Int J Clin Exp Pathol 2016;9(8):8122-8131
www.ijcep.com /ISSN:1936-2625/IJCEP0026170

*Original Article*

# Elevated serum levels of trefoil factor 3 are correlated with severity of sepsis patients

Wei Sun, Jie Qin, Ren-Fei Shan, Yan-An Zhu, Hai-Yong Zhu

*Department of Emergency Medicine, Zhejiang Taizhou Hospital, Linhai, Zhejiang, P. R. China*

Received February 17, 2016; Accepted May 20, 2016; Epub August 1, 2016; Published August 15, 2016

**Abstract:** Trefoil factor 3 (TFF3) is a secreted protein that plays important roles in mucosal protection, gastrointestinal inflammation and cell migration. Furthermore, aberrant expression of TFF3 was reported from patients with gastrointestinal inflammation and solid tumors. We therefore analyzed TFF3 serum concentrations in sepsis patients. A total of 186 sepsis patients (82 with uncomplicated sepsis, 104 with septic shock) were studied retrospectively at intensive care unit (ICU) of our hospital, and 180 healthy controls were recruited for comparison. For each patient, clinical data and laboratory parameters were assessed. Enzyme-linked immunosorbent assay (ELISA) was performed to examine the serum TFF3 concentrations. Patients were followed for approximately 1 year. Our results showed that serum TFF3 levels were significantly elevated at admission to the ICU and after 3 days of treatment in sepsis patients compared to healthy controls. In sepsis patients, TFF3 concentrations were significantly elevated in septic shock compared to uncomplicated sepsis. A positive correlation was observed between the serum TFF3 concentrations and sepsis severity, such as multi-organ failure scores and inflammatory mediators. In all sepsis patients, serum TFF3 levels increased from admission to day 3 of ICU treatment. Furthermore, elevated TFF3 levels at day 3 of ICU were a strong indicator for an unfavorable prognosis. In conclusions, elevated TFF3 serum concentrations are associated with an unfavorable outcome in sepsis patients, and this indicates TFF3 might be a potential prognostic biomarker in early sepsis of ICU treatment.

**Keywords:** Trefoil factor 3 (TFF3), sepsis, septic shock, systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), biomarker

## Introduction

Sepsis and septic shock are major causes of death in patients referred to intensive care units [1] and account for over 25% mortality of in-patients [2]. Sepsis is a systemic inflammatory response syndrome (SIRS) caused by infection [3]. Septic shock is severe type of sepsis with refractory hypotension and multiple organ dysfunction syndrome (MODS), often leading to high mortality rate [4]. The pathophysiology of sepsis is characterized by activation of both pro-inflammatory and anti-inflammatory pathways [5]. These two pathways involve the expression and secretion of a variety of pro-inflammatory and anti-inflammatory cytokines from different immune and parenchymal cells [6]. The immunologic imbalance between pro-inflammatory and anti-inflammatory responses promotes sepsis-associated organ dysfunction and shock, thereby contributing poor outcome [7]. Therefore, it is highly demanded for new biomarkers in the early phase of sepsis that can help to predict prognosis of patients and assist clinical decision-making [8].

The human trefoil factor (TFF) family is small secreted proteins that are mainly expressed in the epithelial cells of the gastrointestinal tract [9]. TFF consists of three small peptide members. TFF3, also known as intestinal trefoil factor (ITF), is mainly expressed in the goblet cells of the intestine as well as the breast, salivary gland, respiratory tract and hypothalamus [10]. TFF3 play important roles in biological functions and pathological processes, such as wound healing [11], mucosal protection [12], inflammation of the gastrointestinal tract [13] and a variety of solid tumors [14]. TFF3 is a secreted protein and serum TFF3 might act as a biomarker for malignancies and ulcerative colitis [15, 16].

Despite the regulatory roles of TFF3 in inflammation and immunity, its functional involvement in sepsis remains to be elucidated. Moreover, TFF3 could be induced during inflammatory joint disease and acts as a link between inflammation and tissue remodeling processes [17]. TFF3 also demonstrates anti-inflammatory effect on experimental necrotizing enterocolitis [18]. Thus TFF3 seems to have both pro-inflammatory and anti-inflammatory effects, which may depend on different context of pathophysiology. Furthermore, the diagnostic and prognostic value of TFF3 in sepsis patients is currently unclear. Therefore, this study we conducted a retrospective investigation on sepsis patients at a medical ICU and measured serum TFF3 concentrations on admission to and 3 day after ICU treatment. The study aimed to address the regulation and diagnostic value of serum TFF3 concentrations in sepsis and septic shock. Finally, we also explored whether serum TFF3 levels can serve as a prognostic factor for survival of sepsis patients.

## Subjects and methods

### *Subjects*

A total of 186 consecutive patients (119 male, 67 female; median age 59 years, range 26-82 years) were included in this study between July 2013 and October 2015 in the general Internal Medicine ICU of Zhejiang Taizhou Hospital. The medium length of stay at the ICU was 8 days (range 1-34 days). Patient clinical data and laboratory parameters were collected. The patients were observed and followed until death or after hospital discharge by directly contacting the patients or their relatives. The sepsis was diagnosed based on the criteria proposed by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference Committee for sepsis [2]. Septic shock was defined as severe sepsis complicated with refractory arterial hypotension (SBP<90 mmHg, or MAP<65 mmHg) which need fluid replacement and vasopressors. A total of 180 healthy blood donors were collected as control subjects with matched age and sex ratios. The study protocol was approved by the ethics committee of Zhejiang Taizhou Hospital (Linhai, Zhejiang Province, China) and conducted in accordance with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from the patient, his or her relatives.

