

## Association between Trefoil Factor 3 and Gastrointestinal Failure in Critically Ill Children

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MODS and intestinal failure and correlated with mortality in this group. Measurement of TFF3 in children with MODS gives an assessment of intestinal function and may have a prognostic role in this setting.

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**Key words:** Trefoil factor 3; Multiorgan dysfunction syndrome; Gastrointestinal failure; Pediatric

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### ABSTRACT

**AIM:** While gut dysfunction has been proposed as an important factor in multiorgan dysfunction syndrome (MODS), there are few objective measures of gut failure. This study aimed to ascertain the key features of intestinal failure in MODS and to determine if serum trefoil factor 3 (TFF3) correlated with intestinal failure in patients with MODS.

**METHODS:** A retrospective assessment of children admitted to a pediatric intensive care unit (PICU) was undertaken to characterize key features of MODS with intestinal failure. A second cohort of children with MODS was enrolled prospectively with serial measurement of serum TFF3 from admission until day 5. TFF3 was also measured in 30 healthy infants.

**RESULTS:** Intestinal failure was associated with higher mortality, longer PICU stay and greater acidosis or hypoxia in the retrospective cohort. In the prospective study, baseline serum TFF3 was higher in the children with MODS and intestinal failure than children with MODS without intestinal failure or healthy control children ( $13.2 \pm 3.1$  ug/mL vs  $1.5 \pm 0.5$  ug/mL vs  $1.8 \pm 0.4$  ug/mL:  $p < 0.01$ ). The mean TFF3 in the children without intestinal failure did not increase over time, while levels in children with MODS and intestinal failure increased progressively ( $p < 0.01$ ). Elevated TFF3 was associated with higher mortality (71 % vs 32%:  $p < 0.01$ ).

**CONCLUSION:** Serum TFF3 was elevated only in children with

### INTRODUCTION

Gastrointestinal failure (GIF) has long been recognized as a common problem among critically ill children and adults<sup>[1-4]</sup>. Several recent studies of GIF in intensive care patients with multiorgan dysfunction syndrome (MODS) have focused on the role of the gut in the pathogenesis of MODS<sup>[5,6]</sup>, developing the concept of the gut as the “motor” of critical illness<sup>[7-9]</sup>. While this concept is intriguing, clinical correlation is difficult given that there are few objective, or easily measurable, variables of gastrointestinal dysfunction. Furthermore, there is no consensus on the clinical definition of GIF in the context of MODS<sup>[10]</sup>. A pediatric consensus statement on MODS did not include this organ system in the proposed classification<sup>[11]</sup>.

In our recent audit of MODS in children admitted to a pediatric intensive care unit (PICU), GIF, defined clinically as the presence of gastrointestinal haemorrhage<sup>[12,13]</sup>, was the third most common organ system to fail<sup>[14]</sup>. Furthermore, GIF was associated with a high mortality rate in this cohort. Others have also noted that GIF in MODS is associated with higher mortality, longer intensive care unit stay and increased ventilator requirements<sup>[1,4]</sup>. Given the importance of the relationship between GIF and mortality, the ability to recognize early changes of GIF should provide opportunities for earlier intervention and consequent improved outcomes.

Trefoil factor 3 (intestinal) (TFF3), a protein secreted by gastric antral and intestinal mucosa, contributes to the maintenance of

mucosal integrity<sup>[15]</sup>. TFF3 plays a critical role in mucosal protection and in restitution after epithelial damage<sup>[16]</sup>. These functional properties are consequent to coordinated promotion of cell migration and inhibition of apoptosis<sup>[17]</sup>.

The expression of TFF3 has not yet been assessed as a potential objective marker of GIF in MODS. The aims of this project were to (i) examine correlates of GIF in MODS through further analysis of the previously collected data; (2) measure levels of TFF3 in a cohort of normal children; and (3) prospectively define the relationship between serum TFF3 concentrations and the development of MODS and clinically diagnosed GIF in children in a PICU setting.

## METHODS

### Retrospective cohort of children in PICU

A retrospective chart audit of all children (aged 2 months to 14 years) admitted to the six bed PICU of the Second Hospital of Lanzhou University, China, from September 2001 to May 2006 was previously conducted to examine the epidemiology of MODS<sup>[14]</sup>. Further analyses of this cohort were undertaken to ascertain differences between patients with MODS with and without GIF.

