# Elevated endothelial dysfunction related biomarker levels indicate the severity and predict the incidence of sepsis

**Gaosheng Zhou**
Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College

**Jingjing Liu**
Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College

**Hongmin Zhang**
Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College

**Xiaoting Wang**
Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College

**Dawei Liu** ( dwliu2016@126.com )
Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College

**Article**

**Keywords:** Biomarker, syndecan-1, soluble Thrombomodulin, Sepsis, Septic shock.

**Posted Date:** July 7th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1803381/v1

# Abstract

**Background:** The present study assessed to investigate the relationship between the serum endothelial dysfunction-related biomarker levels and the severity of organ dysfunction in septic patients.

**Methods:** In total, 105 patients admitted to the Department of Critical Care Medicine were enrolled between September 2020 and November 2021. The levels of serum syndecan-1, soluble thrombomodulin(sTM) were measured by enzyme-linked immunosorbent assay (ELISA); Clinical and laboratory data were also recorded.

**Results:** The enroll patient were divided into three groups: Infection group (n = 28), septic non-shock group (n = 31), and septic shock group (n = 46). Serum syndecan-1level (102.84 ± 16.53 vs. 55.38 ± 12.34 ng/mL), and sTM(6.60 ± 1.44 ng/mL vs. 5.23 ± 1.23 ng/mL, P < 0.01) in the septic group were clearly increased compared with the in infection group respectively. Besides, the serum syndecan-1 level was closely positively correlated with the serum sTM ($r_s$ = 0.712, $r^2$ = 0.507, P < 0.001). Moreover, either serum syndecan-1($r_s$ = 0.687, $r^2$ = 0.472, P < 0.001) or sTM levels ($r_s$ = 0.6, $r^2$ = 0.36, P < 0.01), was significantly positively correlated with the sequential organ failure assessment scores respectively. For the sepsis diagnosis, the results indicate Syndecan-1 (AUC 0.89 ± 0.04, P < 0.001) was more valuable for prediction than sTM (AUC 0.77 ± 0.06, P < 0.001). And for the septic shock diagnosis, the results indicate Syndecan-1 (AUC 0.95 ±0.02, P < 0.001) as the best predictor as well as SOFA score (AUC 0.95 ± 0.02, P < 0.001), compared with sTM (AUC 0.88 ± 0.03, P < 0.001).

**Conclusions:** Serum syndecan-1, sTM levels were associated with the severity of organ dysfunction in septic patients, and serum syndecan-1, sTM levels were good for early identification of septic, particularly septic shock patients.


# Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection associated with significant morbidity and mortality in the world [1]. The pathogenetic mechanism of sepsis is highly complex, endothelial dysfunction is an important factor leading to organ dysfunction[2]. Endothelial cells shift toward a pro-inflammatory, pro-adhesive, and pro-coagulant phenotype during sepsis, moreover, the endothelial cells' functional modifications are initially adaptive but ultimately become harmful leading to multiorgan dysfunction during sepsis[3].

The glycocalyx is the constituent of the endothelial surface layer which regulates vascular permeability, adhesion of leukocytes and platelets, shear stress, and inflammatory processes[4].

Syndecan-1 is a member of the polysaccharide syndecan family, which belongs to the family of transmembrane heparan sulfate proteins in the glycocalyx, and circulating syndecan-1 is a marker of endothelial glycocalyx degradation[5]. Previous studies have shown that syndecan-1, is shed and released

into the bloodstream during severe infection, reflecting glycocalyx damage and hence a superficial endothelial disruption[6,7]. Moreover, studies demonstrate that syndecan-1 shedding is associated with both sepsis presence and severity[8,9]. Besides, thrombomodulin (TM), an integral endothelial cell membrane protein, is a critical player in maintaining vascular thrombi resistance[10]. In addition to its anticoagulant activity, thrombomodulin has anti-inflammatory and cytoprotective effects[11]. Moreover, previous studies have shown that thrombomodulin is enzymatically cleaved from the endothelial cells and released into the bloodstream under direct endothelial cell damage, hereby reflecting profound endothelial dysfunction[12].

As the innermost layer of the arterial wall, the syndecan-1 is affected at the earliest[13]. However, soluble thrombomodulin (sTM) is derived due to direct damage to the endothelial cells, not through secretion, which is appeared relatively late in circulating. Syndecan-1 and sTM all can serve as markers of endothelial cell injury in humans but to varying degrees. Furthermore, the circulating levels of syndecan 1, and sTM were independently associated with mortality in both trauma and MI patients[14,15]. However, there are limited data regarding the relationship between the Syndecan-1, sTM and the severity of septic organ dysfunction, and its predictive value in sepsis. The purpose of the present study was to investigate the relationship between the syndecan-1, sTM, and the severity of organ dysfunction in septic patients, and its predictive value in sepsis.

## Methods

# Participants

This present study was carried out at the Department of Critical Care Medicine between September 2020 and November 2021. The study was approved by the hospital institutional review board (Ethics Approval No. ZS-2774) and was performed following the Declaration of Helsinki. Written informed consent was obtained from all enrolled patients or their families. Patients were screened for enrolment within the first 24 hours of being admitted.

