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ORIGINAL ARTICLE

Enteric bacterial loads are associated with interleukin-6 levels in systemic inflammatory response syndrome patients

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KEYWORDS
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Abstract *Background:* Loss of intestinal integrity is a critical contributor to excessive inflammation following severe trauma or major surgery. In the case of enterocyte damage, intestinal fatty acid-binding protein (IFABP) is released into the extracellular space. Excessive production of interleukin (IL)-6 can induce systemic inflammatory response syndrome (SIRS). However, the correlation of IL-6 with gut barrier failure and bacterial translocation in critically ill patients has not been well characterized.
Purposes: To define the relationship between enteric bacterial loads and IL-6 levels in patients with SIRS.
Methods: Variables related to prognosis and treatment were measured in 85 patients with SIRS upon admission to the emergency room. IL-6 and IFABP were measured using an enzyme-linked immunosorbent assay. Enteric bacterial loads in blood were measured through quantitative real-time polymerase chain reaction with primers specific for enteric bacteria.
Results: Multivariate analysis revealed a positive correlation between enteric bacterial loads and IL-6 levels in blood. Elevated IFABP concentration was associated with low blood pressure, high respiration rate, hyperglycemia, and high Sequential Organ Failure Assessment score. Elevated C-reactive protein concentrations were associated with higher soluble CD14 levels in blood.

Conflict of interest: The authors have declared that no conflict of interest exists.

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Conclusion: Enterocyte damage is associated with hypotension and tachypnia in patients with SIRS. Gut function failure may permit enteric bacteria to enter the blood, thereby elevating IL-6 levels and inducing a systemic inflammatory response, resulting in multiple organ failure.

1. Introduction

Excessive production of proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 by immunocompetent cells can induce systemic inflammatory response syndrome (SIRS).[1] Among these proinflammatory cytokines, IL-6 has a longer half-life than TNF-α and IL-1β do, and blood IL-6 levels remain consistently elevated in people with various diseases.[1] IL-6 has been implicated as being responsible for increased gut mucosal permeability in mice with a condition associated with systemic inflammation, namely, polymicrobial peritonitis induced by cecal ligation and perforation.[2] IL-6 is a pleiotropic cytokine involved in both proinflammatory and anti-inflammatory responses by regulating leukocyte function and apoptosis.[3,4] It is a crucial cytokine associated with inflammatory bowel disease as well as other chronic inflammatory diseases and cancer.[5] However, the correlation of IL-6 with gut barrier failure and bacterial translocation in critically ill patients has not been well characterized.

The intestinal tract acts as a major physical barrier between the microflora and internal host tissue, and it responds to the mucosal innate system through commensal microflora.[6] The mucosal barrier is composed of epithelial apical junction complexes, consisting of tight junctions and adherence junctions.[7] Gut barrier function failure due to a major stress insult permits bacterial and endotoxin translocation, which triggers systemic cytokines and exacerbates a systemic immunoinflammatory response that results in organ failure.[8] Intestinal barrier failure is a crucial issue in the treatment of critically ill patients. Bacterial translocation from the intestinal tract is a major cause of thermal injury-induced sepsis and mortality.[9] Providing enteral nutrients shortly after injury alters the gut flora and protects the immunocompromised, stressed, or thermally injured patients through an unknown mechanism.[10] However, small intestine dysfunction is frequently underdiagnosed and associated with poor prognosis in critically ill patients.[11]

Intestinal fatty acid-binding protein (IFABP), which is a small cytoplasmic protein specifically localized in small bowel enterocytes and involved in fatty acid transport, is normally undetectable in plasma.[12] In healthy adults, small bowel hypoperfusion during submaximal effort was shown to cause acute reduction of enterocyte mass.[13] In the case of enterocyte damage, IFABP is released into the extracellular space, leading to increased concentrations of IFABP in plasma and urine. Patients with septic shock show increased urinary IFABP concentrations, suggesting that the shock condition is associated with enterocyte damage.[14] Failure of the gut mucosal barrier to exclude bacteria and endotoxins from the portal and systemic circulation is responsible for the development of sepsis and multiple organ failure.[8] Although experimental data are compelling, corroborative evidence from studies involving patients with sepsis is scarce.

