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Perioperative altered gut microbiota composition in patients with colorectal cancer: A prospective cohort study

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Abstract:	Objectives: The relationship between surgery and gut microbiota has recently attracted attention, however changes of gut microbiota and the composition are still unclear. The aim of this study was to investigate altered gut microbiota in patients with colorectal cancer in perioperative period. Methods: This prospective, single-center, observational cohort study included 48 patients with colorectal cancer who underwent radical surgery at the Oita University Hospital. Stool samples were collected on the day of and 2 days before surgery, and on postoperative days 1, 3, 7, and after 1 month and 1 year. The primary endpoint of this study was to elucidate gut microbiota composition using 16S rRNA gene sequencing, and the secondary endpoint was to elucidate its association with surgical outcomes. Results: Forty-eight patients were enrolled over a 2-year period from November 2016 to October 2018. Diversity of the gut microbiota decreased to approximately 30% of the preoperative level on the third postoperative day. It recovered to 60% of the preoperative state in the first month and to 80% in the first year. The preoperative gut microbiota was dominated by commensal bacteria (26%), whereas on the first postoperative day, the proportion of facultative anaerobes (46%) increased. Significant

	differences were not observed between the changes in the gut microbiota and any surgical outcomes. Conclusions: Among gut microbiota composition, facultative anaerobes changed to dominant during the perioperative period of colorectal cancer surgery. The results would provide microbial approaches to maintain gut microbiota composition in practice.

Original Article

Perioperative altered gut microbiota composition in patients with colorectal cancer: A prospective cohort study

A short running title: Perioperative gut microbiota in colorectal surgery

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ABSTRACT

Objectives: The relationship between surgery and gut microbiota has recently attracted attention, however changes of gut microbiota and the composition are still unclear. The aim of this study was to investigate altered gut microbiota in patients with colorectal cancer in perioperative period.

Methods: This prospective, single-center, observational cohort study included 48 patients with colorectal cancer who underwent radical surgery at the Oita University Hospital. Stool samples were collected on the day of and 2 days before surgery, and on postoperative days 1, 3, 7, and after 1 month and 1 year. The primary endpoint of this study was to elucidate gut microbiota composition using 16S rRNA gene sequencing, and the secondary endpoint was to elucidate its association with surgical outcomes.

Results: Forty-eight patients were enrolled over a 2-year period from November 2016 to October 2018. Diversity of the gut microbiota decreased to approximately 30% of the preoperative level on the third postoperative day. It recovered to 60% of the preoperative state in the first month and to 80% in the first year. The preoperative gut microbiota was dominated by commensal bacteria (26%), whereas on the first postoperative day, the proportion of facultative anaerobes (46%) increased. Significant differences were not observed between the changes in the gut microbiota and any

surgical outcomes.

Conclusions: Among gut microbiota composition, facultative anaerobes changed to dominant during the perioperative period of colorectal cancer surgery. The results would provide microbial approaches to maintain gut microbiota composition in practice.

Key words: gut microbiota, prospective cohort study, perioperative period

INTRODUCTION

The human gastrointestinal tract harbors various bacterial species[1]—the gut microbiota—that are responsible for the digestion of food, degradation of toxic substances, maturation of intestinal immunity,[2] and development of defense system against pathogens.[3, 4] Recently, the relationship between surgical intervention and changes in the gut microbiota has garnered the attention of the scientific community.[5, 6] , Particularly, previous reports demonstrated that postoperative changed in the gut microbiota after colorectal cancer surgery could affect to increase anastomotic leakage.[7, 8, 9]. However, the underlying mechanisms of the changes in the intestinal microbiota composition during the perioperative period of colorectal cancer remains unclear due to affect of surgical intervention, antibiotics, laxatives, and fasting. Furthermore, few studies have comprehensively investigated the changes in the perioperative intestinal microbiota over mid-to long-term periods.[10, 11]

In this study, we aimed to elucidate the changes in the gut microbiota composition of patients with colorectal cancer, immediately after surgery and during long-term follow-up. Moreover, we investigated the association of changes in gut microbiota composition with postoperative complications.

MATERIALS AND METHODS

Patients and study design

This prospective, single-center, observational cohort study included 48 patients with colorectal cancer who underwent radical surgery at the Oita University Hospital between November 2016 and October 2018. The study was approved by the Ethics Committee of our hospital (IRB number:1083). The inclusion criteria were as follows: colorectal cancer stage 0–III, diagnosed based on pathology; age between 20 and 80 years; function of major organs preserved; no prior chemotherapy or radiation therapy, and ability to undergo mechanical and chemical bowel lavage. The exclusion criteria were as follows: elderly patients over 81years old, those with advanced stage IV cancer, and those with obstructive colorectal cancer.

The primary endpoint of this study was to elucidate the perioperative changes in the gut microbiota composition during mid- to long-term follow-up. The secondary endpoint was to clarify the relationship between patient background, tumor factors, surgical treatment, and postoperative course.