The present study was divided into three groups: the Infection group, septic non-shock group, and septic shock group. The infection group comprised patients admitted to ICU with an active infection. The septic non-shock group comprised of patients who showed an acute change SOFA score ≥ 2 points consequent to the infection and septic shock could be excluded. The septic shock group required the administration of vasopressors and a lactate level > 2 mmol/L on the day of ICU admission [1]. Sepsis/septic shock was diagnosed according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis 3.0) [1]. We excluded patients aged < 18 years, with intensive care unit (ICU) stay of less than 24 h, and those with massive bleeding or pulmonary embolism, heart attack or acute exacerbation of previous heart disease in the previous week, heart surgery in the last week, or lack of informed consent by the patient or their families.

# Data collection

Baseline clinical and laboratory data were carried out within 24 h after ICU admission, including patient age, sex, hemodynamic parameters, blood chemistry, Sequential Organ Failure Assessment (SOFA) scores, and Acute Physiology and Chronic Health Evaluation (APACHE) II scores.

# Blood sample collection

Peripheral blood samples were collected within the first 24 h on ICU admission, then centrifuged immediately. Serum was separated by centrifugation at 2500 g for 15 min and stored immediately at -80 °C until assessment by enzyme-linked immunosorbent assay (ELISA).

# Enzyme-linked immunosorbent assay (ELISA) measurements

Established biomarkers of endothelial glycocalyx and cell damage, respectively. The soluble biomarkers, syndecan-1, and sTM were measured in serum according to the manufactures recommendations by commercially available immunoassays. The Human Syndecan-1 ELISA kit (CUSABIO, Catalog No. CSB-E1498h, Wu Han, China), Human soluble Thrombomodulin ELISA kit (Abcam, Catalog No. ab214029, Cambridge, England).

# Statistical analysis

Data with normally distributed data were expressed as the mean and standard deviation and were compared using the Student's t-test or one-way analysis of variance. Data with non-normally distributed were presented as the median and interquartile intervals and were compared using the Mann-Whitney U test. Categorical variables were compared using the chi-square test and are recorded as proportions. The Correlations of normally distributed data were analyzed using the Pearson method, and Spearman correlation analysis was performed on non-normally distributed data. Statistical analysis was performed using the SPSS 23 (SPSS, IBM, Armonk, NY, USA) and GraphPad Prism 8.0 ( GraphPad Software Inc., San Diego, CA, USA). Two-tailed P values $\leq 0.05$ were considered statistically significant.The sample size was estimated based on a priori power calculation indicating an 80% power to detect a difference of syndecan-1, soluble thrombomodulin(sTM) (efect size 0.7) among different groups at the 0.05 signifcance level using a power and sample size website.

## Results

# 1 General and clinical characteristics of patients

In all, 131 patients were screened for enrolment, and 105 patients were included in this study (Fig. 1). The patients were divided into three groups: Infection group (n = 28), Septic nonshock group (n = 31), and Septic shock group (n = 46). There were no significant differences in age, sex, arteriovenous carbon dioxide partial pressure difference (Pv-a$CO_2$), central venous blood oxygen saturation ($ScvO_2$), mean arterial blood pressure (MAP), central venous pressure (CVP) among the three groups. The patients with

septic shock had significantly higher SOFA scores, lactate, compared with the septic non-shock group. The basic characteristics of all enrolled patients are presented in Table 1.

Table 1 patient characteristics at ICU admission

| Categories | Infection<br>n = 28 | Septic nonshock<br>n = 31 | Septic shock<br>n = 46 | P value |
|---|---|---|---|---|
| Age<br>(yr, mean ± SD) | 62 ± 18 | 59 ± 18 | 62 ± 17 | 0.768 |
| Gender<br>(male, %) | 64.29 | 77.42 | 63.04 | 0.591 |
| APACHEII score | 15(11–16) | 16(14–19) | 17(15–19)* | 0.013 |
| SOFA score | 0(0–2) | 5(4–8)* | 11(9–14)*# | 0.000 |
| HR (bpm) | 96 ± 24 | 106 ± 20 | 110 ± 22* | 0.031 |
| MAP (mmHg) | 89 ± 12 | 91 ± 16 | 84 ± 15 | 0.070 |
| CVP (mmHg) | - | 11 ± 7 | 8 ± 4 | 0.327 |
| Pv-a$CO_2$ (mmHg) | - | 4(1.7–6.9) | 5.1(2.7–6.1) | 0.453 |
| $ScvO_2$ (%) | - | 77.3 ± 9.3 | 75.2 ± 10.3 | 0.716 |
| Lactate (mmol/l) | 1.0(0.8–1.6) | 1.6(1.1–2.3) | 4.0(2.2–7.8)*# | 0.000 |

**Abbreviations**: APACHE II score indicates Acute physiology and Chronic Health Evaluation II score, SOFA sequential organ failure assessment score, HR heart rate, MAP mean arterial blood pressure, CVP central venous pressure, Pv− aCO2 arteriovenous carbon dioxide partial pressure difference, ScvO2 central venous blood oxygen saturation. Data was presented by mean standard deviation, n (%), or median (interquartile range). *P < 0.05 for the comparison between infection and septic nonshock, septic shock. #P < 0.05 for the comparison between septic nonshock and septic shock.

# 2 Serum levels of endothelial damage-related biomarkers in a derivation cohort of patients

To investigate the changes of endothelial damage-related biomarkers in the progression from infection to septic shock, the results were shown in Fig. 2 and Table 2. The serum levels of syndecan-1 and sTM increased progressively from infected patients to septic non-stock patients to septic shock patients. The tendencies of syndecan-1 and sTM levels variation were relatively similar in different groups. Serum

syndecan-1 concentrations on ICU admission were significantly increased in the septic shock group, septic nonshock group, compared with the infection group (Fig. 2).