### *Determination of clinical data and laboratory parameters*

APACHE-II (Acute Physiology and Chronic Health Evaluation II) score was used to evaluation sepsis severity [19]. SOFA (Sequential Organ Failure Assessment) score was used to evaluate extent of organ dysfunction [20]. Serum was obtained at admission to the ICU before therapeutic intervention and 3 days after ICU treatment. All samples were immediately placed on ice, followed by centrifugation at 1000 g for 10 min, and then serum samples were stored at -80°C. C-reactive protein (CRP), Interleukin 6 (IL-6), Tumor necrosis factor-α (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA). Mortality was defined as death on ICU or after discharge from ICU and hospital.

### *Determination of TFF3 serum concentrations by ELISA*

Venous blood was collected from sepsis patients at admission to the ICU and 3 days after ICU treatment, or from healthy controls. Serum was acquired by centrifugation was performed at 1000 g for 10 min, and was stored in aliquots at -70°C until use. The serum TFF3 was measured by Human TFF3 ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's protocol. The absorbance at OD450 wavelength was measured by an ELISA plate reader (Ricso RK201, Shenzhen Ricso Technology Co., Ltd, Shenzhen, Guangdong, China). The serum concentrations TFF3 were determined by the standard curves constructed from recombinant human TFF3 (Range: 0~100 ng/mL).

### *Statistical analysis*

The statistical analysis was performed by the commercially available software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). In the table, data are displayed as median and range. Differences between two groups were determined by an independent t-test in comparing quantitative data, or by Chi-squared test or a Fisher's exact test in comparing categorical data. Multiple comparisons between more than two groups were conducted by Kruskal-Wallis ANOVA and

**Table 1.** Baseline patient characteristics

| Parameter | All patients | Uncomplicated sepsis | Septic shock |
|---|---|---|---|
| Number | 186 | 82 | 104 |
| Age (years) | 59.0 (26-82) | 61.0 (26-82) | 57.0 (26-82) |
| Gender (male/female) | 119/67 | 44/28 | 75/29 |
| APACHE II score | 21.0 (12-33) | 19.0 (12-29) | 25.0 (15-33)* |
| SOFA score | 12.0 (6-18) | 9.0 (6-16) | 13.0 (6-18)* |
| Scr (μmol/L) | 75.1 (45.4-322.6) | 57.1 (45.4-146.8) | 119.1 (46.1-322.6)* |
| WBC ($10^3$/μL) | 14.5 (5.7-36.7) | 13.3 (6.6-22.1) | 15.3 (5.7-36.7)* |
| CRP (mg/dL) | 185.2 (67.3-278.7) | 174.1 (67.3-259.4) | 197.7 (93.8-278.7)* |
| IL-6 (pg/mL) | 397.3 (126.7-1213.2) | 193.2 (127.7-728.7) | 595.7 (212.2-1213.2)* |
| TNF-α (pg/mL) | 35.9 (21.7-105.7) | 32.5 (21.7-73.2) | 42.6 (25.2-105.7)* |
| ICU days | 8.0 (1-34) | 6.5 (1-27) | 19.0 (5-59)* |
| Death during ICU or follow-up (%) | 81 (43.5%) | 27 (32.9%) | 54 (51.9%)* |
| TFF3 at admission (ng/mL) | 16.88 (11.3-23.0) | 15.8 (11.3-22.8) | 17.7 (11.5-23.0)* |
| TFF3 at day 3 (ng/mL) | 24.33 (14.3-38.0) | 21.2 (14.3-33.4) | 26.8 (15.1-38.0)* |

Apache, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; Scr, Serum creatinine; WBC, white blood cell count; CRP, C-reactive protein, IL-6, Interleukin 6; TNF-α, Tumour necrosis factor-α; ICU, intensive care unit. *Compared with the uncomplicated sepsis group, the difference is significant (P<0.05).

Mann-Whitney U test. Correlations between TFF3 and other variables were analyzed using the Spearman correlation tests. The impact of serum TFF3 level on survival of sepsis patients was analyzed by Kaplan-Meier curves and log-rank test calculations. A probability value of *P*<0.05 was considered as statistically significant difference.

## Results

### *Patient characteristics*

The clinical characteristics for each patient at the time of ICU admission are presented in **Table 1**. Among all 186 patients, there are 82 cases of uncomplicated sepsis and 104 cases of septic shock. These two groups are matched in age and gender, with no statistically significant differences in age and gender (P>0.05). As expected, patients with septic shock had greater APACHE II score, SOFA score and WBC, higher serum Scr, CRP, IL-6 and TNF-α levels, longer ICU stay time and higher mortality (P< 0.05).

### *TFF3 serum concentrations are elevated in sepsis patients*

Serum samples of sepsis patients were collected to analyze TFF3 concentrations at admission to the ICU (before therapeutic interventions) and after 3 days of treatment. We compared serum TFF3 levels at ICU admission between different subgroups of sepsis patients. Patients had significantly higher TFF3 serum concentrations as compared to healthy controls (P<0.05) (**Figure 1A**). Patients with high APACHE II scores (≥20) displayed increased serum TFF3 levels (median 17.71 ng/mL, range 11.64-22.96 ng/mL) compared to patients with low APACHE II scores (median 15.53 ng/mL, range 11.30-20.13 ng/mL; P<0.001; **Figure 1B**). Moreover, patients with high SOFA scores (≥10) displayed increased serum TFF3 levels (median 17.60 ng/mL, range 12.11-22.96 ng/mL) compared to patients with low SOFA scores (median 15.91 ng/mL, range 11.30-19.88 ng/mL; P<0.001; **Figure 1C**). We investigated the association between serum TFF3 and septic shock, and found that patients with septic shock displayed increased serum TFF3 levels (median 17.70 ng/mL, range 11.45-22.96 ng/mL) compared to patients with uncomplicated sepsis (median 15.82 ng/mL, range 11.30-22.76 ng/mL; P<0.001; **Figure 1C**; **Table 1**). This indicates that high serum levels of TFF3 are associated with the severity, multiple organ dysfunctions and prognosis of sepsis patients.

