)	7 (13.7)	0.242
Gram-negative	31 (53.4)	29 (56.9)	0.721
Other	12 (20.7)	16 (31.4)	0.203
Septic shock, No. (%)	25 (43.1)	33 (64.7)	0.024
Disease severity, median (IQR)			
SOFA score	9.50 (5.00)	11.00 (5.00)	0.001
APACHE II score	17.00 (7.25)	22.00 (7.00)	<0.001

Data are expressed in n (%) and median (quartile spacing).

IQR: Interquartile range; MAP: Mean artery pressure; NE: Norepinephrine; WBC: White blood cells; PLT: Platelet; Lac: lactic acid; PCT: Procalcitonin; CRP: C-reactive protein; COPD: Chronic obstructive pulmonary disease; CHF: Chronic heart failure; CKD: Chronic kidney disease; SOFA score: Sequential organ failure score; APACHE II score: Acute physiology and chronic health score.

3.4. Serum LMAN2 level in predicting 28-day mortality

The serum LMAN2 level of sepsis survival group and non-survival group was measured. It was found that the serum LMAN2 level of non-survival group was significantly higher than that of survival group ($P < 0.001$, Figure 4A).

The ROC curve was used to predict the effect of serum LMAN2 level on the 28-day mortality of sepsis patients in ICU. ROC analysis showed that the AUC value of LMAN2 was 0.715 ($P < 0.001$, [95% CI] 0.619–0.810). In addition, the role of routine clinical sepsis-related indicators in predicting 28-day mortality was also analyzed: the AUC values of SOFA, APACHE II, PCT and CRP were 0.679 ($P < 0.001$, [95% CI] 0.580–0.778), 0.775 ($P < 0.001$, [95% CI] 0.689–0.862), 0.600 ($P = 0.073$, [95% CI] 0.494–0.706) and 0.518 ($P = 0.750$, [95% CI] 0.408–0.627) respectively. Besides, the significance of combined LMAN2, SOFA and APACHE II on the prognosis of ICU sepsis patients was analyzed: the AUC was 0.779 ($P < 0.001$, [95% CI] 0.689–0.853) (Figure 4B).

ROC curve analysis showed that the best threshold of LMAN2 level for predicting 28-day mortality was 1.28 ng/ml (Table 4). Over 28 days, 40 (78.4) septic patients died in the high-expression group of LMAN2 (>1.28 ng/ml) compared to 11 (21.6) in the low-expression group (Adjust HR = 3.199, [95% CI] 1.617–6.811) (Figure 5). Similarly, the survival time of the high expression group 17 (20.25) d was significantly shorter than that of the low expression group 28 (0) d ($P < 0.001$).

COX regression was used to determine risk factors for 28-day mortality. Serum LMAN2 level (>1.28 ng/ml) and APACHE II score on the day of admission to ICU are independent risk factors of 28-day mortality of sepsis patients after adjustment for age, sex, septic shock, APACHE II score, SOFA score (Figure 6).

4. Discussion

In recent years, attention has been paid to the integrity of glycocalyx increasingly, which is the earliest damaged site in sepsis [17]. The main cause of terminal manifestations in some patients with septic shock is the low responsiveness of blood vessels to NE due to the damage of vascular endothelial glycocalyx [18, 19]. Some studies have shown that the occurrence of sepsis can cause the glycocalyx on the surface of endothelial cells to shed, and the degree of shedding is positively correlated with the severity of sepsis [20, 21]. Therefore, an effective biomarker is essential for the identification and intervention of glycocalyx shedding.