Treatment protocol

Two days before the colorectal surgery, the patients were admitted to the hospital for stool sampling. On the day before the surgery, the patients were made to fast but only clear liquids, orally administered two oral antibiotics (kanamycin 2.25 g and

metronidazole, 750 mg total doses), and prepared for preoperative mechanical bowel lavage (administered 2 L of polyethylene glycol-electrolyte solution). Cefmetazole was administered as a prophylactic antibiotic on the day of the surgery, and its administration continued until the first postoperative day; administration of intestinal bacterial preparations as probiotics was initiated on the first postoperative day; and diet was initiated at the discretion of the attending physician. Stool samples were collected on postoperative days 1, 3, and 7 during the hospital stay, and after 1 month and 1 year of the surgery. Postoperative adjuvant chemotherapy was administered at the discretion of the attending physician.

Fecal sample collection

Regular fecal sample collection for the investigation of postoperative changes in the gut microbiota of patients with colorectal cancer is difficult due to irregular times of defecation after surgery. Therefore, we used the rectal swab stool collection method that enables reliable sample collection on specific dates during the perioperative period. In the patients with ostomy after abdominoperineal resection, the ostomy swabbed was performed.

Stool samples were collected six times on the day before surgery and on postoperative days 1, 3, and 7, and after 1 month and 1 year of surgery, using rectal

swabs; they were treated with guanidine thiocyanate solution (Techno Suruga Laboratory Co., Ltd.) and stored at -80°C in a freezer until further use.

16S rRNA gene sequencing

The bacterial DNA from different swab samples was extracted using the QIAamp DNA Mini Kit (Qiagen, Inc., Germany). The extracted DNA was used to amplify the V3-–V4 region of the 16S rRNA gene of the bacteria to determine the gut bacterial community structure. Primer set 341 F (5'-ACTCCTCCGGGAGGCAGCAG-3') and 806 R (5'-GGACTACGCGGGTATCTA AT-3') was used to amplify the target region, according to a previous report.[12]

The amplification condition was as follows: pre-denaturation at 95°C for 3 min; 25 cycles of denaturation at 95°C for 30 s; annealing at 55°C for 30 s; and extension at 72°C for 30 s; final extension at 72°C extension for 5 min; and storage at 4°C for further analyses. The amplified products were subjected to library preparation and sequencing on an Illumina MiSeq platform according to the manufacturer's instructions (Illumina Technologies, USA).

The raw operational taxonomic unit (OTU) files obtained from the Illumina sequencing instrument (Illumina Technologies) were analyzed using the CLC Metagenomics Workbench. Classifications to representative OTUs were assigned using

the Greengenes database as a reference dataset.[13]

Principal coordinate analysis (PCoA)

PCoA was used to compare the gut microbiota. PCoA is used to investigate similarity in data by analyzing the distance matrix of the data; it enables visualization of differences between data points. Data from all samples were obtained using UniFrac analysis, and the obtained distance matrix was subjected to PCoA to elucidate the intestinal microcosm of each fecal sample and investigate the similarity between biological communities. PCI and PC2 represent the first and second principal components, respectively, and the percentage after the principal.[14]

Clinical data collection and definition

Demographic data included gender, age, body mass index (BMI), medical history, clinical stage, approach, surgical procedures, postoperative fasting period and postoperative complications. The severity of complications was classified based on the Clavien-Dindo classification of surgical complications.

Statistical analysis

Student's *t*-test was used for comparison between two groups; the Bonferroni test was used for comparison between multiple groups; and permutational multivariate analysis of variance (PERMANOVA) was used for statistical analysis of beta diversity.

Statistical significance was set at P <0.05. significant.

RESULTS

Patient characteristics

In this study, 48 patients were enrolled over a 2-year period, from November 2016 to October 2018. The patient demographics and perioperative data are listed in Table 1. The participants included 25 and 23 men and women, respectively, with a median age of 67 years and a median body mass index (BMI) of 23. Colon and rectal resection were performed in 23 and 25 patients, respectively, with laparoscopic, open, and transanal approaches in 41, 3, and 4 patients, respectively. The median start of diet was 4 days. Intestinal bacteria were administered to 47 patients, whereas one patient was not administered intestinal bacteria due to patient's preference. Postoperative adjuvant therapy was administered to 18 patients.

Perioperative changes in gut microbiota

The follow-up of the postoperative samples was 100% until day 7, 98% at 1 month, and 16.6% at 1 year. The perioperative changes in gut microbiota diversity were observed over time; the number of gut bacterial species gradually decreased from 148 (preoperative) to 45 on day 7, and after 1 month of surgery, the number increased to 94,

which was approximately 60% of the preoperative number. In terms of long-term outcome, 1 year after surgery, the number of species recovered to 122, i.e., 80% of the preoperative number (Table 2 and Fig. 1).