### *Kinetics of TFF3 serum concentrations during early ICU treatment*

We next analyzed the kinetics of TFF3 serum concentrations after 3 days of ICU treatment. TFF3 levels remained significant increase in day 3 compared with those at admission

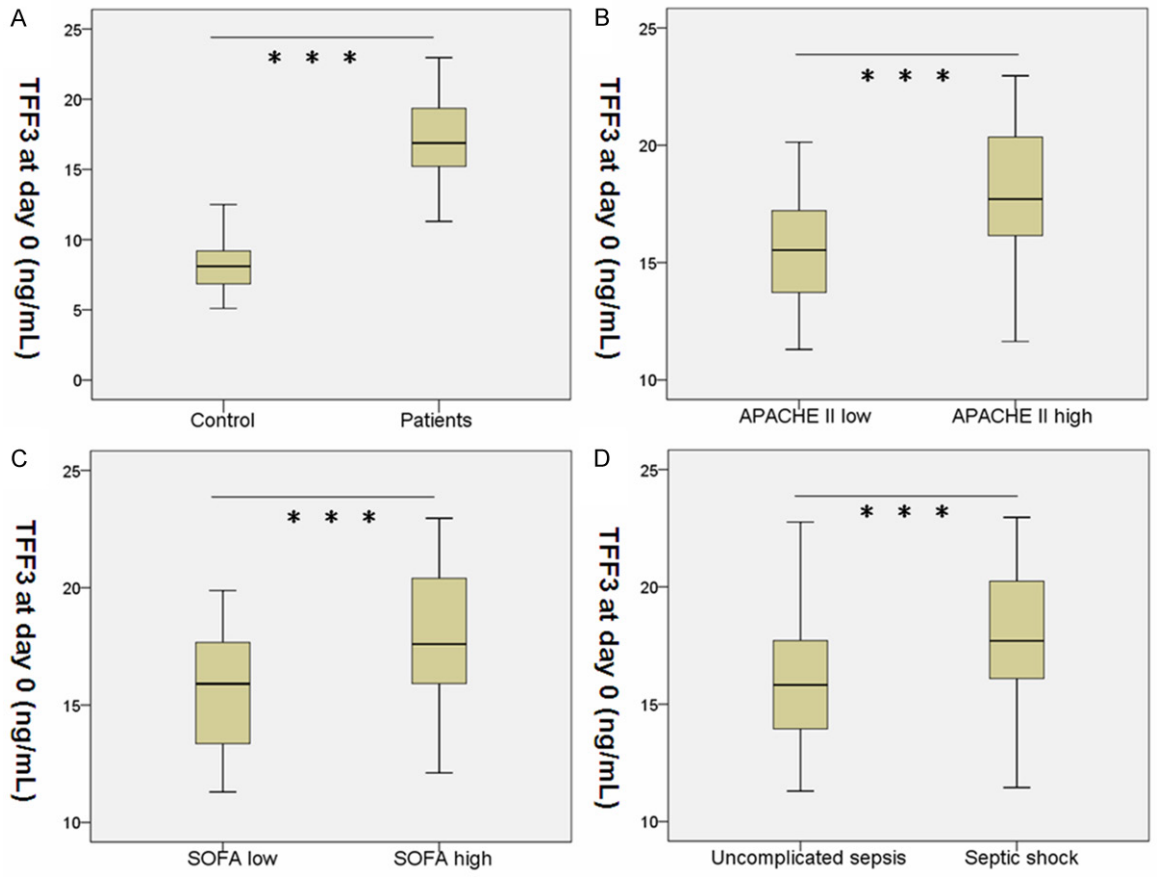


**Figure 1.** Serum TFF3 concentrations of sepsis patients at ICU admission. A. Serum TFF3 concentrations at admission to the ICU were measured by ELISA. Sepsis patients show significantly higher serum TFF3 as compared with healthy controls (P<0.05, t-test). B. Serum TFF3 levels at admission to the ICU are significantly elevated in sepsis patients with high initial APACHE II scores (≥20) compared to patients with low APACHE II scores (<20). C. Serum TFF3 concentrations at admission are significantly higher in patients with high SOFA scores (≥10) compared to patients with low SOFA scores (<10). D. Patients with septic shock displayed increase in TFF3 serum levels at admission compared to patients with uncomplicated sepsis. Box plot are displayed, where the bold line indicates the median per group, the box represents 50% of the values, and horizontal lines indicate minimum and maximum values. *P<0.05, **P<0.01, ***P<0.001.

(**Figure 2A**). We then compared TFF3 levels between different subgroups and found serum TFF3 was significantly increased in patients with higher APACHE II scores, higher SOFA scores and septic shock (**Figure 2B-D**).

*TFF3 levels after 3 days of ICU treatment are correlated to markers of organ function and inflammation*

To determine factors possibly contributing elevated serum TFF3 concentrations in sepsis patients, correlation analyses were performed with various clinical and laboratory parameters. These analyses showed that TFF3 levels after 3 days of ICU treatment were significantly associated sepsis severity. High serum TFF3 levels were closely correlated to APACHE II score and SOFA score (**Figure 3A**, **3B**), which are two important clinical indicators in the assessment of multiple organ dysfunction. High serum TFF3 levels were also correlated to decreased renal function, evidenced by elevated serum creatinine concentrations (**Figure 3C**). Furthermore, serum TFF3 concentrations were closely correlated to systemic inflammation in sepsis patients, such as WBC, CRP, IL-6 and TNF-α (**Figure 3D-G**). Consequently, we found a strong association of TFF2 serum concentrations with ICU stay time (**Figure 3H**).

