Review article

Trefoil Factor 3 Protein and Sepsis – A Review

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Abstract

Sepsis is one of the most common causes of death in hospitalized patients. Disruption of intestinal barrier homeostasis is one of its main hallmarks. Trefoil factor family proteins are known for their role in protecting and repairing the intestinal mucosa. It has been repeatedly shown that the TFF3 protein is involved in maintaining the intestinal barrier. For that reason, it has been studied in the search for objective measures to predict the onset or outcome of sepsis. Several studies have been performed on rodent sepsis models and on sepsis patients, both children and adults. From the limited research available to date, it appears that TFF3 is involved in the pathogenesis of sepsis, but the exact mechanism is not yet clear. Its potential as a sepsis biomarker has so far been low, but more extensive studies on its role in predicting disease severity and outcome, as well as organ dysfunction, may lead to finding specific patient groups or sepsis stages for which it would be suitable.

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Sepsis

Sepsis is one of the leading causes of death in hospitalized patients worldwide [1]. Sepsis is a "life-threatening organ dysfunction caused by a dysregulated host response to infection", as defined by the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) in 2016 [2]. Since most of the research presented here was conducted before 2016, it is important to note the changes made by Sepsis-3. Earlier definitions stated that sepsis is a consequence of the host's systemic inflammatory response syndrome (SIRS) to infection. When this is complicated by organ dysfunction, it is referred to as severe sepsis, which can progress to septic shock. Sepsis-3 considers this continuum misleading, the criteria of SIRS nonspecific, the term severe sepsis redundant, and defines septic shock as a subset of sepsis in which the underlying circulatory and cellular/metabolic abnormalities are so profound as to significantly increase mortality [2].

Septic conditions can be caused by or lead to alterations in intestinal homeostasis [3]. The gastrointestinal tract is under perpetual pressure from potentially destructive agents, such as chemicals, drugs, bacteria, and their products [4]. The intestinal epithelium, a single layer of cells, forms a selective barrier to the external environment, and is critical in protecting the intestinal mucosa from luminal contents [4]. Alterations in homeostasis of the intestinal barrier can lead to increased production of proteolytic enzymes in the intestine, changes in composition of the mucus layer, epithelial apoptosis, increased permeability of epithelial cells, and inflammatory signaling [5–7]. Gut barrier dysfunction in combination with disturbed balance of the microbiome and immune response can lead to remote injury and the development of multiple organ dysfunction syndrome (MODS), and eventually death [3,8–10].

Trefoil factor family proteins

The trefoil factor family owes its name to a characteristic trefoil-like structure consisting of six disulfide-linked cysteine residues in a 38- or 39-amino-acid sequence [11–13]. There are three members of the family: TFF1, formerly known as pS2 [14], and TFF3, formerly ITF [15], have a single trefoil domain, while TFF2, formerly PSP [16], has two. The trefoil domain enables their resistance to digestion by proteases [15–17], which is important because they are secreted by goblet cells into the hostile environment of the intestine.

Minor disruptions of epithelial integrity are common and require prompt intervention in order to restore homeostasis [18]. Trefoil factor family proteins play a role in maintaining mucosal integrity, which is supported by the fact that their accumulation has been shown to be increased after a gastrointestinal tract injury [19–22]. Normal repair of the epithelium requires restitution and regeneration, with restitution being the critical first phase required to reestablish epithelial continuity [23]. Trefoil factor family proteins have been shown to be important for intestinal epithelial restitution [18,24], with TFF3 even being essential [23]. Mice lacking TFF3 fail to restitute the intestinal mucosal barrier after injury, which makes even minor injuries lethal [23]. The TFF3 protein is involved in maintaining the intestinal barrier by reducing intestinal permeability [25–31]. TFF3 preserves the integrity of the intestinal barrier by inhibiting apoptosis induced by inflammatory stress [32]. It is possible that TFF3 protects the gastrointestinal mucosa through its interaction with mucins [24,33].