MODS was defined as failure of at least two organ systems. GIF was defined according to criteria established by the Society of Pediatrics of China, and based upon previously described criteria<sup>[13]</sup>. These are either a blood transfusion requirement of >20mL/kg in 24 hour consequent to gastrointestinal hemorrhage with or without endoscopic confirmation and/or severe abdominal distension, loss of bowel sounds and positive fecal occult blood. For determination of the length of PICU stay, all patients who died within 24 hours of admission to the PICU were excluded. Hypoxia and acidosis at admission to PICU were noted for each subject. Hypoxia was defined as mild if arterial PO<sub>2</sub> was between 60 and 80 mmHg (1 mmHg=0.33 KPa), moderate if between 40 and 60 mm Hg and severe if less than 40 mmHg. Acidosis was defined as mild if pH was between 7.25 and 7.35, moderate if between 7.15 and 7.25 and severe if less than 7.15.

### Prospective patient cohort

A second cohort of patients was enrolled prospectively over a 21 month period to determine the significance of serum TFF3 in children with MODS. Children admitted to the PICU of the Second Hospital of Lanzhou University, China from September 2004 to May 2006 were enrolled. Parents were informed about the study and informed consent obtained.

Clinical data were systematically extracted from the chart using an established template<sup>[14]</sup>. Serum was collected on three occasions: within two hours of PICU admission (Baseline), on day 2-3 of admission (Specimen 2) and on day 4-5 of admission (Specimen 3) if the patient was still alive. The additional volume of blood required for TFF3 determination was collected at times of clinically-indicated blood tests. Serum was stored at -80°C until required for analysis.

### Control group of healthy infants

Thirty healthy infants were recruited from a well child outpatient clinic to establish control values of TFF3. After obtaining parental consent, an additional 2 mls of blood was obtained at the time of clinically-indicated blood tests and then stored at -80°C until required.

### Measurement of serum Trefoil Factor 3

Measurement of serum TFF3 was undertaken using a commercial sandwich ELISA (Abnova Corporation, Walnut, California, USA) following the manufacturer's instructions. Testing was conducted in

triplicate with the operator blinded to the clinical status of the subject.

### Research and Ethical Approvals

The research study and the funding for the project were approved by Lanzhou University, China. At the time of this study, neither the Second Hospital of Lanzhou University nor the Department of Pediatrics of Lanzhou University had formal Research Ethics Boards for review of clinical studies. Following the policies in place at that time, the project was reviewed and approved by the Institutional Review Board of the Second Hospital of Lanzhou University. The study was conducted according to the requirements of the Institutional Research Board. The study was also approved by the Chair of the Department of Pediatrics of the Second Hospital of Lanzhou University. Written consent was obtained from the parents or caregivers of all children prior to inclusion in the study.

### Statistical Analysis

All statistical analyses were completed using SPSS 13.0 software. The relationships between GIF and MODS, and relationships between MODS with GIF with acidosis or hypoxia were analysed using Chi square test. Analysis of variance was utilized for assessment of the levels of the levels of TFF3 in children with MODS with and without GIF. A *p* value of <0.05 was considered statistically significant.

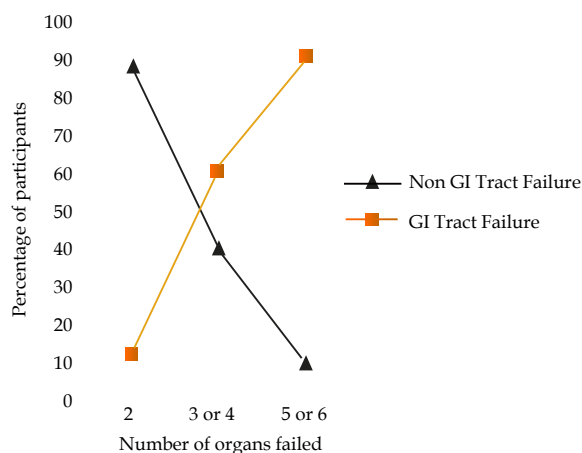
## RESULTS

### Retrospective patient cohort

Further analysis of the data from the 176 patients who met the MODS criteria was undertaken<sup>[14]</sup>. Those with MODS and GIF (*n*=68) were of similar age and gender to those without GIF (*n*=108) (mean age 21 months, range 3 mo to 72 mo vs 45 months, range 2 mo to 168 mo (*p*=0.226), gender ratio 1.7:1 vs 1.5:1 (*p*=0.6).

