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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Association Between Preoperative Muscle Mass and Intraoperative Bacterial Translocation in Patients Undergoing Hepatectomy, Pancreatoduodenectomy, and Esophagectomy

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ABSTRACT

Purpose. This study investigated the correlation between the fecal profile and muscle mass, which has not been elucidated before.

Methods. This study included patients who underwent hepatectomy, pancreatoduodenectomy, or esophagectomy and had fecal samples collected preoperatively and mesenteric lymph nodes (MLNs) harvested intraoperatively to determine the fecal profile and presence of bacteria in the MLNs. Total psoas area (TPA) was measured at the third lumbar vertebra using preoperative computed tomography images. TPA was standardized by body surface area (BSA) using the following equation: standardized TPA (mm^2/m^2) (stTPA) = TPA (mm^2)/BSA (m^2). The fecal concentrations of representative microorganisms and organic acids also were measured.

Results. A total of 127 patients undergoing hepatectomy (n = 48), pancreatoduodenectomy (n = 44), and esophagectomy (n = 35) were included. The fecal levels of predominant obligate anaerobes showed a positive correlation with stTPA, whereas that of pathogenic microorganisms showed a negative correlation with stTPA. The fecal concentrations of total short chain fatty acids (the sum of acetic acid, propionic acid, and butyric acid) also showed a positive correlation with stTPA. The stTPA was significantly lower in patients with positive microorganisms in the MLNs (patients with bacterial translocation) compared to those without microorganisms in the MLNs (p = 0.047).

Conclusions. This study was the first to demonstrate the association between muscle mass and the fecal profile, as well as their association with bacterial translocation.

Despite recent advances in perioperative patient management, highly invasive gastrointestinal surgeries, such as major hepatectomy with extrahepatic bile duct resection, pancreatoduodenectomy, and esophagectomy, remain challenging procedures with a high morbidity rate.[1,2] One of the most common complications after these procedures is infection, including sepsis, intra-abdominal abscess, wound infection, and pneumonia. These complications lead to a prolonged hospital stay and sometimes induce fatal multiple organ dysfunction. Therefore, preventing the occurrence of postoperative infectious complications is a critical issue in patients undergoing highly invasive gastrointestinal surgery.

Previous studies indicated that the preoperative fecal profile is significantly associated with the incidence of postoperative infectious complications.[3–5] Specifically, the fecal organic acid profile, especially fecal concentrations of short chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid, have been shown to have a

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significant impact on the incidence of postoperative infectious complications.[3–6] A decreased fecal concentration of SCFAs reflects the condition of intestinal dysbiosis and impaired intestinal barrier function. In such conditions, the translocation of microorganisms from the gut to lymphatic flow and/or bloodstream (i.e., bacterial translocation) tend to occur, and these events subsequently lead to bacteremia and postoperative infectious complications.[3,7]

Decreased skeletal muscle mass and deteriorated exercise capacity is closely associated with the postoperative complications after gastrointestinal surgeries. Preoperative muscle mass loss has been shown to be associated with postoperative morbidity and mortality in patients undergoing extended hepatectomy with extrahepatic bile duct resection, pancreatoduodenectomy, and esophagectomy.[8–10] It also has been shown that an impaired exercise capacity before surgery is associated with the higher incidence of major complication after hepato-pancreato-biliary surgeries.[11]

Although both the fecal profile and muscle mass have been shown to be associated with the incidence of postoperative complications, the association between the fecal profile and muscle mass is completely unknown. Moreover, the impact of preoperative muscle mass on bacterial translocation has never been investigated before. Clarifying these questions may contribute to improving the preoperative management for patients undergoing highly invasive gastrointestinal surgery. The purpose of the present study, therefore, was to elucidate the association between the preoperative fecal profile and muscle mass in patients undergoing major hepatectomy with extrahepatic bile duct resection, pancreatoduodenectomy, and esophagectomy.