Intestinal failure is one the most frequent complications among patients with sepsis. However, treatment interventions aimed at improving gastrointestinal (GI) perfusion have failed to improve outcomes.[15] We hypothesize that gut barrier function failure due to a major stress insult may permit bacterial translocation, triggering IL-6 release and resulting in organ failure. To test this hypothesis, we examined the IFABP levels, IL-6 levels, enteric bacterial loads, and soluble CD14 (sCD14) in the blood of patients with SIRS and defined the relationship with variables related to prognosis and treatment. The primary objective of this study was to evaluate the changes in blood IL-6 levels in these patients and the relationship of the changes with enteric bacterial loads. The second objective was to identify the factors associated with plasma IFABP concentrations in a population of patients with SIRS. In the future, controlling the contributing factors of enterocyte damage to reduce enteric bacteria translocation in patients with SIRS could be a useful therapeutic strategy for preventing multiple organ failure in patients with severe trauma or those having undergone major surgery.

2. Methods

2.1. Study setting and patients

This prospective study was conducted at the emergency department of the Zuoying Armed Forces General Hospital, Kaohsiung, Taiwan. This study was approved by the local ethics committee (EC-No. ZAFGH 101-06) and conducted in accordance with the guidelines of the Declaration of Helsinki (1964), including current revisions. Patients admitted to the emergency department were screened for the following inclusion criteria: two or more SIRS criteria [temperature, $>38^{\circ}C$ or $<36^{\circ}C$; pulse, >90 beats/min; respiratory rate, >20 breaths/min; or $Paco_2$, <32 mmHg; WBC count, $>12{,}000$ or <4000 cells/μL or $>10\%$ immature (band forms)], age >20 years, and able to give consent. The exclusion criteria for participation in the study were as follows: malignancies; surgery within 72 hours prior to admission; infection with HIV, fungi, or parasites; or inability to sign the consent form. The patients were informed about the trial, and they signed a consent form to confirm their participation. To ensure anonymity, every participant was consecutively assigned an identification number, which was used for further analysis.

2.2. Data collection

Variables at admission to the emergency department were retrospectively collected. The following variables were recorded: age, sex, systolic blood pressure (BP), diastolic BP, mean arterial pressure, respiration rate, blood sugar, and Sequential Organ Failure Assessment (SOFA) score upon emergency room (ER) admission.

2.3. Serum IL-6 and soluble CD14 measurement

Serum IL-6 levels were measured through an enzyme-linked immunosorbent assay (ELISA). The human ELISA kit (eBioscience) was used for the IL-6 assay. The blood was centrifuged at 1000*g*, at 4°C for 15 minutes, and the serum was collected for use. The ELISA plates were coated with 100 μL capture antibody per well at 4°C and maintained overnight. After appropriate washing, 200 μL of assay dilution buffer was added per well at room temperature for blocking for 1 hour. The sample and serial dilutions of standards were added to the plate and incubated at 4°C overnight. After a coat of detection antibody was applied, avidin—HRP was added and incubated at room temperature for 30 minutes. The substrate 3,3′,5,5′-tetramethylbenzidine was added and incubated for 15 minutes. Finally, 2N H_2SO_4 was added to stop the reaction, and the absorbance at 450 nm was measured using an ELISA reader.

Soluble CD14 concentrations in blood were measured through an ELISA (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

2.4. Bacterial DNA isolation and quantitative real-time polymerase chain reaction

Bacterial DNA was isolated from human whole blood by using a kit (GeneMark). Eight volumes of 1× RBC lysis solution was added to the whole blood (200 μL) and incubated at room temperature for 5 minutes. For the real-time polymerase chain reaction (PCR) analysis, 2 ng of experimental sample DNA was added to 20 μL of the reaction mixture containing supermix and 0.2mM each of sense and antisense primers. The primers used to amplify the *wec* F gene are as follows: forward, TGCCGTAAC TTCGGGAGAAGGCA; reverse, TCAAGGCTCAATGTTCAGT GTC. These primers are specific for enteric bacterial species.[16] Amplification was then performed in an iCycler Fluorescence Thermocycler (ABI). Fluorescence data were captured during the 10-second dwell to ensure that primer dimmers were not contributing to the fluorescence signal generated with SYBR Green I DNA dye. The experimental threshold cycle was compared with an *Escherichia coli* bacterial DNA standard curve for quantification.

2.5. IFABP measurement

IFABP is an early and sensitive marker of enterocyte injury. To evaluate the presence and extent of enterocyte injury, serum IFABP concentrations in blood were measured using the ELISA test (R&D Systems) following the manufacturer's instructions.

2.6. Statistical analysis

Data are expressed as the mean ± standard deviation, median and interquartile ranges, and number and percentage. For normally distributed data, continuous variables were compared using the Student *t* test. Pearson's correlation coefficients were used to evaluate the correlations between continuous variables. Linear regression modeling was performed to test for the effect of the different variables on IFABP or IL6 concentrations. Variables with $p < 0.05$ in the univariable linear regression analysis were selected for a multivariate analysis. Statistical analysis was performed using IBM SPSS Statistics Version 18 (IBM Company, Chicago, U.S.A.). Statistical significance was defined at $p < 0.05$ (two-tailed).