The children with MODS and GIF had a longer PICU admission compared to those without GIF (mean length of stay 8.73 days, range 5 to 24 days vs 5.43 days, range 3 to 22 days (*p*<0.001). Overall, infection was the major predisposing cause (73%) of MODS, as previously reported<sup>[14]</sup>. However, infection (especially pneumonia, septicaemia and meningitis) was the predisposing factor in 84% of children with MODS with GIF, compared to 66% in those without (*p*=0.009).

The incidence of GIF was related to the number of failing organs



**Figure 1** Relationship between gastrointestinal failure and the number of failing organ systems in 176 children with MODS managed in a PICU. Retrospective analysis of this group demonstrated that gastrointestinal tract failure was associated with increasing number of other organ systems failing (*p*<0.001). Other organ system failure was classified as 2, 3 or 4, or 5 or 6 failed organs.

( $p < 0.001$ ) (Figure 1). The presence of GIF also was associated with increasing severity of acidosis and hypoxia (Table 1). The fatality rate in children with GIF and MODS was greater in the presence of acidosis (43/56 or 77%) compared with the children without acidosis (6/12 or 50%;  $p = 0.02$ ). Furthermore, the fatality rate with GIF in MODS increased with the presence of hypoxia (46/61 or 75%) compared with the children without hypoxia (3/7, 43%;  $p = 0.02$ ).

**Table 1** The relationship between MODS with gastrointestinal tract failure and acidosis or hypoxia.

	Total number	Number (%) with Gastrointestinal Failure
No Acidosis <sup>1</sup>	48	12 (25%)
Mild Acidosis	58	16 (28%)
Moderate Acidosis	40	20 (50%)
Severe Acidosis	30	20 (67%)
Total with Acidosis <sup>1</sup>	128	56 (44%)
No Hypoxia <sup>2</sup>	37	7 (19%)
Mild Hypoxia	60	15 (25%)
Moderate Hypoxia	53	26 (49%)
Severe Hypoxia	26	20 (77%)
Total with Hypoxia <sup>2</sup>	139	61 (44%)

Retrospective analysis of the relationship between gastrointestinal failure and acidosis or hypoxia was undertaken in 176 children with MODS managed in a PICU. The presence of acidosis<sup>1</sup> or hypoxia<sup>2</sup> were associated with higher rates of gastrointestinal failure ( $p = 0.001$  and  $p < 0.001$  respectively).

### Prospective Patient cohort

Over the period of observation, 153 children were admitted to the PICU. Seventy-four of these children developed MODS, of whom 43 also met the clinical criteria for GIF. Twenty-seven (63%) of the children with MODS and GIF were male, and the mean age of this group was  $22 \pm 10$  months (range 3 to 73 months). Eighteen of the 31 children with MODS without GIF were male and this group had mean age of  $46 \pm 23$  months (range 2 to 168 months). The age and gender of these two groups did not differ ( $p > 0.05$  for both). The mean length of PICU stay for children with MODS and GIF was longer than for those without GIF ( $8.7 \pm 3.3$  days vs  $5.4 \pm 3.3$  days;  $p < 0.001$ ). The 30 healthy children enrolled as a control group had a mean age of  $22 \pm 11$  months (range 3 to 70 months). This group did not differ from the study group in terms of age and gender ( $p > 0.05$  for both).

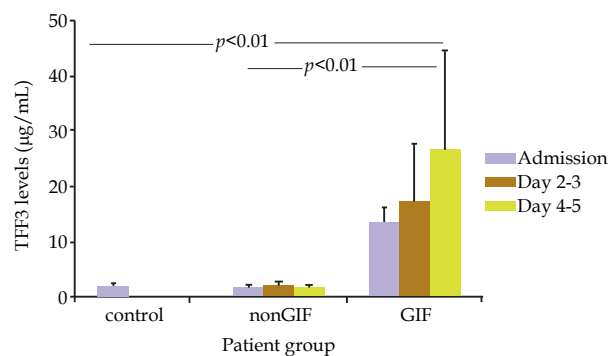
The baseline serum TFF3 concentration was almost 10 times higher in the children with MODS and GIF than in those with MODS without GIF ( $13.24 \pm 3.11$  ug/mL vs  $1.51 \pm 0.47$  ug/mL;  $p < 0.01$ ). TFF3 levels increased over time in the group with GIF ( $p < 0.01$ ) (Figure 2). The mean TFF3 levels in the children with MODS without GIF was similar to that of the control group ( $1.84 \pm 0.40$  ug/mL;  $p > 0.05$ ) and did not change over time (Figure 2).