The levels of syndecan-1 in the septic shock group (102.84 ± 16.53 ng/mL) were increased compared with the septic nonshock group (76.06 ± 10.51 ng/mL), the infection group(55.38 ± 12.34 ng/mL), the difference was statistically significantly(Fig. 2a).

Serum sTM concentrations were significantly increased in the septic shock group, septic non-shock group, compared with the infection group (Fig. 2b). Patients with septic shock had higher sTM levels than septic non-shock group (9.67 ± 3.38 ng/mL vs 6.60 ± 1.44 ng/mL, P < 0.01), and septic non-shock patients had higher sTM levels than infection patients(6.60 ± 1.44 ng/mL vs 5.23 ± 1.23 ng/mL, P < 0.01) (Fig. 2b).

Table 2 Clinical characteristics

| Categories | Infection n = 28 | Septic nonshock n = 31 | Septic shock n = 46 | P value |
|---|---|---|---|---|
| Syndecan-1 (ng/ml) | 55.38 ± 12.34 | 76.06 ± 10.51* | 102.84 ± 16.53*# | 0.000 |
| sTM (ng/ml) | 5.05(4.15-6.24) | 6.82(5.55-7.73)* | 8.58(7.45-10.16)*# | 0.000 |
| OI (mmHg) | 327 ± 112 | 320 ± 144 | 296 ± 134 | 0.561 |
| TB (μmol/L) | 13.7(12.3-28.7) | 13.5(9.4-21.9) | 17.8(12.7-31.0) | 0.093 |
| Cr (μmol/L) | 72(32-109) | 82(58-156) | 90(82-196) | 0.152 |
| Fibrinogen (g/l) | 4.2 ± 1.9 | 4.3 ± 2.0 | 3.9 ± 4.7 | 0.889 |
| Dimer (mg/l) | 3.7(2.6-9.6) | 4.3(2.4-13.1) | 5.4(2.8-9.9) | 0.058 |
| PT (sec) | 14.6(12.6-16.1) | 13.9(12.9-16.0) | 15.4(14.2-20.0) | 0.049 |
| APTT (sec) | 30.7(27.4-35.2) | 31.6(28.5-36.8) | 30.0(26.3-39.2) | 0.896 |
| INR (ratio) | 1.3(1.2-1.5) | 1.1(1.1-1.5) | 1.3(1.2-1.4)# | 0.009 |
| Procalcitonin (ug/L) | 1.1(0.2-10.0) | 1.4(0.6-12.7) | 8.0(1.9-30.3)* | 0.003 |
| PLT (*$10^9$) | 196 ± 79 | 171 ± 99 | 136 ± 93* | 0.022 |

**Abbreviations**: sTM serum thrombomodulin, OI oxygen index, TB total bilirubin, Cr creatinine, PT prothrombin time, APTT activated partial thromboplastin time, INR International Normalized Ratio, PLT platelet. Data was presented by mean standard deviation. *P < 0.05 for the comparison between infection and septic nonshock, septic shock. #P < 0.05 for the comparison between septic nonshock and septic shock.

# 3 Serum syndecan-1 and sTM levels were associated with the organ function, disease severity

To establish whether serum syndecan-1 and sTM levels determine the organ function, disease severity, and its correlations with the SOFA score, APACHE II score on ICU admission, were assessed.

As shown in Fig. 3a, the serum syndecan-1 level was closely positively correlated with the serum sTM ($r_s$ = 0.712, $r^2$ = 0.507, P < 0.001). Moreover, the serum levels of syndecan-1 and sTM were significantly positively correlated with SOFA scores respectively. The serum levels of syndecan-1 were significantly positively correlated with the SOFA scores ($r_s$ = 0.687, $r^2$ = 0.472, P < 0.001, Fig. 3b), similarly, serum sTM levels were positively significantly correlated with the SOFA score ($r_s$ = 0.6, $r^2$ = 0.36, P < 0.01, Fig. 3c). Besides, there is a weak association between the serum levels of syndecan-1 and the APACHE II score ($r_s$ = 0.279, $r^2$ = 0.078, P = 0.006, Fig. 3d). However, our results showed no correlation between the serum levels of sTM and the APACHE II score (r = 0.184, $r^2$ = 0.034, P = 0.073).

To further assess serum syndecan-1 and sTM levels d correlations with organ dysfunction at study enrollment. However, it is clear that neither the serum syndecan1 levels nor sTM, exhibit few significant correlations with oxygen index (OI), total bilirubin (TB), creatinine (Cr), respectively (data not shown).

# 4 Serum syndecan-1 and sTM levels were associated with tissue perfusion

To assess the relationship between endothelial damage and tissue perfusion, the association between the serum syndecan-1, sTM levels and lactate was assessed. As shown in Fig. 4, the serum levels of syndecan-1in all patients admitted to the ICU ($r_s$ = 0.574, $r^2$ = 0.329, P < 0.001, Fig. 4a) , sTM ($r_s$ = 0.458, $r^2$ = 0.210, P < 0.001, Fig. 4b) were significantly positively correlated with lactate respectively, demonstrating that endothelial damage were closely related to tissue perfusion.

# 5 Serum syndecan-1 was associated with coagulation function

Endothelial injury-inducing coagulation abnormalities during sepsis. So the association between the serum syndecan-1, sTM levels, and coagulation function parameters was assessed.

