Serum levels of angiopoietin 2 mRNA in the mortality outcome prediction of septic shock

JUN FENG[1,2*], LILI WANG[1,2*], YIKUAN FENG[1,2], GANG YU[1,2], DAIXING ZHOU[1,2] and JUNSHUAI WANG[1,2]

[1]Emergency Department; [2]Intensive Care Unit, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China

Received May 25, 2021; Accepted January 19, 2022

DOI: 10.3892/etm.2022.11289

Abstract. It has been reported that angiopoietin 2 (Ang-2) plays an integral role in the pathophysiology of sepsis and many other inflammatory diseases. However, the specific role of Ang-2 in septic shock has not been defined. The aim of the present study was to assess the predictive value of serum Ang-2 in patients with septic shock. Clinical data of 85 patients with septic shock and 10 healthy controls admitted to the intensive care unit with a diagnosis of septic shock were collected between January 2020 and October 2020 at Tongji Hospital (Wuhan, China). The serum levels of Ang-2 mRNA were quantified using a quantitative real-time PCR assay. Ang-2, SOFA and APACHE II scores were retrospectively analyzed in relation to 28-day mortality. The area under the receiver operating characteristic (ROC) curve (AUC) was used to discriminate the accuracy of the prediction. Mean Ang-2 mRNA levels in the patients with septic shock were significantly higher than those in the healthy controls ($P<0.05$), and the Ang-2 levels showed a downwards trend over time following treatment. The three indicators (AUCs, SEMs, P-values) were Ang-2 (0.82, 0.03, $P<0.01$), SOFA score (0.76, 0.04, $P<0.01$), and APACHE II score (0.73, 0.04, $P<0.01$). The present study confirmed that Ang-2 mRNA levels were significantly elevated in septic shock. The Ang-2 mRNA level at ICU admission in a patient with septic shock could be a predictive biomarker for mortality.

Correspondence to: Dr Junshuai Wang, Intensive Care Unit, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 JieFang Avenue, Wuhan, Hubei 430030, P.R. China
E-mail: harry107@163.com

*Contributed equally

Key words: septic shock, angiopoietin 2, biomarkers, endothelial damage, accuracy of the prediction

Introduction

Septic shock is defined as a subset of sepsis in which specific and profound circulatory, cellular, coagulation and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone (1,2). Several markers can be detected in the serum of septic patients and they reflect endothelial cell activation and endothelium disruption as well as increased leukocyte-endothelial interactions (3,4). In particular, angiopoietin 2 (Ang-2) is a molecule that is involved in the maintenance of endothelial cell barrier function. Increased Ang-2 is predominantly associated with increased intracellular gap formation and disseminated intravascular coagulation (DIC) (5). For instance, Statz *et al* (6) reported that serum Ang-2 levels were related to the prognosis of patients with sepsis-associated DIC. Overall, prediction of a patient's outcome is of great importance to clinicians in their management of septic shock, and the optimal place of novel vasoactive agents in therapy for circulatory dysfunction should be established with further confidence. In particular, direct biomarkers of endothelial damage could also be of great significance during septic shock.

The Sequential Organ Failure Assessment (SOFA) and Acute Physiology And Chronic Health Evaluation II (APACHE II) scores are the most widely applied prognostic markers, owing primarily to their simplicity, accuracy, and integrity of use in clinical practice (2,7). However, due to variations in aetiology, intervention, and patient characteristics, a more suitable marker for sepsis and septic shock must be established and evaluated. The present study retrospectively investigated Ang-2 mRNA levels in a cohort of patients with septic shock. Typically, this context of septic shock consists of surgical and nonsurgical diseases. We hypothesized that Ang-2 mRNA levels are higher in patients with septic shock than in healthy controls and that Ang-2 mRNA levels will decrease over time if patients receive standard and effective treatment. Accordingly, we hypothesized that Ang-2 mRNA levels and illness severity scores are higher in non-survivors than survivors. For Ang-2 to be used in daily practice, equivalent comparisons with standard currently used predictive clinical scoring systems are needed. Thus, another objective of this study was to validate the predictive value of Ang-2 mRNA levels compared with SOFA and APACHE II scores. Additionally, we hypothesized that Ang-2 is more accurate than illness severity scores in mortality outcome prediction.