Composition of gut microbiota

In preoperative samples, the majority of bacteria were identified as commensal bacteria (26%), including those belonging to genera *Prevotella* (13%) and *Bacteroides* (13%). However, on the first postoperative day, gram-positive cocci (46%), such as *Enterococcus* (23%), *Planococcus* (10%), and *Streptococcus* (3%), were detected. On the third postoperative day, the percentage of gram-positive cocci was the highest (63%). Approximately 1 month after surgery, the gut microbiota began resuming its preoperative structure, and after 1 year, the proportion of commensal bacteria was similar to that before surgery (27%) (Fig. 2).

PCoA

Compared with that during the preoperative period, the flora gradually changed from day 1, exhibiting the maximum difference on days 3 and 7. Thereafter, the gut microbiota composition was similar to that of the preoperative stage during the first year. The UniFrac distance, which represents the difference between samples in PCoA, was the largest (0.83) on days 3 and 7 and decreased from 1 month till 1 year after

surgery, indicating that the flora composition was becoming similar to the preoperative flora composition (Fig. 3).

Surgical outcomes

We observed the following postoperative complications: two cases of bowel obstruction, one case of anastomotic leakage, and one case of wound infection. Changes in the gut microbiota were examined in terms of patient background, tumor factors, surgical treatment, and postoperative course; however, significant differences were not observed between the groups.

DISCUSSION

In this prospective cohort study, we identified comprehensive changes in the intestinal microbiota composition of patients with colorectal cancer during perioperative periods, using 16S rRNA gene sequencing. We observed that the intestinal microbiota composition significantly changed immediately after surgery, with the maximum changes observed on days 3 and 7. The composition began returning to its preoperative state after 1 month, and returned to the preoperative state after 1 year even though small samples. To the best of our knowledge, this is the first study to elucidate the distribution of gut microbiota during both postoperative short- and long-term follow-up periods in

patients with colorectal cancer.

In studies on gut microbiota, several bacterial species cannot be identified using culture-based methods; therefore, metagenomic analyses using fecal samples are being performed.[15] Metagenomic analysis of the gut microbiota can be categorized into the following two main types: whole-genome metagenomic analysis of the pre-genome of the microbiota and metagenomic analysis of the 16S rRNA gene.[16] The former exhibits less PCR bias and can analyze functional genes, but the accuracy of taxonomy is lower than that by 16S rRNA gene metagenomic analysis. This method is also less popular because it is costly. Conversely, 16S rRNA gene metagenomics is diverse and useful for identifying microorganisms at the genus level.[1] Therefore, we used 16S rRNA gene metagenomics to identify changes in the gut microbiota composition during the mid- to long-term perioperative period of patients with colorectal cancer.

In this study, gram-negative rod commensal bacteria, such as *Prevotella* and *Bacteroides*, dominated preoperatively, whereas the proportion of gram-positive cocci, such as *Enterococcus*, increased postoperatively. One year after the surgery, the gut microbiota composition of the patients recovered to a state similar to that before the surgery. Changes in the gut microbiota in the early postoperative period were similar to those observed in other studies on gut microbiota during the perioperative period of

colorectal cancer. However, for the first time, this study revealed that the gut microbiota composition returned to its preoperative state as the postoperative period increased.

Factors influencing gut microbiota during colorectal cancer surgery include surgical intervention, antibiotics, bowel cleansing agents, oral butyrate preparations, and underlying diseases. Reddy et al. also investigated the prevalence of Enterobacteriaceae after various combinations of mechanical bowel cleansing, and administration of neomycin and/or synbiotics.[17] A similar concept, i.e., hydration and nutritional solution supplementation within 2 hours of a standby procedure, is already being used in hospitals. Patients with preoperative oral carbohydrate supplementation have a shorter hospital stay, an improved metabolic profile, and a reduced inflammatory response.[18] However, the gut microbiota composition is directly related to postoperative complications, and the direct relationship between preoperative treatment and gut microbiota changes has not been demonstrated in the present study. The gut microbiota is also susceptible to external factors, such as nutrition. Therefore, preoperative and postoperative diet should also be defined.[3] Furthermore, higher rate of postoperative enteritis has been reported in patients who undergo laparoscopic surgery, and an association has been reported between intestinal immunity and CO_2 insufflation during laparoscopic surgery.[19, 20] In addition to lifestyle, diseases such as obesity and diabetes

affect intestinal bacteria.[21-23] If lifestyle and underlying diseases are similar before and after surgery, the gut microbiota composition could return to its preoperative state.