By correlation analysis, the serum syndecan-1 level was weakly correlated with the PT ($r_s$ = 0.286, $r^2$ = 0.082, P = 0.003), INR ($r_s$ = 0.337, $r^2$ = 0.114, P = 0.0004), and there was no apparent correlation between serum syndecan-1 levels and APTT ($r_s$ = 0.063, P = 0.526), D-dimer ($r_s$ = 0.173, P = 0.087), Fib ($r_s$ = -0.054, P = 0.584). However, no significant correlations were found between serum sTM levels and PT ($r_s$ = 0.096,

P = 0.331), APTT ($r_s$ = 0.038, P = 0.704), D-dimer ($r_s$ = 0.040, P = 0.698), Fib ($r_s$ = - 0.190, P = 0.053), INR ($r_s$ = 0.191, P = 0.051) (data not shown).

# 6 The ability of endothelial damage biomarkers to predict the incidence of sepsis and septic shock

The results are presented in Table 3.1, 3.2, and Fig. 5. We calculated the cut-off value, sensitivity, and specificity of Syndecan1 and sTM as predictors of sepsis or septic shock for the patients admitted to the ICU using the ROC curve.

For the sepsis diagnosis (Fig. 5a, Table 3.1), the results indicate SOFA score as the best predictor (AUC 0.99 ± 0.01, P < 0.001), and Syndecan-1 (AUC 0.89 ± 0.04, P < 0.001) was more valuable for prediction than sTM (AUC 0.77 ± 0.06, P < 0.001), PCT (AUC 0.59 ± 0.07, P = 0.262) ,APACHE II score (AUC 0.65 ± 0.07, P = 0.052).

For the septic shock diagnosis (Fig. 5b, Table 3.2), the results indicate Syndecan-1 (AUC 0.95 ±0.02, P < 0.001) as the best predictor as well as SOFA score (AUC 0.95 ± 0.02, P < 0.001), compared with sTM (AUC 0.88 ± 0.03, P < 0.001), Lac (AUC 0.85 ± 0.04, P < 0.001), PCT (AUC 0.67 ± 0.05, P < 0.001), APACHE II score (AUC 0.65 ± 0.06, P = 0.01).

Table 3.1 Receiver operating characteristic (ROC) analysis for Syndecan1 and sTM in Sepsis.

| | AUC ± SE | P value | Cut-off value | Sensitivity% | Specificity% |
|---|---|---|---|---|---|
| Syndecan1 | 0.89 ± 0.04 | < 0.001 | 66.58ng/ml | 83.87 | 82.14 |
| sTM | 0.77±0.06 | < 0.001 | 5.08ng/ml | 90.32 | 57.14 |

Table 3.2 Receiver operating characteristic (ROC) analysis for Syndecan1 and sTM in Septic shock.

| | AUC ± SE | P value | Cut-off value | Sensitivit% | Specificity% |
|---|---|---|---|---|---|
| Syndecan1 | 0.95 ± 0.02 | < 0.001 | 87.14 ng/ml | 86.96 | 96.61 |
| sTM | 0.88 ± 0.03 | < 0.001 | 7.43 | 64.43 | 84.75 |
| **Abbreviations:** sTM serum thrombomodulin. | | | | | |

# Discussion

The present study indicated the following points. First, serum syndecan-1 and soluble thrombomodulin (sTM) levels were increased among all the patients in the three groups. The levels of syndecan-1 and sTM gradually increased as the disease progresses, suggesting that the endothelial injury was gradually

aggravated. The levels of syndecan-1 and sTM were significantly higher in the patients with septic shock compared to the infection and sepsis-nonshock groups.

Moreover, we revealed that serum syndecan-1 and sTM levels were elevated with the severity of the disease. Notably, serum syndecan-1 and sTM levels were positively closely related to the SOFA score, respectively. Furthermore, it is more interesting that either serum syndecan-1 or sTM levels were positively significantly correlated with lactate. Besides, the value of syndecan-1 and sTM as a predictor of the incidence of sepsis or septic shock on the day of ICU admission was well demonstrated.

Endothelial dysfunction is common in adult ICU patients, especially in critical patients due to infection. Though it has been evident for decades that endothelial injury is a hallmark of sepsis, new data keep emerging that further reveal the pathophysiology of endothelial damage in sepsis and its association with disease severity, including the applicability of biomarkers for outcome[16,17]. But the conclusions tend to vary in these studies.

Anand et al. reported that there were significant correlations between syndecan-1 levels level with the severity of the disease[18]. Deng et al. found that patients with sepsis have significantly higher serum sTM levels which were positively correlated with the severity of the disease[19].

In the present study, we revealed that the serum levels of syndecan-1 and sTM were elevated with the severity of the disease, which was in accordance with previous findings, but the molecular mechanism remains undefined.

Syndecan-1 is a proteoglycan found in the endothelial glycocalyx. Circulating syndecan-1 is a marker of endothelial glycocalyx degradation, which reflects superficial endothelial damage[20]. However, the mechanism underlying endothelial glycocalyx during sepsis is still not clear. Metalloproteinases (MMPs) are activated in inflammatory states by reactive oxygen species (ROS) and pro-inflammatory cytokines, which are known to cleave proteoglycans directly from the endothelial cell membrane in sepsis[4]. The degraded glycocalyx layer leads to an increase in permeability to plasma proteins and fluids, causing interstitial leakage. Several studies have demonstrated elevated syndecan-1 levels as a marker of glycocalyx degradation in patients with sepsis[21,22].

