CLINICAL OUTCOME STUDY OF CRITICALLY-ILL SEPTIC PATIENTS GIVEN TAURINE SUPPLEMENTED ENTERAL NUTRITION

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ABSTRACT: Background: Sepsis is a significant public health concern and is the main cause of death in surgical intensive care units (ICUs). Patients with sepsis have features consistent with immunosuppression. Taurine is rapidly emerging as one of the more interesting amines which has been reported to have immune-modulatory effect through its action on cytokines. Purpose: To determine the effect of using Immune-Enhancing enteral nutritional feed containing taurine compared to Standard enteral nutritional feed on clinical outcomes of ICU septic population. Methods: This was a prospective, randomized, controlled study. A total of 45 patients were randomly divided into 3 groups: Group 1 (n=15) received Standard Enteral Nutrition feed for two weeks, Group 2 (n=15) received Immune-Enhancing Enteral Nutrition feed containing 10 mg/kg/day of Taurine for two weeks and Group 3 (n=15) received Immune-Enhancing Enteral Nutrition feed containing 30 mg/kg/day of Taurine for two weeks. Parameters measured were serum levels of Interleukin-6, Interleukin-10, C-reactive protein and Total leukocyte count. Results: The current study showed that Taurine had immune-modulatory effect by significantly decreasing the level of pro-inflammatory Interleukin-6 and increasing the level of anti-inflammatory Interleukin-10. Taurine was found effective in improving the clinical outcomes of sepsis patients. Conclusion: Taurine administered enterally at a dose of 30 mg per kg per day to sepsis patients had immune-modulatory effect and improved their clinical outcomes and their quality of life.

INTRODUCTION: Sepsis is a significant public health concern. 1, 2 It is the main cause of death in surgical intensive care units (ICUs), with a continuously increasing incidence and a mortality rate ranging from 30% to 60%, depending on sepsis severity and the days of hospital stay. 3 Treatment of sepsis adds tremendous costs on our health care systems. 1 Therefore, both an early diagnosis and a timely prognosis of sepsis are of utmost importance to control efficacy of antibiotic and surgical therapy, to manage further diagnostics and interventions, and to optimize cost containment by adequate resource allocation. 4 That’s why emergency physicians are required to rapidly and accurately diagnose sepsis, evaluate its severity, and provide appropriate therapy for septic patients. 5

Current knowledge implies that sepsis represents a state of inflammatory mediators’ over-expression, after various noxious insults, especially bacterial infections. Enhanced pro-inflammatory cytokine and inhibited anti-inflammatory cytokine
concentrations are considered as an important component of the pathophysiology of sepsis. The standard treatment of sepsis consists of antibiotics, surgical treatment of the inflammatory focus, monitoring of the patients in intensive care units, normalization of metabolic disturbances, and proper nutrition. Patients with sepsis have features consistent with immunosuppression, including a loss or delayed hypersensitivity, an inability to clear infection, and a predisposition to nosocomial infections.

Therefore, the possibility of modifying the immunological reaction has become an attractive challenge for many scientists in their studies on sepsis; one way of modifying the immunological cascade is through immunonutrition, where Immunonutrition can be defined as modulation of either the activity of the immune system, or modulation of the consequences of activation of the immune system, by nutrients or specific food items fed in amounts above those normally encountered in the diet.

In this regard, most interest has focused on amino acids such as taurine, arginine, glutamine, fatty acids, ribonucleotides and certain trace elements. Taurine (Tau) is rapidly emerging as one of the more interesting and ubiquitous of the amines. Taurine (2-amino-ethane sulphinic acid) is a naturally occurring β-amino acid with a sulphonic acid group replacing the characteristic carboxylic group of amino-acids. Tau plasma levels decrease in response to pathological conditions such as sepsis, suggesting an increased need.

In recent years, a number of therapeutic benefits have been proposed for Tau supplementation including; treatment for diabetes, hypertension, heart failure, retinal degeneration and skeletal muscle disorders. Tau also has an important role in osmoregulation, modulation of cellular calcium levels and bile acid conjugation. Moreover, it was found to have antineoplastic effects, anti-oxidant and neuro-protective effects.