Patients and methods

Patients and data collection. In this retrospective study, clinical data of patients admitted to the intensive care unit (ICU) with a diagnosis of septic shock were collected between January 2020 and October 2020 at Tongji Hospital (Wuhan, Hubei, China). Patients underwent laboratory tests, including but not limited to, routine blood tests, biochemical items (including liver and renal function tests), coagulation factors and inflammation indicators such as C-reactive protein (CRP) and procalcitonin (PCT). To measure Ang-2 mRNA levels, citrated whole blood samples were collected upon ICU admission at baseline (day 0), as well as on days 3, 5 and 7 for those remaining in the ICU at those times. Plasma samples were stored at -80˚C prior to analysis. The baseline clinical characteristics of the patients are provided in Table I.

Diagnosis and treatment. Septic shock was diagnosed according to the consensus of Sepsis-3 published in February 2016 (1). Clinical criteria for septic shock can be identified by a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mmHg and having a serum lactate level >2 mmol/l despite adequate volume resuscitation (2). The SOFA score was calculated based on available clinical information and measured respiratory, hematological, hepatic, cardiovascular, central nervous system and renal function in order to describe clinical status. The APACHE II system uses a point score based upon initial values of 12 routine physiological measurements, age and previous health status to provide a general measure of the severity of disease. A higher SOFA or APACHE II score is indicative of a worse prognosis (Table I). All patients received standard medical or surgical treatment, if needed. The standard medications included but were not limited to antibiotics, vasopressors to maintain MAP ≥65 mmHg, respiratory support including mechanical ventilation, and continuous renal replacement therapy (CRRT) if indicated. The patients underwent laboratory tests, including but not limited to, routine blood tests, biochemical items, coagulation factors and inflammation indicators such as C-reactive protein (CRP) and procalcitonin (PCT).

Patients with advanced cancer, serious cardiovascular disease, trauma with haemorrhagic shock and other severe illnesses with a high risk of short-term mortality were excluded from the study.

RNA isolation and cDNA synthesis. Cell-free RNA (cf.-RNA) was isolated from 2 ml blood samples using a commercially available RNA simple kit (Tiangen) according to the manufacturer's instructions. The amount of total RNA was quantified by the absorbance at 260 nm using an N-5000 spectrophotometer (Yoke Technologies). RNA integrity was analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

Complementary DNA (cDNA) was synthesized from cf.-RNA with mRNA-specific stem-loop reverse transcription (RT) primers by the First-Strand cDNA Synthesis Kit (Tiangen) using the oligo(dT) primers included in the kit according to the manufacturer's protocol. A 20-μl reaction mixture for each sample was prepared containing 5 μl $MgCl_2$, 2 μl 10X PCR buffer, 2 μl dNTPs, 2 μl oligo-deoxythymidine primer, 5 μl RNase-free dH_2O, 2 μl ribonuclease inhibitor, 1 μl of Moloney murine leukaemia virus reverse transcriptase and 1 μg of total RNA diluted in 1 μl and placed into a 0.2 ml thin-wall PCR tube. The reverse transcription (RT) reaction was performed at 42˚C for 50 min and inactivated by heating to 90˚C for 10 min. The first-strand cDNA was cooled on ice and then stored at 0˚C until PCR was performed.

Reverse transcription-quantitative PCR (RT-qPCR). qPCR was performed on an ABI Prism® 7000 PCR instrument (Applied Biosystems China Inc.) using program parameters provided by the manufacturer. GAPDH was used as an internal control for normalization. A total of 100 μl of PCR mixture was prepared containing 0.2 mmol/l dNTPs and 2.5 U *Taq*-polymerase (Tiangen) with the corresponding paired primers at a concentration of 0.2 μmol/l of each primer. The following primers for RT-PCR were designed using Primer Express software (version 5.0; PREMIER Biosoft International): Ang-2-Fwd, CAGATTTTGGACCAGACCAGTG; Ang-2-Rev, ACTGTA TGTTGGATGATGTGCTTG; GAPDH-Fwd, GAAGGTGAA GGTCGGAGTC; and GAPDH-Rev, GAAGATGGTGAT GGGATTTC. The PCR program was performed according to the manufacturer's instructions: denaturation (95˚C for 10 min) followed by amplification (35 cycles at 95˚C for 60 sec, 60˚C for 45 sec and 72˚C for 30 sec). The ΔΔcq value and relative quantity (RQ) were calculated by SDS 1.4 version software (Applied Biosystems; Thermo Fisher Scientific, Inc.), $RQ=2^{-\Delta\Delta cq}$ (8). Each sample was tested in triplicate, and the differences in RNA concentration were normalized in each sample by quantitation of the transcript of the housekeeping gene GAPDH. All Ang-2 concentrations are expressed relative to GAPDH.