3. Results

3.1. Baseline and parameters of participants

Between January 1, 2010 and January 1, 2011, 98 patients were included in the study. Thirteen patients were excluded because of missing data. Thus, 85 patients were enrolled in the study. The main characteristics of the studied patients are listed in Table 1. Chest disease was diagnosed in 23 patients (27%). Among those patients, the most common diagnosis was pneumonia. Cardiovascular disease was diagnosed in four patients (5%). Urinary tract infection was diagnosed in 18 patients (21%). Among these patients, the most common diagnosis was urosepsis. Intra-abdominal infection was observed in six patients (7%). Burns were diagnosed in eight patients (9%). GI tract bleeding was diagnosed in three patients (3.5%). Soft tissue infection was diagnosed in 15 patients (18%). Eight patients were categorized as "others" (4 with acute renal failure, 2 with pulmonary edema, and 2 with ileus). Seven patients (8%) died in the intensive care unit (ICU). Seventy-eight patients (92%) survived.

3.2. Enteric bacteria primers in quantitative assessment of bacterial translocation

This protocol involves primers that amplify a region of *wec* F, a gene that encodes a transferase, which is crucial for creating the repetitive trisaccharide unit of the enterobacterial (EB) common antigen. These EB primers amplify DNA from enteric bacterial species, including *E. coli*, *Klebsiella pneumoniae*, *Salmonella* spp., *Enerobacter* spp., *Serratia* spp., and *Shigella* spp.[16] To determine whether these primers could be used to quantitatively measure enteric bacterial loads, we serially spiked human blood samples with increasing amounts of *K. pneumoniae*. After DNA isolation, we compared the amount of enteric bacterial DNA to a known *E. coli* standard (Figure 1A) through quantitative real-time PCR using the EB primers. The load of bacterial DNA in the spiked blood correlated significantly with the *E. coli* standard (Figure 1B), suggesting that these primers can be used to quantitatively measure the presence of enteric bacteria. We also confirmed that EB primers amplify DNA from *E. coli*. and *K. pneumoniae*, but not from Gram-positive organisms (data not shown).

Table 1 Baseline characteristics of the study populations at SER admission.

Variable	$n = 85$
Age (yr)	67 [56–83]
Sex	
Male	54 (63)
Female	31 (37)
Systolic blood pressure (mm Hg)	99 [78–119]
Diastolic blood pressure (mm Hg)	55 [46–67]
Mean blood pressure (mm Hg)	71 [55–84]
Respiration rate (breaths/min)	23 [20–29]
Heart rate (beats/min)	110 [96–119]
C-reactive protein (mg/L)	9.2 [3.7–17.1]
IL-6 (pg/ml)	120.6 [46.8–398]
sCD14 (pg/ml)	2543 [397–3797]
IFABP (pg/ml)	24.01 [4.92–135.7]
Blood sugar (mg/dl)	168 [137–268]
APACHE II	19 [10–27]
SOFA	7 [1–9]
Lactate	2.39 [1.84–5.81]
Mortality	7 [8]
Survivor	78 [92]
Disease category	
Chest diseases	23 [32]
CV diseases	4 [5]
UTI	18 [21]
IAI	6 [7]
Burn	8 [9]
GI bleeding	3 [4]
Soft tissue infection	15 [17]
Others	8 [9]

n (%); median [interquartile range].
IFABP = intestinal fatty acid-binding protein.
APACHE II = acute physiology and chronic health evaluation II.
SOFA = Sequential Organ Failure Assessment.
sCD14 = soluble CD14.
UTI = urinary tract infection.
IAI = intra-abdomen infection.
CV = cardiovascular.
GI = gastrointestinal.

3.3. Enteric bacterial translocation correlated positively with blood IL-6 levels in patients with SIRS

We evaluated the bacterial translocation of patients with SIRS patients upon admission to the ER by measuring the bacterial loads of enteric organisms in blood. Using a linear regression analysis, we compared the enteric bacterial loads with IL-6 in blood measured in the patients upon admission to the ER. The blood IL-6 levels ranged between 100 and 1000 pg/mL in surviving patients with sepsis.[17] The average blood IL-6 levels in our patients were 120.6 pg/mL. The results of the multivariate regression analysis demonstrated a positive correlation between the enteric bacterial loads and IL-6 levels (Table 2), suggesting that the elevated IL-6 levels correlated positively with the enteric bacterial loads in these patients.

