Medicine®

OPEN

Association between endotoxemia and enterocyte injury and clinical course in patients with gram-positive septic shock
A posthoc analysis of a prospective observational study

Motohiro Sekino, MD, PhD[a],[*], Hiroyuki Funaoka, PhD[b], Shuntaro Sato, PhD[c], Takashi Egashira, MD[a], Haruka Inoue, MD[d], Rintaro Yano, MD, PhD[a], Sojiro Matsumoto, MD[a], Taiga Ichinomiya, MD, PhD[e], Ushio Higashijima, MD[a], Shuhei Matsumoto, MD, PhD[a], Tetsuya Hara, MD, PhD[e]

Abstract

Endotoxemia often occurs in patients with gram-positive infections. The possible mechanism is thought to be bacterial translocation after enterocyte hypoperfusion injury. However, the association between endotoxemia and enterocyte injury among patients with gram-positive septic shock has never been assessed. The aim of this study was to evaluate the association between endotoxemia and enterocyte injury in gram-positive septic shock patients and to evaluate the association among endotoxemia, subsequent clinical course, and other related factors.

This was a posthoc analysis of a prospective observational study that evaluated the capability of intestinal fatty acid-binding protein (I-FABP), an indicator of enterocyte injury, to predict mortality. Among 57 patients in septic shock, those whose causative microorganisms were gram positive were included. The correlation between endotoxin activity (EA), which indicates endotoxemia, and I-FABP levels upon admission to the intensive care unit (ICU), the clinical course, and other related factors were evaluated.

A total of 21 patients were examined. One-third of the patients presented with high EA levels at the time of ICU admission. However, there was no significant correlation between EA and I-FABP levels (Spearman $\rho = 0.002$, $P = .993$). Additionally, high EA levels were not associated with abdominal complications after ICU admission or mortality. Similarly, high EA levels were not associated with severity scores, inotropic scores, or lactate levels upon ICU admission, which were previously reported to be factors related to high EA levels.

In this posthoc analysis, no correlation was observed between endotoxemia and enterocyte injury among patients in gram-positive septic shock. Additionally, high EA levels were not associated with the clinical course and reported factors related to endotoxemia. Although our results need to be validated in a large prospective cohort study, hypoperfusion enterocyte injury might not be a cause of endotoxemia in these patients. Thus, if there is no correlation between EA and I-FABP levels, other mechanisms that induce high EA levels among patients with gram-positive septic shock should be elucidated.

Abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation, EA = endotoxin activity, ICU = intensive care unit, I-FABP = intestinal fatty acid-binding protein, MOF = multiple organ failure, PMX-DHP = polymyxin B-direct hemoperfusion, SOFA = Sequential Organ Failure Assessment.

Keywords: endotoxin, endotoxin activity assay, hypoperfusion, intensive care unit, intestinal ischemia, sepsis

[a] Division of Intensive Care, Nagasaki University Hospital, Sakamoto, Nagasaki, [b] SB Bioscience Co, Ltd, Suita, Osaka, [c] Clinical Research Center, [d] Department of Anesthesiology, Nagasaki University Hospital, [e] Department of Anesthesiology, Nagasaki University Graduate School of Biomedical Science, Sakamoto, Nagasaki, Japan.

[*] Correspondence: Motohiro Sekino, Division of Intensive Care, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan (e-mail: m-sekino@nagasaki-u.ac.jp).

1. Introduction

Endotoxin, or lipopolysaccharide, is a main component in the membranes of gram-negative bacteria and plays a key role as an activator of innate immunity and inflammatory responses leading to multiple organ failure (MOF) and, eventually, poor patient outcome.[1,2] It is well-known that there is a close association between endotoxemia and gram-negative infections, and between the endotoxin levels and the risk of MOF after intensive care unit (ICU) admission.[3,4] Therefore, the removal of endotoxin by an absorption column is receiving increasing attention as a novel therapeutic option for gram-negative septic shock patients.[5] On the contrary, it has also been reported that endotoxemia often occurs in patients with gram-positive infections.[4,6,7] However, the impact on mortality and the cause of endotoxemia have never been elucidated. The possible mechanism of endotoxemia in these cases is thought to be translocation of gut microbial flora and endotoxin secondary to intestinal hypoperfusion.[4,5] This hypothesis is supported by evidence of high levels of endotoxin detected in patients after cardiac surgery with cardiopulmonary bypass and patients following multiple trauma.[8,9] However,

these studies did not investigate the presence of intestinal hypoperfusion injury.