Another factor that may affect the gut microbiota after surgery is postoperative adjuvant chemotherapy; however, in the present study, we did not find any significant changes in the gut microbiota between patients with and without chemotherapy even though a few cases with 1-year follow-up. Furthermore, in a previous study, the proportion of *Lactobacillus* spp. and *Bifidobacterium* spp. in the gut microbiota increased postoperatively in patients treated with synbiotics, but a postoperative increase in potentially pathogenic bacteria was not observed.[24] In another study, the incidence of infection-related complications was lower in the treated group than in the control group.[25] Further clinical studies on the effects of synbiotics and probiotics should be conducted.

The present study has several limitations. First, patient selection bias could not be ruled out owing to the exclusion of elderly patients, those with advanced stage IV cancer, and those with obstructive colorectal cancer. The effects of these factors on gut microbiota were not investigated in our study. Second, the patients who had not used their bowels for a long period, such as those with a covered stoma after colorectal cancer surgery, were not included. To clarify the relationship between bowel non-usage

and changes in the gut microbiota, cases with covered stoma should be included. Third, pre and postoperative diets were not predefined. As there is a close relationship between diet and gut microbiota, diet may have influenced the changes in the gut microbiota. Larger studies with a wider range of eligible patients should be performed to clarify the relationship between preoperative treatment and changes in gut microbiota.

CONCLUSIONS

In this study, we elucidated comprehensive changes in the gut microbiota composition in patients with colorectal cancer during postoperative periods. The gut microbiota changes in the early postoperative period peaked on the third postoperative day, with an increase in the proportion of gram-positive cocci, which are considered "harmful bacteria." As the postoperative period progressed from 1 month to 1 year, the gut microbiota composition returned to its preoperative state. Cohort studies with larger sample size and probiotic intervention are needed to clarify the clinical significance of changes in gut microbiota composition with long-term follow-up after colorectal cancer surgery.

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DISCLOSURE

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Disclosures. Takao Hara, Tsuyoshi Etoh, Takayuki Aiba, Shinichiro Empuku, Takahiro Hiratsuka, Yohei Kono, Tomonori Akagi, Shigeo Ninomiya, Yoshitake Ueda, Hidefumi Shiroshita and Masafumi Inomata have no conflicts of interest or financial ties to disclose for this article.

Author Contributions:

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Tsuyoshi Etoh: Conceptualization, Formal analysis, Investigation, Writing-review & editing, Funding acquisition.

Takayuki Aiba, Shinichiro Empuku, Takahiro Hiratsuka, Yohei Kono, Tomonori Akagi,

Shigeo Ninomiya: Data curation.

Hidefumi Shiroshita: Validation.

Yoshitake Ueda: Methodology.

Masafumi Inomata: Supervision.

All authors have given final approval for the version to be published.

REFERENCES

1. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312(5778):1355-9. doi:10.1126/science.1124234.
2. Krezalek MA, Alverdy JC. Current opinion in clinical nutrition and metabolic care: nutrition and the gastrointestinal tract. Curr Opin Clin Nutr Metab Care. 2016;19(5):347-52. doi:10.1097/MCO.0000000000000299.
3. Stavrou G, Kotzampassi K. Gut microbiome, surgical complications and probiotics. Ann Gastroenterol. 2017;30(1):45-53. doi:10.20524/aog.2016.0086.
4. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009;136(1):65-80. doi:10.1053/j.gastro.2008.10.080.
5. Weisburger JH, Bandaru SR, Narisawa T, Wynder EL. Germ-free status and colon tumor induction by N-methyl-N'-nitro-N-nitrosoguanidine. Proceedings of the Society for Experimental Biology and Medicine. 1975.
6. Gagniere J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E, Bringer MA, Pezet D, Bonnet M. Gut microbiota imbalance and colorectal cancer. World J Gastroenterol. 2016;22(2):501-18. doi:10.3748/wjg.v22.i2.501.
7. Snijders HS, Wouters MW, van Leersum NJ, Kolfschoten NE, Henneman D, de Vries AC, Tollenaar RA, Bonsing BA. Meta-analysis of the risk for anastomotic leakage, the postoperative mortality caused by leakage in relation to the overall postoperative mortality. Eur J Surg Oncol. 2012;38(11):1013-9. doi:10.1016/j.ejso.2012.07.111.
8. Shogan BD, Belogortseva N, Luong PM, Zaborin A, Lax S, Bethel C, Ward M,

Muldoon JP, Singer M, An G, Umanskiy K, Konda V, Shakhsheer B, Luo J, Klabbers R, Hancock LE, Gilbert J, Zaborina O, Alverdy JC. Collagen degradation and MMP9 activation by *Enterococcus faecalis* contribute to intestinal anastomotic leak. Sci Transl Med. 2015;7(286):286ra68. doi:10.1126/scitranslmed.3010658.

9. Shogan BD, Smith DP, Christley S, Gilbert JA, Zaborina O, Alverdy JC. Intestinal anastomotic injury alters spatially defined microbiome composition and function. Microbiome. 2014, 2:35. doi: 10.1186/2049-2618-2-35.