METHODS

Patients

This study included patients who underwent hepatectomy with extrahepatic bile duct resection, pancreatoduodenectomy, or esophagectomy with thoracotomy (from 2007 to 2012) and had fecal samples collected preoperatively and MLNs harvested intraoperatively to determine the fecal profile and presence of bacteria in the MLNs. All patients received antibiotic prophylaxis as a single intravenous drip infusion 30 min before surgery. The antibiotics were administered according to the kinetic half-lives of the antibiotics during the operation to maintain optimal concentrations in the blood and tissue (every 3 h in most cases). The fecal profile and the results of bacterial detection in the MLNs have been already shown in previous reports.[4,7,12,13] Some of the studied patients were also included in the clinical trial to test the effects of preoperative synbiotic treatment on the incidence of bacterial translocation.[4,13] Written, informed consent for participation was obtained from each patient before enrollment in this study, which was approved by the Human Research Review Committee of the Nagoya University Hospital (approved number: 2018-0441).

Assessment of Muscle Mass by CT Scan Images

Preoperative truncal muscle mass was assessed by the sum of the cross-sectional areas of the right and left psoas muscles (total psoas muscle area [TPA]). TPA was measured at the level of the third lumbar vertebra on the first image where both vertebral spines are visible (almost same level of umbilicus) as described previously.[8,14] The measured TPA was standardized by the body surface area (BSA) using the following equation: standardized TPA (mm^2/m^2) (stTPA) = TPA (mm^2)/BSA (m^2).[15,16] In this formula, the BSA was calculated using the Du Bois equation; $BSA = 0.007184 \times \text{body weight (kg)}^{0.425} \times \text{Height (cm)}^{0.725}$.[17]

Sampling of Feces

The fecal samples were collected 1 or 2 days before surgery. Approximately 1 g of feces was collected in a test tube that contained 2 ml of RNAlater® (Ambion, Austin, TX) for the determination of the bacterial count. The samples were held at room temperature for 10 min before storage at − 20 °C. The microorganisms were counted by the 16S and 23S rRNA-targeted RT-qPCR using the Yakult Intestinal Flora-SCAN (YIF-SCAN®), which is more sensitive than the conventional culture method for detecting microorganisms.[18,19] The second sample was collected in an empty test tube to determine fecal organic acid concentrations.

Sampling of MLNs During Surgery

MLNs were harvested with fresh surgical instruments under sterile conditions from the jejunal mesentery, which was not included in the area of lymph node dissection related to tumor removal. To avoid bacterial contamination, the intraperitoneal cavity was fully washed with 3 L of warm saline before sampling, and samples were harvested through the newly excised mesenteric serosa. In patients undergoing major hepatectomy with extrahepatic bile duct resection, an MLN sample was harvested before the preparation of hepaticojejunal reconstruction after the removal of a tumor. In patients undergoing pancreatoduodenectomy, an MLN sample was harvested after completing the pancreatoduodenectomy and before the

preparation of reconstruction. In patients undergoing esophagectomy, an MLN sample was harvested after the removal of a tumor and before the restoration of bowel continuity. The MLN samples were collected into a test tube containing 1 ml of RNAprotect™ Bacterial Reagent (Qiagen, Hilden, Germany) and were held at room temperature for 5 min before storage at − 80 °C.

Measurement of Bacterial Counts in MLNs and Feces

The YIF-SCAN® to measure the bacterial counts in feces and MLNs has already been described elsewhere.[12,19,20] The number of representative fecal microorganisms and the microorganisms that were considered pathogenic based on previous studies were counted.[5,6,21] Samples of feces and MLNs collected at the authors' institution were sent to the Yakult Central Institute for analysis. The patient's identity and clinical information were unknown to the technician performing the analysis.

Measurement of Fecal Organic Acid Concentrations

Feces were homogenized in four volumes of 0.15 μmol/l perchloric acid and allowed stand at 4 °C for 12 h. The homogenate was placed in an Eppendorf tube and centrifuged at 20,400×g at 4 °C for 10 min. Then, the resulting supernatant was passed through a filter with a pore size of 0.45 μm (Millipore Japan, Tokyo). The sample was analyzed for organic acids by high-performance liquid chromatography as previously described using a Waters system (432 Conductivity Detector; Waters, Milford, MA) equipped with 2 columns (Shodex Rspack KC-811; Showa Denko, Tokyo).[22]