Intestinal fatty acid-binding protein (I-FABP) is a small (14–15 kDa) cytosolic protein specifically expressed by enterocytes in the small intestinal mucosa.[10] I-FABP is a useful marker of enterocyte injury, especially in the early stages of small bowel ischemia[11] and necrotizing enterocolitis.[12] Recently, the association between endotoxemia and enterocyte injury, as assessed by I-FABP, has been investigated in patients undergoing cardiopulmonary bypass[13] and patients after cardiac arrest.[14] However, to the best of our knowledge, the association between endotoxemia and enterocyte injury among patients in gram-positive septic shock has never been studied. Enterocyte injury might contribute to the pathophysiology of endotoxemia in such patients, and enterocyte injury and/or endotoxemia might be a potential therapeutic target.

The aim of this study was to evaluate the association between endotoxemia at the time of ICU admission and enterocyte injury based on I-FABP levels among patients in gram-positive septic shock. We also aimed to elucidate the association between endotoxemia and the subsequent clinical course, abdominal complications, and other factors related to endotoxemia.

2. Materials and methods

2.1. Study design and oversight

This study was a posthoc analysis of a prospective observational study on the capability of I-FABP levels to predict mortality, and the association between macroscopic tongue ischemia and enterocyte injury among patients in septic shock.[15,16] The data of the original study were used to address novel clinical questions with new statistical tests. The original study was conducted in a general ICU at Nagasaki University Hospital (Nagasaki, Japan) from May 2012 to March 2015. Ethics approval of the original study and this posthoc analysis was provided by the Institutional Review Board of Nagasaki University Hospital (Nos: 12042382 and 18082010), and written informed consent was obtained from the patients' relatives in the original study.

2.2. Study population

Over a period of 2 years 11 months, 100 adult (aged ≥18 years) patients with septic shock who received mechanical ventilation were prospectively screened for inclusion, and a total of 57 patients were enrolled in the original study.[15,16] Patients with confirmed or strongly suspected intestinal ischemia and/or necrosis as a source of infection, a history of previous small intestine surgery, chronic small bowel disease, pregnancy, uncontrolled bleeding, or in the terminal stage of a comorbid condition were excluded. Among the 57 patients in septic shock, this posthoc analysis included patients with confirmed gram-positive septic shock, and excluded patients whose causative microorganisms were gram-negative, fungi, mixed flora, and unknown according to the results of cultures (blood culture and culture from the source of infection) before and/or at ICU admission.

2.3. Data collection

For this study, we used the database of the original study.[15,16] Baseline values were recorded upon ICU admission and within the first 24 hours of admission, and included the following: age,

gender, Acute Physiology and Chronic Health Evaluation (APACHE) II score, Sequential Organ Failure Assessment (SOFA) score, inotropic score,[17] interventions during ICU stay for septic shock management including vasopressin, low-dose hydrocortisone, continuous renal replacement therapy, and polymyxin B-direct hemoperfusion (PMX-DHP),[5] which absorb circulating endotoxin, site of infection, causative organism, and bacteremia status. Additionally, the patients' estimated glomerular filtration rates (eGFRs) were calculated using the serum creatinine (Cr) level at the time of ICU admission according to the following equation for Japanese patients: eGFR (mL/min/1.73 m^2) = $194 \times Cr^{-1.094} \times age^{-0.287}$ (if female × 0.739).[18]

Blood chemistry tests were conducted on ICU admission to gauge the severity of septic shock, including evaluations of arterial lactate, procalcitonin, and serum N-terminal pro-B-type natriuretic peptide (NT-proBNP). Lactate levels were measured using an ABL800 FLEX system (Radiometer Medical, Copenhagen, Denmark). Procalcitonin and NT-proBNP levels were determined from electrochemiluminescence immunoassays (Roche Diagnostics, Indianapolis, IN). All these data were determined at Nagasaki University Hospital.