Statistical analysis. All statistical analyses were performed using GraphPad Prism (version 7.0 for Windows, GraphPad Software). Quantitative data are expressed as mean ± standard deviation (SD). Mann-Whitney U test was used to compare the medians between survivors and non-survivors. One-way ANOVA was used to assess the differences in Ang-2 levels between baseline (Day 0), and Day 3, Day 5 and Day 7. The Tukey-Kramer post hoc test was performed for pairwise comparison between groups. The t-tests were applied to compare the means between healthy controls and patients and the means between survivors and non-survivors. A χ^2 test or Fisher's exact test was performed for comparison of qualitative data. Receiver operating characteristic (ROC) procedures were used to predict mortality outcomes, and the areas under the ROC curves (AUCs) were calculated. The DeLong method was used to test the statistical significance of the difference between ROC curves (Ang-2, SOFA score and APACHE II score). All analyses were 2-tailed, with P-values <0.05 considered to indicate a statistically significant difference.

Results

Clinical features of the septic shock patients. Clinical data of 85 patients with septic shock and 10 healthy controls were collected for the study. Differences in sex and age were not significant between the patients and the control group. The serum lactate and prothrombin time (PT) were significantly

Table I. Main clinical characteristics of the patients with sepsis.

Parameters	Healthy controls (n=10)	All patients (n=85)	Survivors (n=65)	Non-survivors (n=20)	P-value
Mean age (range) (years)[a]	53 (30-68)	55 (30-80)	55 (30-80)	57 (33-69)	0.820
Sex (male/female)[a]	7/3	55/30	40/25	15/5	0.300
Ang II level (ratio)[b]	1±0.2	10.5±3.7	7.8±2.9	12.6±2.7	<0.001
PCT (ng/ml)[b]	0.3±0.1	16.39±13.1	10.92±8.13	21.86±15.95	0.007
CRP (ng/ml)[b]	5±2.1	135.7±59.58	139.6±63.69	127.85±44.71	0.360
Albumin (g/l)[b]	43.3±3.2	26.3±3.7	26.3±3.3	24.1±3.9	0.030
Lactate (mmol/l)[b]	0.8±0.5	4.8±2.5	3.4±1.6	6.1±2.9	<0.001
PT (sec)[b]	12±1.1	13.5±6.2	14.3±3.0	20.5±5.6	<0.001
Fibrinogen (g/l)[b]	2.8±1.5	1.6±0.8	2.0±0.7	1.1±0.7	<0.001
SOFA score[b]	-	8.6±3.7	6.7±2.4	11.0±2.9	<0.001
APACHE II score[b]	-	16.3±7.4	12.1±4.5	22.2±6.5	<0.001
Length of ICU stay (mean ± SD, days)[b]	-	14.6±8.6	11.2±6.8	17.3±10.8	0.025

[a]Data are expressed as median (range) or number; [b]Data are expressed as mean ± SD. PCT procalcitonin; CRP C-reactive protein; PT, prothrombin time; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology And Chronic Health Evaluation II; Ang II, Angiopoietin II. P-value is calculated between survivors and non-survivors (significant for P<0.05).

Table II. Infection localization of septic shock.