*Elevated TFF3 are associated with overall survival of sepsis patients*

Based on the significant correlation of serum TFF3 with multiple organ dysfunction and infl-

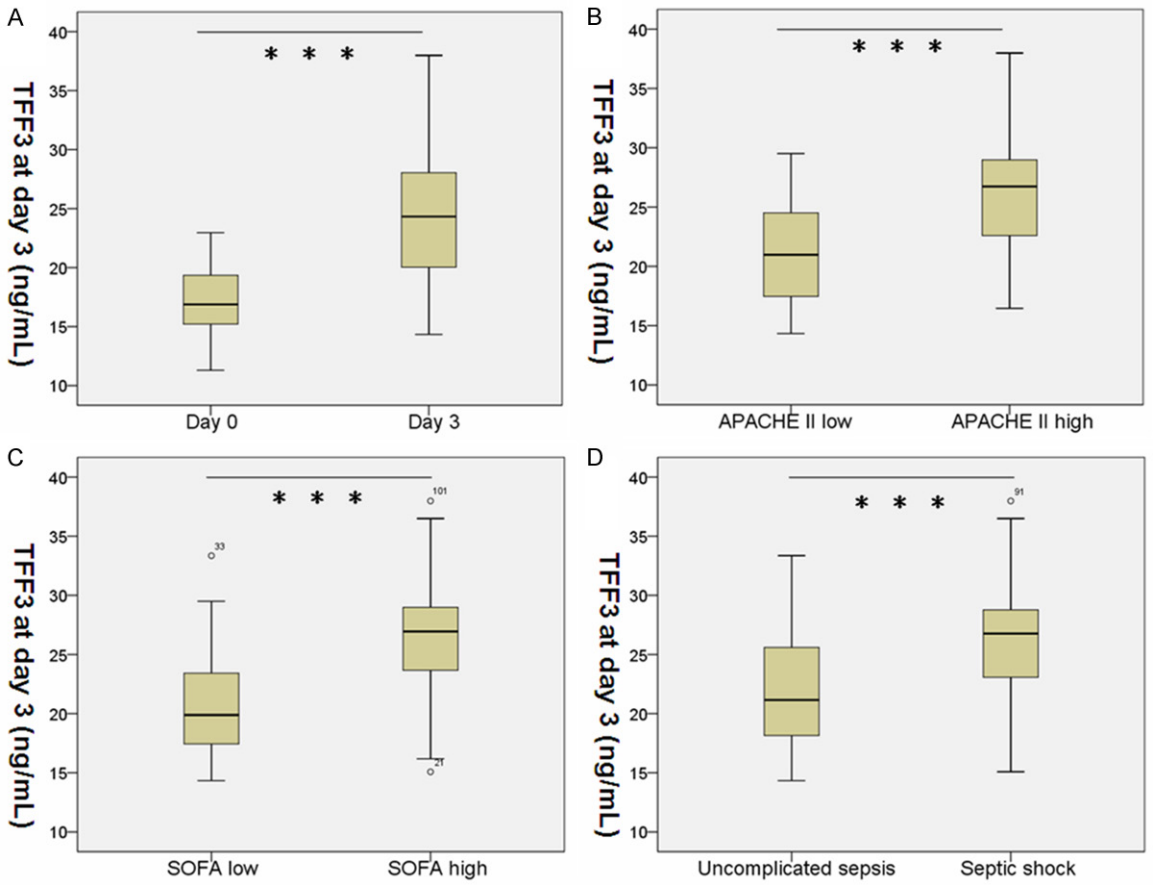


**Figure 2.** Serum TFF3 concentrations of sepsis patients after 3 days of ICU treatment. A. Serum TFF3 concentrations after 3 days of ICU were measured by ELISA. In all the sepsis patients, serum TFF3 levels after 3 days are significantly higher as compared to the values at ICU admission ($P<0.05$, t-test). B. Serum TFF3 levels after 3 days to the ICU are significantly elevated in sepsis patients with high APACHE II scores (≥20) compared to patients with low APACHE II scores (<20). C. Serum TFF3 concentrations after 3 days are significantly higher in patients with high SOFA scores (≥10) compared to patients with low SOFA scores (<10). D. TFF3 serum levels after 3 days are increased in patients with septic shock compared to patients with uncomplicated sepsis. Box plot are displayed, where the bold line indicates the median per group, the box represents 50% of the values, and the open circles indicate outlier values, and horizontal lines indicate minimum and maximum values. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

ammatory markers, we hypothesized that TFF3 might be a prognosis factor in sepsis patients. We therefore compared serum TFF3 concentrations at admission and after 3 days of ICU in survivors and non-survivors of sepsis patients. Serum TFF3 at admission was slightly higher in non-survivors than in survivors, but with no significant difference (**Figure 4A**). However, non-survivors displayed higher serum TFF3 concentrations at day 3 compared to survivors (**Figure 4B**). To further investigate whether serum TFF3 at day 3 is correlated with mortality of sepsis patients, we performed Kaplan-Meier survival analysis. We selected serum TFF3 24.33 ng/mL (median concentrations of TFF in sepsis patients) as cutoff, and patients with TFF3≥ 24.33 ng/mL were considered as high TFF3 group. Patients with TFF3 level ≥24.33 ng/mL demonstrated significantly poor survival and higher mortality compared to patients with TFF3 level <24.33 ng/mL (Log Rank = 6.704, $P<0.05$, **Figure 4C**).