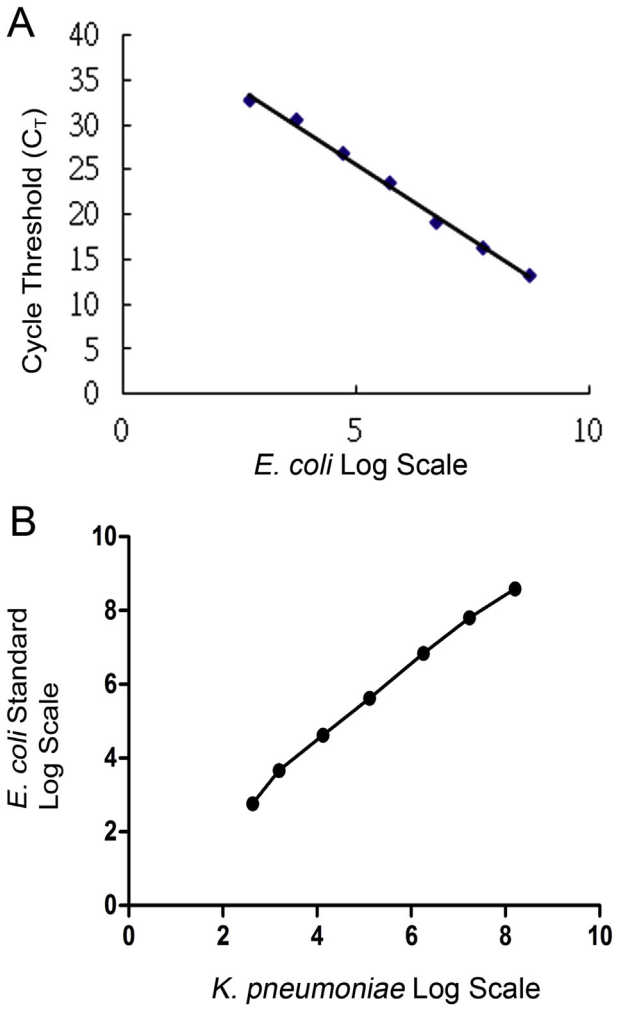

Figure 1 EB primers are useful in the quantitative measurement of enteric bacterial loads. (A). Real-time PCR standard curve obtained by plotting the threshold cycle values against an *Escherichia coli* log scale. (B) Human blood was spiked with serially increasing inocula of *Klebsiella pneumoniae*. Subsequently, DNA was isolated, and real-time PCR was performed with EB primers to compare the quantities of amplicons with the quantity of an *E. coli* DNA standard. We found a significant correlation between the *E. coli* standard and the DNA isolated from the spiked blood. Ct = cycle threshold; EB = enterobacterial; PCR = polymerase chain reaction.

3.4. Hypotension was associated with enterocyte injury in patients with SIRS

The results of a linear regression analysis between IFABP concentrations and BP upon admission to the ER indicated that the patients with low systolic pressure had significantly elevated IFABP concentrations ($r = 0.361$, $p = 0.003$; Figure 2A). Patients with low diastolic BP also had significantly elevated IFABP concentrations ($r = 0.319$, $p = 0.012$; Figure 2B). Similarly, patients with low mean BP exhibited increased IFABP concentrations ($r = 0.359$, $p = 0.003$; Figure 2C). Moreover, the plasma IFABP concentrations in

Table 2 Results of multivariate regression analysis for the contributing factors of IL-6.

	β	*p* value	*r*
Enteric bacterial loads	0.531	0.001*	0.533
sCD14	.049	NS	
SBP	−.110	NS	
DBP	.118	NS	
Glucose	−.089	NS	
APACH II	.375	NS	
SOFA	.166	NS	
RR	.213	NS	
IFABP	.021	NS	

* = statistically significant
SBP = systolic blood pressure.
DBP = diastolic blood pressure.
RR = respiration rate.
IFABP = intestinal fatty acid-binding protein.
APACHE II = acute physiology and chronic health evaluation II.
SOFA = Sequential Organ Failure Assessment.
sCD14 = soluble CD14.
NS = not significant.

patients with a systolic BP of <90 mmHg were higher than those in patients with a systolic BP of >90 mmHg ($p < 0.05$; Figure 2D). These results suggest that hypotension is associated with enterocyte injury, and patients with a systolic BP of <90 mmHg show increased enterocyte damage.

3.5. Tachypnea was associated with enterocyte damage in patients with SIRS

The results of a linear regression analysis between IFABP concentrations and the respiration rate upon admission to the ER indicated that IFABP concentrations increased with the respiration rate ($r = 0.3$, $p = 0.021$; Figure 3A). Moreover, plasma IFABP concentrations were significantly higher in patients with a respiration rate of >20 breaths/min than in those with a respiration rate of <20 breaths/min ($p < 0.05$; Figure 3B). These results suggest that tachypnea is associated with enterocyte injury, and enterocyte damage is significantly higher in patients with a respiration rate of >20 breaths/min.