Parameters	All patients (n=85)	Survivors, (n=65)	Non-survivors (n=20)	P-value
Surgical intervention	22 (25.9)	17 (26.2)	5 (22.7)	0.250
Infection location				
Pulmonary	26 (30.6)	18 (27.7)	8 (40)	0.410
Abdominal	20 (23.5)	15 (23.1)	3 (15)	0.540
Liver abscess	3 (3.5)	2 (3.1)	1 (5)	0.560
Biliary tract	3 (3.5)	2 (3.1)	1 (5)	0.560
Urinary tract	17 (20)	14 (21.5)	3 (15)	0.750
Trauma	8 (9.4)	7 (10.8)	1 (5)	0.670
Central nervous system	5 (5.9)	3 (4.6)	2 (10)	0.590
Undefined	3 (3.5)	2 (3.1)	1 (5)	0.560

Data are expressed as n (%). P-value is calculated between survivors and non-survivors (significant for P<0.05).

higher in the non-survivors (6.1±2.9 mmol/l vs. 3.4±1.6 mmol/l; and 20.5±5.6 sec vs. 14.3±3.0 sec, P<0.001, respectively). However, serum albumin and fibrinogen levels were significantly higher in the survivors (26.3±3.3 g/l vs. 24.1±3.9 g/l; and 2.0±0.7 g/l vs. 1.1±0.7 g/l, P=0.03 and P<0.001, respectively). Serum procalcitonin (PCT) and C-reactive protein (CRP) are generally accepted indicators of inflammation, but only PCT differed significantly between the survivors and the non-survivors (10.92±8.13 ng/ml vs. 21.86±15.95 ng/ml, P=0.007). The non-survivors had higher SOFA (11.0±2.9 vs. 6.7±2.4) and APACHE II (22.2±6.5 vs. 12.1±4.5) scores (P<0.001). Overall, the survivors had a shorter length of ICU stay (11.2±6.8 days vs. 17.3±10.8 days, P=0.025). Data are presented in Table I.

Both surgical and nonsurgical diseases can result in septic shock. However, no significant difference was found in regards to the mortality between the two groups (P=0.25). Among sequential sites of infection, the lung, abdomen and urinary tract were the most frequent sources of infection, with proportions of 30.6, 23.5 and 20%, respectively. There were no significant differences in mortality in each category (P=0.410, 0.540, and 0.750, respectively) (Table II), with P<0.05 indicating a significant difference.

Downward trend of serum Ang-2 levels over time. The mean Ang-2 mRNA levels in all patients with septic shock at baseline (Day 0), Day 3, Day 5, and Day 7 were compared to those in the

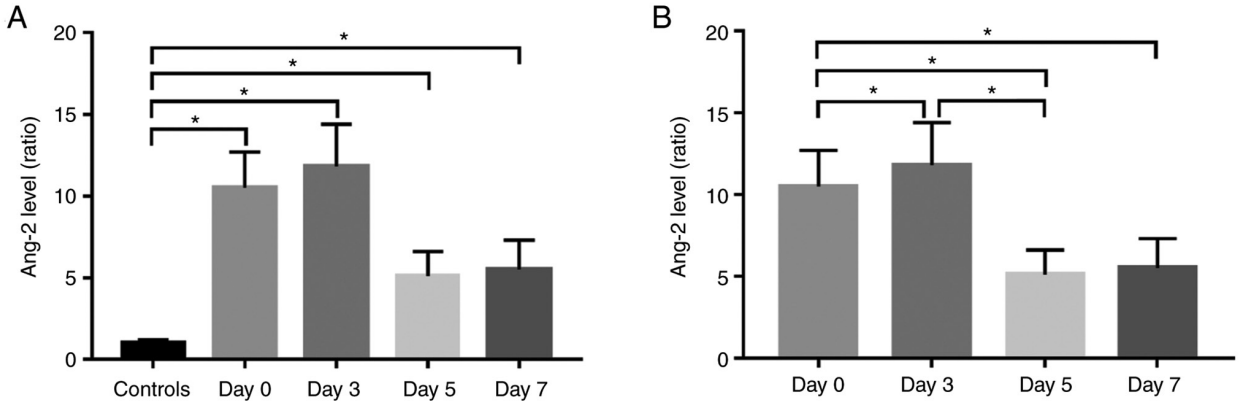

Figure 1. (A) Significant increase of Ang-2 levels in septic shock patients and (B) the downward trend over time. *P<0.001. Ang-2, angiopoietin 2.