In 1994, Filedler and others isolated the lectin mannose-binding 2 (LMAN2) from dog kidney cells for the first time, which is one of the components of lipid raft on epithelial cell membrane [22]. However,

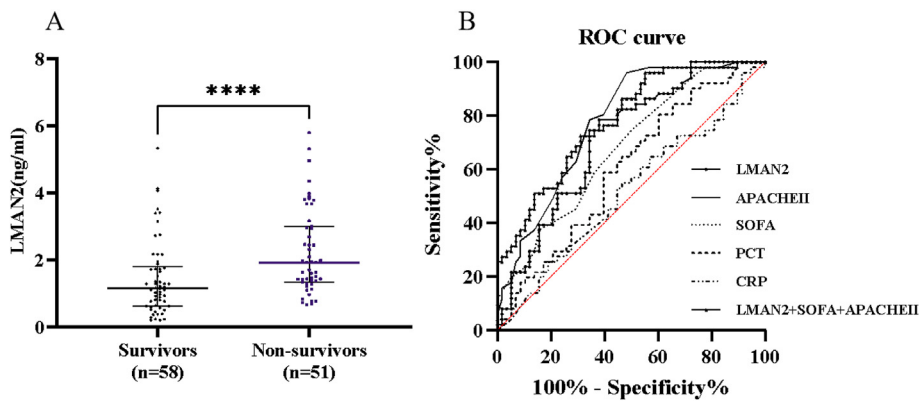


Figure 4. Prediction of 28-day mortality in patients with sepsis by serum LMAN2 level. (A) Comparison of serum LMAN2 level between the sepsis survival group and the death group, $P < 0.0001$, the difference is statistically significant; (B) the receiver operating curve (ROC) of LMAN2, SOFA score, APACHEII score, PCT, CRP and LMAN2 combined with SOFA score and APACHEII score at admission predicts the 28-day mortality of patients with sepsis. Area under the ROC curve: LMAN2 = 0.715 ($P < 0.001$), SOFA score = 0.679 ($P = 0.001$), APACHE II score = 0.775 ($P < 0.001$), PCT = 0.600 ($P = 0.073$), CRP = 0.518 ($P = 0.750$)), LMAN2 combined with SOFA score and APACHE II score is 0.779 ($P < 0.001$).

Table 4. ROC curve analysis for the prediction of 28-day mortality.

	AUC	P value	Cut-off value	Youden index	Sensitivity (%)	Specificity (%)
LMAN2	0.715	<0.001	1.28	0.405	78.43	62.07
SOFA	0.679	<0.001	9	0.245	74.51	50.00
APACHE II	0.775	<0.001	17	0.478	96.08	51.72

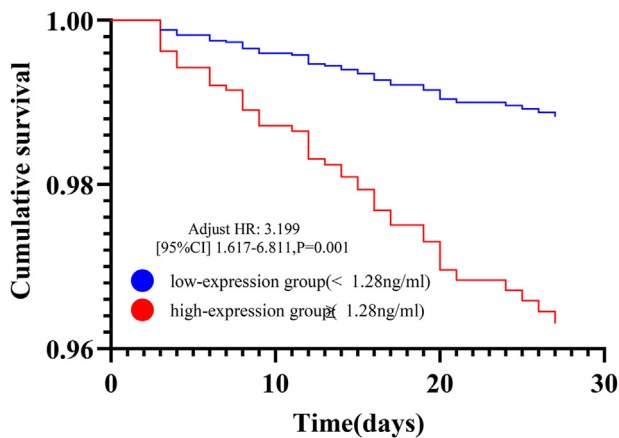


Figure 5. COX regression survival curves for the LMAN2 high and low expression groups, adjusted for age, sex, SOFA score, APACHE II score, and septic shock.

glypican-1, the only proteoglycan expressed on endothelial cell membrane in glycocalyx, specifically binds glypican-1 to lipid raft through C-terminal glycosyl phosphatidylinositol (GPI) anchor [23, 24]. Therefore, it is speculated that LMAN2 is involved in the shedding of glycocalyx. In this study, serum LMAN2 level in septic shock group was higher than that in sepsis group, and the AUC of serum LMAN2 levels to predict septic shock was 0.699 ($P < 0.001$, [95% CI] 0.600–0.797), while the AUC of PCT and CRP were 0.73 ([95% CI] (0.63–0.83)), 0.53 ([95% CI] 0.42–0.65) respectively [25]. In addition, serum LMAN2 level was

positively correlated with SOFA score, APACHE II score, and the changes in serum LMAN2 level with increasing number of organ failures, indicating that higher serum LMAN2 level may indicate more severe sepsis. In previous studies, sepsis caused glycocalyx shedding on endothelial cell surface, and the shedding degree was positively correlated with the severity of sepsis [20, 21]. Glycocalyx shedding can lead to impaired vascular tension, platelet adhesion, protein extravasation and tissue edema [26]. To sum up, it may prove the correlation between serum LMAN2 level and clinical related indexes of sepsis severity in this paper, and speculate that LMAN2 is involved in the shedding of vascular endothelial glycocalyx. The increase of serum LMAN2 level in early stage of clinical sepsis may suggest the existence of glycocalyx injury.

