**Name of Journal:** *World Journal of Gastrointestinal Oncology*

**Manuscript NO:** 66044

**Manuscript Type:** ORIGINAL ARTICLE

***Basic Study***

**Dysbiosis of the duodenal microbiota as a diagnostic marker for pancreaticobiliary cancer**

Sugimoto M *et al.* Duodenal microbiota in pancreaticobiliary cancer

Mitsuru Sugimoto, Kazumichi Abe, Tadayuki Takagi, Rei Suzuki, Naoki Konno, Hiroyuki Asama, Yuki Sato, Hiroki Irie, Ko Watanabe, Jun Nakamura, Hitomi Kikuchi, Mika Takasumi, Minami Hashimoto, Tsunetaka Kato, Ryoichiro Kobashi, Takuto Hikichi, Hiromasa Ohira

**Mitsuru Sugimoto, Kazumichi Abe, Tadayuki Takagi, Rei Suzuki, Naoki Konno, Hiroyuki Asama, Yuki Sato, Hiroki Irie, Ko Watanabe, Jun Nakamura, Hitomi Kikuchi, Mika Takasumi, Minami Hashimoto, Tsunetaka Kato, Ryoichiro Kobashi, Hiromasa Ohira,** Department of Gastroenterology, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan

**Takuto Hikichi,** Department of Endoscopy, Fukushima Medical University Hospital, Fukushima 960-1295, Japan

**Author contributions:** Sugimoto M wrote the paper and designed and performed the research and laboratory experiments; Abe K provided advice on the laboratory experiments and research; Takagi T, Suzuki R, Konno N, Asama H, Sato Y, Irie H, Watanabe K, Nakamura J, Kikuchi H, Takasumi M, Hashimoto M, Kato T, and Kobashi R provided clinical advice; Hikichi T supervised the report; Ohira H supervised the report and writing of the paper.

**Corresponding author: Mitsuru Sugimoto, MD, PhD, Assistant Professor, Doctor,** Department of Gastroenterology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima 960-1295, Japan. kitachuuou335@yahoo.co.jp

**Received:** March 22, 2021

**Revised:** July 10, 2021

**Accepted:** September 16, 2021

**Published online:**

**Abstract**

BACKGROUND

Pancreaticobiliary cancer (PB Ca) is a lethal disease, and a useful diagnostic marker is urgently needed. A correlation between the human microbiota and malignant gastrointestinal diseases was recently reported.

AIM

To investigate the efficacy of the duodenal microbiota for diagnosing PB Ca.

METHODS

We recruited 22 patients with benign pancreaticobiliary diseases (benign group) and 12 patients with PB Ca (malignant group). The duodenal microbiota of each patient was analyzed by the 16S rDNA terminal restriction fragment length polymorphism method. Patient characteristics, tumor markers, and relative abundances of the duodenal microbiota were compared between the benign and malignant groups.

RESULTS

Cancer antigen 19-9 (CA19-9), *Bifidobacterium*, *Clostridium* cluster XVIII, and *Prevotella* levels differed significantly between the benign and malignant groups. *Clostridium* cluster XVIII had the greatest area under the receiver operating characteristic curve (AUC) among the four factors with respect to diagnosing PB Ca (cutoff value: 3.038%; sensitivity: 58.3%; specificity: 95.2%; AUC: 0.81). The combination of *Clostridium* cluster XVIII (cutoff value: 3.038%) and CA19-9 Levels (cutoff value: 18.8 U/mL) showed 91.7% sensitivity and 71.4% specificity for diagnosing PB Ca.

CONCLUSION

The duodenal microbiota may be useful for PB Ca screening.