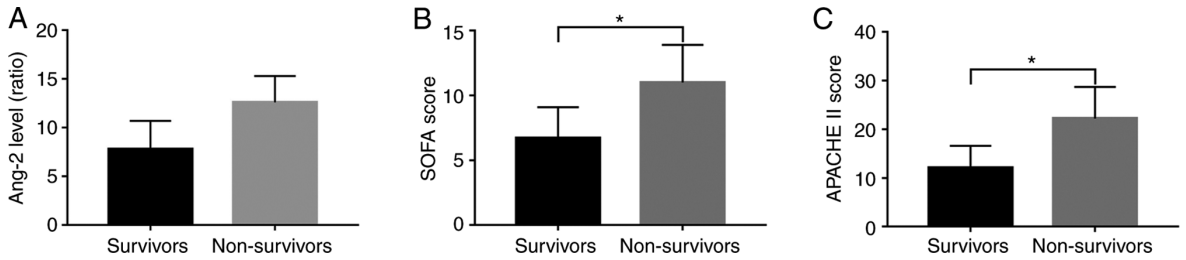

Figure 2. Septic shock is correlated with significantly higher (A) Ang-2 levels and (B) SOFA and (C) APACHE II scores in the non-survivors than in the survivors. *P<0.001. Ang-2, angiopoietin 2; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation.

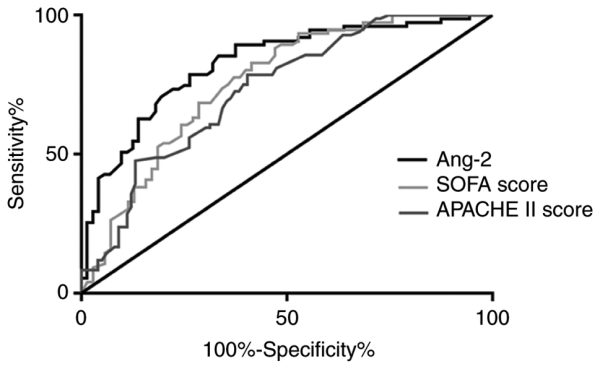

Figure 3. ROC curves of three predictors (Ang-2, SOFA and APACHE II scores) for mortality outcome. Ang-2, angiopoietin 2; SOFA, Sequential Organ Failure Assessment; APACHE, Acute Physiology and Chronic Health Evaluation.

normal controls. Mean Ang-2 levels in patients with septic shock at Day 0, Day 3, Day 5, and Day 7 were significantly higher than those in the controls (Fig. 1A, P<0.001). However, the Ang-2 mRNA level at Day 3 was significantly elevated compared to the baseline (Day 0) (P<0.001), and decreased rapidly at Day 5 and 7 (P<0.001). The Ang-2 mRNA levels were measured in healthy controls at 1.0±0.2 and in patients at 10.5±2.2 at Day 0, 11.8±2.6 at Day 3, 5.1±1.5 at Day 5, and 5.5±1.8 at Day 7. Generally, the serum Ang-2 levels showed a significant downward trend over time after standard treatment (Fig. 1B, P<0.001).

Ang-2 level, SOFA and APACHE II scores indicate illness severity and prognosis. Of the 85 patients for which mortality data were reported, 65 survived and 20 died, giving a 28-day mortality rate of 23.57%. The mean Ang-2 mRNA levels at admission and baseline clinical measures of illness severity, including SOFA and APACHE II scores, were significantly lower in survivors than in non-survivors (Fig. 2A-C, P<0.001). The Ang-2 mRNA level in the survivors was 7.8±2.9 compared to 12.6±2.7 in the non-survivors. SOFA score was 6.7±2.4 in the survivors compared to 11.0±2.9 in the non-survivors. The APACHE II score was 12.1±4.5 in the survivors compared to 22.2±6.5 in the non-survivors (Table I, P<0.001).

Ang-2 level predicts the outcome of septic shock. Fig. 3 shows the ROC curves for predicting the 28-day mortality based on baseline parameters. The different parameters (AUC, SEM, and P-values) for Ang-2 (0.82, 0.03, P<0.01), for SOFA score (0.76, 0.04, P<0.01), and APACHE II score (0.73, 0.04, P<0.01) were calculated. This indicates that the Ang-2 level at admission to the ICU may be more accurate than current clinical illness scores at predicting 28-day mortality in patients with septic shock.

