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Synbiotics reduce infectious complications by improving the intestinal milieu and enhancing the immune function in critically ill emergency surgical patients

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Objective: To determine the effects of synbiotics upon the intestinal milieu, immune response, and infection in critically ill patients.
Methods: Thirty-two critically ill patients, who had undergone emergency surgery, were divided into two groups, those who started receiving enteral synbiotics (*Lactobacillus casei* strain Shirota and *Bifidobacterium breve* strain Yakult plus galactooligosaccharides; group S, n = 20) and those who did not receive enteral synbiotics (the control, group C, n = 12) within 48 hours of admission to the ICU and continuing for 14 days.
Results: Baseline values were comparable between the two groups. NK (natural killer) cell activity and lymphocyte counts significantly increased in group S. C-reactive protein concentrations significantly decreased in both groups. White blood cell counts significantly decreased in group S, but remained unchanged in group C. Group S had more *Bifidobacterium* and *Lactobacillus* and less *Pseudomonas* than group C in the stool samples after the trial. Concentrations of fecal organic, acetic, and propionic acids were significantly greater and the incidences of infectious complications were significantly lower in group S (25% vs. 75%, $P < 0.01$). The ICU stay was significantly shorter for group S. None of the patients died or developed adverse events.
Conclusions: Synbiotics enhanced host immune function in critically ill emergency surgical patients, improved the intestinal milieu, and decreased the incidence of infectious complications.

Key words: synbiotics, probiotics, prebiotics, intensive care, infection

Introduction

Despite advances in surgical techniques and intensive care medicine, patients admitted to intensive care units (ICU) frequently become susceptible to multiple infectious complications that significantly increase morbidity and mortality rates and add to hospital costs. Critically ill patients in the ICU can easily become immunocompromised, and their normal flora often becomes replaced with pathogens due to multiple factors, among which broad-spectrum antibiotics are the most important. The consequences include the development of nosocomial infections and antibiotic resistance.[1]

Bacteria can translocate across the gastrointestinal epithelium during critical illness, which when combined with impaired immune function can result in infection.[2] Increased intestinal permeability, intestinal microbial imbalance, and host immunodeficiency are important triggers of bacterial translocation.[3]

Probiotics are viable bacteria that benefit the host by improving the intestinal microbial balance and are widely used in food supplements.[4] Synbiotics are a combination of probiotics and prebiotics. Prebiotics are non-digestible food ingredients that stimulate the growth or activity, or both, of bacteria in the digestive system that is beneficial to the host. They can improve the intestinal microbial milieu and activate host immune function, which helps to prevent bacterial translocation.[5-8] Synbiotic therapy reportedly decreases septic complications under extreme conditions, such as liver transplantation,[9] major surgery,[5,6] and trauma.[10,11]

Patients in the ICU intuitively seem to be ideal candidates for synbiotic therapy. However, the clinical value of synbiotics for critically ill emergency surgical

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patients remains unclear because few clinical studies have been conducted.

The present prospective randomized study investigates the effect of synbiotics on intestinal microflora and organic acids, as well as on immune responses and infection, in patients admitted to the ICU after emergency surgery.

Materials and Method

Patients and protocol

The Ethics Committee for Medical Research at the University of Kitasato approved the study protocol, and written informed consent was obtained from all the participating patients.

Patients admitted to the ICU of the Kitasato University Hospital between October 2007 and July 2009 were eligible for the study if they met the following inclusion criteria: admission to the ICU from the tertiary emergency center of the Kitasato University Hospital, trauma or emergency surgical patients and fed enterally within 72 hours of admission. Exclusion criteria comprised: age <15 years, unable to receive nutrition via the gastrointestinal tract, hemodynamically unstable within 48 hours of admission, and not expected to survive for 7 days due to an uncorrectable medical condition.

A total of 40 admitted patients who provided written informed consent (or patients whose relatives or legal representatives provided written informed consent) to participate in the study were randomly allocated to either the group that received synbiotics (group S, n = 22) or to the control group that did not receive synbiotics (group C, n = 18). Eight of the patients were excluded from the trial before its completion due to there being insufficient data in 4 and the other 4 were discharged from our hospital leaving 20 and 12 patients in groups S and C, respectively.