## Discussion

In this study, we assessed serum TFF3 concentrations upon admission to the ICU and at day 3 after ICU in sepsis patients. Our results showed that serum TFF3 concentrations were much higher in the sepsis patients than in the healthy controls. Moreover, serum TFF3 levels were higher in septic shock compared to uncomplicated sepsis patients, which suggests that sepsis may promote TFF3 production and TFF3

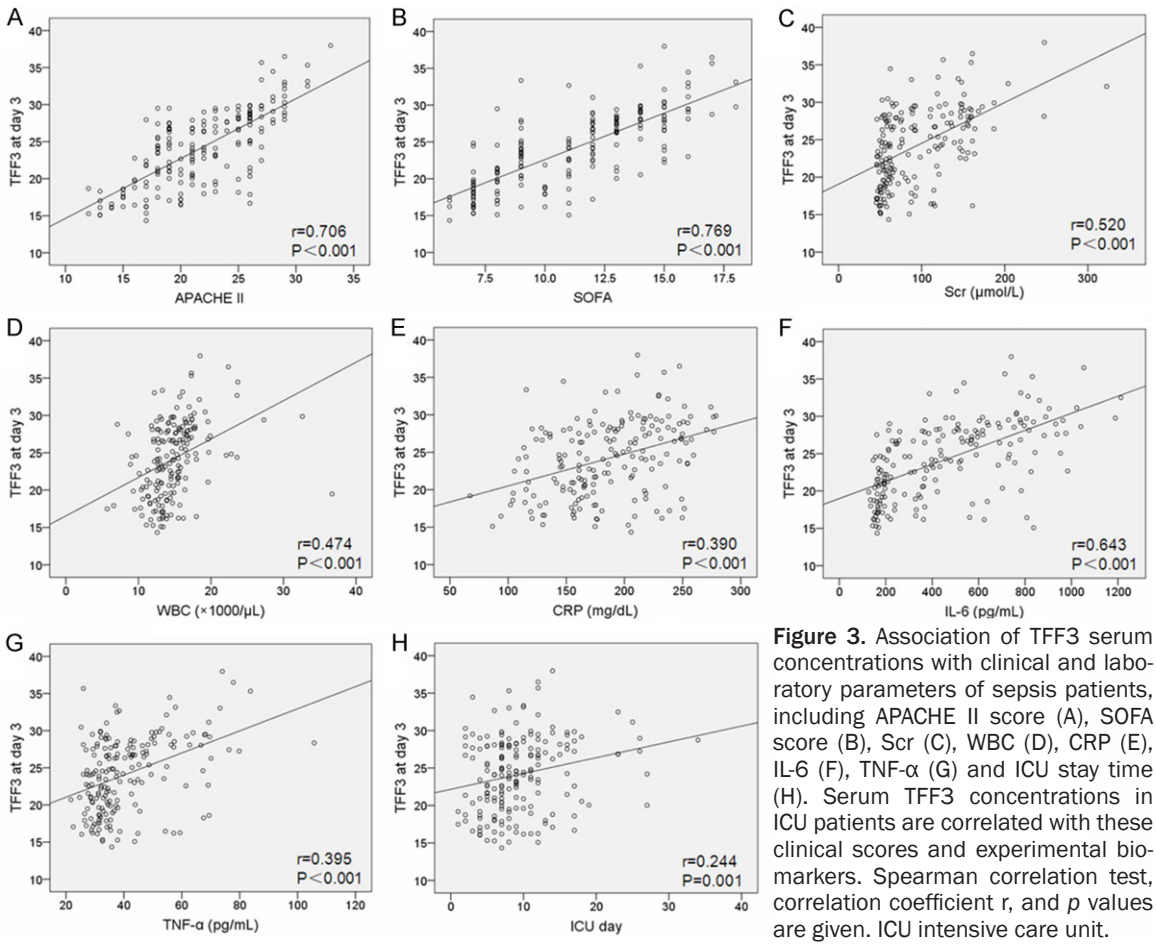


**Figure 3.** Association of TFF3 serum concentrations with clinical and laboratory parameters of sepsis patients, including APACHE II score (A), SOFA score (B), Scr (C), WBC (D), CRP (E), IL-6 (F), TNF-α (G) and ICU stay time (H). Serum TFF3 concentrations in ICU patients are correlated with these clinical scores and experimental biomarkers. Spearman correlation test, correlation coefficient r, and *p* values are given. ICU intensive care unit.

contribute the progression of sepsis to septic shock. Serum TFF3 concentrations increased from ICU admission to day 3 after ICU, and serum TFF3 at day 3 were found to closely correlated with the APACHE II score, SOFA score, WBC, CRP, IL-6 and TNF-α. This suggests that TFF3 may regulate organ dysfunction and inflammatory responses in the initiated and deteriorated phases of sepsis. Furthermore, non-survivors displayed higher serum TFF3 concentrations compared to survivors at day 3 but not at ICU admission, and elevated serum TFF3 were associated with long ICU stay time and higher mortality of sepsis patients. This suggests serum TFF3 might be a potential prognostic biomarker in early phase of sepsis.

