Original Article

Impact of fecal short-chain fatty acids on prognosis in critically ill patients

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Aim: This study aimed to evaluate the relationship between fecal organic acids and mortality in critically ill patients.

Methods: This retrospective study included 128 patients who fulfilled the criteria of systemic inflammatory response syndrome and had a serum C-reactive protein level of greater than 10 mg/dL. Patients were treated in the intensive care unit for more than 2 days. Patients were divided into two groups: survivors and non-survivors. We measured and compared eight kinds of fecal organic acids between the two groups. We focused on the minimum and maximum value of each fecal organic acid and evaluated prognostic factors by using classification and regression tree (CART) and multivariate logistic regression analyses.

Results: We included 90 patients as survivors and 38 as non-survivors. The CART analysis revealed that the dominant factors for mortality were the minimum values of propionate and acetate and the maximum values of lactate and formic acid. In the evaluation of the minimum values of fecal organic acids, propionate was significantly associated with increased mortality (odds ratio, 0.11 [95% confidence interval, 0.024–0.51]; $P = 0.005$), acetate (0.047 [0.005–0.49]; $P = 0.01$), and age (1.048 [1.015–1.083]; $P = 0.004$). In the evaluation of the maximum values, lactate was significantly associated with increased mortality (5.21 [2.024–13.42], $P = 0.001$) and age (1.050 [1.017–1.084]; $P = 0.003$).

Conclusion: An altered balance of fecal organic acids was significantly associated with mortality in critically ill patients.

Key words: Acetate, critical illness, prognosis, propionate, short-chain fatty acid

INTRODUCTION

THE GUT IS the largest immune organ of the human body and also an important target organ for various kinds of stress caused by severe insults such as infection, trauma, and shock.[1,2] These stresses are considered to have an important role in promoting infectious complications and multiple organ dysfunction syndrome from the viewpoints of deteriorated intestinal epithelium, the immune system, and commensal bacteria. Gut dysfunction is now recognized as a cause for the promotion of diseases.[3,4]

Lipids have been a focus, not only as energy sources but also as immune-modulating substrates.[5] Unlike long- and medium-chain fatty acids, short-chain fatty acids (SCFAs), which mainly consist of acetate, propionate, and butyrate with two to four carbon atoms, are principally derived from the fermentation of carbohydrates and amino acids by anaerobic microorganisms. Short-chain fatty acids are used mainly by intestinal epithelial cells as energy substrates, and they influence the motility of the intestinal tract and increase intestinal blood flow. We previously reported on altered gut flora and fecal organic acids in critically ill patients and showed that these patients had significantly lower levels of

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fecal organic acids, especially SCFAs, than healthy volunteers.[6,7]

Despite the evidence implicating the importance of SCFAs in the gut, the effect of SCFAs on prognosis in critically ill patients has not yet been clarified. In this study, we measured eight kinds of fecal organic acids, including SCFAs, in critically ill patients and investigated the effect of SCFAs on their prognosis.

METHODS

Patients

THIS RETROSPECTIVE STUDY enrolled patients who: (i) fulfilled the criteria of systemic inflammatory response syndrome (SIRS) according to the American College of Chest Physicians and the Society of Critical Care Medicine,[8] (ii) had a serum C-reactive protein (CRP) level greater than 10 mg/dL, (iii) were admitted to the Department of Traumatology and Acute Critical Medicine, Osaka University Graduate School of Medicine, Osaka, Japan, during the period November 2003–January 2008, (iv) were treated in the intensive care unit for more than 2 days. We used CRP ≥10 mg/dL to focus on cases with severe inflammation because CRP is mainly used as a marker of inflammation. We excluded patients from whom we could not collect any fecal samples during hospitalization. Continuous enteral feeding was initiated with 500 kcal/day and gradually increased depending on the patient's condition. We used Impact (Novartis Nutrition, Minneapolis, MN, USA) as an enteral nutritional product for all patients in our intensive care unit and used 25–30 kcal/kg ideal body weight as the goal for the administered dose. Fourteen healthy volunteers provided fecal samples as controls for examinations of fecal organic acids concentrations. This study was approved by the Institutional Review Board of Osaka University (approval no. 13217), and informed consent was obtained from each patient's family.