All received concomitant therapy with antibiotics, inotropes, enteral or peripheral parenteral nutrition, and proton pump inhibitors. β-lactam antibiotics were used for 1－4 days after ICU admission in all patients. After that, patients who acquired infectious complications received antibiotics sensitive to causative bacteria until the infections improved.

Patients who were discharged from the ICU early were excluded from the study.

All synbiotics were obtained from Yakult Honsha (Tokyo). Biolactis powder (3 g/day) containing 1.5×10^9－2.1×10^{10} live *Lactobacillus casei* strain Shirota/g, BBG-01 (3 g/day) containing 1×10^9 live *Bifidobacterium breve* strain Yakult/g and galactooligosaccharides (Oligomate S-HP; 15 g/day) were administered to group S via a nasogastric feeding tube or orally for 14 days (starting on day 2 of ICU admission to day 15).

Both groups received enteral nutrition within 72 hours of ICU admission.

Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated from data obtained during the 24 hours after ICU admission.

Stool samples from all the patients were also examined after the trial.

Blood was sampled before and after the trial (on days 1－2 and 15－16, respectively) to measure standard laboratory parameters as well as C-reactive protein (CRP), white blood cell counts (WBC), total lymphocyte counts (TLC), and natural killer (NK) cell activity.

The activities of NK cells in peripheral blood mononuclear cells (PBMC) were measured using a ^{51}Cr release assay. In the mononuclear cell fraction, the activity of NK cells was assessed by specific target lysis against chromium-labeled K562 tumor cells. Briefly, 10^4 target ^{51}Cr-labelled K562 cells were mixed with effector PBMC in 96 round-bottomed tissue culture plates at an effector-to-target ratio of 100:1. Chromium release was assessed after 4 hours of incubation at 37℃ by measuring gamma-emmision.[12]

Fecal bacteriologic examination

Fecal samples were weighed immediately after defecation, suspended in 9 volumes of RNA later (Ambion Inc., Austin, TX, USA), and then incubated for 10 minutes at room temperature.

Fecal homogenate (200 μl) was added to 1 ml of sterilized phosphate buffer solution (PBS) and then centrifuged at 5,000 $\times$ g for 10 minutes to stabilize RNA (or DNA). The supernatant was discarded and the pellet was stored at -80℃ until RNA (or DNA) was isolated as described elsewhere.[13-15] The nucleic acid fraction was suspended in 1 mL of nuclease-free water (Ambion).

A standard curve was generated based on reverse transcription-quantitative polymerase chain reaction (RT-qPCR) data (using the threshold cycle [C_T], the cycle number when threshold fluorescence was reached) and the corresponding cell count in standard strains diluted as described elsewhere,[13,14] and visualized by staining with 4,6-diamidino-2-phe-nylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA).[16] To determine what bacteria were present in samples, three serial dilutions of an extracted RNA sample were analyzed by RT-qPCR, and the CT values in the linear range of the assay were applied to the standard curve generated in the

same experiment to obtain the numbers of bacterial cells corresponding to each nucleic acid sample and then these were converted to numbers of bacteria per sample. The specificity of the RT-qPCR assay using group- or species-specific primers was determined as described previously.[13,14] Quantitative analysis of *L. casei* strain Shirota has been described previously.[15]

Measurements of fecal organic acid concentrations and pH

Portions of weighed, homogenized stool samples were mixed with four volumes of 0.15 M perchloric acid and reacted at 4℃ for 12 hours. The mixture was separated by centrifugation at 20,000 × g for 10 minutes at 4℃, and then the supernatant was passed through a 0.45-μm membrane (Millipore Japan, Tokyo) and sterilized. Organic acid concentrations in the samples were measured using an HPLC (Waters high-performance liquid chromatography) system (Waters 432 Conductive Detector; Waters, Milford, MA, USA) and a Shodex RSpak KC-811 column (Showa Denko, Tokyo).[17] We calculated the concentrations of organic acids based on a standard curve prepared from a mixture of 1 to 20 mM succinic, lactic, formic, acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. The glass electrode of a D-51 pH meter (Horiba Seisakusho, Tokyo) was directly inserted into the homogenized stool samples to measure pH.