A newly discovered topic of interest for functions of taurine is the modification of the endogenous inflammatory response; the attenuation of the elaboration of pro-inflammatory mediators and up-regulation of anti-inflammatory factors. On the basis aforementioned, we investigated, in the present study, the effects of using Immun-enhancing enteral nutritional feed containing Taurine compared to Standard enteral nutritional feed on clinical outcomes of ICU septic population in order to find out possible mechanism of action of taurine on inflammatory responses.

**MATERIALS AND METHODS:**

**Patients and setting:**

This is a prospective, randomized, controlled study that was conducted on 45 adult critically-ill septic patients staying for more than 24 h in the ICU department, Qasr Al-Aini hospital, Cairo, Egypt in the period from June 2013 to March 2014. We included in the study patients diagnosed with sepsis both clinically and by laboratory findings based on the 1991 ACCP/SCCM Sepsis Directory and the diagnostic criteria advanced by the 2001 International Sepsis Definition Conference, exhibiting two or more of the following signs: (1) temperature >38°C or <36°C, (2) pulse rate of >90 beats/min, (3) respiratory rate of >20 breaths/min or hyperventilation with a partial pressure of arterial carbon dioxide (PaCO2) of <32 mmHg, or (4) white blood cell (WBC) count of >12,000/mm3 or <4000/mm3. Patients included had no history of chronic gastrointestinal disease, no total parenteral nutrition and enteral feedings starting within the first 12 h. All enrolled patients had a Sequential Organ Failure Assessment score (SOFA) of less than 12.

We excluded from the study, patients who were under 18 years, patients who received anti-inflammatory drugs or corticosteroids before admission, patients who had immunosuppressive illness or had received massive blood transfusion and patients who were on do-not-resuscitate status or cardiac arrest. We also excluded patients with diabetes, chronic respiratory insufficiency, cardiovascular insufficiency and kidney and liver diseases (biopsy proven cirrhosis or a serum total bilirubin of > 3.0 mg/dl).

Patients were randomly divided into three groups; group 1 (n=15) included patients who received Standard Enteral Nutrition feed for two weeks, group 2 (n=15) included patients who received...
Immune-Enhancing Enteral Nutrition feed containing 10 mg/kg/day of Taurine for two weeks and group 3 (n=15) included patients who received Immune-Enhancing Enteral Nutrition feed containing 30 mg/kg/day of Taurine for two weeks. Approval for the study protocol for both the scientific and the ethical aspects was obtained from the committee of Ethics of Faculty of Pharmacy, Ain Shams University and the Scientific Committee for Clinical Research of Al Qasr Al-Aini hospital. The study was performed in accordance with the Declaration of Helsinki and an informed consent was obtained directly from each patient/legal representative before enrollment.

**Feeding protocol:**
The rate of administering the diet was gradually increased from 30 ml/h for the first 24 to 48 hours then increased to full feeding depending on the passage of flatus and bowel action. All patients reached their nutritional goal within 72 h. Daily supply of the main nutritional substances in standard enteral nutritional feed amounted on average to: 10.8±1.3 g nitrogen, 208±24.4 g glucose, 66±7.7 g fat (including 101.6±11.9 g of protein and 1693±198 kcal). The supply of calories and nitrogen did not differ significantly between the three studied groups.

The standard method of delivery of EN was by way of a fine bore silicone feeding tube. Where appropriate, as decided by the attending clinician, EN was alternatively administered via a percutaneous endoscopic gastrostomy (PEG), a surgically placed gastrostomy tube, or a feeding jejunostomy.

The provision of the nutritional support was overseen and closely monitored by a dedicated nutrition team. A senior dietician, specialist nutrition nurse were responsible for prescribing, monitoring and recording intakes and complications on a twice-daily basis. All access sites for nutritional support were inspected twice daily.

**Data collected:**
Full history taking and meticulous physical examination on admission. Baseline demographics, past medical history, and medications were obtained from patients’ charts.

Routine cultures of blood, sputum, urine, and suspected sites of infection were obtained. All patients were managed by conventional supportive measures for critically ill septic patients including antibiotics, fluids, oxygen therapy, and ventilatory support whenever required.