Discussion

In the critical setting, the upregulation of angiopoietin 2 (Ang-2) is a physiologic and potentially lifesaving response and is significantly associated with the severity of disease. Ang-2 has been proven to increase in the context of sepsis (6,9,10). Ang-2 mRNA is directly or indirectly induced by infection across a broad spectrum of diseases (9,11). Several features of endothelial function are significantly correlated with the severity or outcome of sepsis, such as endothelial-originated

anticoagulants, represented by protein C; indicators of endothelial injury, represented by vWF; and regulators of vascular function and permeability, represented by Ang-2 (12,13). In the present study, elevated Ang-2 expression was confirmed by measuring the serum Ang-2 mRNA level in the patients with septic shock and by correlation with survival outcome. Both surgical and nonsurgical patients were enrolled, and patients presenting with multiple infection sites (lung, abdomen and urinary tract) were included. As determined by the present study, a downward trend of Ang-2 mRNA levels over time after intensive treatment indicated an improvement in circulation deterioration. Through continuous intensive and effective treatment in the ICU, the majority of patients improved and were discharged from the ICU. However, longer stays in the ICU indicated a more severe illness, which influenced the serum Ang-2 mRNA level on Day 7 compared with Day 5.

Sepsis is defined as a life-threatening multiple organ dysfunction caused by a dysregulated host response to infection (14). A National Heart, Lung and Blood Institute report also proposed the unifying concept of sepsis as a severe endothelial dysfunction syndrome that causes multiple organ failure in response to intravascular or extravascular microbial agents, which calls for a better understanding of endothelial function (15). Accumulating evidence suggests that the breakdown of endothelial barrier function plays a central role in the pathogenesis of sepsis (14,16,17). Although microcirculatory dysfunction may arise to various degrees in most clinical conditions, automatic regulatory mechanisms of microvascular function are the most severely impaired during sepsis, suggesting that microcirculatory dysfunction is a predominant pathophysiological manifestation of sepsis syndrome (18). Systemic and localized infections can induce disruptions of the integrity of the microcirculation in multiple organs. The severity of the infection is due to an activation cascade that leads to auto-amplification of cytokine production: the cytokine storm (16,19). Septic shock can lead to ischemic and inflammatory injuries to the lungs, kidney, heart, brain, and other organs and is a barrier to advances in the medical and surgical management of multiple diseases.

Genetic and molecular studies spanning thousands of human subjects have linked imbalances of Ang-2 levels to major adverse clinical events arising from bacterial sepsis and other severe infections (9,11). Ang-2 acts on endothelial cells, increasing endothelial permeability (20). Under normal physiological conditions, Ang-2 levels are low but upregulated during inflammation or cancer. Upon Ang-2 binding, Tie2 heterodimerizes with Tie1, and they can both form complexes with integrins. Ang-2 can also bind to integrins independently of Tie2, inducing endothelial cell migration and sprout formation (21,22). In addition, Ang-2 can induce a rapid loss of the endothelial glycocalyx in human sepsis. Inhibition of Ang-2 or Tie2 activation completely abolished endothelial glycocalyx damage (23). These studies on the regulation of the vascular endothelium have raised optimism concerning the development of methods to affect the progression of shock and multiorgan dysfunction.

In recent years, increasing empirical evidence has shown that extensive cross-talk between inflammation and coagulation systems plays a pivotal role in the pathogenesis of microvascular failure and subsequent multiple organ failure as a result of severe infection (24,25). For clinical operationalization, patients with septic shock can be identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and serum lactate level greater than 2 mmol/l (>18 mg/dl) in the absence of hypovolemia. This combination is associated with hospital mortality rates greater than 40% (19). The high morbidity and mortality of sepsis are the consequences of multiple organ dysfunction/failure, including acute respiratory distress syndrome (ARDS) or disseminated intravascular coagulation (DIC), which are due primarily to septic injury and dysfunction of the microvasculature, especially microvascular endothelial cells. Septic microvascular dysfunction of both pulmonary and systemic vascular beds is of clinical importance, as it occurs early in the course of sepsis (26,27).