Besides, thrombomodulin (TM) is an endothelial cell transmembrane glycoprotein that is only released upon direct endothelial disruption[23]. soluble thrombomodulin (sTM) is released into plasma during inflammation, presumably due to cleavage from endothelial cells by neutrophil-derived enzymes[24,25]. Unlike syndecan-1, sTM is derived due to direct damage to the endothelial cells, not through secretion. Thus, the level of sTM was associated with serious cell membrane breakage and a large number of apoptotic/necrotic cells in sepsis. Regardless, either the levels of syndecan-1 or sTM can reflect the severity of endothelial damage during sepsis. syndecan-1 and sTM were used as biomarkers of endothelial dysfunction in the present research proving the above conclusion once more.

Furthermore, endothelial dysfunction ultimately contributes to ending multi-organ damage during sepsis or septic shock. Considering the close relationship between endothelial function and organ function during sepsis[1], we also explored the correlation between serum endothelial dysfunction biomarker levels and the SOFA score. We also found that serum syndecan-1 and sTM level was closely related to the SOFA score. Previous studies have shown that plasma syndecan-1 and sTM increased progressively and significantly across groups with increasing infectious severity and correlated significantly with organ failure as measured by the sequential organ failure assessment (SOFA) score in a patient with varying degrees of infectious disease[26].

Similarly, there was a strong association between epitheliopathy and organ failure in a large multicenter study of 1103 critically ill patients predominantly suffering from sepsis[17], demonstrating that patients with sepsis had higher plasma levels of syndecan-1 and sTM than non-infected patients. These results are thus consistent with the above idea and support our conclusion.

Besides, previous studies indicated that high syndecan-1 and sTM levels independently predicted liver and renal failure, respectively, and high sTM was further associated with an increased risk of development of multiple organ failure[27]. However, further analysis in this research shows that no significant correlations were found between syndecan-1, sTM levels, and organ function-related biomarker levels in septic patients. Moreover, endothelial dysfunction shifts toward a proapoptotic, proinflammatory, pro-adhesive, and procoagulant phenotype during sepsis, so, the central role of endothelial dysfunction in the cross-talk between inflammation coagulation has also been recognized[28,29]. Previous studies found an independent association between high circulating syndecan-1 levels and coagulopathy in a smaller cohort of 184 patients with severe sepsis or septic shock. Nevertheless, the correlations between endothelial dysfunction and coagulation found in this present research are relatively weak and it remains to be determined.

Sepsis-induced organ dysfunction is associated with an inflammatory and coagulation response of the endothelium as described above. Beyond this, previous studies showed that endothelial dysfunction may also result in decreased blood perfusion and therefore may aggravate lactic acidemia[30]. Microvascular obstruction and systemic endothelial dysfunction are associated with plasma lactate in patients with falciparum malaria independently[31]. In the present study, we revealed that the serum levels of syndecan-1 and sTM were significantly correlated with lactate, which was in accordance with previous findings, indicating the endothelial damage was closed correlated with tissue perfusion. Perfusion abnormalities, which were caused by endothelial dysfunction, may be another important cause of sepsis-related organ failure in sepsis. Besides, our previous study showed that mitochondrial dysfunction has been proposed as an important cause of sepsis-related organ failure in sepsis[32]. Thus, endothelial function induction by mitochondrial dysfunction might be an additional mechanism during sepsis.

Moreover, although clinicians know that endothelial activation and dysfunction play a critical role in the pathophysiology of sepsis and represent an important therapeutic target to reduce sepsis mortality, the gap between basic research and clinical applications of endothelial activation and dysfunction remains

wide. It is therefore important to identify endothelial damage early in sepsis or septic shock. An elevation of serum syndecan-1 and sTM levels could be used to alert clinicians that endothelial injury was existed, in clinical treatment, we should put more emphasis on endothelial function and trying to protect endothelial cells from injury in the process of sepsis in the further.

# limitations

We acknowledge that this study has several limitations. First, our sample size was limited and replication studies with a larger sample size will be needed. Second, the findings described here are observational and, hence, no causality can be inferred. Third, caution should be taken when interpreting these results. The well-known high heterogeneity of septic shock patients might contribute to this. Fourth, we only analyzed blood from the initial draw, and we did not follow the biomarker dynamics over time. Fifth, we did not include a healthy control group, the Serum syndecan-1 and sTM levels at baseline need to measure.

## Conclusions

The syndecan-1 and sTM levels are positively associated with organ damage in patients with sepsis and septic shock. Moreover, serum syndecan-1 and sTM could be promising biomarkers for the early diagnosis of sepsis, particularly septic shock patients.

## Abbreviations

APACHE II score indicates Acute physiology and Chronic Health Evaluation II score, SOFA sequential organ failure assessment score, HR heart rate, MAP mean arterial blood pressure, CVP central venous pressure, $P_{v\text{-}a}CO2$ arteriovenous carbon dioxide partial pressure difference, ScvO2 central venous blood oxygen saturation. sTM serum thrombomodulin, OI oxygen index, TB total bilirubin, Cr creatinine, PT prothrombin time, APTT activated partial thromboplastin time, INR International Normalized Ratio, PLT platelet.

## Declarations


### *Acknowledgments*

None.


### *Authors' contributions*

G Z designed the study, performed the statistical analysis, and drafted the manuscript. J L Collected serum samples and collated the clinical and laboratory data. H Z contributed to the analytic strategy and statistical analyses. X W conceived and designed the study, and revised the manuscript. D L designed the

study and revised the manuscript. All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. All authors read and approved the final manuscript.