Definition of infection

The course of each patient was recorded daily in detail and infectious complications were recorded for up to 28 days after ICU admission. Pneumonia was defined as a characteristic pulmonary infiltrate on chest radiographs accompanied by leukocytosis. Meningitis was defined as a clinical syndrome (fever, hemodynamic instability, altered mental status, meningismus) in association with culture of a recognized pathogen from the cerebrospinal fluid. Bacteremia was diagnosed when microbes were isolated from a single blood culture. CR-BSI (catheter-related bloodstream infection) was defined regardless of the microorganism as positive peripheral blood cultures associated with the same organism being isolated from catheter-tip cultures. Wound infection was defined as spontaneous or surgically released purulent discharge that was positive for bacteria in cultures.

Statistics

Results are expressed as means ± SD. Data were statistically analyzed using the Fisher exact probability test and paired and unpaired Student *t*-tests as appropriate. $P < 0.05$ was considered statistically significant.

Results

After starting the trial, 8 of the 40 patients were excluded because data were insufficient in 4 and the other 4 were discharged from our hospital before study completion. Of the 32 patients who completed the trial, 20 and 12 comprised the synbiotic (S) and control (C) groups, respectively.

Table 1 describes the patients' characteristics. Age, gender, APACHE II scores and the ratio of trauma to non-trauma did not significantly differ between the two groups. All trauma patients underwent emergency surgery or interventional radiology. All non-trauma patients underwent emergency surgery. All of the patients tolerated enteral feeding well.

The activities of NK cells were similar between the groups before the trial (22.8 ± 16.8% vs. 24.5 ± 15.5%) but increased more in group S than in group C after the trial (37.3 ± 14.9% vs. 27.3 ± 18.4%), although the difference did not reach statistical significance. The NK cell activity significantly increased in group S ($P < 0.001$) during the trial but remained unchanged in group C (Figure 1A).

The TLC significantly increased in group S (before vs. after: 865 ± 319 vs. 1,139 ± 394/μl, $P < 0.01$) but remained unchanged in group C (1,012 ± 387 vs. 1,046 ± 354/μl) (Figure 1B).

The CRP value decreased significantly in groups S and C from 10.8 ± 5.7 to 2.5 ± 2.9 mg/dl ($P < 0.001$) and from 13.8 ± 6.3 to 3.6 ± 2.4 mg/dl ($P < 0.001$), respectively (Figure 2C). The WBC also significantly decreased in group S from 9,555 ± 3,258 to 6,275 ±

Table 1. Baseline characteristics of patients

	Synbiotic group (n = 20)	Control group (n = 12)
Age (y)	51.9 ± 21.4	55.2 ± 22.5
Gender (m/f)	7/13	8/4
APACHE II score	18.9 ± 7.6	20.6 ± 5.9
Trauma	15	6
Nontrauma	5	6
SAH, CH	3	3
Cerebral infarction	0	1
Colon perforation	1	1
Esophageal perforation	1	0
Necrotizing faciitis	0	1

SAH, subarachnoid hemorrhage; CH, cerebral hemorrhage
No intergroup differences were found.