**Sampling and laboratory analysis:**
Blood samples from adult patients with sepsis were obtained and were centrifuged and stored in deep freeze at -80 °C. Serum C-reactive protein (CRP), Total leukocyte count (TLC) were assessed at baseline of the study (day 0) and at the end of the study (day 14). Interleukin-6 (IL-6) and Interleukin-10 (IL-10) serum levels were measured at baseline of the study, day 5, day 10 and day 14.

**Statistical Methods:**
IBM SPSS statistics (V. 22.0, IBM Corp., USA, 2013) was used for data analysis. Values were expressed as the mean ± standard deviation (SD), and in case of a skewed distribution as the median and range. The results were estimated as statistically significant when p-values<0.05.

**RESULTS:**
Demographic comparison between the three groups as represented in Table 1 showed no statistically significant difference in age (p= 0.997), gender (p= 0.913) or weight (p= 0.998) which ensured the avoidance of any baseline cofounders.

<table>
<thead>
<tr>
<th>TABLE 1. DEMOGRAPHIC DATA; COMPARISON OF AGE, GENDER AND WEIGHT BETWEEN THE 3 GROUPS [MEAN± STANDARD DEVIATION]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n=15)</td>
</tr>
<tr>
<td>Age (Years)</td>
</tr>
<tr>
<td>Gender N (%)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Weight (kg)</td>
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</tbody>
</table>
Serum concentrations of IL-6 in the three studied groups:
IL-6 serum levels were determined by ELISA. Serum levels of IL-6 were increased in the three studied groups at baseline with no statistically significant difference between the three groups. (p=0.887). At the end of the study (day 14), the effect of taurine on IL-6 serum levels in group 3 using the large taurine dose level (30mg/kg/day) dramatically suppressed the elevation of IL-6 with statistically significant difference between the three studied groups (p= 0.015). IL-6 decreased significantly in group 3 at the end of the study when compared by group 1 and group 2 (p=0.01), (p=0.016) respectively. The delta change between baseline and end of the study of serum level of IL-6 showed statistically significant difference between the 3 groups (p=0.006) where there was significant decrease in IL-6 levels in group 3 when compared by group 1 and group 2 (p=0.004), (p=0.003) respectively as shown in Table 2.
On the other hand, the delta change between baseline and day 5 of the study showed no statistically significant difference between the 3 studied groups (p= 0.079), similarly, the delta change between baseline and day 10 of the study showed no statistically significant difference between the 3 groups (p=0) as demonstrated in Fig 1.

Serum concentrations of IL-10 in the three studied groups:
We also detected serum levels of IL-10 by ELISA. As represented in Table 2, at baseline of the study, there was no statistical significant difference in serum IL-10 levels between the three groups (p=0.73). At the end of the study, taurine’s effect on IL-10 serum levels led to increase in serum IL-10 levels with more increase in the third group compared to the other 2 groups, but this increase was not statistically significant (p=0.3).
Additionally, as represented in Fig.2, the delta change between baseline and end of the study showed no statistically significant difference between the 3 studied groups (p=0.2), in the same concern, there was no statistically significant difference when comparing group 1 and group 3 (p=0.08) also when comparing group 2 and group 3 (p=0.56).