Clinical suspicion of bowel ischemia after ICU admission was evaluated by 2 independent critical care physicians. Reasons for suspicion included newly developed abdominal distention, persistent or newly developed lactic acidosis and/or shock, and gastrointestinal bleeding. Information was also collected regarding nonocclusive mesenteric ischemia (NOMI) and life-threatening abdominal complications that needed surgical intervention as determined by a radiologist and/or surgeon. Patient outcomes, including all-cause 28-day mortality and inpatient mortality, were also recorded.

2.4. Endotoxin activity assay

Endotoxin levels at ICU admission were measured using the chemiluminescent endotoxin activity (EA) assay (Spectral Diagnostics, Toronto, ON, Canada), as described previously, which were considered reliable compared to the chromogenic limulus amebocyte lysate assay.[4,19] Briefly, 50 μL samples of whole blood were incubated in duplicate with saturating concentrations of an anti-lipid A IgM antibody, and stimulated with opsonized zymosan. The resulting respiratory burst activity was detected as light release from the lumiphor luminol by a chemiluminometer (Berthold technologies, Bad Wildbad, Germany). By measuring basal and maximally stimulated responses, EA levels can be expressed in relative units derived from the integral of the basal and stimulated chemiluminescent responses. EA levels were classified as "low" (0.0–0.39), "intermediate" (0.40–0.59), and "high" (≥0.60). EA levels of 0.4 and 0.6 are approximately equivalent to endotoxin concentrations of 500 and 1500 pg/mL, respectively.[20] The EA levels were measured at Nagasaki University Hospital.

2.5. Serum I-FABP measurement

The I-FABP was used as a marker of enterocyte injury caused by intestinal hypoperfusion and ischemia. In the original study, blood samples were collected upon ICU admission (day 0) and again on days 1 to 7, 10, and 14 to examine the serum I-FABP levels. In this study, we used only the data obtained at the time of ICU admission because I-FABP is rapidly eliminated by kidney and renal replacement therapy after intensive care

treatment[15,21]; namely, reliability of I-FABP as a marker of enterocyte injury is highest at ICU admission. The details of the measuring method were described previously.[15] Briefly, all measurements were conducted at a single laboratory (DS Pharma Biomedical Co, Ltd, Osaka, Japan) using a human I-FABP-specific enzyme-linked immunosorbent assay (DS Pharma Biomedical Co, Ltd).[22] Previous studies applying the same technique determined that the mean serum I-FABP level in healthy volunteers was 1.1 ± 0.9 (range, 0.1–5.5) ng/mL,[22] and the optimal cutoff point for diagnosing intestinal ischemia was 9.1 ng/mL.[11] Attending critical care physicians were blinded to patient I-FABP results, therefore the results had no effect on clinical treatment decision making.

2.6. Statistical analysis

Baseline characteristics are presented as medians and interquartile ranges for quantitative variables and as frequencies for categorical variables. The correlation between EA and I-FABP levels at the time of ICU admission was evaluated using Spearman rank correlation coefficient, ρ. We then compared the incidence of clinically suspected bowel ischemia, actual life-threatening abdominal complications, and patient outcomes between patients with EA < 0.6 (low intermediate) and those with EA ≥ 0.6 (high). Additionally, we compared the APACHE II scores, SOFA scores, inotropic scores, lactate, and Cr levels, which are reported factors related to endotoxemia evaluated by EA levels.[4,7,23] NT pro-BNP[24] and procalcitonin,[25] which have been reported to be prognostic biomarkers for sepsis, were also compared. The association between variables was assessed using Wilcoxon rank sum testing and Fisher exact test.

All tests were 2-sided, and $P < .05$ was considered statistically significant. Statistical analyses were conducted using JMP pro 14 (SAS Institute Inc, Cary, NC).

3. Results

3.1. Study population

A total of 21 patients in gram-positive septic shock were selected for this posthoc analysis (Fig. 1). The patient' baseline characteristics are presented in Table 1. The median APACHE II score and SOFA score were relatively high at 30 (range: 26–35.5) and 13 (range: 10.5–15), respectively. Sites of infection were found in 7 cases (33.3%) with soft tissue/bone disease, 4 cases (19.0%) with cardiovascular disease, 4 cases (19.0%) with abdominal disease, 3 cases (14.3%) with lung/thorax disease, and 3 cases (14.3%) with other pathologic sites. Causative microorganisms with EA levels were as follows: 12 cases (57.1%) of Staphylococcus aureus (including 5 cases with methicillin-resistant S aureus) with median EA levels of 0.45 (range: 0.14–0.77), 5 cases (23.8%) of Streptococcus species (including 3 cases with S pneumonia, 1 case with S pyogenes, and 1 case with S agalactiae) with median EA