Fecal samples and determination of fecal organic acid concentrations

We collected fecal samples serially from all patients. The fecal samples were transported at −20°C to the Yakult Central Institute (Tokyo, Japan). We measured eight kinds of organic acid in the feces by high-performance liquid chromatography. Feces were homogenized in 1 mL distilled water. The homogenate was then placed in an Eppendorf tube and centrifuged at 20,400 *g* for 10 min at 4°C. A mixture of 0.9 mL of the resulting supernatant and 0.1 mL of 1.5 mol/L perchloric acid was mixed well in a glass tube and allowed to stand at 4°C for 12 h. The suspension was then passed through a filter with a pore size of 0.45 μm (Nihon Millipore, Tokyo, Japan). The sample was analyzed for organic acids by high-performance liquid chromatography as previously described[9] using a Waters 432 Conductivity Detector (Waters, Milford, MA, USA) equipped with two columns (Shodex KC-811; Showa Denko, Tokyo, Japan). The concentrations of fecal organic acids were calculated with the use of external standards. The reproducibility and stability of these measurements were shown previously.[10] We retrospectively evaluated the minimum and maximum value of each fecal organic acid measured during hospitalization and determined prognostic factors by classification and regression tree (CART) analysis.

Surveillance and definition of complications

Bacterial infection was diagnosed in accordance with the definitions of the Centers for Disease Control and Prevention.[11] Body temperature was measured continuously. Surveillance cultures from blood and sputum were undertaken routinely for each patient. In cases of suspected infection, laboratory testing, chest X-ray, and computed tomography scanning were carried out as necessary. Bacteremia was defined as a positive blood culture.

Statistical analysis

Continuous variables expressed as the median (25th and 75th percentiles) were compared by the Mann–Whitney *U*-test. Categorical variables expressed as number (%) were compared by the χ^2-test unless the expected counts in any of the cells were below 5, in which case the Fisher exact test was used.

We used CART analysis, which is a binary recursive partitioning using non-parametric approaches, to identify key fecal organic acids and their cut-off values for mortality.[12] We also used multivariate logistic regression analysis to quantitatively evaluate the effect of the covariates that were suggested by CART analysis.

The most important prognostic factors were selected, and a predictive model was developed as follows. First, the minimum and maximum values of each fecal organic acid were selected from all patients. The maximum value was the highest value and the minimum value was the lowest value measured during hospitalization. Second, the Mann–Whitney *U*-test was applied to discover variables with potential prognostic value, and covariates with $P > 0.20$ were excluded. Finally, CART was used to create a tree using the minimum and maximum data values of all fecal organic acids. The effects of the minimum and the maximum values of the fecal organic acids that were selected as key prognostic factors by

Table 1. Characteristics of critically ill patients included in this study

	Total (n = 128)	Survivors (n = 90)	Non-survivors (n = 38)	P-value
Age, years	63 (50–74)	60 (43–71)	70 (61–80)	**0.001**
Sex (male)	85 (66)	58 (64)	27 (71)	0.470
ICU stay, days	30 (18–54)	30 (19–53)	28 (16–55)	0.650
APACHE II score on admission	15.5 (12.0–21.0)	15.0 (11.3–21.0)	17.5 (14.0–20.8)	0.310
Origins of SIRS				
Sepsis	87 (68)	55 (61)	32 (84)	1.000
Trauma	28 (22)	24 (27)	4 (11)	
Burn	12 (9)	10 (11)	2 (5)	
Unknown	1 (1)	1 (1)	0 (0)	
Bacteremia	50 (39)	21 (23)	29 (76)	**<0.001**
Number of antibiotic types	4 (2–6)	4 (2–6)	5 (4–8)	**0.003**
Duration of antibiotic use, days	18 (10–33)	15 (10–28)	25 (12–43)	**0.034**

Categorical values are expressed as number (%); continuous values are expressed as median (25th–75th percentiles). Bold values indicate statistical significance.
APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; SIRS, systemic inflammatory response syndrome.

CART analysis, and age, sex, and Acute Physiology and Chronic Health Evaluation (APACHE) Ⅱ score on admission were analyzed with the multivariable logistic regression models.