<table>
<thead>
<tr>
<th>TABLE 2: IL-6, IL-10 IN THE 3 GROUPS [MEDIAN (RANGE)]</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 day 0</td>
<td>140 (55-537)</td>
<td>109 (55-716)</td>
<td>122 (56-609)</td>
<td>0.887</td>
</tr>
<tr>
<td>IL-6 day 5</td>
<td>148 (51-436)</td>
<td>139 (59-739)</td>
<td>107 (30-473)</td>
<td>0.523</td>
</tr>
<tr>
<td>IL-6 day 10</td>
<td>137 (48-401)</td>
<td>106 (68-702)</td>
<td>63 (20-307)</td>
<td>0.06</td>
</tr>
<tr>
<td>IL-6 day 14</td>
<td>121 (37-392)</td>
<td>116 (25-635)</td>
<td>55 (6-163)</td>
<td>0.01* Gp2 vs. 3</td>
</tr>
<tr>
<td>IL-6 (0,14) dC</td>
<td>-0.096 (-0.8-0.4)</td>
<td>-0.17 (-0.77-1.1)</td>
<td>-0.5 (-0.9-0.1)</td>
<td>0.004* Gp1 vs. 3</td>
</tr>
<tr>
<td>IL-6 (0,14) dC</td>
<td>0.56 Gp2 vs. 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 day 0</td>
<td>27 (4-151)</td>
<td>17 (2-269)</td>
<td>22 (4-127)</td>
<td>0.73</td>
</tr>
<tr>
<td>IL-10 day 5</td>
<td>35 (12-103)</td>
<td>27 (12-246)</td>
<td>29 (7-129)</td>
<td>0.78</td>
</tr>
<tr>
<td>IL-10 day 10</td>
<td>30 (17-97)</td>
<td>27 (7-249)</td>
<td>35 (11-116)</td>
<td>0.82</td>
</tr>
<tr>
<td>IL-10 day 14</td>
<td>37 (20-102)</td>
<td>38 (9-171)</td>
<td>47 (24-185)</td>
<td>0.3</td>
</tr>
<tr>
<td>IL-10 (0,14) dC</td>
<td>0.4 (-0.6-8.25)</td>
<td>1.04 (-0.5-4.14)</td>
<td>1.14 (-0.26-5)</td>
<td>0.08 Gp1 vs. 3</td>
</tr>
<tr>
<td>IL-10 (0,14) dC</td>
<td>0.56 Gp2 vs. 3</td>
<td></td>
<td></td>
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</tbody>
</table>

FIG.1: IL-6 (DELTA CHANGE, dC) IN THE 3 GROUPS
Sepsis Biomarker:
CRP is a commonly used sepsis biomarker. Its levels were measured at baseline of the study, where the CRP serum levels were dramatically increased in the three studied groups denoting the diagnosis of sepsis and its hyper inflammatory state but there was no statistically significant difference between the three groups ($p=0.976$). The delta change between baseline and end of the study showed statistically significant difference where there was more decrease of CRP serum levels in group 3 when compared by group 1 and group 2 ($p=0.04$), ($p=0.045$) respectively as shown in Table 3 and Fig.(3).

Total leukocyte count: (TLC)
Serum TLC levels were measured at baseline of the study and there was no significant difference between the three groups ($p=0.986$). As demonstrated in Table 3 and Fig. (4), at the end of the study, there was a decrease in the serum TLC levels in the three groups but with no statistically significant difference between them ($p=0.884$).

**TABLE 3: CRP, TLC [MEDIAN (RANGE)]**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP day 0</td>
<td>50 (13-201)</td>
<td>51 (4-211)</td>
<td>53 (5-211)</td>
<td>0.976</td>
</tr>
<tr>
<td>CRP day 14</td>
<td>38 (11-88)</td>
<td>39 (10-88)</td>
<td>36 (10-71)</td>
<td>0.466</td>
</tr>
<tr>
<td>CRP(0.14) dC</td>
<td>-0.22 (-0.84-1.07)</td>
<td>-0.27 (-0.83-8.75)</td>
<td>-0.36 (-0.88-1)</td>
<td>0.04* Gp1 vs. 3, 0.045* Gp2 vs. 3</td>
</tr>
<tr>
<td>TLC day 0</td>
<td>15.7 ± 3.9</td>
<td>16 ± 5.5</td>
<td>16.2 ± 6.8</td>
<td>0.986</td>
</tr>
<tr>
<td>TLC day 14</td>
<td>11.6 ± 2.8</td>
<td>11.9 ± 4.4</td>
<td>11.4 ± 3.2</td>
<td>0.884</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein, TLC: Total Leukocyte Count, Gp: Group

**FIG.2: IL-10 (DELTA CHANGE, dC) IN THE THREE GROUPS**

**FIG.3: CRP (DELTA CHANGE, dC) BETWEEN BASELINE AND END OF THE STUDY IN THE 3 GROUPS**
**DISCUSSION:** Many previous literatures in the field of sepsis have a uniform agreement that it is an enormously complex clinical syndrome that arises from the activation of an innate host response to danger, causing cell injury and organ dysfunction.\(^{28}\) Despite rapid progress in health care over the past decades, sepsis continues as a leading cause of death in many intensive care units.\(^{29}\)