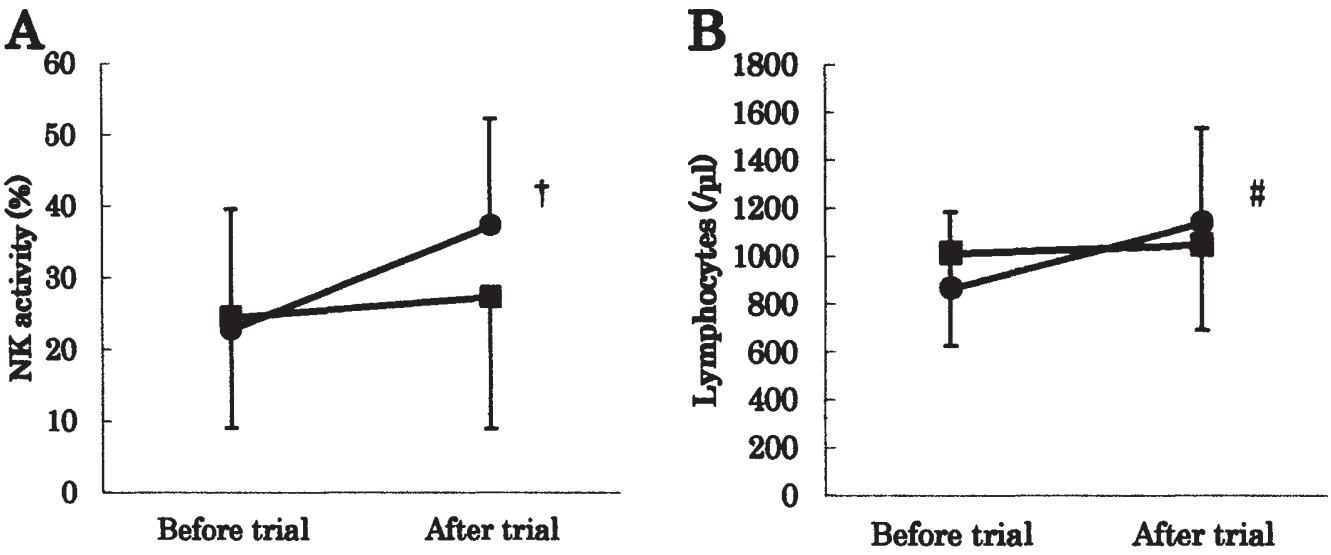

Figure 1. Natural killer (NK) cell activity (**A**) and lymphocyte counts (**B**) before and after trial in group S (received synbiotics, solid circles) and group C (control, solid squares).

The NK cell activities were similar between the groups before the trial but increased more in group S than that in group C after the trial, although the difference did not reach a statistical significance. The NK cell activity significantly increased in group S during the trial, but remained unchanged in group C. The TLC (total lymphocyte counts) significantly increased in group S but remained unchanged in group C.

†P < 0.001 vs. before the trial. #P < 0.01 vs. before the trial. Results are means ± SD.

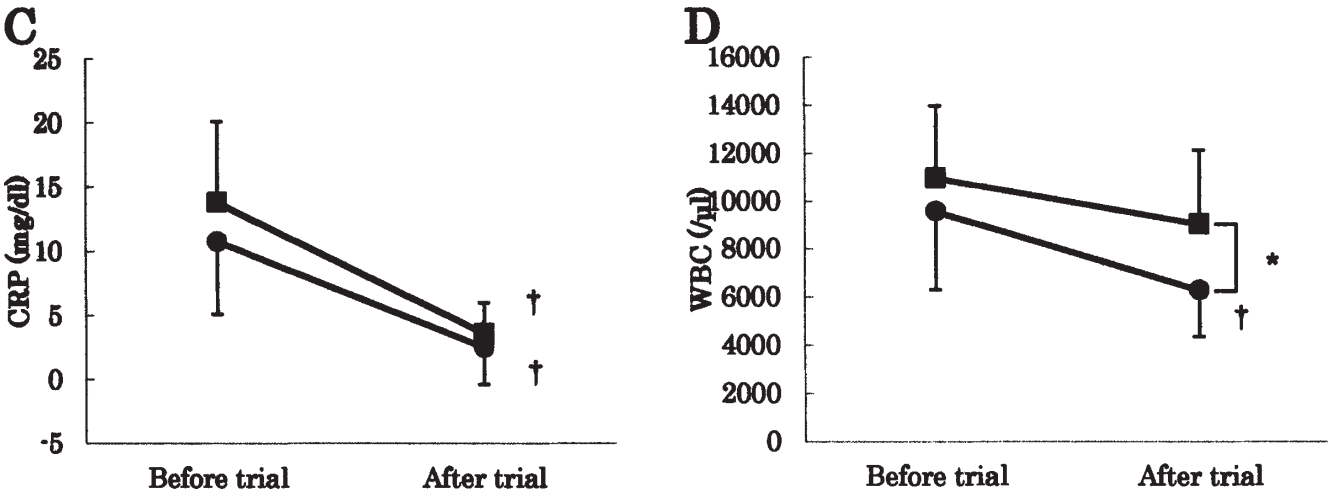

Figure 2. Levels of C-reactive protein (CRP) (C) and white blood cell counts (WBC) (D) before and after trial in group S (received synbiotics, solid circles) and group C (control, solid squares).