Sepsis and its course depend on many factors, including the site and type of infection, genetic predisposition, demographic characteristics, underlying conditions, immune system status, disease progression, treatment, etc., which inherently makes sepsis a very heterogeneous disease [34–37]. In search of objective measures that could predict the onset or outcome of such a complex disease, some research, summarized in this article, has been conducted on proteins belonging to the trefoil factor family [38–47], in particular the TFF3 protein [38–42,45–47].

TFF3 in rodent models of sepsis

Since intestinal barrier disruption has a major impact on the development of sepsis, Jiang et al. studied the changes in the intestinal mucosal immune barrier in septic rats induced by cecal ligation and puncture (CLP) [38]. CLP is the most widely used technique for inducing sepsis in animals [48]. The intestinal mucosa of septic rats exhibited lower TFF3 mRNA expression compared with control groups. The expression levels decreased with time, namely 3, 6, or 12 hours after induction of sepsis. In parallel with the gradual decrease in TFF3 expression, apoptosis increased with time and was higher than in control animals. Under these conditions, bacterial DNA in the blood was increased, i.e., bacterial translocation increased [38].

Later, a group from the same hospital performed the CLP experiment on mice [39]. This time, they divided the animals into 6-hour, 24-hour, and 48-hour groups, depending on how long after induction of sepsis they were sacrificed, and they measured TFF3 protein accumulation. Interestingly, the TFF3 protein level was slightly increased at the 6-hour time point compared with the control group, while the other two time groups showed a significant decrease in the TFF3 protein level. In addition, a significantly lower number of goblet cells was observed in all three groups of treated animals compared with the control group. The researchers did not observe rapid repair of intestinal disruption, which they suggest could be explained by the reduction of TFF3, along with a reduction in goblet cell migration to the site of injury and TGF- β 1 accumulation, as well as an increase in pro-inflammatory cytokines and pro-apoptotic caspase-3 protein levels [39].

Using an LPS (bacterial lipopolysaccharide) induced sepsis model in rats, Shi et al. [40] demonstrated a delay in intestinal mucosal repair during sepsis. They focused on the molecular mechanism through which protein disulfide isomerase a1 (PDIA1) affects the formation of TFF3 dimers and how they change during sepsis; they hypothesized that PDIA1 and TFF3 dimer levels are important contributors to intestinal mucosal destruction and lack of repair, and that PDIA1 is the critical catalyst for TFF3 dimerization. They confirmed a close correlation between PDIA1 and TFF3 dimerization using several methods, including protein modeling. They indicated that TFF3 monomers are unlikely to dimerize in the absence of PDIA1. They noted a decreasing trend in the expression of TFF3 (monomer and dimer) and PDIA1, as well as impaired PDIA1 function in both rat and cell models of sepsis, suggesting that a lack of both may be an important factor in intestinal mucosal damage and impaired repair in sepsis, as impaired PDIA1 function may lead to decreased formation of TFF3 dimers, ultimately resulting in more intestinal damage and less repair.

TFF3 in pediatric patients with sepsis

In search of a new objective diagnostic marker for gastrointestinal failure, a pediatric intensive care unit in China studied serum TFF3 levels of children with MODS, where one group had gastrointestinal failure (GIF), and the other did not [41]. TFF3 levels were associated with both GIF and mortality. Serum TFF3 levels were nearly ten times higher in children with MODS and GIF than in children with MODS and without GIF, and they increased over time. In the group without GIF, serum TFF3 levels remained the same over time and were similar to those of the healthy control group. A total of 71% of the children with MODS, GIF, and high TFF3 died, compared with 32% of the children with normal TFF3 and without GIF. This suggests that early measurement of serum TFF3 levels in hospitals may serve as a prognostic factor, but, as the authors themselves note, the levels should be measured over a longer period in order to see exactly how they change. It should also be noted that this study was conducted in only one hospital in China; it would need to be confirmed in other facilities.