3.6. High blood sugar levels were associated with enterocyte injury in patients with SIRS

The association of hyperglycemia with increased mortality in patients with SIRS is unclear. The results of a linear regression analysis between IFABP concentrations and blood sugar upon admission to the ER suggested that the patients with increased blood sugar levels had significantly elevated IFABP concentrations ($r = 0.291$, $p < 0.05$). We tested several values in the linear regression analysis and found that 230 mg/dL is the optimum cutoff value. Moreover, plasma IFABP concentrations were significantly higher in

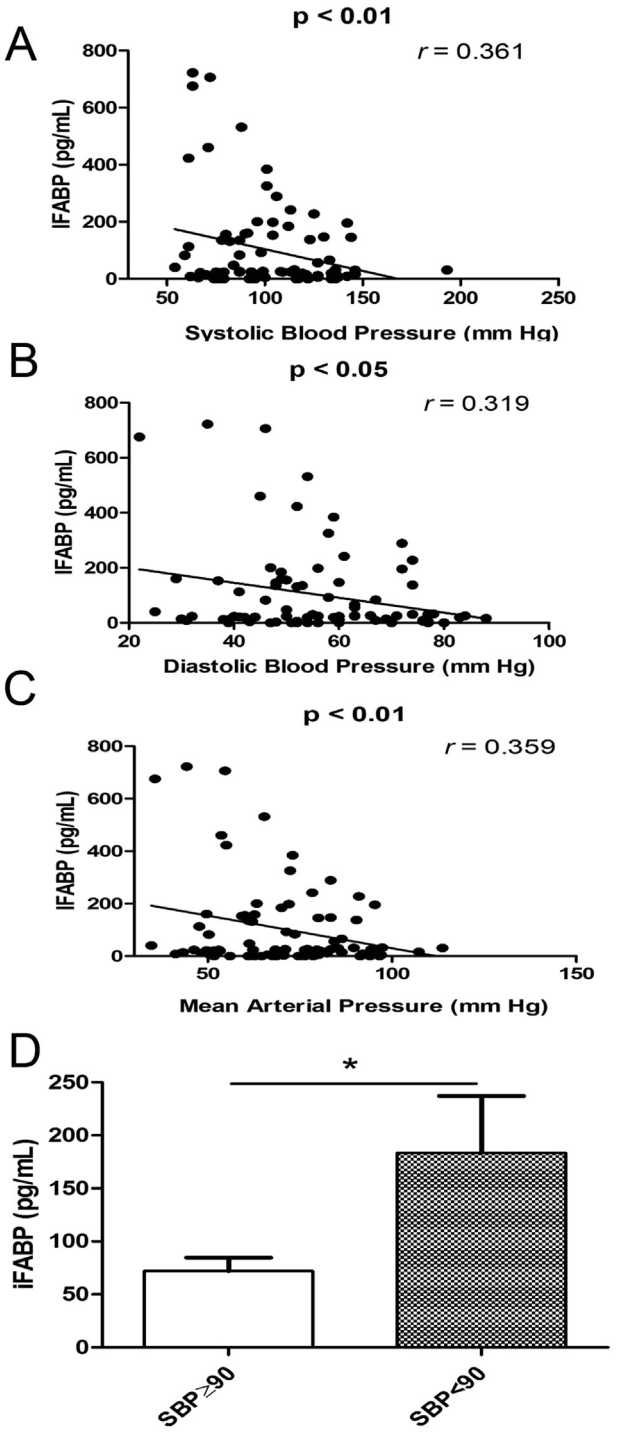

Figure 2 Lower BP was associated with elevated IFABP levels in patients with SIRS. (A). Patients with low systolic pressure had elevated IFABP levels. (B) Patients with low diastolic BP upon admission to the ER also had elevated IFABP levels. (C) Patients with low mean BP exhibited elevated IFABP levels. (D) Serum IFABP levels were higher in patients with a systolic BP of <90 mmHg upon to the ER than in those with a systolic BP ≥90 mmHg. *$p < 0.05$. BP = blood pressure; ER = emergency room; IFABP = intestinal fatty acid-binding protein; SIRS = systemic inflammatory response syndrome.