**Key Words:** Pancreaticobiliary cancer; Diagnostic marker; Duodenal microbiota; *Clostridium* cluster XVIII; Cancer antigen 19-9

Sugimoto M, Abe K, Takagi T, Suzuki R, Konno N, Asama H, Sato Y, Irie H, Watanabe K, Nakamura J, Kikuchi H, Takasumi M, Hashimoto M, Kato T, Kobashi R, Hikichi T, Ohira H. Dysbiosis of the duodenal microbiota as a diagnostic marker for pancreaticobiliary cancer. *World J Gastrointest Oncol* 2021; In press

**Core Tip:** Recently, a correlation between the human microbiota and malignant gastrointestinal diseases was reported. In this report, the efficacy of the duodenal microbiota for diagnosing pancreaticobiliary cancer (PB Ca) was investigated. The combination of *Clostridium* cluster XVIII (cutoff value: 3.038%) and cancer antigen 19-9 Levels (cutoff value: 18.8 U/mL) showed 91.7% sensitivity and 71.4% specificity for diagnosing PB Ca. In conclusion, the duodenal microbiota may be useful for PB Ca screening.

**INTRODUCTION**

Pancreaticobiliary cancer (PB Ca) is a lethal disease[[1](#_ENREF_1" \o "Siegel, 2018 #1)]. Surgery is the only radical treatment for pancreatic cancer, but unfortunately, many pancreatic cancer patients have advanced-stage lesions or other organ metastases, and they thus are not candidates for surgery[[2](#_ENREF_2" \o "Geer, 1993 #2)]. For those who can undergo surgical treatment, the 5-year survival rate is reported to be 20%-30%[[3](#_ENREF_3" \o "Picozzi, 2017 #1582),[4](#_ENREF_4)].

In general, biliary tract cancer is difficult to diagnose. Conventional diagnostic methods include evaluating tumor markers [cancer antigen 19-9 (CA19-9) or carcinoembryonic antigen (CEA)], biliary biopsy, biliary juice cytology, and brush cytology, but the diagnostic power of these methods is not sufficient[[5-19](#_ENREF_5" \o "Uchida, 2008 #5)]. It has been reported that serum CA19-9 elevation is observed in 85% of patients with cholangiocarcinoma, though elevation of this marker can also be found in benign obstructive jaundice. Similarly, elevated serum CEA, which is not seen in obstructive jaundice, occurs in only 30% of patients with cholangiocarcinoma[[20](#_ENREF_20" \o "Khan, 2002 #20)]. Therefore, effective diagnostic methods for the early diagnosis of PB Ca are urgently needed. Recently, a correlation between the human microbiota and malignant gastrointestinal diseases was reported[[21-26](#_ENREF_21" \o "Kostic, 2012 #21)]. In addition, oral and salivary microbiota communities have been reported to be effective in diagnosing pancreatic cancer or predicting the onset of pancreatic cancer[[27-29](#_ENREF_27)], and the risk of pancreatic cancer is reportedly increased in patients with a history of periodontal disease[[30](#_ENREF_30)]. Furthermore, serum antibodies against oral microbiota are reported to be a risk factor for the onset of pancreatic cancer[[31](#_ENREF_31" \o "Michaud, 2013 #715)]. However, the mechanism by which this dysbiosis leads to pancreatic cancer is unknown, especially as the pancreas is relatively distant from the mouth.

Thus, we hypothesized that the duodenal microbiota would be more efficient than the oral microbiota for diagnosing PB Ca because the duodenum is closer to the bile duct and pancreas than the oral cavity. The aim of this study was to determine the efficacy of the duodenal microbiota for diagnosing PB Ca.

**MATERIALS AND METHODS**

***Ethical approval***

This study was approved by the Institutional Review Board of Fukushima Medical University.