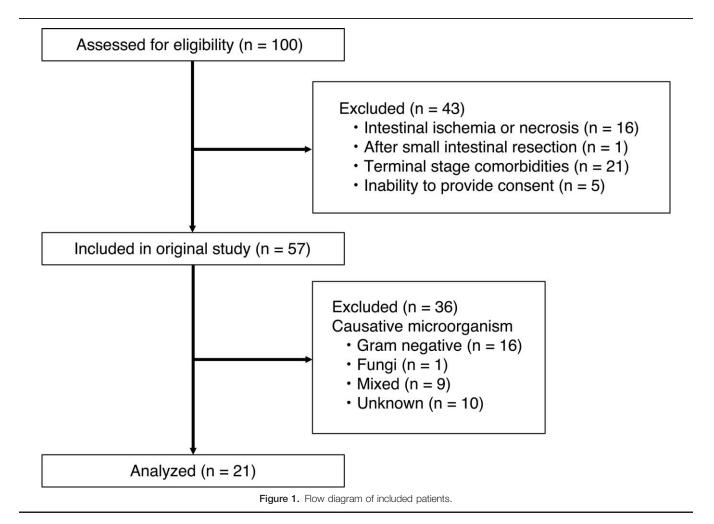Figure 1. Flow diagram of included patients.

Table 1

Summary of baseline characteristics.

Characteristics	Value
Age, yrs	74 (65.5–80.5)
Male sex, n (%)	9 (42.9)
APACHE II score	30 (26–35.5)
SOFA score	13 (10.5–15)
Inotropic score*	35 (29–54)
Laboratory data at ICU admission	
White blood cells, $\times 10^3/mm^3$	14.8 (4.7–21.8)
Platelets, $\times 10^3/mm^3$	8.4 (4.2–14.4)
Creatinine, mg/dL	2.4 (1.2–3.8)
Lactate, mmol/L	2.9 (2.3–8.1)
Procalcitonin, ng/mL	30.1 (9.4–129.0)
NT-proBNP, ng/mL	14,807 (3114.5–37,078)
eGFR†, mL/min/1.73 m²	18.5 (11.3–38.8)
Intervention after ICU admission	
Vasopressin, n (%)	9 (42.9)
Low-dose hydrocortisone, n (%)	17 (81.0)
Continuous renal replacement therapy, n (%)	18 (85.7)
Polymyxin B-direct hemoperfusion, n (%)	10 (47.6)

Data are presented as medians (interquartile range) or n (%).
APACHE = Acute Physiology and Chronic Health Evaluation, SOFA = Sequential Organ Failure Assessment, NT-proBNP = N-terminal pro-B-type natriuretic peptide, eGFR = estimated glomerular filtration rate.
* Inotropic score calculated as (dopamine dose × 1) + (dobutamine dose × 1) + (epinephrine dose × 100) + (norepinephrine dose × 100), where all doses are expressed as micrograms per kilogram per minute.
† eGFR calculated as $194 \times Cr^{-1.094} \times age^{-0.287}$ (if female × 0.739).

levels of 0.59 (range: 0.3–0.86), 2 cases (9.5%) of *Enterococcus faecium* with EA levels of 0.56 and 0.65, 1 case (4.8%) of *Clostridium paraputrificum* with EA levels of 0.53 and 1 case (4.8%) of *Propionibacterium acnes* with EA levels of 0.60. Bacteremia was observed 16 cases (76.2%) in this study. Soft tissue/bone was a major site of infection and *S aureus* was a major causative microorganism.

3.2. Correlation between EA level and I-FABP upon ICU admission

The EA levels at ICU admission were as follows: high levels (≥0.60) of EA were observed in 7 cases (33.3%), intermediate levels (0.40–0.59) in 10 cases (47.6%), and low levels (0.0–0.39) in 4 cases (19.0%) of patients. The correlation between EA levels and I-FABP levels upon ICU admission is shown in Figure 2. The EA levels were not correlated with the I-FABP levels (Spearman $\rho = 0.002$, $P = .993$).