It has proved that intestinal barrier dysfunction contributes to the development of uncontrolled SIRS [21], thereby playing an important role in the development of sepsis, MODS and septic shock [22]. Intestinal injury occurs in the early phase of sepsis [23], and this indicates it may act as a contributing factor to the progression of sepsis. Intestinal mucosa after severe sepsis showed high inflammation and declined goblet cell function, thus affecting the repair of damaged intestinal barrier [24]. TFF3 is produced by goblet cell, and could protect and enhance intestinal barrier function by activating PI3K/Akt pathway and stimulation of intestinal goblet cells [25, 26]. Our results showed higher serum TFF3 in sepsis patients compared with healthy controls, and there are association of serum TFF3 with sepsis severity and organ dysfunction. Moreover, serum TFF3 levels of sepsis patients increased from at ICU admission to 3 day after ICU treatment. It seems that TFF3 might act as a biomarker for sepsis severity rather than a contributing factor to deterioration of sepsis. Higher serum TFF3 levels indicate severe damage to multiple organs especially intestinal cells. In fact, in sepsis animals TFF3 expression was increased after onset of sepsis, with impaired immunological function of the intestinal mucosa [24, 27]. Higher TFF3

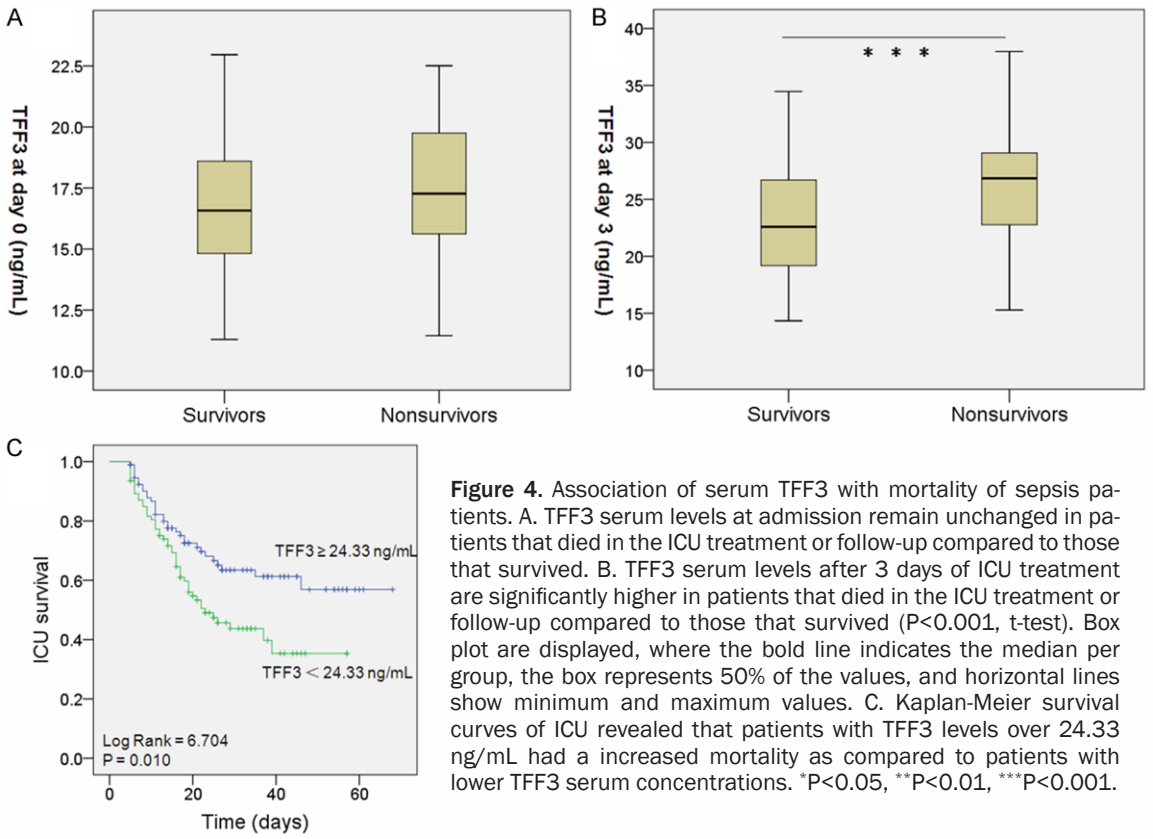


**Figure 4.** Association of serum TFF3 with mortality of sepsis patients. A. TFF3 serum levels at admission remain unchanged in patients that died in the ICU treatment or follow-up compared to those that survived. B. TFF3 serum levels after 3 days of ICU treatment are significantly higher in patients that died in the ICU treatment or follow-up compared to those that survived ($P<0.001$, t-test). Box plot are displayed, where the bold line indicates the median per group, the box represents 50% of the values, and horizontal lines show minimum and maximum values. C. Kaplan-Meier survival curves of ICU revealed that patients with TFF3 levels over 24.33 ng/mL had a increased mortality as compared to patients with lower TFF3 serum concentrations. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

may exert protective effect on intestinal mucosa, which is supported by reports that LPSE preconditioning increased mucosal expression of TFF3 and lessened intestinal mucosa injury by ischemia and reperfusion in a hemorrhagic shock rat model [28]. Other two member of TFF family, TFF1 and TFF2, were also found to be increased in sepsis pediatric patients and correlated with sepsis severity and MODS [29, 30]. Our study firstly reported that TFF3 is increased in sepsis patients.

Sepsis is characterized by profound imbalance of the immune system in response to infection and/or organ damage, which determine the prognosis of sepsis patients. Excessive inflammatory response, especially the adaptive immune response, was activated in the early phase of sepsis, but prolonged immunosuppressive state was occurred with the development of sepsis, with a high probability of septic shock and mortality [31, 32]. In our study, serum TFF3 concentrations of sepsis patients increased from ICU admission to day 3 after ICU, which is in accordance with report that after CLP the injury of intestinal immune function was progressively increased. In our study, serum TFF3 concentrations were correlated with pro-inflammatory cytokines, such as CRP, IL-6 and TNF-α. These cytokines can enhance intestinal tight junction permeability and damage the integrity of the intestinal barrier [33]. Furthermore, these pro-inflammatory cytokines decrease TFF3 expression and production in intestinal cells, and cause more severe intestinal injury, such as ischemia and hypoxia, ischemia-reperfusion injury, thereby leading to excessive release of many inflammatory cytokines [34, 35]. Then a positive feedback is formed between intestinal barrier dysfunction and inflammatory response, making MODS and septic shock inevitable outcome for sepsis. Therefore, biomarkers in the early phase of sepsis are important for rapid decision-making, so as to adopt early intervention and increase the prognosis of sepsis patients.