***Patients***

We assessed 34 patients with pancreaticobiliary disease who visited our hospital over two years. Twenty-two patients were diagnosed with benign pancreaticobiliary diseases (benign group) [chronic pancreatitis: 6; intraductal papillary mucinous neoplasm (IPMN): 5; gallbladder adenomyomatosis: 3; autoimmune pancreatitis: 3; benign common bile duct (CBD) stricture of unknown origin: 2; serous cystic neoplasm: 2; and CBD stone: 1] (Table 1). The other 12 patients were diagnosed with PB Ca (malignant group) (pancreatic cancer: 9; bile duct cancer: 3). The patients provided written informed consent to participate in this study. For all pancreatic cancer cases, the lesion was located in the head. Eight pancreatic cancer patients were diagnosed by endoscopic ultrasound-guided fine needle aspiration. One pancreatic cancer patient was diagnosed with intraductal papillary mucinous carcinoma with evident worsening of the lesion by imaging. Benign diseases were diagnosed by no histological malignancy or unchanging lesions after a clinical course of at least six months. Furthermore, the IPMN patients in the benign group did not have high-risk stigmata or worrisome features[[32](#_ENREF_32" \o "Tanaka, 2017 #1825)]. The cases of bile duct cancer were diagnosed by biliary biopsy or surgery. According to the cytology grade, classes IV and V were diagnosed as malignancies. The stage of PB Ca was determined based on the UICC classification, ver. 8.

The patients did not receive antibiotic agents for at least a week prior to duodenal juice collection, and they did not receive steroids at all.

***Sample collection and DNA extraction***

An endoscope was used under sedation with midazolam. The endoscope was advanced to the duodenum, and 0.5-1.0 mL of duodenal juice was collected through a catheter and stored at -20°C. The endoscope used was Q260 and Q260H, and the catheter was a PR-109Q-1 or PR-104Q-1 (Olympus, Tokyo, Japan).

Bacterial DNA was extracted from duodenal juice samples in accordance with a previous report by Takahashi *et al*[[33](#_ENREF_33" \o "Takahashi, 2014 #1177)].

***Terminal restriction fragment length polymorphism***

Terminal restriction fragment (T-RF) length polymorphism (T-RFLP) was performed by TechnoSuruga Laboratory (Shizuoka, Japan) according to Nagashima’s methods[[34](#_ENREF_34" \o "Nagashima, 2003 #61),[35](#_ENREF_35)]. The 16S rRNA gene was amplified from the extracted DNA using the primers 5’ FAM-labeled 516F (5’-TGCCAGCAGCCGCGGTA-3’) and 1510R (5’- GGTTACCTTGTTACGACTT-3’) and HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany) with a Thermal Cycler Dice (Takara, Shiga, Japan). The amplification program used was as follows: preheating at 94°C for 15 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 2 min; and a terminal extension at 72°C for 10 min. DNA amplification was verified by electrophoresis of the polymerase chain reaction (PCR) products (2 μL) through a 1.0% agarose gel with Tris-acetate-EDTA buffer. The amplified DNA was purified by a MultiScreen PCR96 Filter Plate (Millipore, Billerica, MA, United States).

The purified PCR product (3 μL) was digested with 10 U of Fast Digest *BseL*I (*Bsl*I) (Thermo Fisher Scientific) in a total volume of 15 μL at 37°C for 10 min. The restriction digestion products (0.5 μL) were mixed with 0.1 μL of a DNA fragment-length standard size marker and 10 μL of deionized formamide. The standard size marker was MapMarker X-Rhodamine Labeled 50-1000 bp (Bio Ventures, Murfreesboro, TN, United States). The samples were denatured at 95°C for 2 min and then placed immediately on ice. The T-RF length was established using an ABI PRISM 3130xl genetic analyzer (Thermo Fisher Scientific), and the length and peak area were determined using the genotyping software GeneMapper (Thermo Fisher Scientific). The fragment sizes were estimated using the Local Southern method in GeneMapper software (Thermo Fisher Scientific). If the peak height was less than 50 fluorescence units, the T-RF was excluded from the analysis. The fragments were resolved to one base pair by manual alignment of the size standard peaks from different electropherograms, and the predicted T-RFLP patterns of the 16S rDNA of known bacterial species were obtained using publicly available sequences. T-RFs were divided by operational taxonomic units (OTUs), and bacterial classification was performed according to the ratio of each OTU per total OTU area. The OTUs were identified by correspondence to a database of human intestinal flora (https://www.tecsrg.co.jp/t-rflp/index.html).