Recording of Clinical Data and Postoperative Complications

Preoperative prognostic nutritional index (PNI) and neutrophil-to-lymphocyte ratio (NLR), as prognostic indicators of cancer patients, were calculated using blood test data obtained 1 or 2 days before surgery.[23–25] PNI was assessed using the following equation as described previously: PNI = 10 × serum albumin (g/dl) + 0.005 × total lymphocyte count in the peripheral blood (/mm^3).[26]

Detailed daily postoperative courses were recorded after surgery by an independent research assistant. A postoperative pancreatic fistula and delayed gastric emptying were classified according to the criteria of the International Study Group of Pancreatic Surgery.[27,28] Posthepatectomy liver failure and bile leakage were defined according to the criteria of the International Study Group of Liver Surgery.[29,30]

Statistical Analysis

Statistical analysis was performed using JMP® version 11 for Windows® (SAS Institute, Cary, NC). Continuous data were expressed as the median and inter quartile range and were analyzed using the nonparametric Wilcoxon rank-sum test. The χ^2 test or Fischer's exact test was used for the analysis of categorical variables. The correlation between the two variables was determined by Spearman's rank correlation coefficient. p value < 0.050 was considered statistically significant.

RESULTS

Preoperative and Intraoperative Factors

Between 2007 and 2012, 157 patients were eligible for inclusion criteria in this study. Among them, 17 patients who were scheduled for hepatectomy with extrahepatic bile duct resection, 6 patients who were scheduled for pancreatoduodenectomy, and 7 patients who were scheduled for esophagectomy were excluded because of unresectable tumor, refusal for participation, and sampling failure of MLNs during surgery. Therefore, a total of 127 patients (hepatectomy with extrahepatic bile duct resection, $n = 48$; pancreatoduodenectomy, $n = 44$; esophagectomy, $n = 35$) were subjected for the analysis. The median age of the study patients was 66.9 years, and 95 patients (75%) were male (Table 1). The median body mass index (BMI) was 21.2 kg/m^2. Most of the patients had malignancies, such as bile duct cancer (53%), pancreatic cancer (13%), and esophageal cancer (26%). All of the patients had PS values of 0 or 1. The median stTPA was 1110 mm^2/m^2. The median operation time was 560 min, and the median intraoperative blood loss was 1225 ml. A total of 56 patients (44%) received allogeneic blood transfusion.

Postoperative Factors

In every surgical procedure, major complications with Clavien-Dindo grade ≥ 3 occurred in approximately 40% of patients (Supplementary Table 1). The most common complication was infection in every procedure. Other commonly observed complications were bile leakage (25%) and liver failure (29%) in hepatectomy; pancreatic fistula (34%) and delayed gastric emptying (18%) in pancreatoduodenectomy; and pneumonia (26%) in esophagectomy. Two patients who had hepatectomy (4%) died after surgery.

TABLE 1 Preoperative factors

Preoperative factors	*n* = 127
Age (year)	66.9 (59.6–72.5)
Gender (male/female)	95/32
Body mass index (kg/m^2)	21.2 (19.9–22.9)
Comorbidity, *n* (%)	
Diabetes mellitus	24 (19)
Hypertension	39 (31)
Ischemic heart disease	6 (5)
Chronic pulmonary disease	4 (3)
Diagnosis, *n* (%)	
Bile duct cancer	67 (53)
Pancreatic cancer	16 (13)
IPMN	7 (6)
Chronic pancreatitis	2 (2)
Esophageal cancer	35 (26)
PS (0/1/2–4)	116/11/0
Serum albumin (g/dl)	3.6 (3.3–3.9)
Neutrophil count (/μ)	3100 (2400–3900)
Lymphocyte count (/μ)	1400 (1000–1800)
PNI	43 (39–48.5)
NLR	2.3 (1.6–3.0)
Body composition indexes	
BSA (m^2)	1.59 (1.46–1.70)
TPA (mm^2)	1789 (1368–2182)
TPA/BSA (= stTPA) (mm^2/m^2)	1110 (905–1293)

Continuous data are expressed as median (interquartile range)

IPMN intraductal papillary mucinous neoplasm, *PS* physical status, *PNI* prognostic nutritional index, *NLR* neutrophil lymphocyte ratio, *BSA* body surface area, *TPA* total psoas area, *stTPA* standardized total psoas area

Fecal Levels of Microorganisms and Organic Acids

Obligate anaerobes, such as *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, *Bifidobacterium*, and *Atopobium* cluster, were the predominant microorganisms in feces (Fig. 1a). The median total fecal organic acid concentration was 91.9 μmol/g of feces (Fig. 1b). Most of the fecal organic acids were SCFAs, such as acetic acid, propionic acid, and butyric acid.