All reported P-values were two-sided, and a P-value of <0.05 was considered to indicate statistical significance. All statistical analyses were undertaken using IBM SPSS statistics version 19 (Armonk, NY, USA).

RESULTS

Patient characteristics

CHARACTERISTICS OF THE patients are shown in Table 1. There were 90 survivors and 38 non-survivors. The two groups did not differ significantly in terms of sex or APACHE II score. Age in the non-survivors was significantly older than that in the survivors (P = 0.001). The principal origin of SIRS was sepsis in 87 of the 128 patients in the two groups. Significantly more types of antibiotics were used in the non-survivors than in the survivors during hospitalization (P = 0.003), and the duration of antibiotic use in the non-survivors was significantly longer than that in the survivors (P = 0.034). The incidence of bacteremia was significantly higher in the non-survivors than that in the survivors (76% vs. 23%, P < 0.001).

Organic acid profiles

Timing of fecal sample collection is shown in Table 2. We collected 456 fecal samples for SCFA analysis. Fecal samples were collected evenly throughout hospitalization in both the survivors and the non-survivors. The minimum and maximum values of each fecal organic acid and the results of univariate analysis are shown in Tables 3 and 4. In terms of the minimum values, total organic acids, acetate, propionate, and isovaleric acid were significantly decreased in the non-survivors. In terms of the maximum values, lactate, formic acid, and succinic acid were significantly increased in the non-survivors. Otherwise, isovaleric acid and acetate were significantly decreased in the non-survivors.

Additionally, CART and multivariate logistic regression analyses of the predominant covariates were carried out for

Table 2. Timing of fecal sample collection in critically ill patients

	Survivors	Non-survivors	Total
Week 1	50	30	80
Week 2	43	34	77
Week 3	36	30	66
Week 4	30	18	48
Week 5	26	15	41
Week 6	17	14	31
Week 7	11	8	19
Week 8	16	9	25
Week 9	7	7	14
Week 10	6	3	9
>11 weeks	36	10	46
Total	278	178	456

Table 3. Minimum values of fecal organic acids in each group of critically ill patients

	Survivors	Non-survivors	*P*-value
Total organic acids	56.1 ± 4.1	35.5 ± 5.1	**0.005**
Acetate	34.5 ± 2.7	19.5 ± 2.9	**0.001**
Propionate	7.8 ± 0.9	3.3 ± 0.9	**0.004**
Butyrate	3.3 ± 0.5	1.9 ± 0.7	0.058
Succinic acid	1.9 ± 0.6	2.3 ± 0.9	0.435
Lactate	1.3 ± 0.4	3.3 ± 1.0	0.061
Formic acid	0.5 ± 0.1	0.6 ± 0.3	0.513
Isovaleric acid	1.3 ± 0.3	0.2 ± 0.2	**0.011**
Valeric acid	0.5 ± 0.2	0.1 ± 0.1	0.112

Values are expressed as mean ± SE (μmol/g of feces). Bold values indicate statistical significance.

Table 4. Maximum values of fecal organic acids in each group of critically ill patients

	Survivors	Non-survivors	*P*-value
Total organic acids	96.1 ± 5.2	87.5 ± 7.6	0.475
Acetate	59.3 ± 3.0	48.4 ± 4.9	**0.037**
Propionate	15.9 ± 1.3	12.5 ± 1.6	0.133
Butyrate	7.5 ± 0.7	6.4 ± 1.2	0.179
Succinic acid	7.2 ± 1.3	10.9 ± 2.2	**0.035**
Lactate	7.2 ± 1.4	21.8 ± 4.7	**<0.001**
Formic acid	2.7 ± 0.4	4.7 ± 1.1	**0.024**
Isovaleric acid	3.4 ± 0.4	2.0 ± 0.6	**0.028**
Valeric acid	1.6 ± 0.3	0.7 ± 0.3	0.086

Values are expressed as mean ± SE (μmol/g of feces). Bold values indicate statistical significance.

the minimum and maximum values of the fecal organic acids. For the minimum values, the primary split variable was determined to be the minimum value of propionate, and the cut-off value was 9.7 μmol/g of feces. The secondary split variable was determined to be the minimum value of acetate, and the cut-off value was 2.5 μmol/g of feces. The mortality in each partition and the diagnostic characteristics of this tree are shown in Figure 1.