A group from Brno [42] found that the serum TFF3 protein level can distinguish children with SIRS, sepsis, severe sepsis, septic shock, and MODS (according to the 2005 International Pediatric Sepsis Consensus Conference [49]) from control subjects, but the TFF3 level was not adequate for distinguishing between the stages

of septic state prior to the development of severe organ dysfunction. In all patients, the TFF3 level was significantly higher compared with the control group, and in MODS patients, it was significantly higher compared with the rest of the patients. No temporal effect on TFF3 protein level was observed.

The same group also examined TFF1 [43] and TFF2 [44] protein levels in the serum of septic children. They found that TFF1 levels did not discriminate between different septic states up to the development of significant septic shock and organ dysfunction, and TFF2 levels up to organ dysfunction, but the discriminatory power is too low to serve as a diagnostic tool. Both could be used to distinguish septic patients from controls, and TFF2 could additionally differentiate survivors from non-survivors, as its levels were significantly higher in non-survivors [43].

Interestingly, in a study focusing on biomarkers for distinguishing necrotizing enterocolitis (NEC) from septic bowel manifestations, no difference was found between preterm infants with sepsis and control groups [46]. NEC patients had significantly higher TFF3 levels than septic patients, and no association was found between TFF3 and nonspecific systemic inflammation indicators, which suggests that there exists only an association with intestinal damage, rather than the systemic inflammatory response [46]. In addition, TFF3 has been proposed as a potential new therapy for NEC because it was shown that treatment of rat NEC models with TFF3 alleviated the intestinal tissue injury [45].

TFF3 in adult patients with sepsis

A study was conducted on adult intensive care unit (ICU) patients diagnosed with sepsis [47]. The severity of the condition was determined using the APACHE II (acute physiology and chronic health evaluation) score [50], and the extent of organ dysfunction was determined using the SOFA (Sequential Organ Failure Assessment) score [51]. The ELISA assay was used for measurement of septic patients' TFF3 protein levels; they were measured on admission to the ICU and after 3 days of treatment in the ICU. Serum TFF3 levels were significantly higher in septic patients than in control subjects, and they increased with time. Patients with higher APACHE II scores had increased TFF3 concentrations, as did patients with higher SOFA scores. Patients with septic shock, which is defined as severe sepsis complicated with refractory arterial hypotension requiring fluid replacement and vaso¬pressors, had higher TFF3 levels than those with uncomplicated sepsis. While serum TFF3 levels on admission were only slightly higher in patients with a fatal outcome, TFF3 levels were significantly higher three days later in non-survivors compared with survivors. High TFF3 levels correlated with renal dysfunction, systemic inflammation, long ICU stay, and higher mortality. Overall, these results suggest that high serum TFF3 levels are associated with the severity of organ dysfunction and a worse prognosis for the septic patient. The researchers suggest that TFF3 could serve as a prognostic biomarker in the early stages of sepsis, but a larger-scale study is needed first.

In a different study [52], serum TFF3 protein levels were determined in patients with abdominal sepsis, postoperative gastrointestinal patients, and healthy volunteers. TFF3 levels were elevated in patients with abdominal sepsis compared with healthy volunteers, as well as postoperative gastrointestinal patients (no differences were found in the latter two). In contrast to the study presented above [47], TFF3 protein levels were maintained for four days in sepsis patients. Meanwhile, serum TFF3 levels in the postoperative group increased on day 2 and reached sepsis patients' levels on day 4 post-op, suggesting that plasma TFF3 levels increase in response to GI injury of a larger extent or longer duration. TFF3 was higher in patients belonging to both the sepsis and the post-op group who were admitted with shock than those without shock. They found markedly increased TFF3 levels in patients with three or more organs in failure, and a correlation with renal dysfunction. TFF3 levels were higher in abdominal sepsis patients with a fatal outcome; however, TFF3 levels at admission were not a good predictor of survival.