3.3. I-FABP level upon ICU admission and the clinical course and related factors in patients with EA < 0.6 and EA ≥ 0.60

The I-FABP levels at the time of ICU admission, the incidence of bowel ischemia suspected clinically after ICU admission, NOMI, life-threatening abdominal complications, and mortality in patients with EA < 0.6 (n = 14) and EA ≥ 0.60 (n = 7) are
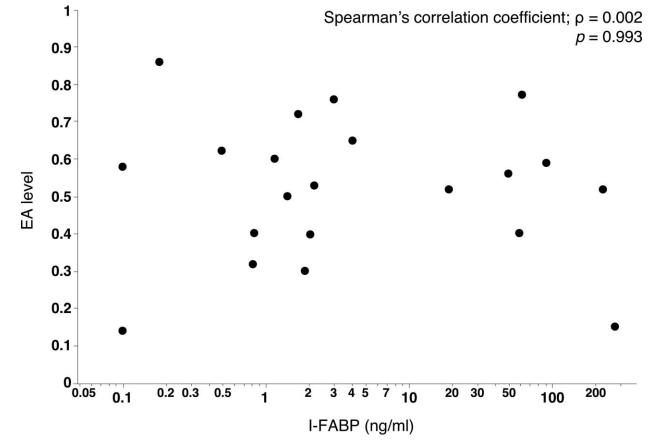

Figure 2. Correlation between endotoxin activity (EA) levels and intestinal fatty acid-binding protein (I-FABP) at the time of admission to the intensive care unit. The EA levels were not correlated with the I-FABP levels.

Table 2

Intestinal fatty acid-binding protein level upon intensive care unit admission and clinical course.

Characteristics	EA < 0.6 (n=14)	EA ≥ 0.6 (n=7)	P-value
I-FABP, ng/mL	2.1 (0.8–67.0)	1.6 (0.5–4.0)	.526
Bowel ischemia suspected clinically, n (%)	8 (57.1)	1 (14.3)	.159
Reason to suspect bowel ischemia			
Lactic acidosis and/or shock,[*] n (%)	8 (64.3)	1 (14.3)	.159
Abdominal distension,[†] n (%)	7 (50.0)	1 (14.3)	.174
Gastrointestinal bleeding, n (%)	1 (7.1)	0 (0)	1.000
Nonocclusive mesenteric ischemia, n (%)	2 (14.3)	0 (0)	.533
Other abdominal complication,[‡] n (%)	1 (7.1)	0 (0)	1.000
Total abdominal complications,[§] n (%)	3 (21.4)	0 (0)	.521
28-d mortality, n (%)	6 (42.9)	2 (28.6)	.656
Inpatient mortality, n (%)	7 (50.0)	2 (28.6)	.642

Data are presented as medians (interquartile range) or n (%). P-values are determined by Wilcoxon rank sum test or Fisher exact test.
EA = endotoxin activity, I-FABP = intestinal fatty acid-binding protein.
[*] Defined as persistent or newly developed lactic acidosis and/or hypotension.
[†] Defined as newly developed abdominal distention.
[‡] Defined as life-threatening abdominal complication requiring surgical intervention.
[§] Defined as nonocclusive mesenteric ischemia + other abdominal complication.

shown in Table 2. There were no significant differences between the 2 groups. Additionally, the APACHE II scores, SOFA scores, inotropic scores, the lactate and Cr levels (which are factors related to EA activity), and procalcitonin and NT pro-BNP levels (which are reported to be prognostic biomarkers of sepsis) of the 2 groups are shown in Table 3. There were also no significant differences between the 2 groups.

4. Discussion

In this posthoc analysis, one-third (7/21) of the patients in gram-positive septic shock presented with high EA levels at the time of ICU admission. However, there was no significant correlation between EA and I-FABP levels. Additionally, high EA levels were not associated with abdominal complications after ICU admission or with mortality. Similarly, high EA levels were not associated with APACHE II scores, SOFA scores, inotropic scores, or lactate and Cr levels at the time of ICU admission, which are factors related to high EA levels.