During 1979-2000, the total sepsis-related mortality rose from 22 to 44 per 100,000 annual mortality in the United States alone.\(^{30}\)

One of the ways to improve the immunity and to lower the number of sepsis complications was the introduction of enteral immunonutrition.\(^{31}\) Authors showed that the supplementation of early enteral immunonutrition to critically-ill patients have positive immune-modulatory effects, where immunonutrition enhances the host response and induces a switch from acute phase to constitutive proteins.\(^{32}\)

In recent years, several compounds have been examined from the perspective of immunomodulation. Taurine, is a sulfur-containing amino acid.\(^{33}\) It is one of the most abundant amino acids in the human body, but it is classified as a conditionally essential amino acid because the body cannot synthesize sufficient amounts of taurine during extreme stress, such as sepsis.\(^{29}\) Moreover, Tau has been studied as an immunomodulator.\(^{33}\) However, to our best knowledge, taurine has not been reported as the ancillary drug for sepsis.

In sepsis, the metabolic balance of amino acids deteriorates which correlates with poor prognosis. Altered balance of the aminogram is significantly associated with mortality in patients with sepsis, where sepsis, severe trauma and infection cause large decrease in plasma taurine concentrations which may indicate supplementation.\(^{34}\)

Recent attention has been directed in sepsis to the role of cytokines. Cytokines have been widely assessed as potential biomarkers in sepsis for years, as they are important mediators in the pathophysiology of this disease, and most are produced immediately after sepsis onset.\(^{8}\)

In the pathogenesis of sepsis, cytokines can be classified as pro-inflammatory (e.g., Interleukin-6) and anti-inflammatory (e.g., Interleukin-10) according to their inflammatory activities. While pro-inflammatory cytokine production (IL-6, IL-1) is an essential part of the immune response, the excessive production of these molecules may result in increased morbidity and mortality.\(^{35}\) IL-6 is considered a notable element in the cytokine network during sepsis.\(^{36}\) Previous reports indicated that IL-6 concentration often closely correlated with the severity of sepsis.\(^{37}\) IL-6 stimulates hepatic protein synthesis during acute phase responses and acts as an endogenous pyrogen. Because of these functions IL-6 has been described as an alarm hormone.\(^{38}\) Immune-modulatory effect was assessed in our current study, where serum levels of IL-6 were measured in the three groups at baseline of the study, day 5, day 10 and at the end of the study (day 14).
IL-6 serum levels were elevated at baseline of our study indicating hyper inflammatory sepsis diagnosis where IL-6 serum levels are markedly increased in patients with sepsis at the time of admission to the intensive care unit as reported by Hack et al.  

Our study showed that the administration of taurine at a dose of 30 mg/kg/day to group 3 has resulted in significant decrease in the serum levels of IL-6 at the end of the study when compared to the other 2 groups. As well as, the delta change between baseline and end of the study showed significant decrease of IL-6 in group 3 compared by group 1 and 2. This is in agreement with other studies demonstrating the immune-modulatory effect of taurine decreasing the level of pro-inflammatory cytokines, which is a sign of good prognosis and decreased sepsis mortality, where Tau’s protective effects have been demonstrated in some inflammatory conditions.

One of the proposed protective mechanisms of taurine is its reaction with hypochlorous acid, produced via myeloperoxidase pathway, to produce taurine Chloramine (Tau-Cl), a powerful anti-inflammatory agent.

Where Park et al reported that the production of IL-6 was inhibited by Tau-Cl. These data demonstrate the ability of Tau-Cl to modulate the immune response and suggest a central role for taurine and its chloramine in regulating the immune response. In the same concern, Zaki et al reported in their study of the therapeutic effect of taurine on arthritic patients that taurine chloramine inhibits secretion of some pro-inflammatory cytokines such as IL-6 which is crucial in pathogenesis of inflammatory diseases as rheumatoid arthritis.