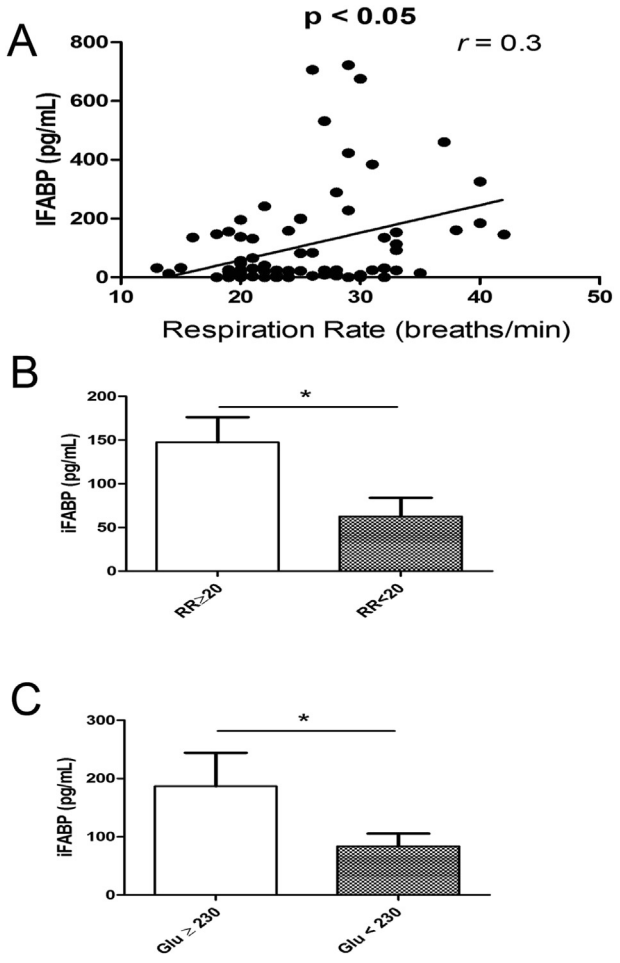

Figure 3 Higher respiration rate and hyperglycemia were associated with the elevated IFABP levels in the patients with SIRS. (A) Patients with a high respiration rate showed elevated IFABP levels. (B) Serum IFABP levels were higher in the patients with a respiration rate of ≥20 breaths/min than in those with a respiration rate of <20 breaths/min upon admission to the ER. (C) Plasma IFABP concentrations were higher in patients with a blood sugar level of ≥230 mg/dL than in those with a blood sugar of <230 mg/dL upon admission to the ER. *$p < 0.05$. ER = emergency room; IFABP = intestinal fatty acid-binding protein; SIRS = systemic inflammatory response syndrome.

patients with blood sugar levels ≥230 mg/dL than in those with blood sugar levels of <230 mg/dL ($p < 0.05$; Figure 3C).

These results indicate that hyperglycemia is associated with enterocyte injury, and enterocyte damage significant increases in patients with blood sugar levels of ≥230 mg/dL.

3.7. Enterocyte damage was associated with SOFA score

To examine the relationship between enterocyte injury and multiple organ failure, we calculated the SOFA scores upon admission to the ER. The results of a linear regression analysis between IFABP concentrations and SOFA scores indicated that the IFABP concentrations increased with the SOFA score ($r = 0.331$, $p = 0.007$; Figure 4). These results

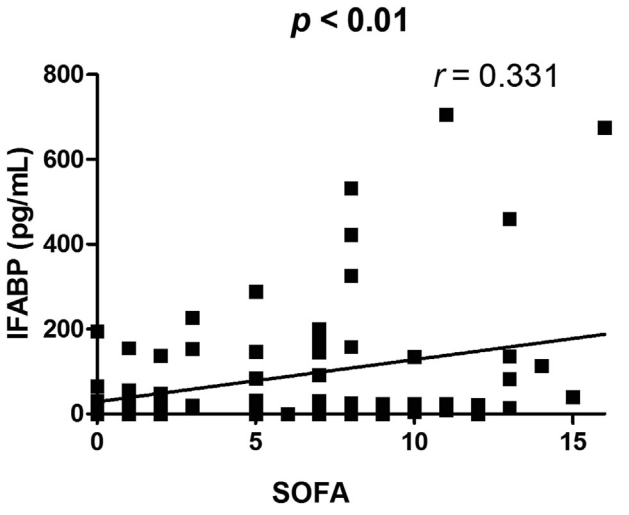

Figure 4 Higher SOFA scores correlated significantly with elevated IFABP levels in the patients with SIRS ($r = 0.319$, $p = 0.007$). IFABP = intestinal fatty acid-binding protein; SOFA = Sequential Organ Failure Assessment; SIRS = systemic inflammatory response syndrome.

suggest that higher IFABP concentrations indicate multiple organ failure in patients with SIRS.