10. Lassen K, Soop M, Nygren J, Cox PB, Hendry PO, Spies C, von Meyenfeldt MF, Fearon KC, Revhaug A, Norderval S, Ljungqvist O, Lobo DN, Dejong CHC. Consensus review of optimal perioperative care in colorectal surgery: Enhanced Recovery After Surgery (ERAS) Group recommendations. Arch Surg. 2009;144(10):961-9.

11. Liu Z, Qin H, Yang Z, Xia Y, Liu W, Yang J, Jiang Y, Zhang H, Yang Z, Wang Y, Zheng Q. Randomised clinical trial: the effects of perioperative probiotic treatment on barrier function and post-operative infectious complications in colorectal cancer surgery - a double-blind study. Aliment Pharmacol Ther. 2011;33(1):50-63. doi:10.1111/j.1365-2036.2010.04492.x.

12. He T, Cheng X, Xing C. The gut microbial diversity of colon cancer patients and the clinical significance. Bioengineered. 2021;12(1):7046-60. doi:10.1080/21655979.2021.1972077.

13. http://greengenes.lbl.gov.

14. C L. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71(12):8228-35.

doi:10.1128/AEM.71.12.8228-8235.2005.

15. Amann RI, Ludwig W, Schleifer KH. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 1995;59:143-69.

16. Morgan XC, Huttenhower C. Chapter 12: Human microbiome analysis. PLoS Comput Biol. 2012;8(12):e1002808. doi:10.1371/journal.pcbi.1002808.

17. Reddy BS, Macfie J, Gatt M, Larsen CN, Jensen SS, Leser TD. Randomized clinical trial of effect of synbiotics, neomycin and mechanical bowel preparation on intestinal barrier function in patients undergoing colectomy. Br J Surg. 2007;94(5):546-54. doi:10.1002/bjs.5705.

18. Mathur S, Plank LD, McCall JL, Shapkov P, McIlroy K, Gillanders LK, Merrie AE, Torrie JJ, Pugh F, Koea JB, Bissett IP, Parry BR. Randomized controlled trial of preoperative oral carbohydrate treatment in major abdominal surgery. Br J Surg. 2010;97(4):485-94. doi:10.1002/bjs.7026.

19. Inomata M, Kusano T, Etoh T, Akagi T, Shibata T, Ueda Y, Tojigamori M, Shiroshita H, Noguchi T, Shiraishi N, Kitano S. Comparing incidence of enterocolitis after laparoscopic and open low anterior resection for stage II:III rectal cancer. Asian J Endosc Surg. 2014;7:214-21. doi: https://doi.org/10.1111/ases.12100.

20. Kusano T, Etoh T, Inomata M, Shiraishi N, Kitano S. CO(2) pneumoperitoneum increases secretory IgA levels in the gut compared with laparotomy in an experimental animal model. Surg Endosc. 2014;28(6):1879-85. doi:10.1007/s00464-013-3408-3.

21. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S,

Prifti E, Vieira-Silva S, Gudmundsdottir V, Pedersen HK, Arumugam M, Kristiansen K, Voigt AY, Vestergaard H, Hercog R, Costea PI, Kultima JR, Li J, Jorgensen T, Levenez F, Dore J, Meta HITc, Nielsen HB, Brunak S, Raes J, Hansen T, Wang J, Ehrlich SD, Bork P, Pedersen O. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015;528(7581):262-6. doi:10.1038/nature15766.

22. Ruth EL, Fredrik B, Turnbaugh P, Catherine AL, Robin DK, Jeffrey IG. Obesity alters gut microbial ecology. PNAS. 2005;102(32):11070–5.

23. Bouter KE, van Raalte DH, Groen AK, Nieuwdorp M. Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. Gastroenterology. 2017;152(7):1671-8. doi:10.1053/j.gastro.2016.12.048.

24. Tanaka K, Yano M, Motoori M, Kishi K, Miyashiro I, Ohue M, Ohigashi H, Asahara T, Nomoto K, Ishikawa O. Impact of perioperative administration of synbiotics in patients with esophageal cancer undergoing esophagectomy: a prospective randomized controlled trial. Surgery. 2012;152(5):832-42. doi:10.1016/j.surg.2012.02.021.

25. Lederer AK, Pisarski P, Kousoulas L, Fichtner-Feigl S, Hess C, Huber R. Postoperative changes of the microbiome: are surgical complications related to the gut flora? A systematic review. BMC Surg. 2017;17(1):125. doi:10.1186/s12893-017-0325-8.

Table 1. Patient characteristics and postoperative outcomes.