Currently, during the first week of ICU treatment for sepsis patients, rapid diagnostic and therapeutic management still remains a major challenge, and it is immensely important for the outcome of sepsis [36]. Therefore, the use of

novel biomarkers may significantly improve the treatment and prognosis of sepsis patients. Serum TFF3 is a good biomarker for gastrointestinal, breast, prostate cancers and ulcerative colitis [15, 16, 37, 38]. Our results showed higher serum TFF3 concentrations in non-survivors compared to survivors at day 3 but not at ICU admission, and higher serum TFF3 group demonstrated higher mortality. Therefore, serum TFF3 offers a novel prognostic biomarker of sepsis to guide treatment decisions at early phase of ICU treatment.

Our study has provided evidence that TFF3 could act as a diagnostic tool in the prognostic judgment of sepsis patients during the early phase of ICU treatment. A larger independent cohort investigation was needed to confirm this study. Moreover, molecular pathogenesis of TFF3 in sepsis remains unclear. TFF3 seems to have both pro-inflammatory and anti-inflammatory effects. Furthermore, IL-4 and IL-13 can up-regulate TFF3 expression in the mucus-producing HT-29 cells [39]. The IL-4 mRNA expression in PBMCs of sepsis patients was significantly higher in survivors than in non-survivors [40]. The serum IL-13 concentration 3 day after admission was significantly higher in the shocked group compared no shocked group [41]. Both IL-4 and IL-13 are anti-inflammatory cytokines, and their regulation with TFF3 in sepsis deserves further study.

In conclusion, our study identifies TFF3 as a biomarker in sepsis patients to assess disease severity and prognosis. TFF3 serum levels are elevated in sepsis patients compared to healthy controls both at admission and after 3 days of ICU treatment, elevated TFF3 levels at day 3 of ICU treatment demonstrate significant correlations with organ dysfunctions and inflammatory response and are a strong independent predictor for high mortality.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Jie Qin, Department of Emergency Medicine, Zhejiang Taizhou Hospital, 150 Ximen Street, Linhai 317000, Zhejiang, P. R. China. Tel: +086-13586151070; E-mail: qinjzj@163.com

## References

[1] Stevenson EK, Rubenstein AR, Radin GT, Wiener RS, Walkey AJ. Two decades of mortality trends among patients with severe sepsis: A comparative meta-analysis. Crit Care Med 2014; 42: 625-631.

[2] Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL; International Surviving Sepsis Campaign Guidelines Committee; American Association of Critical-Care Nurses; American College of Chest Physicians; American College of Emergency Physicians; Canadian Critical Care Society; European Society of Clinical Microbiology and Infectious Diseases; European Society of Intensive Care Medicine; European Respiratory Society; International Sepsis Forum; Japanese Association for Acute Medicine; Japanese Society of Intensive Care Medicine; Society of Critical Care Medicine; Society of Hospital Medicine; Surgical Infection Society; World Federation of Societies of Intensive and Critical Care Medicine. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008; 36: 296-327.

[3] Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. Am Coll Chest Phys 1992; 101: 1644-1655.

[4] Esper AM and Martin GS. Extending international sepsis epidemiology: the impact of organ dysfunction. Crit Care 2009; 13: 120.

[5] Angus DC and van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013; 369: 840-851.

[6] Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol 2013; 13: 862-874.

[7] Zhang H. Immune-imbalance in sepsis. Minerva Anestesiol 2000; 66: 435-437; discussion 438.

[8] Liu X, Ren H, Peng D. Sepsis biomarkers: an omics perspective. Front Med 2014; 8: 58-67.

[9] Wright NA, Poulsom R, Stamp G, Van Noorden S, Sarraf C, Elia G. Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease. Gastroenterology 1993; 104: 12-20.