For the maximum values of fecal organic acids, the primary split variable was determined to be the maximum value of lactate, and the cut-off value was 11.3 μmol/g of feces. The secondary split variable was determined to be the maximum value of formic acid, and the cut-off value was 7.1 μmol/g of feces. The mortality in each partition and the diagnostic characteristics of this tree are shown in Figure 2.

The diagnostic characteristics of the tree for minimum value showed a sensitivity of 87.5% and specificity of 74.1%, whereas those of the tree for maximum value showed a sensitivity of 91.7% and specificity of 76.7%.

For the minimum values of fecal organic acids, multivariate logistic regression analysis revealed that age and the minimum values of propionate and acetate were significant prognostic factors for mortality (Table 5). For the maximum values, age and maximum lactate were identified as significant prognostic factors for mortality (Table 6).

DISCUSSION

THIS STUDY PROVIDES the first in vivo evidence, to our knowledge, that the altered balance of fecal organic acids is associated with mortality in critically ill patients. A recent study has shown that SCFAs, particularly acetate and propionate, bind the G-protein-coupled receptor 43 (GPR43) in adipose tissue. Stimulation of GPR43 by SCFAs was necessary for the anti-inflammatory responses because GPR43-deficient mice showed exacerbated inflammation in models of colitis, arthritis, and asthma. The SCFA–GPR43 interactions profoundly affected intestinal and systemic inflammatory responses.[13] The low concentrations of acetate and propionate observed in non-survivors in the present study could reduce the anti-inflammatory effect. Fukuda *et al.*[14] showed that acetate produced by protective bifidobacteria improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection. Furthermore, Asahara *et al.*[15] showed a positive correlation between fecal acetic acid level and tight-junction-related gene expression in the intestinal epithelium in their experiment using an infected mouse model.

The maximum values of lactate and formic acid were also selected as prognostic factors (Fig. 2). Our group previously showed that when patients had severely acidic feces, fecal lactate, succinic acid, and formic acid were significantly increased over those of patients with normal feces. We also showed that abnormal fecal pH was associated with the incidence of bacteremia and mortality.[16] The increase of lactate and formic acid can make feces severely acidic, and severely acidic feces could injure intestinal epithelial cells and worsen the patient's condition. The present results indicate that not only a lack of SCFAs but also an accumulation of lactate and formic acid might be associated with disruption of the gastrointestinal environment in critically ill conditions, which leads to bacterial translocation.

There was a significant difference between the two groups in age, number of antibiotic types, and duration of antibiotic use in this study. Previous studies have suggested that changes in the composition of bacterial species and genes in