### *Funding*

This work was supported by capital clinic research and demonstration application of diagnosis and treatment project (No. Z201100005520038).


### *Availability of data and materials*

Data are available on request from the corresponding author.

### *Ethics approval and consent to participate*

The study was approved by the PUMCH institutional review board (Ethics Approval No.ZS-2774). All subjects provided written informed consent.

### *Consent for publication*

The patients gave written informed consent for clinical data use and publication.

### *Competing interests*

The authors declare that there is no conflict of interest.


### *Author Details*

Department of Critical Care Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China.

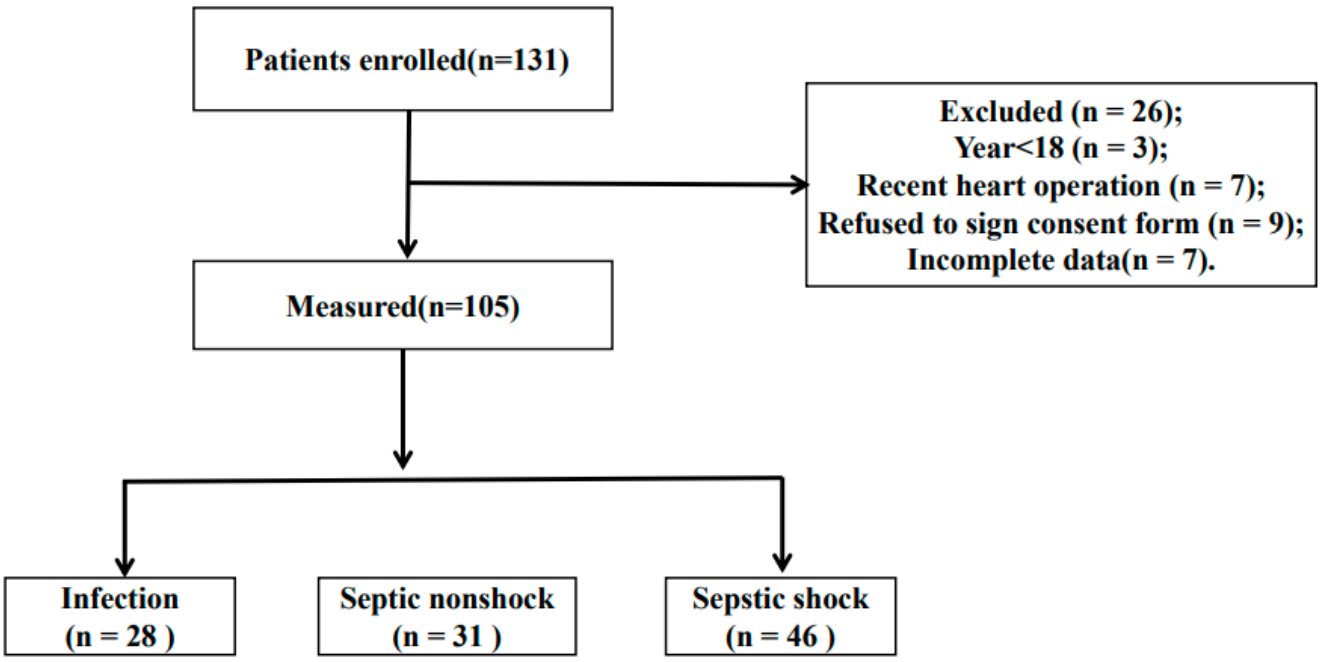

## References


1. *Singer, M. et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 315, 801–810 (2016).*
2. *Ince, C. et al. THE ENDOTHELIUM IN SEPSIS. Shock 45, 259–270 (2016).*
3. *Joffre, J., Hellman, J., Ince, C. & Ait-Oufella, H. Endothelial Responses in Sepsis. Am J Respir Crit Care Med 202, 361–370 (2020).*
4. *Uchimido, R., Schmidt, E. P. & Shapiro, N. I. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. Crit Care 23, 16 (2019).*
5. *Dogné, S. & Flamion, B. Endothelial Glycocalyx Impairment in Disease: Focus on Hyaluronan Shedding. Am J Pathol 190, 768–780 (2020).*