The frequency of endotoxemia, specifically with high EA levels, in cases of gram-positive sepsis was reported to be 32%[4] to 40%.[7] These data are almost equivalent to our findings of 33%. The possible mechanism is thought to be a translocation of the gut microbial flora and endotoxin.[4,5] However, only a few studies have been published that investigated the association between EA and I-FABP levels in patients following cardiac arrest[14] and cardiopulmonary bypass.[13] The highest EA levels, found on ICU days 0 to 3, were positively correlated with the highest urinary I-FABP levels in the 21 successfully resuscitated patients.[14] On the contrary, there was no correlation between EA and I-FABP levels in 16 patients with congenital heart disease after cardiac surgery that required cardiopulmonary bypass.[13] In a recent report that investigated the association between cardiopulmonary bypass, enterocyte injury, and endotoxemia in 34 patients, prolonged cardiopulmonary bypass was found to be a risk factor for high I-FABP levels and endotoxemia assessed by the limuls amebocyte assay.[26] However, the correlation between I-FABP and endotoxin levels was not described in the manuscript. In summary, there are only a few studies investigating the association between endotoxemia and intestinal hypoperfusion injury. Definitive conclusions have not yet been reached regarding this matter, even for critically ill patients after cardiac arrest and cardiopulmonary bypass.

To the best of our knowledge, this is the first report investigating a correlation between EA and I-FABP levels among patients in gram-positive septic shock. However, we found no correlation between EA and I-FABP levels measured at the time of ICU admission. Furthermore, high EA levels were not associated with mortality, severity score, or abdominal complications after ICU admission, which have been reported to be risk factors for elevated EA levels. The fact that one-third of the patients presented with high EA levels at the time of ICU admission means

Table 3

Intestinal fatty acid-binding protein level upon intensive care unit admission and related factors.

Characteristics	EA < 0.6 (n=14)	EA ≥ 0.6 (n=7)	P-value
APACHE II score	30 (27.8–36)	26 (25–36)	.431
SOFA score	12.5 (9.8–15.5)	13 (11–15)	.871
Inotropic score[*]	35 (27.3–55)	35 (30–53)	1.000
Lactate, mmol/L	3.05 (2.28–9.4)	2.6 (1.4–3.9)	.432
Creatinine, mg/dL	2.41 (1.403–4.125)	1.33 (1.16–3.61)	.535
Procalcitonin, ng/mL	72.015 (12.333–140.875)	9.15 (7.39–42.53)	.129
NT-proBNP, ng/mL	14,845 (2927.3–42,070.8)	14,807 (5432–19,824)	.971

Data are presented as medians (interquartile range).
APACHE = Acute Physiology and Chronic Health Evaluation, EA = endotoxin activity, NT-proBNP = N-terminal pro-B-type natriuretic peptide, SOFA = Sequential Organ Failure Assessment.
[*] Inotropic score calculated as (dopamine dose × 1) + (dobutamine dose × 1) + (epinephrine dose × 100) + (norepinephrine dose × 100), where all doses are expressed as micrograms per kilogram per minute.

that the cause happened prior to the ICU admission. Previous studies that have investigated the usefulness of I-FABP for diagnosing acute intestinal ischemia reported a cutoff point of 9.1 ng/mL.[11] Applying those data to our study, one-third (7/21) of the patients exceeded the cutoff point, indicating that intestinal hypoperfusion and enterocyte injury had occurred upon or prior to ICU admission, similar to intestinal ischemia and necrosis. However, there was only 1 patient who was categorized as having a high EA level. Additionally, among 14 patients who presented with normal levels of I-FABP (mean 1.1 ± 0.9 [range, 0.1–5.5] ng/ mL),[22] 6 of them presented with high EA levels. From the results of our study, we believe that enterocyte injury might not be the cause of high EA levels. In other words, high EA levels might not be indicative of enterocyte injury, intestinal ischemia, and bacterial/toxin translocation. As far as we know, there have been no previous reports that have investigated the association between EA levels and true intestinal ischemia or necrosis diagnosed on contrast computed tomography, gastrointestinal endoscopy, and/or surgical intervention. We may need to elucidate the correlations among these patients first, and then, if there is no correlation between endotoxemia and intestinal ischemia, we should reveal alternative mechanisms and factors related to elevated EA levels among patients in gram-positive septic shock. This will require additional studies in the future using a much larger study population. We believe revealing the pathophysiology of endotoxemia in these patients might help identify a potential therapeutic target. For example, the removal of endotoxin, namely PMX-DHP, might become an effective therapeutic option even in patients with gram-positive septic shock.[6] Additionally, if endotoxemia was caused by bacterial translocation from enterocyte injury and intestinal ischemia, antibiotic therapy targeting intestinal bacteria might have to be added for better outcomes. Clinical practice guidelines regarding gram-positive septic shock patients might change by clarifying the mechanism of endotoxemia.