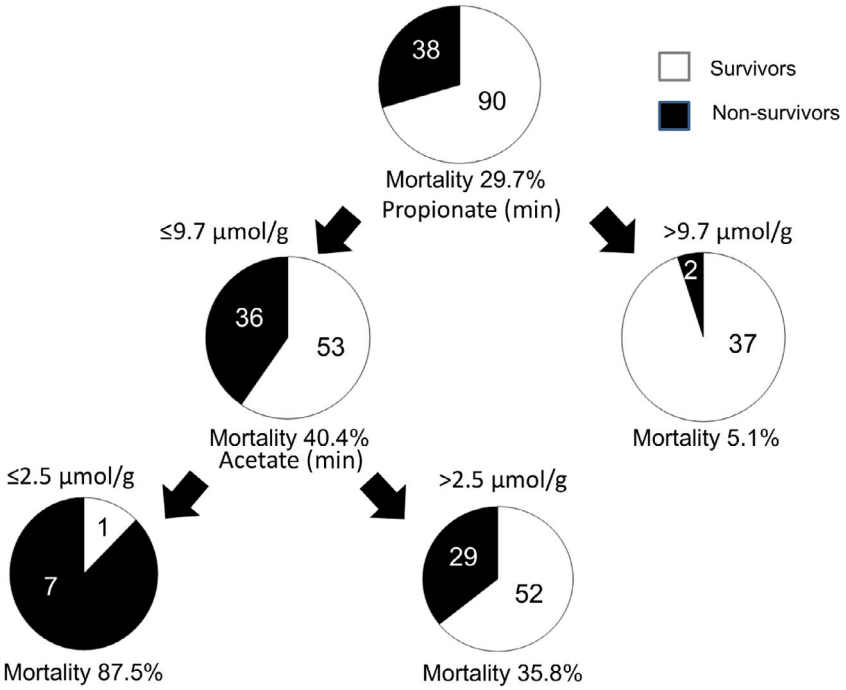

Fig. 1. Charts showing mortality in critically ill patients, partitioned by the minimum value of fecal organic acids using classification and regression tree analysis. min, minimum.

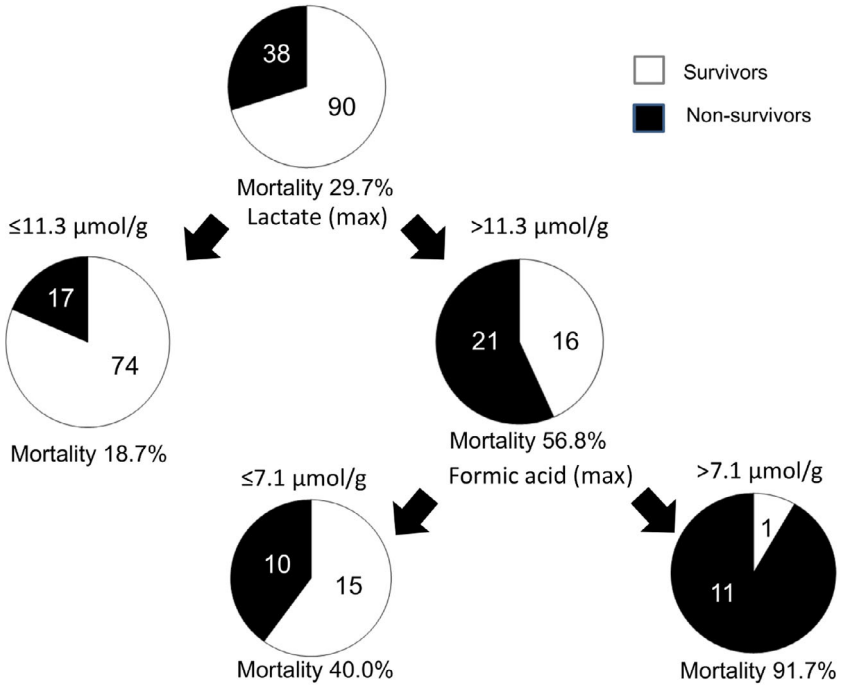

Fig. 2. Charts showing mortality in critically ill patients, partitioned by the maximum value of fecal organic acids using classification and regression tree analysis. max, maximum.

Table 5. Results of multivariate logistic regression analysis in critically ill patients using the minimum value of fecal organic acids

	Coeff (β)	SE	*P*-value	Odds ratio	95% confidence interval	
					Lower limit	Upper limit
Mortality						
Age	0.047	0.017	**0.004**	1.048	1.015	1.083
Sex	0.839	0.553	0.129	2.313	0.783	6.838
APACHE II	−0.023	0.033	0.489	0.977	0.915	1.043
Propionate (min)	−2.199	0.778	**0.005**	0.111	0.024	0.510
Acetate (min)	−3.054	1.192	**0.010**	0.047	0.005	0.488

Bold values indicate statistical significance.
APACHE, Acute Physiology and Chronic Health Evaluation; Coeff (β), coefficient; min, minimum; SE, standard error.