Factors	Patients (n=48)
Gender (male/female)	25/23
Age* (years)	67 (44–80)
Body mass index (kg/m^2)*	23 (17–36)
Prognostic nutritional index*	49 (28–61)
Medication history	
Hypotensive drugs	19
Hypoglycemic drugs	11
Proton pump inhibitors	11
Clinical stage	
0	4
I	13
II	10
III	21
Approach	
Laparoscopic	41
Open	3
Transanal	4
Procedures	
Local resection	5
Colectomy	18

Anterior resection	21
(with ileostomy)	(5)
Abdominoperineal resection	4
Postoperative fasting period[*] (days)	4 (1–44)
Postoperative complications [†]	
Anastomotic leakage	1
Surgical site infection	1
Ileus	2
Neurogenic bladder	3
Late ureteral injury	1
Cerebral infarction	1
Venous thromboembolism	1
Cellulitis at the instillation site	1

[*]Median (range); [†]Clavien-Dindo grade II or higher

1

	Pre	1D	3D	7D	1M	1Y
Facultative anaerobes	8.5	46.8	59.2	53.7	8.9	12.4
Enterococcus	1.1	22.8	36.2	37.2	1.4	2.0
Enterobacteriaceae	1.3	7.7	3.4	6.0	2.8	2.5
Staphylococcus	0.1	9.6	11.4	4.4	0.1	0.1
Lactobacillus	1.2	0.5	2.4	2.3	1.5	1.3
Corynebacterium	1.5	6.1	5.8	3.6	1.7	4.1
Fusobacterium	3.2	0.1	0.0	0.1	1.4	2.5
Obligate anaerobes	39.7	8.9	3.7	15.0	45.3	44.4
Bacteroides	13.0	2.0	1.1	4.0	10.6	12.0
Prevotella	12.7	1.0	0.4	2.9	9.8	15.5
Finegoldia	6.2	0.8	0.4	0.8	11.8	7.3
Parabacteroides	1.3	1.3	1.3	6.4	4.2	1.5
Peptoniphilus	4.9	0.9	0.2	0.4	6.0	7.0
Bifidobacterium	1.5	2.7	0.3	0.6	2.7	0.7
Clostridium	0.1	0.2	0.0	0.0	0.1	0.4
Obligate aerobes	4.2	15.0	26.0	14.2	4.8	2.1
Planococcaceae	0.5	10.1	17.0	5.7	0.4	0.0
Streptococcus	3.7	3.2	6.3	5.7	4.4	2.1
Pseudomonas	0.0	1.7	2.6	2.8	0.0	0.0
Others	47.6	29.3	11.1	17.1	41.0	41.2
Total	100	100	100	100	100	100

2 **Table 2. Genus level changes in gut microbiota composition during follow-up.**

3 Pre: preoperative day; D: postoperative day; M: postoperative month; Y: postoperative

year

FIGURE LEGENDS

Figure 1. Changes in microbial diversity

Figure 2. Change in the proportion of different bacterial genera comprising the gut microbiota

A: Proportion of each genus

B: Bacteriological classification according to oxygen requirement

Figure 3. Principal coordinate analysis

A: Each point shows the diversity of gut microbiota in each fecal sample

B: UniFrac distance during pre and postoperative stages, which represents the difference between samples in principal coordinate analysis

Original Article

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A short running title: Perioperative gut microbiota in colorectal surgery

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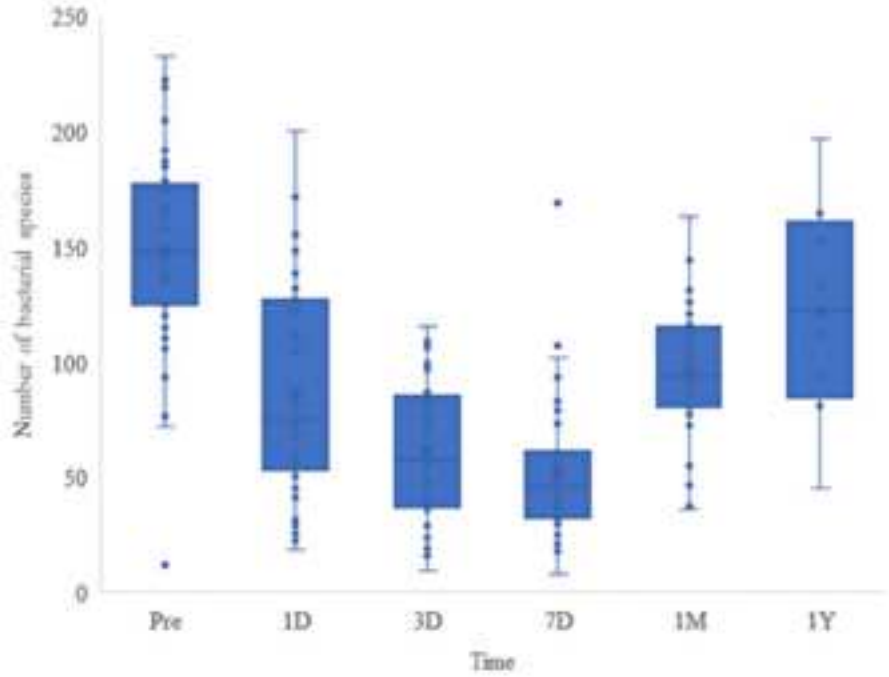