6. *Ikeda, M. et al. Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. J Crit Care 43, 48–53 (2018).*
7. *Smart, L., Bosio, E., Macdonald, S., Dull, R., Fatovich, D. M., Neil, C. & Arendts, G. Glycocalyx biomarker syndecan-1 is a stronger predictor of respiratory failure in patients with sepsis due to pneumonia, compared to endocan. J Crit Care 47, 93–98 (2018).*
8. *Nelson, A., Berkestedt, I., Schmidtchen, A., Ljunggren, L. & Bodelsson, M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock 30, 623–627 (2008).*
9. *Sallisalmi, M., Tenhunen, J., Yang, R., Oksala, N. & Pettilä, V. Vascular adhesion protein-1 and syndecan-1 in septic shock. Acta Anaesthesiol Scand 56, 316–322 (2012).*
10. *La Mura, V., Tripodi, A., Tosetti, G., Cavallaro, F., Chantarangkul, V., Colombo, M. & Primignani, M. Resistance to thrombomodulin is associated with de novo portal vein thrombosis and low survival in patients with cirrhosis. Liver Int 36, 1322–1330 (2016).*
11. *Shrestha, B., Ito, T., Kakuuchi, M., Totoki, T., Nagasato, T., Yamamoto, M. & Maruyama, I. Recombinant Thrombomodulin Suppresses Histone-Induced Neutrophil Extracellular Trap Formation. Front Immunol 10, 2535 (2019).*
12. *Ishii, H., Uchiyama, H. & Kazama, M. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. Thromb Haemost 65, 618–623 (1991).*
13. *Marechal, X. et al. Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. Shock 29, 572–576 (2008).*
14. *Johansson, P. I., Stensballe, J., Rasmussen, L. S. & Ostrowski, S. R. High circulating adrenaline levels at admission predict increased mortality after trauma. J Trauma Acute Care Surg 72, 428–436 (2012).*
15. *Ostrowski, S. R., Pedersen, S. H., Jensen, J. S., Mogelvang, R. & Johansson, P. I. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. Crit Care 17, R32 (2013).*
16. *Pons, S., Arnaud, M., Loiselle, M., Arrii, E., Azoulay, E. & Zafrani, L. Immune Consequences of Endothelial Cells' Activation and Dysfunction During Sepsis. Crit Care Clin 36, 401–413 (2020).*
17. *Johansen, M. E. et al. Profound endothelial damage predicts impending organ failure and death in sepsis. Semin Thromb Hemost 41, 16–25 (2015).*
18. *Anand, D., Ray, S., Srivastava, L. M. & Bhargava, S. Evolution of serum hyaluronan and syndecan levels in prognosis of sepsis patients. Clin Biochem 49, 768–776 (2016).*
19. *Yue, L., Deng, X., Yang, M. & Li, X. Elevated B-type natriuretic peptide (BNP) and soluble thrombomodulin (sTM) indicates severity and poor prognosis of sepsis. Ann Palliat Med 10, 5561–5567 (2021).*
20. *Piotti, A. et al. Endothelial damage in septic shock patients as evidenced by circulating syndecan-1, sphingosine-1-phosphate and soluble VE-cadherin: a substudy of ALBIOS. Crit Care 25, 113 (2021).*

21. *Huang, X. et al. Plasma Endothelial Glycocalyx Components as a Potential Biomarker for Predicting the Development of Disseminated Intravascular Coagulation in Patients With Sepsis. J Intensive Care Med 36, 1286–1295* (*2021*).
22. *Saoraya, J., Wongsamita, L., Srisawat, N. & Musikatavorn, K. Plasma syndecan-1 is associated with fluid requirements and clinical outcomes in emergency department patients with sepsis. Am J Emerg Med 42, 83–89* (*2021*).
23. *Giri, H., Panicker, S. R., Cai, X., Biswas, I., Weiler, H. & Rezaie, A. R. Thrombomodulin is essential for maintaining quiescence in vascular endothelial cells. Proc Natl Acad Sci U S A 118*, (*2021*).
24. *Boehme, M. W., Deng, Y., Raeth, U., Bierhaus, A., Ziegler, R., Stremmel, W. & Nawroth, P. P. Release of thrombomodulin from endothelial cells by concerted action of TNF-alpha and neutrophils: in vivo and in vitro studies. Immunology 87, 134–140* (*1996*).
25. *Lohi, O., Urban, S. & Freeman, M. Diverse substrate recognition mechanisms for rhomboids; thrombomodulin is cleaved by Mammalian rhomboids. Curr Biol 14, 236–241* (*2004*).
26. *Ostrowski, S. R., Haase, N., Müller, R. B., Møller, M. H., Pott, F. C., Perner, A. & Johansson, P. I. Association between biomarkers of endothelial injury and hypocoagulability in patients with severe sepsis: a prospective study. Crit Care 19, 191* (*2015*).
27. *Johansson, P. I., Stensballe, J. & Ostrowski, S. R. Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. Crit Care 21, 25* (*2017*).
28. *Joffre, J. & Hellman, J. Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation. Antioxid Redox Signal 35, 1291–1307* (*2021*).
29. *Levi, M. & van der Poll, T. Inflammation and coagulation. Crit Care Med 38, S26-34* (*2010*).
30. *El-Hattab, A. W., Hsu, J. W., Emrick, L. T., Wong, L. J., Craigen, W. J., Jahoor, F. & Scaglia, F. Restoration of impaired nitric oxide production in MELAS syndrome with citrulline and arginine supplementation. Mol Genet Metab 105, 607–614* (*2012*).
31. *Hanson, J. et al. Microvascular obstruction and endothelial activation are independently associated with the clinical manifestations of severe falciparum malaria in adults: an observational study. BMC Med 13, 122* (*2015*).
32. *Liu, J., Zhou, G., Chen, R., Tong, Z., Zhang, H., Wang, X. & Liu, D. Mitochondrial Sirt3 serves as a biomarker for sepsis diagnosis and mortality prediction. Sci Rep 12, 10414* (*2022*).

# Figures




## Figure 1

Screening flowchart of patients.