3.8. Soluble CD14 was associated with C-reactive protein

To examine the relationship between sCD14 and inflammation, C-reactive protein levels were measured upon admission to the ER. The results of a linear regression analysis between sCD14 concentrations and C-reactive protein levels indicated a significantly positive correlation between them ($r = 0.476$, $p < 0.0001$; Figure 5).

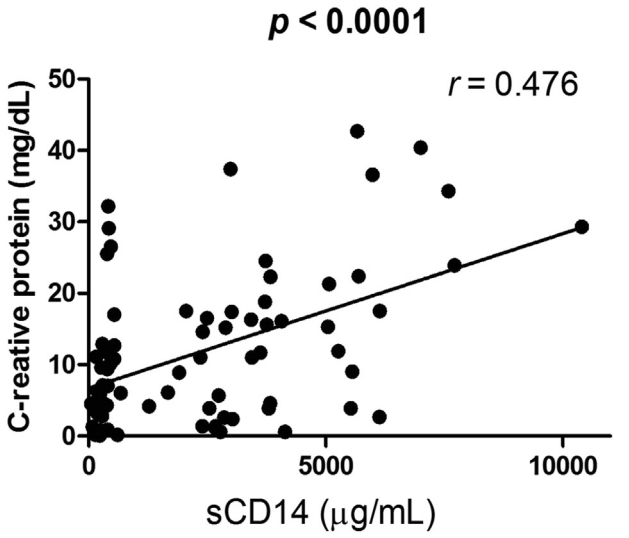

Figure 5 Elevated C-reactive protein concentrations were associated with high sCD14 levels in patients with SIRS. sCD14 = soluble CD14; SIRS = systemic inflammatory response syndrome.

4. Discussion

The role of gut injury and inflammation in major trauma-induced SIRS and multiple organ dysfunction syndrome has been known for decades.[18] Cross-talk between commensal intestinal microflora and systemic innate immunity remains undefined. Deitch[19] discovered that the systemic spread of gut-derived factors leading to lung injury and systemic inflammation occurs through the intestinal lymphatics. Bacterial fragments and toxic components, such as lipopolysaccharides, lipoteichoic acid, endotoxin, superantigen, and enterotoxin invasion, can induce inflammatory cytokine secretion and trigger a systemic inflammatory response.[20] Patients with end-stage renal disease have significantly higher IL-6 levels when bacterial DNA is present.[21] Furthermore, blood IL-6 levels are also associated with the survival of patients with burns and sepsis.[22] Oda et al[23] reported that IL-6 levels are elevated in patients with SIRS or sepsis and extremely high in patients with septic shock. However, blood IL-6 levels upon admission into ER do not differ significantly between survivors and nonsurvivors.[23] Moreover, Giannoudis et al[24] reported that elevated IL-6 is a reliable diagnostic indicator of a SIRS state. A cutoff value of 200 pg/dL was shown to be significantly diagnostic of a SIRS state.[24] We previously demonstrated that thermal injury does not induce high IL-6 levels in mice on the 2nd day after the injury. IL-6 levels were significantly elevated in mice subjected to a thermal injury and then treated with antibiotics, suggesting that bacterial translocation induced by thermal injury followed by antibiotic treatment may increase IL-6 levels in blood.[25] High blood IL-6 levels correlated with hyperglycemia and difficulties in glucose control in septic patients.[17] Our data further indicate that enteric bacterial loads in blood correlate positively with IL-6 levels in the blood of patients with SIRS. This suggests that IL-6 levels in blood may be an indicator of enteric bacterial translocation in these patients. In addition, enteric bacterial translocation may induce a pulmonary or systemic inflammatory response through IL-6 levels in blood. In summary, our results indicate that a failure of gut barrier function due to mesenteric ischemia permits bacterial and endotoxin translocation, which may triggers systemic IL-6 levels and exacerbate a systemic inflammatory response that results in organ failure.