Association Between Fecal Levels of SCFAs and Microorganisms

There were significant correlations between the fecal concentration of total SCFAs (sum of acetic acid, propionic acid, and butyric acid) and fecal counts of obligate anaerobes, such as *Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, *Bifidobacterium*, and *Atopobium* cluster (Fig. 2).

Association Between the Muscle Mass and Fecal Levels of Microorganisms/Organic Acids

Overall, predominant obligate anaerobes showed a positive correlation with stTPA (Fig. 3a). In contrast, pathogenic facultative anaerobes such as *Enterobacteriaceae* and *Enterococcus* showed a negative correlation with stTPA. Notably, the fecal counts of *Clostridium perfringens* (a pathogenic microorganism of toxic enteritis) showed a significant negative correlation with stTPA ($p = 0.030$).

All three SCFAs showed a positive correlation with stTPA (Fig. 3b). Accordingly, the total concentration of SCFAs and the total concentration of organic acids also showed a positive correlation with stTPA.

Factors Associated with the Incidence of Bacterial Translocation

The presence of microorganisms in the MLN samples was detected in 17 patients (35%) in hepatectomy, 11 patients (25%) in pancreatoduodenectomy, and 13 patients (37%) in esophagectomy. Detected bacterium in the MLN were predominantly obligate anaerobes. Various preoperative and intraoperative factors were compared between the patients with positive and negative microorganisms in the MLNs (Table 2). Consequently, only stTPA showed a significant association with the positivity of microorganisms in the MLNs. The stTPA was significantly lower in patients with positive microorganisms in the MLNs compared to those with negative microorganisms in the MLNs ($p = 0.047$). Moreover, the patients with positive microorganisms in the MLNs showed a significantly higher incidence rate of postoperative infectious complications compared to those with negative microorganisms in the MLNs (54% vs. 35%, $p = 0.044$; Table 2).

DISCUSSION

In this study, the authors found that there is a certain correlation between the muscle mass and the fecal profile. Fecal concentrations of predominant obligate anaerobes and SCFAs showed a positive correlation with stTPA. Although the exact mechanism was not clarified in this study, the results implied that the fecal profile may be associated with a maintenance of adequate muscle mass. Moreover, it was first shown that there is a correlation between bacterial translocation and preoperative muscle mass. According to the univariate analysis including