Studies conducted on human subjects are summarized in Table 1.

Table 1. Summary of studies regarding TFF3 in the context of sepsis conducted so far on human subjects

Patient subgroups	Controls	Method	Main findings of the study	Biomarker potential	Ref.
			Pediatric patients		
MODS patients with or without GIF	Healthy controls	ELISA of serum TFF3	Higher serum TFF3 was associated with GIF and with mortality.	Early measurement of serum TFF3 levels may prove useful as a prognostic factor.	[41]
Patients with sepsis, severe sepsis, septic shock, and MODS	Patients undergoing surgery without signs of infection	quantitative enzyme immunoassay of serum TFF3	All septic patients had higher serum TFF3 levels compared to controls with no signs of infection. MODS patients had higher TFF3 levels than other patient groups. Higher TFF3 levels were associated with higher mortality.	TFF3 levels could not differentiate between various septic conditions in patients until a marked organ dysfunction developed.	[42]
			Adult patients		
Patients with sepsis and septic shock	Healthy blood donors	ELISA of serum TFF3	TFF3 concentrations were much higher in sepsis patients than in healthy controls. High serum TFF3 levels were associated with the severity of sepsis and mortality. TFF3 levels were higher in septic shock than uncomplicated sepsis patients.	Serum TFF3 might serve as a potential prognostic biomarker in the early phase of sepsis.	[47]
Abdominal sepsis, postoperative gastrointestinal patients	Healthy volunteers	Luminex multiplex assay of serum TFF3	TFF3 levels were elevated in patients with abdominal sepsis compared with healthy volunteers, as well as postoperative gastrointestinal patients. TFF3 was higher in patients belonging to both the sepsis and the post-op group who were admitted with shock than those without shock. TFF3 levels were higher in abdominal sepsis patients with a fatal outcome.	TFF3 levels at admission were not a good predictor of patient survival.	[52]

The potential of TFF3 as a biomarker for sepsis

Research to date has only scratched the surface of the issue of TFF3 in the context of sepsis. Although the exact mechanisms and details remain to be elucidated, it is evident from both rodent and human data that TFF3 is involved in the pathophysiology of sepsis. In rodent models, lower intestinal TFF3 levels were associated with septic conditions and decreased intestinal repair, while in humans, higher serum TFF3 levels correlated with disease severity. It is difficult to compare the currently available human and rodent data since they are scarce, describe different things, and so far do not offer any mechanistic explanations. A link between TFF3 and sepsis possibly lies in sepsis-induced dysfunction of the intestinal barrier. Intestinal permeability and apoptosis [9], as well as both pro- and anti-inflammatory cytokine production [53] are the hallmarks of sepsis. TFF3 is essential for intestinal epithelial restitution [23], and it preserves the integrity of the intestinal barrier by inhibiting apoptosis induced by inflammation [32]. The expression of TFF3 can be regulated by inflammatory signals and vice versa [54]. The association between TFF3 activation and some known interleukins produced in sepsis, such as IL-6 [55,56], IL-10 [57], IL-8 [58,59], TNF α [60], IFN γ, and IL-12 [61], was previously reported. Perhaps a balance between cytokine and TFF3 activation in sepsis determines the progression and outcome of the disease.

The major limitation of sepsis research conducted so far were the small and demographically homogeneous patient groups for such a heterogeneous disease. Further and more extensive studies are needed to explore the molecular role of TFF3 in the pathogenesis of sepsis, the mechanisms of intestinal damage and repair, the immune response, apoptosis, and the temporal evolution of its effect. It remains to be seen whether sepsis promotes TFF3 production or whether TFF3 promotes sepsis progression, and at what stage of the disease it makes the greatest contribution. Based on the information available to date, TFF3 is not a good biomarker for sepsis in general, but further research into its potential for predicting disease severity and outcome, as well as organ dysfunction, may lead to finding specific patient populations or stages of sepsis for which it is appropriate.

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