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

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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].
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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 vasoressors. 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
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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-\( \alpha \) 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 (\( \geq 20 \)) 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 1A**). Moreover, patients with high SOFA scores (\( \geq 10 \)) 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 1B**). 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...
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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-
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Ammonia 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...
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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 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.
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may exert protective effect on intestinal muco-
sa, 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 cor-
related 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 inflam-
matory response, especially the adaptive
immune response, was activated in the early
phase of sepsis, but prolonged immunosup-
pressive state was occurred with the develop-
ment 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 func-
tion 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 dam-
age the integrity of the intestinal barrier [33].
Furthermore, these pro-inflammatory cytokines
decrease TFF3 expression and production in
intestinal cells, and cause more severe intesti-
nal injury, such as ischemia and hypoxia, isch-
emia-reperfusion injury, thereby leading to
excessive release of many inflammatory cyto-
kines [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 therape-
utic management still remains a major chal-
lenge, and it is immensely important for the
outcome of sepsis [36]. Therefore, the use of
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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.

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