The CRP decreased significantly in groups S and C. The WBC significantly decreased in group S but remained unchanged in group C during the trial.

*P < 0.01 between groups. †P < 0.001 vs. before the trial. Results are means ± SD.

1,916/μl ($P < 0.001$), but remained unchanged in group C during the trial (from 10,967 ± 3,000 to 9,017 ± 3,124/μl) (Figure 2D).

The number of total bacteria in the fecal flora did not significantly differ between the two groups. The numbers of *Bifidobacterium* were significantly greater in group S than in group C. Group S had more total *Lactobacillus* and less *Pseudomonas* than did group C, but the differences were not statistically significant (Table 2).

The concentration of total fecal organic acids, particularly acetic and propionic acids was significantly greater in group S than that in group C (Table 3).

The rate of infectious complications was significantly reduced in group S for up to 28 days after ICU admission (group S vs. group C: 25% vs. 75%, $P < 0.01$). The duration of mechanical ventilation did not significantly differ between the groups (6.7 ± 6.3 vs. 8.2 ± 8.0 days). The cumulative duration of antibiotic therapy was shorter in group S than that in group C (7.8 ± 7.1 vs. 11.8 ± 7.7 days), but the difference did not reach statistical significance. The ICU stay was significantly shorter for group S than that for group C (17.7 ± 7.7 vs. 23.0 ± 5.8 days) (Table 4).

None of the patients died while in hospital or developed adverse events or side effects of any severity during the study period. Specifically, bowel ischemia or

Table 2. Fecal microflora after synbiotic trial

	Synbiotic group (n = 20)	Control group (n = 12)	P
Total bacteria	10.1 ± 0.7	10.0 ± 0.6	0.329
Clostridium coccoides group	9.1 ± 0.9	8.7 ± 1.4	0.207
C. leptum subgroup	8.8 ± 1.4	8.7 ± 1.2	0.395
Bacteroides fragilis group	8.9 ± 1.3	9.1 ± 0.8	0.313
Bifidobacterium	9.3 ± 1.2	7.9 ± 1.7	0.006
Atopobium cluster	8.5 ± 1.2	8.7 ± 1.3	0.353
Prevotella	6.2 ± 2.1	5.7 ± 1.4	0.217
C. perfringens	4.3 ± 1.5	3.6 ± 1.1	0.107
Total *Lactobacillus*	8.1 ± 1.0	7.0 ± 2.0	0.054
Enterobacteriaceae	7.2 ± 1.2	7.5 ± 1.5	0.317
Enterococcus	8.4 ± 1.1	8.7 ± 1.0	0.271
Staphylococcus	6.2 ± 1.6	6.5 ± 1.2	0.201
Pseudomonas	4.1 ± 1.5	5.0 ± 2.0	0.071
L. casei strain Shirota	8.1 ± 0.6	<5.7	<0.001

Values are mean ± SD ($\log_{10}$ cells/g of feces)

Table 3. Fecal organic acid concentrations after synbiotic trial

	Synbiotic group (n = 20)	Control group (n = 12)	P
Total organic acids	120.0 ± 45.0	79.8 ± 24.7	0.001
Succinic acid	10.0 ± 23.9	1.1 ± 2.0	0.058
Lactic acid	4.2 ± 6.1	2.8 ± 6.8	0.266
Formic acid	0.3 ± 0.5	1.0 ± 2.6	0.171
Acetic acid	71.5 ± 24.3	46.3 ± 15.0	0.002
Propionic acid	21.0 ± 10.5	15.8 ± 6.3	0.044
Butyric acid	7.4 ± 6.2	5.4 ± 4.5	0.168
Isovaleric acid	4.0 ± 2.8	4.7 ± 2.7	0.242
Valeric acid	2.1 ± 1.8	3.0 ± 2.5	0.109
pH	7.3 ± 1.0	7.3 ± 0.6	0.411

Values are mean ± SD (μmol/g of feces)

Table 4. Infectious complications after ICU admission, cumulative length of antibiotic therapy and lengths of ventilation and ICU stay