The key pathophysiology underlying this life-threatening disease is the preponderant inflammatory host response to pathogen infections leading to the overexpression of inflammatory mediators. Sepsis-induced acute lung injury (ALI) is characterized by injury and dysfunction of the pulmonary microvasculature and pulmonary microvascular endothelial cells (28). Septic pulmonary microvascular dysfunction is primarily induced by the effects of septic inflammation on pulmonary microvascular endothelial cells (12,27). Sepsis-associated coagulopathy is also a result of the inflammation-induced activation of coagulation pathways. Although large clinical trials of sepsis-related anticoagulant therapies failed to show survival benefits, post hoc analysis of databases showed beneficial effects of anticoagulants in subgroups of patients with early sepsis-induced disseminated intravascular coagulation (29). As stated above, the pathophysiological changes in sepsis are entirely mediated by microvascular dysfunction.

As septic shock can occur across a spectrum of severities, it is of great importance to quantify disease severity in any study population. Our data showed that among the 85 septic patients involved in surgical and nonsurgical diseases, the lung, abdomen and urinary tract were the most frequent sites of infection, with proportions of 30.6, 23.5 and 20%, respectively. However, there were no significant differences in the mortality rate among the infection sites. Surgical intervention had no impact on mortality. Sequential Organ Failure Assessment (SOFA) scores were calculated daily in the ICU and are recommended as clinical criteria, combining the axes of the respiratory, hematologic, hepatic, cardiovascular, and central nervous systems, and renal function, to describe the clinical status. Organ dysfunction can be indicated by an increase in the SOFA score of 2 points or more, associated with an in-hospital mortality greater than 10% (30). The Acute Physiology and Chronic Health Evaluation (APACHE II) scoring system consists of three parts: the acute physiology score, age, and chronic health evaluation, and it is the most widely applied predictive model in the ICU. Likewise, the APACHE II score can be used as an indicator of sepsis severity. A higher APACHE II score is indicative of a worse prognosis. The APACHE II and SOFA scores incorporate similar but not identical criteria with which to identify septic shock. In the present study, the non-survivors were found to have higher SOFA and APACHE II scores ($P<0.001$).

In a prospective study, Ang-2 expression was found to be significantly upregulated in sepsis-associated coagulopathy, and this biomarker was used to stratify patients with sepsis into non-overt DIC and overt DIC (6). Studies have also shown

that Ang-2 is a promising biomarker with which to evaluate the gastrointestinal status and function in acute pancreatitis and it could serve as a useful tool for clinicians evaluating disease severity and prognosis (31,32). The results of the present study are consistent with the previous literature. The ROC curves for predicting 28-day mortality based on the baseline parameters of Ang-2, SOFA and APACHE II scores were calculated in the present study. Higher mortality was associated with higher Ang-2 mRNA levels as well as SOFA and APACHE II scores. Consequently, the Ang-2 mRNA level was found to be a valuable disease severity indicator and a predictive biomarker for mortality outcomes.

However, the inflammatory indicator of C-reactive protein (CRP) did not differ between the survivors and the non-survivors in this study. A possible explanation for this phenomenon is that prolonged septic shock leads to severe endothelial dysfunction and extensive leukocyte insufficiency as well as consumption of inflammatory factors. In general, the indeterminacy of clinical applications of synthetic human Ang-2 requires more clinical evidence.

In summary, the present study confirmed that the serum Ang-2 mRNA level is a prognostic indicator that is superior to SOFA and APACHE II scores currently used in the ICU. Since sepsis is not a specific illness but rather a syndrome encompassing a still uncertain pathology, additional studies to explore therapeutic interventions in regards to serum angiopoietin 2 are of great importance for the clinical management of septic shock.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

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

Authors' contributions

JF and LW designed the study and reviewed the manuscript, and share first authorship. YF and GY performed the laboratory examinations, acquired the majority of the data and drafted the manuscript. DZ and JW confirmed the authenticity of all the raw data, analyzed the data and revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Data collection and analysis were undertaken in compliance with the approval and supervision of the Tongji Hospital Research Institutional Review Board (approval no. TJ-IRB20211252; Wuhan, China). The local ethics committee classified this study as a retrospective study based on data analysis, and patient consent was not required.

Patient consent for publication

Patient consent for publication was not necessary because this retrospective study did not contain any personal information that could lead to the identification of the patient.

Competing interests

The authors declare that they have no competing interests.

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