Finally, this study found that serum LMAN2 level on the day of admission to ICU is related to the 28-day mortality of septic patients. Serum LMAN2 level was found to be an independent predictor of 28-day mortality of septic patients, and serum LMAN2 level combined with SOFA and APACHE II can improve the ability to predict 28-day mortality of septic patients. It takes 5–7 days to recover from glycocalyx injury, and it takes longer to restore hemodynamic stability [27], which may explain that serum LMAN2 level is an independent risk factor for predicting 28-day mortality in sepsis patients.

This study illustrates the association between LMAN2 and sepsis. However, this study has some limitations: Firstly, the sample of the study in this article is relatively small and is a single-center research, which may require multi-center study to further verified. Secondly, the combination of LMAN2, SOFA score and APACHE II score can improve the prediction accuracy of 28-day mortality in septic patients. However, compared with only using APACHE II score to predict the 28-day

Characteristic	High expression No.(%)	Low expression No.(%)	Unadj.HR (95% CI)	P	Adj.HR (95% CI)	P
Age			1.01(0.99-1.03)	0.250	1.01(0.99-1.03)	0.210
Sex(Male)	40(62.5)	31(68.9)	1.23(0.68-2.21)	0.500	1.54(0.84-2.92)	0.196
Septic shock(Yes)	23(35.9)	28(62.2)	2.04(1.15-3.63)	0.020	1.36(0.74-2.56)	0.330
APACHE II score			1.12(1.07-1.17)	<0.001	1.11(1.04-1.18)	0.002
SOFA score			1.18(1.07-1.23)	<0.001	0.99(0.89-1.10)	0.150
LMAN2(>1.28ng/ml)			3.36(1.72-6.56)	<0.001	3.20(1.62-6.81)	0.001

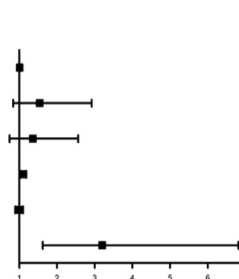


Figure 6. Multivariate COX regression model for 28-day mortality in patients with sepsis, adjusted for age, sex, septic shock, APACHE II score and SOFA score.

mortality of sepsis patients, the difference is not statistically significant. In the future, it may be necessary to explore whether the combination of LMAN2 and other biomarkers can further improve the predictive value of 28-day or even 90-day mortality of sepsis patients. Thirdly, as mentioned earlier, serum LMAN2 level increases with increasing number of organ failure. However, in this study, we only measured serum LMAN2 level on the first day of ICU admission in sepsis patients and not dynamically. In our future studies, we will try to find out the relationship between the dynamics of serum LMAN2 level and sepsis progression in sepsis patients and whether serum LMAN2 level changes with remission or cure of sepsis meaning that LMAN2 may be one of the targets of sepsis treatment. Finally, this article does not elucidate the deeper mechanisms of LMAN2 in the development of sepsis.

5. Conclusions

This study shows that high serum LMAN2 level may indicate septic shock and is associated with an unfavorable prognosis for sepsis patients.

Declarations

Author contribution statement

Junjie Bao: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Min Shao: Conceived and designed the experiments.

Miao Liu: Contributed reagents; materials, analysis tools or data.

Shi Chen: Analyzed and interpreted the data; Performed the experiments.

Jun Yuan: Analyzed and interpreted the data; Wrote the paper.

Limian Cao: Contributed reagents, materials, analysis tools or data.

Yutao Zha, Jiejie Qiao and Qigang Yang: Analyzed and interpreted the data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e11409>.

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