Another anti-inflammatory derivative of taurine; taurrolidine was reported by Marcinkiewicz et al to have protective effects in various experimental models of synovitis.

Our study’s results are in agreement with the finding that low serum levels of IL-6 have been shown to be the best single predictor of decreased mortality in patients with sepsis.

A study by Srisangthong et al done on 203 sepsis patients admitted to the ICU showed that IL-6 levels decreased significantly in patients who survived; in contrast no significant change was shown in serum IL-6 levels of non survivors.

Another cytokine was measured in our study which was Interleukin-10; (IL-10) is a cytokine with anti-inflammatory properties, expressed by many cells of innate and adaptive immune response. IL-10 has a crucial role in limiting the immune response to infection thereby preventing inflammation and excessive damage to the host.

IL-10 has multiple functions. IL-10, in collaboration with prostaglandin E2, inhibits pro-inflammatory cytokine synthesis. It plays an important role in the control of immune reactions during systemic infections. IL-10 also decreases the synthesis of IL-1 and IL-6.

In our study, IL-10 serum levels were increased at the end of the study in the three studied groups with more increase in the third group of the large taurine dose level (30 mg/kg/day) but there was no statistically significant difference between the 3 groups. Also the delta change between baseline and end of the study showed no significant difference between the 3 groups.

Results of our study align with the results reported by Slotwinski et al, who showed in their study of the use of early enteral immunonutrition in critically ill patients that there was no significant change in IL-10 levels between both the immunonutrition treated group and the control group.

At the end of the study, IL-10 serum levels increase in group 3 explained one of the reasons of the significant decrease of IL-6 in the same group receiving the highest dose of taurine (30 mg/kg/day) and having the highest IL-10 serum levels at the end of the study as IL-10 decreases the synthesis of the pro-inflammatory IL-6. CRP is an acute-phase protein produced in the liver in response to infection, injury, or inflammation; it is a commonly used marker for sepsis diagnosis. The typical acute response in adult humans includes increased CRP serum levels, where elevated CRP levels are indicators of acute phase reaction and bad prognosis.
Many studies showed that a high serum CRP concentration in patients within 24 hours of admission to hospital is indicative of sepsis and can differentiate between septic patients and non-infectious Systemic Inflammatory Response Syndrome (SIRS).\(^{50}\) In the same concern, Yousef et al showed that CRP level on admission was higher in sepsis group compared to non-infectious SIRS group.\(^{51}\)

In our study, CRP serum levels were elevated at baseline of the study for the three groups which is an indicator of diagnosis of sepsis in all the three groups’ patients with no statistical significant difference between the three groups. On the other hand, the delta change between the CRP serum levels of baseline and end of the study showed statistically significant difference between the three studied groups with the significant decrease in the third group when compared by group 1 and group 2.

CRP production is a part of a larger picture of the acute phase response. This is principally regulated by the cytokine IL-6 \(^{52}\), which can explain the significant decrease of IL-6 serum levels in group 3 which correlates also with the significant decrease of CRP in the same group leading to suppression of the hyper pro-inflammatory phase caused by increase of IL-6 and regulating the acute phase response caused by elevated CRP levels.

CRP serum levels decrease can also be explained by the use of antibiotics in the three groups as a basic treatment for sepsis which causes suppression of the inflammatory response and the acute phase and decrease in serum levels of CRP. TLC was also measured in our study in the three studied groups at baseline and at the end of the study. TLC serum levels were found to decrease in the three study groups but with no statistically significant difference between them.

CONCLUSION: In conclusion, this is the first study to report that taurine possesses a protective role in the sepsis process. The present study findings demonstrated that a therapeutic effect of taurine is mediated through restoring the balance between pro-inflammatory and anti-inflammatory cytokines. The data further support that the balance between pro- and anti-inflammatory factors during sepsis is necessary for better prognosis and improving the quality of life for septic patients, and they provide a novel mechanism through which taurine participates in septic responses.

Further investigations are needed on the precise mechanisms as well as taurine's function as an immunomodulator in the management of sepsis. The present study provided experimental evidence for exploiting taurine as an immunonutrient, bringing hope to a large number of sepsis patients.

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