Seventy-one percent of the children with MODS and GIF with elevated TFF3 (mean  $13.24 \pm 3.11$ ) died. In contrast, the fatality rate of the group with MODS without GIF and normal TFF3 (mean  $1.51 \pm 0.47$ ) was just 32% ( $p < 0.01$ ).

## DISCUSSION

In this study of critically ill children in a PICU in China the combination of MODS and GIF in these children resulted in longer PICU stay and higher mortality rate. Furthermore, when assessed prospectively, serum TFF3 was associated both with GIF and with mortality.

Laboratory and clinical research strongly suggests that the gastrointestinal tract plays a pivotal pathogenic role in MODS. Shock with resulting gut hypoperfusion is an important inciting event, with the reperfused gut being a source of proinflammatory mediators that



**Figure 2** Levels of Trefoil Factor 3 (TFF3) in 74 children with MODS with and without gastrointestinal failure (GIF). TFF3 was measured at admission, after 2-3 days and after 4-5 days in 74 children admitted to a PICU as well as a group of healthy control children. Mean levels in the 43 children with MODS and GIF were greater than in the 31 children without GIF ( $p < 0.01$ ) at each time point, and also greater than in the 30 children in the control group ( $p < 0.01$ ). TFF3 levels rose over time in those with MODS and GIF ( $p < 0.01$ ). Error bars indicate the standard deviation.

can amplify the early systemic inflammatory response syndrome (SIRS) and thus contribute to MODS<sup>[18-21]</sup>. Early gut hypoperfusion results in reduced motility (e.g. intestinal ileus) that sets the stage for progressive gut dysfunction. The proximal gut then acts as a reservoir for pathogens and toxins that contribute to late sepsis-associated MODS<sup>[20]</sup>. Subsequent infections may lead to further worsening of this gut dysfunction. Thus, the gut can be both an instigator and a victim of MODS<sup>[20]</sup>. At present even though GIF in MODS represents a major risk of adverse outcome, diagnosis of GIF depends upon clinical manifestations (abdominal distention, decreased or absent bowel sounds and gastrointestinal haemorrhage)<sup>[12,13]</sup>. Clearer definitions of GIF are clearly required.

TFF3 plays a key role in the maintenance of mucosal integrity, with both protection of the epithelium from injury and promotion of repair once injury has occurred<sup>[16,17]</sup>. It is rational, therefore, to consider serum TFF3 concentrations as an indicator of GIF in MODS. Indeed, the findings from the prospective component of the current study support serum TFF3 concentration being a useful objective marker of GIF in MODS. Serum TFF3 concentrations correlated with GIF and with mortality in these unwell children. The early measurement of serum TFF3 levels may prove useful as a prognostic factor in this setting.

Serum TFF3 concentrations are elevated in patients with inflammatory bowel disease compared to healthy controls<sup>[22]</sup>. Recent animal studies suggest that TFF3 may be an important therapeutic target in gut disease, such as in necrotizing enterocolitis<sup>[23]</sup>. However, further understanding of the role and responses of TFF3 is required before TFF3 can be considered for therapeutic roles.

There are limitations in this study of serum TFF3 in critically ill children with MODS and GIF. Firstly, this study included critically ill children in one pediatric intensive care unit in a Chinese city. These results may not be directly applicable to other settings. Infection was the major predisposing factor to MODS in this PICU. This pattern is seen also in MODS in other PICUs in developing countries with advancing economies, but infection may not be such an important factor in industrialized countries<sup>[14]</sup>. Therefore, this study needs to be replicated in other PICUs, including those in industrialized countries to firstly determine the prevalence of GIF in MODS in PICUs where infections are less common predisposing factors and also to verify the association between serum TFF3 concentration and GIF in the context of MODS.

In addition, this study utilized a rigorous definition of GIF in

MODS. It is not yet clear if TFF3 is elevated in the setting of MODS with less severe perturbations of gastrointestinal function. Furthermore, this study did not include measurement of serum TFF3 beyond 4 to 6 days after admission. It would be important to further define the characteristics of TFF3 over longer periods of time, including following recovery from GIF.

In conclusion, this study has demonstrated that GIF greatly alters outcome in children with MODS. Furthermore, TFF3 levels correlated with GIF and outcome, suggesting that measurement of this protein could provide important prognostic information in these children. Further studies are now required to define additional elements of the roles of this protein.

## ACKNOWLEDGMENTS

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