	Synbiotic group (n = 20)	Control group (n = 12)	P
Patients with any infectious complications (n)	5 (25.0%)	9 (75.0%)	0.008
Pneumonia	0	2	
Meningitis	0	2	
CRBSI[a]	2	2	
Bacteremia	2	2	
Cholangitis	1	0	
Wound infection	0	1	
Cumulative length of antibiotic therapy (days)	7.8 ± 7.1	11.8 ± 7.7	0.069
Length of ventilation (days)	6.7 ± 6.3	8.2 ± 8.0	0.285
ICU length of stay (days)	17.7 ± 7.7	23.0 ± 5.8	0.024

[a]Catheter related blood stream infection

bacteremia due to *Lactobacillus* (contain *L. casei* strain Shirota) or *Bifidobacterium* (contain *B. breve* strain Yakult) did not arise.

Discussion

The present findings suggest that synbiotics enhanced host immune function by improving intestinal microflora and organic acids, which subsequently decreased the rates of infectious complications among critically ill emergency surgical patients. Critically ill patients are at very high risk of developing infectious complications that can lead to multiple organ failure and impair prognosis. Several experimental and clinical studies have suggested that an inexpensive and practical therapy such as the enteral administration of probiotics could help to improve immune competence and reduce the incidence of nosocomial infections in the ICU.[1,7,18] Kotzampassi et al.[10] showed in a randomized control trial that synbiotics confer benefits upon critically ill trauma patients. The rates of infection, SIRS, severe sepsis and mortality were significantly reduced in patients treated with synbiotics. The length of the stay in the ICU and the duration of mechanical ventilation were also significantly reduced relative to a placebo. Alberda et al.[18] examined the effects of probiotics in a randomized, double-blind, placebo-controlled trial of critically ill patients. The patients responded to viable probiotics with a significantly larger increase in systemic IgA and IgG concentrations than those who received a placebo or sonicates. Several probiotic trials of critically ill patients have found that probiotics do not affect mortality and/or ICU-acquired infections.[19,20] Although a recent systematic review found no evidence to support the use of probiotics in adult ICU patients,[19] the inclusion of patients with various diseases and different probiotics among the studies limits the applicability of this conclusion.

There were no reports demonstrating effects of prebiotics to infection. When administered in combination with probiotics, prebiotics may enhance the survival of probiotic strains as well as stimulate the activity of the host's endogenous bacteria. The effectiveness of probiotics is similar to that of synbiotics, however, synbiotics are more effective to the intestinal flora. The purpose of this study was to investigate the effectiveness of synbiotics in critically ill, emergency surgical patients.

The present study found that WBC counts and CRP concentrations that serve as a marker of systemic inflammation were significantly decreased in group S. Our results suggest that synbiotics can favorably modify systemic inflammatory responses. The prevention of infectious complications by synbiotics led to a shorter stay in the ICU and a shorter cumulative duration of antibiotic therapy.

In the present study, CRP decreased significantly in both groups during the trial, whereas WBC significantly decreased in group S, but remained unchanged in group C. This result might be due to the difference of the response speed between WBC and CRP. WBC counts increased more rapidly than did CRP concentrations in patients with infections, consequently post-trial WBC counts were higher in the control group in which more infectious complications occurred. Elevated levels of CRP are associated with infection; however, levels of CRP may also be elevated in patients with trauma, surgery, or inflammatory disease. A decreased CRP level can provide support for the improving infection,

inflammation, and decreasing morbidity.

An important finding arising from the present study is that synbiotics enhanced NK cell activity and total lymphocyte counts. Sugawara et al.[5] investigated the effects of synbiotics in patients undergoing surgery to treat biliary cancer in a randomized controlled trial. Their results showed that preoperative synbiotics enhanced NK cell activity and lymphocyte counts. Sheih et al.[12] studied the effect of probiotic lactic acid bacteria on natural immunity in healthy volunteers. Their study showed increases in the phagocytic capacity of peripheral blood polymorphonuclear leukocytes and in the tumoricidal activity of NK cells, which act as cytolytic effectors and play a pivotal role in the innate immune system. Synbiotics can enhance systemic cellular immune responses and might help to promote natural immunity. Nagao et al.[21] reported enhancement of NK cell activity by oral intake of fermented milk containing *Lactobacillus*. *Lactobacillus* has been found to induce IL-12 production by macrophages; in turn, IL-12 stimulates T cells to secrete INF-γ, while both IL-12 and INF-γ augment NK cell activity. Many of the probiotic effects are mediated via immune regulation, in particular by control of the balance of proinflammatory and anti-inflammatory cytokines.