Figure 1

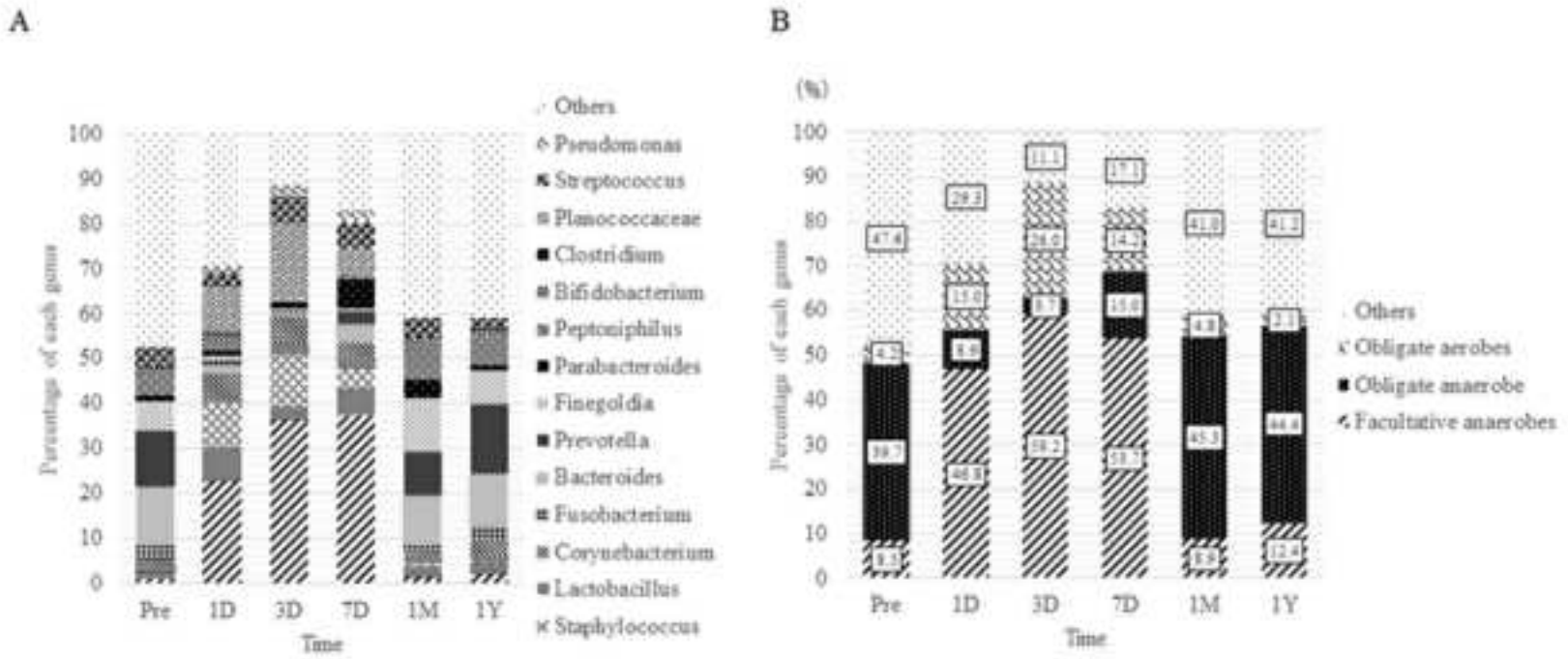

Figure 2

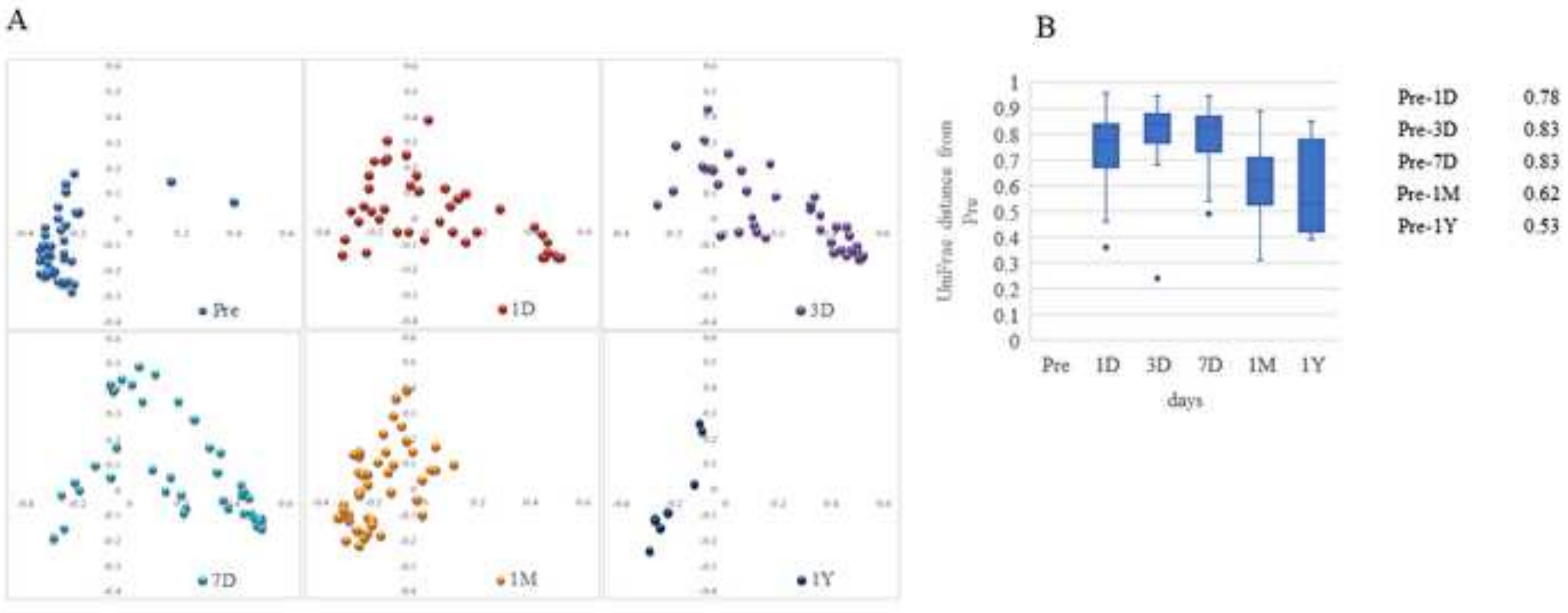

Figure 3

Table 1. Patient characteristics and postoperative outcomes.

Factors	Patients (n=48)
Gender (male/female)	25/23
Age* (years)	67 (44–80)
Body mass index (kg/m^2)*	23 (17–36)
Prognostic nutritional index*	49 (28–61)
Medication history	
Hypotensive drugs	19
Hypoglycemic drugs	11
Proton pump inhibitors	11
Clinical stage	
0	4
I	13
II	10
III	21
Approach	
Laparoscopic	41
Open	3
Transanal	4
Procedures	
Local resection	5
Colectomy	18

Anterior resection	21
(with ileostomy)	(5)
Abdominoperineal resection	4
Postoperative fasting period[*] (days)	4 (1–44)
Postoperative complications [†]	
Anastomotic leakage	1
Surgical site infection	1
Ileus	2
Neurogenic bladder	3
Late ureteral injury	1
Cerebral infarction	1
Venous thromboembolism	1
Cellulitis at the instillation site	1

[*]Median (range); [†]Clavien-Dindo grade II or higher

	Pre	1D	3D	7D	1M	1Y