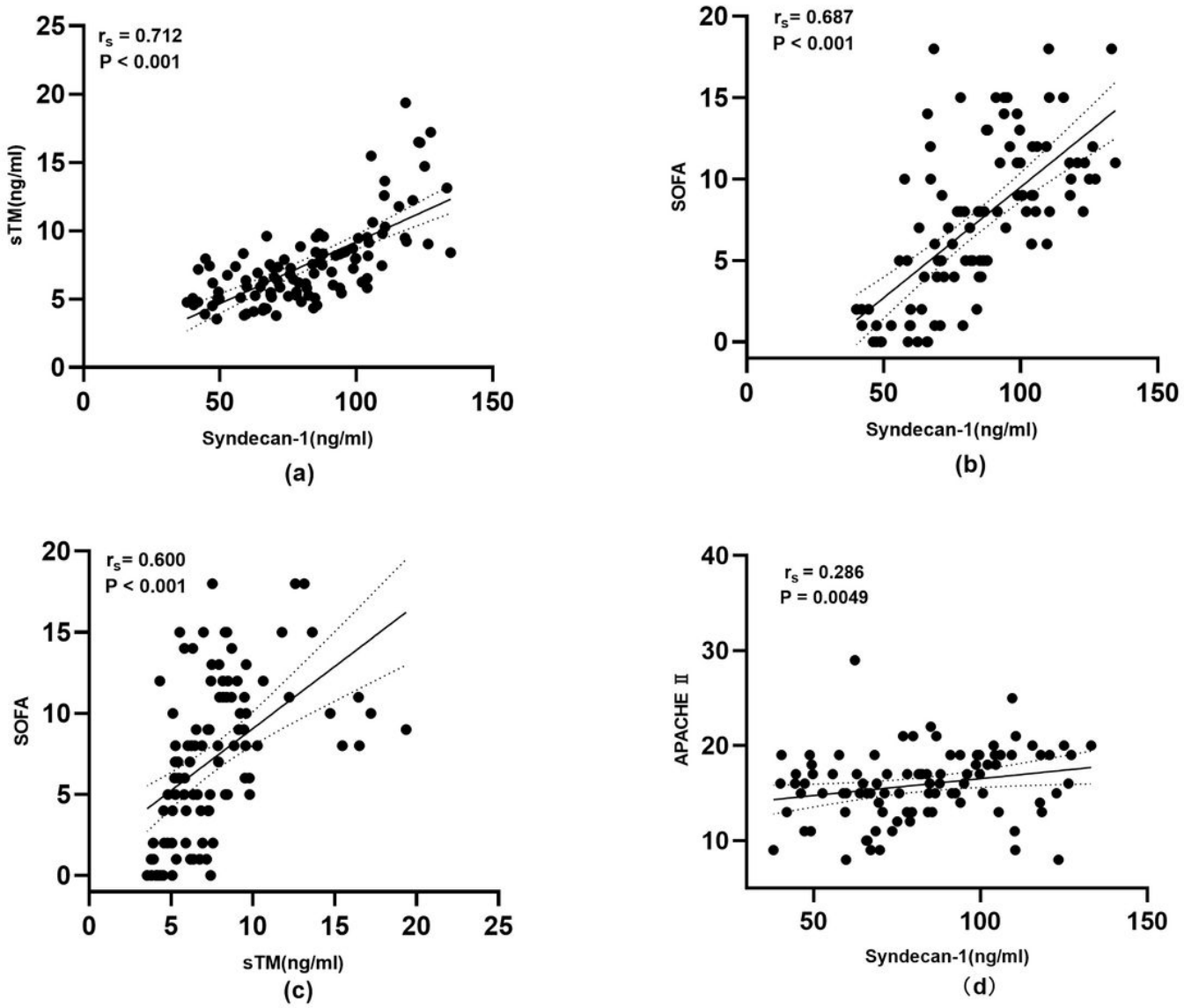
## Figure 2

Comparison of serum endothelial damage-related biomarker levels in different groups.

(a) Serum syndecan-1 level in the septic shock group(102.84 ± 16.53 ng/mL) vs. the septic nonshock group (76.06 ± 10.51 ng/mL) vs. the infection group(55.38 ± 12.34 ng/mL).

(b) Serum sTM level in the septic shock group(9.67 ± 3.38 ng/mL) vs. the septic nonshock group (6.60 ± 1.44 ng/mL) vs. the infection group(5.23 ± 1.23 ng/mL).

*P < 0.01, Septic non-shock group vs Infection group, **P < 0.01, Septic non-shock group vs Septic shock group, ***P < 0.01, Septic shock group vs Infection group.




## Figure 3

***Correlation analysis.*** Serum syndecan-1 level was positively correlated with sTM level in all patients admitted to the ICU, $r_s$ = 0.712, $r^2$ = 0.507, P < 0.001. Serum syndecan-1 level was positively correlated with SOFA score in all patients admitted to the ICU, $r_s$ = 0.687, $r^2$ = 0.472, P < 0.001. Serum sTM level was positively correlated with SOFA score in all patients admitted to the ICU,$r_s$ = 0.279, $r^2$ = 0.078, P = 0.006. Abbreviations: sTM serum thrombomodulin, SOFA sequential organ failure assessment score, ICU intensive care unit.

## Figure 4

***Serum syndecan-1 and sTM levels were associated with tissue perfusion.*** Serum syndecan-1 level was positively correlated with lactate level in all patients admitted to the ICU, $r_s = 0.574$, $r^2 = 0.329$, $P < 0.001$. Serum sTM level was positively correlated with lactate level in all patients admitted to the ICU, $r_s = 0.458$, $r^2 = 0.210$, $P < 0.001$. Abbreviations: sTM serum thrombomodulin, ICU intensive care unit.

## Figure 5

***Receiver operating characteristic curve analysis.*** The ability of Syndecan1 and sTM on ICU admission to predict the incidence of sepsis (a) or septic shock (b). Abbreviations: sTM serum thrombomodulin, ICU intensive care unit, ICU intensive care unit, APACHE II score Acute Physiology and Chronic Health Evaluation II score, SOFA sequential organ failure assessment score, Lac lactate, PCT Procalcitonin.