[10] May FE and Westley BR. Trefoil proteins: their role in normal and malignant cells. J Pathol 1997; 183: 4-7.
[11] Jiang GX, Zhong XY, Cui YF, Liu W, Tai S, Wang ZD, Shi YG, Zhao SY, Li CL. IL-6/STAT3/TFF3 signaling regulates human biliary epithelial cell migration and wound healing in vitro. Mol Biol Rep 2010; 37: 3813-3818.
[12] Sun Z, Liu H, Yang Z, Shao D, Zhang W, Ren Y, Sun B, Lin J, Xu M, Nie S. Intestinal trefoil factor activates the PI3K/Akt signaling pathway to protect gastric mucosal epithelium from damage. Int J Oncol 2014; 45: 1123-1132.
[13] Ciacci C, Di Vizio D, Seth R, Insabato G, Mazzacca G, Podolsky DK, Mahida YR. Selective reduction of intestinal trefoil factor in untreated coeliac disease. Clin Exp Immunol 2002; 130: 526-531.
[14] May FE and Westley BR. Trefoil proteins: their role in normal and malignant cells. J Pathol 1997; 183: 4-7.
[15] Xiao L, Liu YP, Xiao CX, Ren JL, Guleng B. Serum TFF3 may be a pharamcodynamic marker of responses to chemotherapy in gastrointestinal cancers. BMC Clin Pathol 2014; 14: 26.
[16] Srivastava S, Kedia S, Kumar S, Pratap Mouli V, Dhingra R, Sachdev V, Tiwari V, Kurrey L, Pradhan R, Ahuja V. Serum human trefoil factor 3 is a biomarker for mucosal healing in ulcerative colitis patients with minimal disease activity. J Crohns Colitis 2015; 9: 575-579.
[17] Rösler S, Haase T, Claassen H, Schulze U, Schicht M, Riemann D, Brandt J, Wohlrab D, Müller-Hilke B, Goldring MB, Sel S, Varoga D, Garreis F, Paulsen FP. Trefoil factor 3 is induced during degenerative and inflammatory joint disease, activates matrix metalloproteinases, and enhances apoptosis of articular cartilage chondrocytes. Arthritis Rheum 2010; 62: 815-825.
[18] Shi L, Zhou PH, Xi JL, Yu HG, Zhang BH. Recombinant human trefoil factor 3 ameliorates bowel injury: its anti-inflammatory effect on experimental necrotizing enterocolitis. Int J Pept 2014; 2014: 634135.
[19] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985; 13: 818-829.
[20] Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med 1998; 26: 1793-1800.
[21] Hotchkiss RS and Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003; 348: 138-150.
[22] Chang JX, Chen S, Ma LP, Jiang LY, Chen JW, Chang RM, Wen LQ, Wu W, Jiang ZP, Huang ZT. Functional and morphological changes of the gut barrier during the restitution process after hemorrhagic shock. World J Gastroentero 2005; 11: 5485-5491.
[23] Derikx JP, Poeze M, van Bijnen AA, Buurman WA, Heineman E. Evidence for intestinal and liver epithelial cell injury in the early phase of sepsis. Shock 2007; 28: 544-548.
[24] Chang RM, Wen LQ, Chang JX, Fu YR, Jiang ZP, Chen S. Repair of damaged intestinal mucosa in a mouse model of sepsis. World J Emerg Med 2013; 4: 223-228.
[25] Lin N, Xu LF, Sun M. The protective effect of trefoil factor 3 on the intestinal tight junction barrier is mediated by toll-like receptor 2 via a PI3K/Akt dependent mechanism. Biochem Biophys Res Commun 2013; 440: 143-149.
[26] Bhatia S, Prabhu PN, Benefiel AC, Miller MJ, Chow J, Davis SR, Gaskins HR. Galacto-oligosaccharides may directly enhance intestinal barrier function through the modulation of goblet cells. Mol Nutr Food Res 2015; 59: 566-573.
[27] Jiang LY, Zhang M, Zhou TE, Yang ZF, Wen LQ, Chang JX. Changes of the immunological barrier of intestinal mucosa in rats with sepsis. World J Emerg Med 2010; 1: 138-143.
[28] Chang R, Wang Y, Chang J, Wen L, Jiang Z, Yang T, Yu K. LPS preconditioning ameliorates intestinal injury in a rat model of hemorrhagic shock. Inflamm Res 2014; 63: 675-682.
[29] Žurek J and Fedora M. Trefoil factor 1 as a marker of mucosal damage of the gastrointestinal tract in children with sepsis. Biomarkers 2013; 18: 338-342.
[30] Zurek J, Kýr M, Vavřina M, Fedora M. Trefoil factor 2 expression and its significance as a predictor of severity of sepsis in children. Peptides 2013; 46: 1-5.
[31] Li J, Li M, Su L, Wang H, Xiao K, Deng J, Jia Y, Han G, Xie L. Alterations of T helper lymphocyte subpopulations in sepsis, severe sepsis, and septic shock: a prospective observational study. Inflammation 2015; 38: 995-1002.
[32] Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, Srivastava A, Swanson PE, Green JM, Hotchkiss RS. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA 2011; 306: 2594-2605.
[33] Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight

junction permeability in intestinal epithelium. J Biol Chem 2011; 286: 31263-31271.
[34] Dossinger V, Kayademir T, Blin N, Gött P. Down-regulation of TFF expression in gastrointestinal cell lines by cytokines and nuclear factors. Cell Physiol Biochem 2002; 12: 197-206.
[35] Loncar MB, Al-azzeh ED, Sommer PS, Marinovic M, Schmehl K, Kruschewski M, Blin N, Stohwasser R, Gött P, Kayademir T. Tumour necrosis factor alpha and nuclear factor kappaB inhibit transcription of human TFF3 encoding a gastrointestinal healing peptide. Gut 2003; 52: 1297-1230
[36] Gaieski DF, Mikkelsen ME, Band RA, Pines JM, Massone R, Furia FF, Shofer FS, Goyal M. Impact of time to antibiotics on survival in patients with severe sepsis or septic shock in whom early goal-directed therapy was initiated in the emergency department. Crit Care Med 2010; 38: 1045-1053.
[37] May FE and Westley BR. TFF3 is a valuable predictive biomarker of endocrine response in metastatic breast cancer. Endocr Relat Cancer 2015; 22: 465-479.
[38] Perera O, Evans A, Pertziger M, MacDonald C, Chen H, Liu DX, Lobie PE, Perry JK. Trefoil factor 3 (TFF3) enhances the oncogenic characteristics of prostate carcinoma cells and reduces sensitivity to ionising radiation. Cancer Lett 2015; 361: 104-111.
[39] Blanchard C, Durual S, Estienne M, Bouzakri K, Heim MH, Blin N, Cuber JC. IL-4 and IL-13 up-regulate intestinal trefoil factor expression: requirement for STAT6 and de novo protein synthesis. J Immunol 2004; 172: 3775-3783.
[40] Wu HP, Wu CL, Chen CK, Chung K, Tseng JC, Liu YC, Chuang DY. The interleukin-4 expression in patients with severe sepsis. J Crit Care 2008; 23: 519-524.
[41] Collighan N, Giannoudis PV, Kourgeraki O, Perry SL, Guillou PJ, Bellamy MC. Interleukin 13 and inflammatory markers in human sepsis. Br J Surg 2004; 91: 762-768.