SIRS is defined as an acute host reaction to various stimuli including both infectious and noninfectious causes. The definition of SIRS is based on physiological parameters including body temperature, heart rate, respiration rate (of oxygen saturation), and abnormal leukocytes counts (leukocytosis, an elevation of immature neutrophils or leukopenia). GI dysfunction is common in critically ill patients. Fifty-nine percent of critically ill patients have at least one GI symptom during their stay in ICUs.[26] However, evaluating the small bowel is difficult; it is a deep organ, and critically ill patients are frequently unable to inform clinicians about digestive complaints. Therefore, small bowel dysfunction may sometimes be occult or misdiagnosed. The role of gut injury and inflammation in major trauma-induced SIRS and multiple organ dysfunction syndrome have been known for decades without clear clinical evidence. The gut origin hypothesis suggests that gut barrier function failure due to a major stress insult permits bacterial and endotoxin translocation, which triggers splanchnic cytokines to perpetuate and exacerbate a systemic immunoinflammatory response that may result in organ failure.[27] However, gut barrier dysfunction could not be directly linked to sepsis.[27] Our data indicate that enterocyte damage upon admission to the ER is common in patients with SIRS and increased enterocyte damage related to their SOFA scores. In summary, our data suggest that serum IFABP may be a reliable indicator of multiple organ failure in patients with SIRS, and that increased enterocyte damage indicates multiple organ failure. From this viewpoint, increased IFABP may indicate intestinal barrier failure and subsequent bacterial translocation in critically ill patients. Using therapeutic strategies such as early enteral feeding or vasopressor agents to decrease enterocyte damage could be useful in preventing multiple organ failure in critically ill patients.

The pathophysiology of enterocyte damage in critically ill patients may be mediated by nonocclusive mesenteric ischemia. Acute mesenteric ischemia is defined as a recent and rapid imbalance between the demand and the delivery of oxygen and nutrients to the splanchnic area.[28] We found that low systolic BP, a cause of nonocclusive mesenteric ischemia, was associated with high IFABP concentrations in the investigated patients. These results were further corroborated by the negative correlation between diastolic BP and IFABP concentration. Moreover, the results of the multivariate analysis indicate that mean BP correlates negatively with IFABP concentration. In summary, our data suggest that low BP in patients with SIRS may induce enterocyte damage through mesenteric ischemia. Moreover, serum IFABP concentrations are significantly elevated in patients with systolic BP below 90 mmHg, suggesting that maintaining the systolic BP above 90 mmHg is crucial for preventing enterocyte damage and subsequent organ failure in critically ill patients.

A recent study found that patients with chronic obstructive pulmonary disease (COPD) have altered intestinal permeability at rest. Furthermore, performing activities of daily living significantly increased plasma IFABP concentrations in patients with COPD but not in a group of controls. These findings indicate that patients with COPD are prone to have altered GI tract function.[29] However, the correlation between respiratory function and intestinal barrier failure in critically ill patients has not been characterized. Deitch[19] reported that the systemic spread of gut-derived factors leading to lung injury and systemic inflammation occurs through the intestinal lymphatics. Our data further suggest that tachypnea is associated with enterocyte damage. Because the respiratory rate was checked upon admission to the ER after the enterocyte damage had already occurred, we speculate that tachypnea is a result, rather than a cause, of enterocyte damage in patients with SIRS.

Gut-derived endotoxin is a contributing factor for the development of low-grade inflammation, which is a hallmark for type 2 diabetes development.[30] Diabetes mellitus is a chronic, progressive, medically incurable disease, and is predominantly an intestinal disease. Increased intestinal

permeability, altered intestinal microbiota, and subsequent metabolic endotoxemia have been suggested to be causal factors in type 2 diabetes.[31] However, patients with type 2 diabetes do not shown higher intestinal permeability compared with healthy controls.[32] Hyperglycemia and insulin resistance are virtually universal in sepsis. Hyperglycemia is potentially harmful because it acts as a procoagulant,[33] induces apoptosis,[34] impairs neutrophil function, impairs wound healing, and is associated with an increased risk of death. However, the appropriate target glucose ranges in patients with sepsis are unknown. Furthermore, insulin therapy in ICU patients does not cause a significant difference in mortality.[35] Our data suggest that hyperglycemia is associated with enterocyte damage in patients with SIRS. Increased enterocyte damage may cause increased intestinal permeability and subsequent metabolic endotoxemia in patients with hyperglycemia. Moreover, our data suggest that enterocyte damage is significantly higher in patients with blood sugar levels of $\geq$230 mg/dL. In the future, maintaining blood sugar at $<$230 mg/dL may assist in preventing an increase in intestinal permeability and subsequent metabolic endotoxemia in patients with SIRS.

In summary, enterocyte injury is a crucial factor contributing to excessive inflammation. Gut barrier function failure may permit enteric bacteria to enter the blood, thereby triggering an increase in systemic IL-6 levels. Hypotension may be a major factor for enterocyte damage in patients with SIRS admitted to the ER. Respiration rate correlates positively with enterocyte damage. Maintaining blood sugar levels at $<$230 mg/dL may help prevent enterocyte damage in patients with SIRS. C-reactive protein concentrations correlate positively with sCD14 levels. Using therapeutic strategies to alleviate hypotension and tachypnea may further prevent intestinal complications, bacterial translocation, and subsequent multiple organ failure in patients with severe trauma.

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