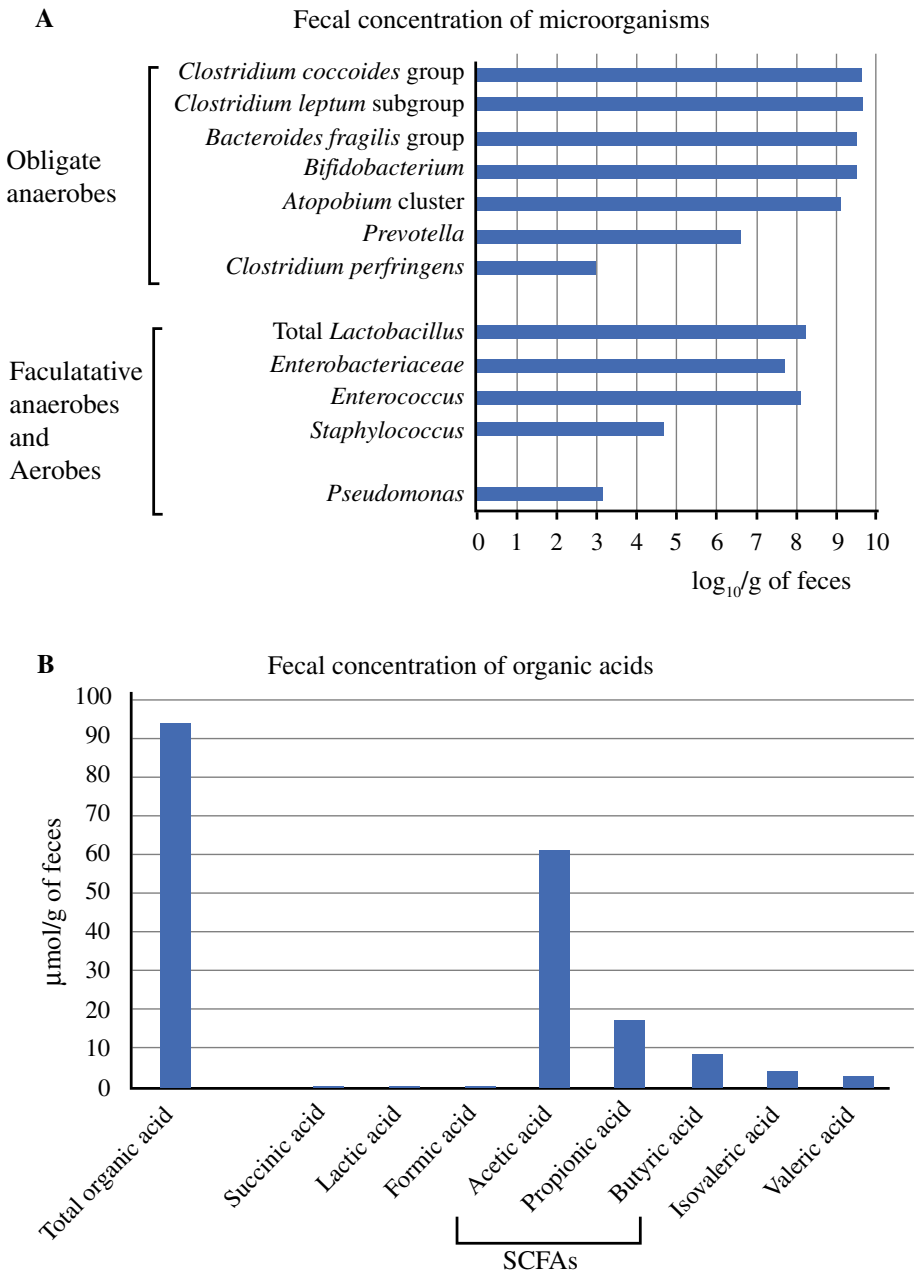


FIG. 1 The fecal levels of representative microorganisms (**a**) and organic acids (**b**). *SCFAs* short chain fatty acids

multiple preoperative and intraoperative factors, stTPA was the only factor that showed the association with the positivity of microorganisms in the MLN.

As indicated in this study, there is a significant positive correlation between the fecal levels of predominant microorganisms (obligate anaerobes) and that of SCFAs. In the normal fecal profile, undigested dietary fiber is metabolized to the SCFAs by intestinal microorganisms. The produced SCFAs not only serve as an energy source for the host but also improve intestinal barrier function. Our previous study indicated that the fecal concentration of SCFAs closely predicts the incidence of bacterial translocation during major hepatectomy with extrahepatic bile duct resection.[3] Interestingly, this study indicated a positive correlation between stTPA and the fecal levels of predominant microorganisms (obligate anaerobes) as well as SCFAs. Moreover, stTPA was significantly lower in patients who developed bacterial translocation during surgery. However, it is still unclear which is more important. In addition, the mechanism of mutual interaction between the fecal profile and muscle mass is still unknown. It can be speculated that an impaired microenvironment, even if it is not clinically evident, may constantly induce bacterial translocation, and a small number of microorganisms flowing into the systemic circulation may elicit a continuous inflammatory response in the host. Sustained