There are several potential limitations to this study. First, the nature of posthoc analysis presents inherent limitations. Additionally, the number of patients was very small. Our findings need to be validated in a much larger prospective cohort study. However, it might be difficult to prospectively identify a large number of patients with gram-positive septic shock because the causative microorganism is rarely identified upon ICU admission. Second, the serum level of I-FABP is influenced by renal function.[21] Previous studies that evaluated serum I-FABP did not take this into account despite the fact that critically ill patients often suffer from renal problems.[27] In this study, the patients' mean eGFR was 18.5 (range: 11.3–38.8) mL/min/1.73 m^2, and the eGFR was distributed in a relatively narrow range. Although it may have had little impact on our results, the influence of renal function on I-FABP levels should be considered in future studies.

5. Conclusion

In this posthoc analysis, one-third of the patients with gram-positive septic shock presented with high EA levels at the time of ICU admission. However, our analysis could not identify an association between endotoxemia and enterocyte injury among these patients. Additionally, high EA levels were not associated with the patient's clinical course and factors reported that were related to endotoxemia. Although our results need to be validated in a large prospective cohort study, hypoperfusion enterocyte

injury might not be a cause of endotoxemia. In that case, if there is no correlation between EA and I-FABP levels, other mechanisms inducing high EA levels among patients in gram-positive septic shock should be elucidated. Revealing the pathophysiology of endotoxemia in these patients might help identify a potential therapeutic target.

Author contributions

Conceptualization: Motohiro Sekino, Hiroyuki Funaoka, Tetsuya Hara.
Data curation: Motohiro Sekino, Haruka Inoue, Rintaro Yano, Sojiro Matsumoto, Taiga Ichinomiya, Ushio Higashijima, Shuhei Matsumoto.
Formal analysis: Shuntaro Sato.
Funding acquisition: Motohiro Sekino.
Investigation: Motohiro Sekino, Haruka Inoue, Rintaro Yano, Sojiro Matsumoto, Taiga Ichinomiya, Ushio Higashijima, Shuhei Matsumoto.
Methodology: Motohiro Sekino, Shuntaro Sato, Tetsuya Hara.
Project administration: Motohiro Sekino, Tetsuya Hara.
Resources: Motohiro Sekino, Hiroyuki Funaoka, Tetsuya Hara.
Software: Motohiro Sekino, Shuntaro Sato.
Supervision: Motohiro Sekino, Tetsuya Hara.
Validation: Motohiro Sekino, Hiroyuki Funaoka, Shuntaro Sato, Takashi Egashira, Haruka Inoue, Rintaro Yano, Sojiro Matsumoto, Taiga Ichinomiya, Ushio Higashijima, Shuhei Matsumoto, Tetsuya Hara.
Visualization: Motohiro Sekino, Shuntaro Sato.
Writing – original draft: Motohiro Sekino.
Writing – review & editing: Motohiro Sekino, Hiroyuki Funaoka, Shuntaro Sato, Takashi Egashira, Haruka Inoue, Rintaro Yano, Sojiro Matsumoto, Taiga Ichinomiya, Ushio Higashijima, Shuhei Matsumoto, Tetsuya Hara.