Facultative anaerobes	8.5	46.8	59.2	53.7	8.9	12.4
Enterococcus	1.1	22.8	36.2	37.2	1.4	2.0
Enterobacteriaceae	1.3	7.7	3.4	6.0	2.8	2.5
Staphylococcus	0.1	9.6	11.4	4.4	0.1	0.1
Lactobacillus	1.2	0.5	2.4	2.3	1.5	1.3
Corynebacterium	1.5	6.1	5.8	3.6	1.7	4.1
Fusobacterium	3.2	0.1	0.0	0.1	1.4	2.5
Obligate anaerobes	39.7	8.9	3.7	15.0	45.3	44.4
Bacteroides	13.0	2.0	1.1	4.0	10.6	12.0
Prevotella	12.7	1.0	0.4	2.9	9.8	15.5
Finegoldia	6.2	0.8	0.4	0.8	11.8	7.3
Parabacteroides	1.3	1.3	1.3	6.4	4.2	1.5
Peptoniphilus	4.9	0.9	0.2	0.4	6.0	7.0
Bifidobacterium	1.5	2.7	0.3	0.6	2.7	0.7
Clostridium	0.1	0.2	0.0	0.0	0.1	0.4
Obligate aerobes	4.2	15.0	26.0	14.2	4.8	2.1
Planococcaceae	0.5	10.1	17.0	5.7	0.4	0.0
Streptococcus	3.7	3.2	6.3	5.7	4.4	2.1
Pseudomonas	0.0	1.7	2.6	2.8	0.0	0.0
Others	47.6	29.3	11.1	17.1	41.0	41.2
Total	100	100	100	100	100	100

Table 2. Genus level changes in gut microbiota composition during follow-up.

Pre: preoperative day; D: postoperative day; M: postoperative month; Y: postoperative

year