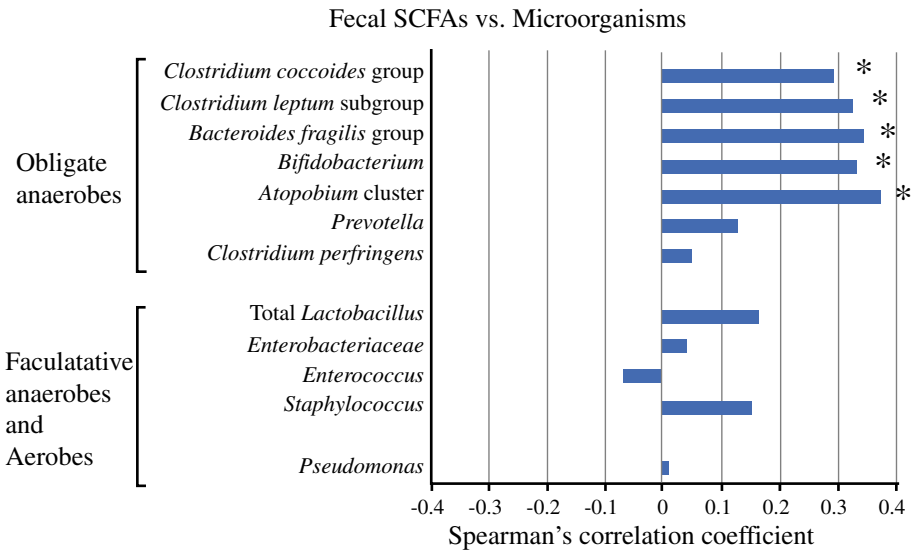
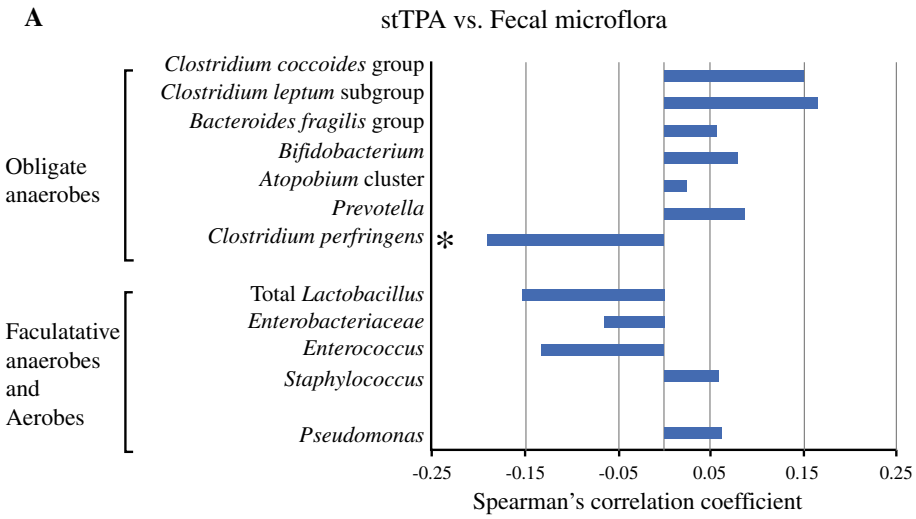


FIG. 2 Association between the fecal concentrations of short chain fatty acids (SCFAs) and representative microorganisms. *$p < 0.05$ by Spearman's rank correlation coefficient



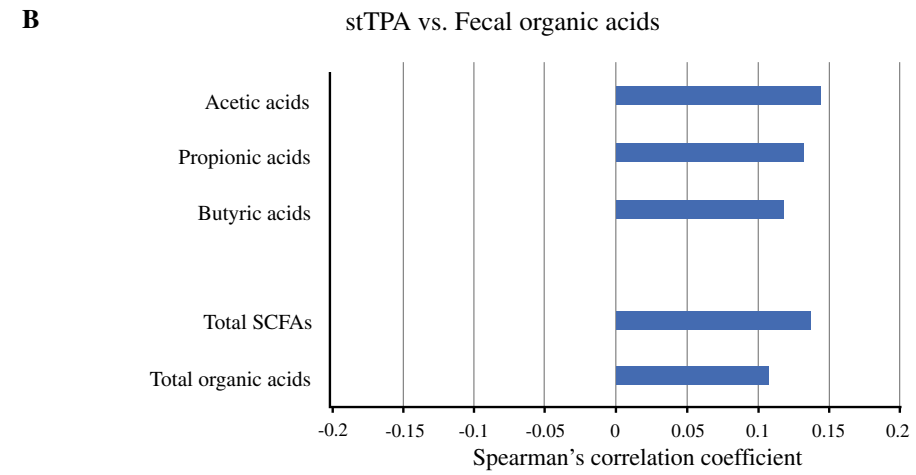


FIG. 3 Association between the standardized total psoas area (stTPA) and fecal levels of representative microorganisms (**a**) and fecal organic acids (**b**). *SCFAs* short chain fatty acids. *$p < 0.05$ by Spearman's rank correlation coefficient