References

[1] Beutler BA. TLRs and innate immunity. Blood 2009;113:1399–407.
[2] Klein DJ, Derzko A, Foster D, et al. Daily variation in endotoxin levels is associated with increased organ failure in critically ill patients. Shock (Augusta, Ga) 2007;28:524–9.
[3] Marshall JC, Walker PM, Foster DM, et al. Measurement of endotoxin activity in critically ill patients using whole blood neutrophil dependent chemiluminescence. Crit Care (London, England) 2002;6:342–8.
[4] Marshall JC, Foster D, Vincent JL, et al. Diagnostic and prognostic implications of endotoxemia in critical illness: results of the MEDIC study. J Infect Dis 2004;190:527–34.
[5] Ronco C, Klein DJ. Polymyxin B hemoperfusion: a mechanistic perspective. Crit Care (London, England) 2014;18:309.
[6] Adamik B, Smiechowicz J, Kubler A. The importance of early detection of endotoxemia. Innate Immun 2016;22:503–9.
[7] Bottiroli M, Monti G, Pinciroli R, et al. Prevalence and clinical significance of early high endotoxin activity in septic shock: an observational study. J Crit Care 2017;41:124–9.
[8] Klein DJ, Briet F, Nisenbaum R, et al. Endotoxemia related to cardiopulmonary bypass is associated with increased risk of infection after cardiac surgery: a prospective observational study. Crit Care (London, England) 2011;15:R69.
[9] Charbonney E, Tsang JY, Li Y, et al. Endotoxemia following multiple trauma: risk factors and prognostic implications. Crit Care Med 2016;44:335–41.

[10] Pelsers MM, Namiot Z, Kisielewski W, et al. Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue distribution and clinical utility. Clin Biochem 2003;36:529–35.

[11] Matsumoto S, Sekine K, Funaoka H, et al. Diagnostic performance of plasma biomarkers in patients with acute intestinal ischaemia. Br J Surg 2014;101:232–8.

[12] Heida FH, Hulscher JB, Schurink M, et al. Intestinal fatty acid-binding protein levels in necrotizing enterocolitis correlate with extent of necrotic bowel: results from a multicenter study. J Pediatr Surg 2015;50:1115–8.

[13] Pathan N, Burmester M, Adamovic T, et al. Intestinal injury and endotoxemia in children undergoing surgery for congenital heart disease. Am J Respir Crit Care Med 2011;184:1261–9.

[14] Grimaldi D, Guivarch E, Neveux N, et al. Markers of intestinal injury are associated with endotoxemia in successfully resuscitated patients. Resuscitation 2013;84:60–5.

[15] Sekino M, Funaoka H, Sato S, et al. Intestinal fatty acid-binding protein level as a predictor of 28-day mortality and bowel ischemia in patients with septic shock: a preliminary study. J Crit Care 2017;42:92–100.

[16] Sekino M, Funaoka H, Sato S, et al. Association between macroscopic tongue ischemia and enterocyte injury and poor outcome in patients with septic shock: a preliminary observational study. Shock (Augusta, Ga) 2018;50:530–7.

[17] Cruz DN, Antonelli M, Fumagalli R, et al. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. JAMA 2009;301:2445–52.

[18] Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–92.

[19] Romaschin AD, Harris DM, Ribeiro MB, et al. A rapid assay of endotoxin in whole blood using autologous neutrophil dependent chemiluminescence. J Immunol Methods 1998;212:169–85.

[20] Mignon F, Piagnerelli M, Van Nuffelen M, et al. Effect of empiric antibiotic treatment on plasma endotoxin activity in septic patients. Infection 2014;42:521–8.

[21] Okada K, Sekino M, Funaoka H, et al. Intestinal fatty acid-binding protein levels in patients with chronic renal failure. J Surg Res 2018;230:94–100.

[22] Funaoka H, Kanda T, Kajiura S, et al. Development of a high-specificity sandwich ELISA system for the quantification of human intestinal fatty acid-binding protein (I-FABP) concentrations. Immunol Invest 2011;40:223–42.

[23] Paternoster G, Rubino G, Balducci A, et al. Association between endotoxin activity and acute kidney injury in cardiac patients undergoing cardiopulmonary bypass. Blood Purif 2014;37:27–32.

[24] Wang F, Wu Y, Tang L, et al. Brain natriuretic peptide for prediction of mortality in patients with sepsis: a systematic review and meta-analysis. Crit Care (London, England) 2012;16:R74.

[25] Lee CC, Chen SY, Tsai CL, et al. Prognostic value of mortality in emergency department sepsis score, procalcitonin, and C-reactive protein in patients with sepsis at the emergency department. Shock (Augusta, Ga) 2008;29:322–7.

[26] Adamik B, Kubler A, Gozdzik A, et al. Prolonged cardiopulmonary bypass is a risk factor for intestinal ischaemic damage and endotoxaemia. Heart Lung Circ 2017;26:717–23.

[27] Uchino S, Kellum JA, Bellomo R, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA 2005;294:813–8.