We used *B. breve* strain Yakult and *L. casei* strain Shirota as probiotics and galactooligosaccharides as prebiotics in the present study. Levels of *Bifidobacterium* and *Lactobacillus* were greater, whereas those of *Pseudomonas* were lower in group S than in group C. *Bifidobacterium* and *Lactobacillus* are predominant in the gut and confer various health benefits. These probiotic bacteria increase short-chain fatty acid concentrations in feces and potentially upregulate the systemic immune response.[22] Shimizu et al.[7] reported that synbiotics (*B. breve* strain Yakult, *L. casei* strain Shirota and galactooligosaccharides) maintain the gut flora and milieu and decrease the incidence of septic complications in patients with severe systemic inflammatory response syndrome. Regardless, increasing beneficial bacteria and decreasing harmful bacteria are important for maintaining host defenses, especially during recovery from critical illness. Intestinal microflora are important influences on the host immune system and ideally provide a natural defense against invading pathogens.[23] Microflora include beneficial, opportunistic and harmful bacteria. Beneficial intestinal flora protect the intestinal tract from the proliferation of harmful bacteria that manifest pathogenicity when host resistance is decreased.[24]

Total fecal concentrations of organic acids, particularly acetic and propionic acids, were significantly greater in group S than those in group C. Beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* ferment carbohydrate to produce organic acids, among which acetic acid that are considered short chain fatty acids are utilized as energy substrates mainly by intestinal epithelial cells. Short chain fatty acids also affect the motility of the intestinal tract and increase intestinal blood flow.[5,7,25] Our results indicate that maintaining levels of *Bifidobacterium* and *Lactobacillus* after initial administration maintains a favorable intestinal environment.

In the present study, during intensive care, patients who acquired infectious complications received antibiotics sensitive to causative bacteria until the infections improved. Because the rate of infectious complications was significantly higher in group C than that in group S, the cumulative duration of antibiotic therapy was longer in group C (not statistically significant). Therefore, the potential effect of antibiotic use on the intestinal flora cannot be excluded. In this study, however, more *Bifidobacterium* and *Lactobacillus* and less *Pseudomonas* in fecal flora were induced by administration of synbiotics; and, furthermore, the NK cell activity and the TLC significantly increased in the synbiotic group. Therefore, the administration of synbiotics reduced infectious complications, and this effect probably results from enhancing immune function through improving the intestinal microbial balance and milieu.

None of our patients developed adverse events or side effects during the study period. Whelan et al.[2] systematically reviewed the safety of probiotics in patients receiving nutritional support. Twenty case reports of adverse events in 32 patients described infections due to *Lactobacillus rhamnosus* GG or *Saccharomyces boulardii*. Risk factors included central venous catheters and disorders associated with increased bacterial translocation. Many probiotics have been safely administered to patients receiving nutritional support, although some probiotic products increase the risk of complications in specific types of patients such as those undergoing transplantation and those with pancreatitis.[2]

Although our findings were derived from a small patient cohort with various diseases, they nevertheless suggest that administering synbiotics to critically ill patients after emergency surgery could be an effective approach to preventing infectious complications. The type and concentration of the synbiotics, duration of therapy and route of administration were not comparable in most other trials, but synbiotics offer numerous practical benefits including low-cost preparation, long

shelf life and straightforward administration. In the era of increasing bacterial resistance and the relative exhaustion of the antibiotic pipeline, the emergence of novel strategies using synbiotics could become important for critically ill patients recovering.

Conclusions

The administration of synbiotics combined with early enteral nutrition can reduce infection complications in critically ill patients after emergency surgery. This beneficial effect may result from enhancing immune function through improving the intestinal microbial balance and milieu. The therapy presented herein including probiotic *L. casei* and *B. breve*, together with prebiotic galactooligosaccharides, shows promise in the management of critically ill patients in the ICU.

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