TABLE 2 Possible risk factors of bacterial translocation

Factors	Microorganisms in the MLN		*p* value
	Negative (*n* = 86)	Positive (*n* = 41)	
Age (year)	66 (60–72)	67 (60–74)	0.578
Gender (male/female)	66/20	29/12	0.466
Body mass index (kg/m^2)	21.2 (20.1–22.9)	21.2 (19.1–22.8)	0.626
Comorbidity, *n* (%)			
Diabetes mellitus	13 (15)	11 (27)	0.115
Hypertension	25 (29)	14 (34)	0.562
Ischemic heart disease	4 (5)	2 (5)	0.955
Chronic pulmonary disease	4 (5)	0	0.161
PNI	42.8 (39–47.5)	43.5 (39.5–48.8)	0.418
NLR	2.41 (1.56–3.04)	2.22 (1.55–2.87)	0.669
TPA/BSA (= stTPA) (mm^2/m^2)	1168 (918–1338)	1055 (815–1246)	**0.047**
Operation time (min)	569 (501–648)	537 (459–712)	0.436
Blood loss (ml)	1092 (792–1718)	1500 (847–2173)	0.098
Allogeneic blood transfusion, *n* (%)	35 (41)	21 (51)	0.264
Postoperative infectious complication, *n* (%)	30 (35)	22 (54)	**0.044**

Statistically significant values ($p < 0.05$) are given in bold

Continuous data are expressed as median (interquartile range)

MLN mesenteric lymph node, *PNI* prognostic nutritional index, *NLR* neutrophil lymphocyte ratio, *TPA* total psoas area, *BSA* body surface area, *stTPA* standardized total psoas area

inflammatory conditions deplete muscle mass (a storehouse of protein), because the inflammatory response requires a large amount of protein.

With respect to the association between the fecal levels of microorganisms and stTPA, there was a significant negative correlation between the fecal counts of *Clostridium perfringens*, which is a pathogenic microorganism of toxic enteritis, and stTPA. Thus, it can be hypothesized that patients with low skeletal muscle mass are more susceptible to toxic enteritis induced by *Clostridium perfringens*. It also can be speculated that myonecrotic toxins produced by *Clostridium perfringens* may be absorbed from the intestine and deteriorate muscle mass.[31,32] The association between the fecal concentration of specific pathogenic microorganisms and total skeletal muscle mass also should be more thoroughly investigated in a future study.

This study indicated that both the fecal profile and muscle mass are correlated to bacterial translocation and subsequent infectious complications. How can these factors be improved before surgery? Previous studies at the authors' institution indicated that preoperative administration of synbiotics (a combination of probiotics and prebiotics) improved the fecal profile and prevented postoperative infectious complications.[5,6] Another study indicated that prehabilitation (a combination of preoperative exercise and amino acid supplement intake) increased muscle mass and exercise capacity even in cancer patients.[33] In this regard, the combination of synbiotic administration and prehabilitation before surgery may be one of the preferable ways to prevent postoperative infectious complications. It also should be investigated whether the improvement of the fecal profile by synbiotic administration further enhances muscle gain during prehabilitation.

This study has several limitations. The study included three different surgical procedures. All three procedures are highly invasive with a high incidence rate of postoperative complications. In this regard, the studied patients are not a representative population in gastrointestinal surgery. The major complication in each procedure also is different because of the different types of invasiveness. Further, large-scale, prospective studies should be planned to clearly elucidate the association between the fecal profile and muscle mass and their impact on the incidence of bacterial translocation induced by surgical stress. As mentioned, we only found a correlation between the fecal profile and muscle mass, and the mechanism of this observation is still unknown. Moreover, an effective strategy to improve the fecal profile and muscle mass before surgery have not been elucidated. Further mechanistic study is expected to clarify the mutual interaction between the fecal profile and maintenance of muscle mass.

CONCLUSIONS

This is the first study to show an association between muscle mass measured by CT scan images and the fecal profile. The results also indicate that there is an association

between patients with low muscle mass and bacterial translocation as measured during surgery. Further investigation is required to elucidate the mechanism of the impact of muscle mass on the fecal profile and body composition.

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