Table 6. Results of multivariate logistic regression analysis in critically ill patients using the maximum value of fecal organic acids

	Coeff (β)	SE	*P*-value	Odds ratio	95% confidence interval	
					Lower limit	Upper limit
Mortality						
Age	0.049	0.016	**0.003**	1.050	1.017	1.084
Sex	1.008	0.538	0.061	2.739	0.954	7.863
APACHE II	−0.003	0.034	0.925	0.997	0.933	1.065
Lactate (max)	1.651	0.483	**0.001**	5.212	2.024	13.424
Formic acid (max)	1.186	0.607	0.051	3.274	0.997	10.748

Bold values indicate statistical significance.
APACHE, Acute Physiology and Chronic Health Evaluation; Coeff (β), coefficient; max, maximum value; SE, standard error.

the intestinal flora occur with aging.[17] It is also reported that the genes related to SCFA production were lost from the intestinal flora and that glycolytic ability was decreased with aging.[18] These changes could be one of the reasons why aging was detected as an important factor related to mortality in the present study. There is no doubt that antibiotics are important, but they adversely affect the intestinal flora. Some systemically administered antibiotics are excreted in the bile and secreted in the upper gastrointestinal tract, and upon reaching the colon, they cause serious damage to the intestinal flora.[19] Short-chain fatty acids could also be reduced with the destruction of the intestinal flora, which might have worsened the prognosis.[20]

Short-chain fatty acids are mainly used by intestinal epithelial cells as energy substrates, and some are absorbed into the portal flow to the liver and used as systemic energy sources. They can also influence the motility of the intestinal tract, absorption of electrolytes, and pancreatic secretion and can increase intestinal blood flow. In the present study, total organic acids including SCFAs were significantly decreased in the critically ill patients compared with those in the healthy controls (data not shown). One reason would be the altered gut flora that produce SCFAs. The commensal gut flora in humans plays an essential role in homeostasis and protection from injury in the gut.[21,22] Under critically ill conditions, it is difficult to maintain normal gut flora.[6] Not only does the stress derived from diseases such as trauma and burns affect the balance of the gut microbiota, but also the stress caused by the treatment with various therapeutic agents such as histamine H_2 receptor blockers, catecholamines, and broad-spectrum antibiotics can influence the gut flora. We reported that critically ill patients had 100 to 10,000 times fewer total anaerobes, *Bifidobacterium*, and *Lactobacillus* and 100 times greater *Staphylococcus* when compared with counts in healthy volunteers.[6] These data indicate that the balance of gut flora is significantly disturbed in critically ill patients. The concentrations of fecal organic acids decreased significantly in the non-survivors, as shown in Table 3, and we believe that this was likely because the number of good bacteria (*Bifidobacterium* and *Lactobacillus*) had decreased. Similarly, the cause of the reduction of short-chain fatty acids in the present study may

have been the reduction of the SCFA-producing bacteria such as *Clostridium*, *Eubacterium*, *Peptococcus*, and *Fusobacterium*.[23,24]

Synbiotics are the combination of probiotics and prebiotics. The clinical effects of synbiotics have been reported in the fields of pediatric surgery, abdominal surgery, and intensive care.[25] In our preliminary report, critically ill patients treated with synbiotics maintained the necessary amounts of fecal organic acids, including SCFAs, and had fewer incidences of enteritis, pneumonia, and bacteremia than those without synbiotics.[26] These reports suggest that the maintenance of gut flora and SCFAs would be a promising intestinal therapy. Synbiotics were given to 76 patients in this study. In the synbiotics-treated group, maximum values of fecal organic acids (acetic acid, propionic acid, succinic acid, lactic acid, and formic acid) increased significantly, but minimum values did not increase (only the minimum value of lactic acid decreased significantly). However, there was no fixed protocol for the treatment with synbiotics in this study, the treatment start date was variable, and the average hospital day of starting treatment was day 6 (1–31), so it was difficult to evaluate the effects of synbiotics in this study. Evaluating the impact of synbiotics on fecal organic acids and prognosis in critically ill patients will be a future task.

Our study has some limitations. This was a retrospective study, and thus the timing of feces sampling was different between patients. The number of samples was too small to make conclusions about the temporal patterns.

In conclusion, the minimum values of fecal acetic acid and propionate in the non-survivors were significantly lower than those in the survivors. The altered balance of fecal organic acids was associated with mortality in critically ill patients. Thus, the maintenance of SCFAs could be a target for future intestinal therapy.

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DISCLOSURE

Approval of the research protocol: This study was approved by the Institutional Review Board of Osaka University.
Informed consent: Informed consent was obtained from each patient's family.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.
Conflict of interest: None.

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