***Analyzed traits***

Patient characteristics and tumor markers (age, sex, reduction in body weight ≥ 5 kg within 6 mo prior to duodenal juice sampling, intake of proton pump inhibitors, CA19-9) were compared between the two groups. The body weight marker was selected for the following reasons. The composition ratio of the microbiota has been reported to be different between subjects with obesity and those with a normal body mass index[[36](#_ENREF_36" \o "Ley, 2006 #1598)]. Because the intake of high-fat foods influences the quantity and composition of bile acid, the intestinal bacterial flora might change[[37](#_ENREF_37" \o "Yatsunenko, 2012 #1597)]. The relative abundances of duodenal microbiota members (*Bacteroides*, *Bifidobacterium*, Lactobacillales, *Prevotella*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, and others) were compared between the benign and malignant groups.

***Statistical analysis***

Normally distributed continuous variables were compared using Student’s *t* test and nonnormally distributed continuous variables using the Mann-Whitney *U* test. Nominal variables were compared with Fisher’s exact test. A receiver operating characteristic (ROC) curve was employed to compare the accuracy of the biomarkers. The relevance among several markers was investigated by spearman's rank correlation coefficient. The *P* value < 0.05 was considered statistically significant.

These statistical analyses were performed using the EZR platform (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of R-commander that was designed to perform functions that are frequently used in biostatistics[[38](#_ENREF_38" \o "Kanda, 2013 #65)].

**RESULTS**

Among the patient characteristics and tumor markers, age and CA19-9 Levels were significantly different between the benign and malignant groups (mean ± standard deviation, age: 63.3 ± 12.2 *vs* 73.0 ± 8.3 years, *P* value = 0.016; median (range), CA19-9: 5.4 (2.0-54.8) *vs* 22.8 (2.0-9893.2), *P* value = 0.03) (Table 2).

Comparison of microbiome components revealed *Bifidobacterium*, *Clostridium* cluster XVIII, and *Prevotella* to be significantly different between the benign and malignant groups (median (range), *Bifidobacterium*: 0 (0-0.5)% *vs* 0.3 (0-4.5)%, *P* value < 0.05; *Clostridium* cluster XVIII: 1.3 (0-3.9)% *vs* 3.6 (0.8-14.9)%, *P* value = 0.006; *Prevotella*: 2.2 (0-25.9)% *vs* 0.1 (0-10.9)%, *P* value = 0.04) (Figure 1 and Table 3).

To determine the influence of age on CA19-9 Levels and microbiome composition, these factors were compared between the subgroup of patients < 69 years and the subgroup of those ≥ 69 years (the median age of all patients was 69 years). According to the results, CA19-9 Levels, *Bifidobacterium*, *Clostridium* cluster XVIII, and *Prevotella* were not influenced by age (Table 4).

We assessed the ability of the microbiota to diagnose PB Ca by calculating the area under the ROC curve (AUC) and found that *Clostridium* cluster XVIII had the highest AUC (cutoff value: 3.038%, sensitivity: 58.3%, specificity: 95.2%, AUC: 0.81) among the three microbiome components (*Bifidobacterium*, *Clostridium* cluster XVIII, *Prevotella*) and CA19-9 Levels (Figure 2).

The combination of *Clostridium* cluster XVIII (cutoff value: 3.038%) and CA19-9 Levels (cutoff value: 18.8 U/mL) was also examined as a marker to diagnose PB Ca; the sensitivity of this combination was 91.7% (11/12), and the specificity was 71.4% (15/21) (Table 5). CA19-9 data were missing for one patient in the benign group.

**DISCUSSION**

In this study, we investigated which members of the duodenal microbiota could aid in diagnosing PB Ca and found *Clostridium* cluster XVIII to be more useful than CA19-9 Levels and other bacteria for diagnosing PB Ca. Notably, the combination of *Clostridium* cluster XVIII and CA19-9 Levels showed high sensitivity, indicating that this combination is valuable for screening patients for PB Ca.

As mentioned above, the oral microbiota has been considered to be a biomarker in pancreatic cancer. First, Michaud *et al*[[30](#_ENREF_30" \o "Michaud, 2007 #30)] reported that a history of periodontal disease was a risk factor for pancreatic cancer. After that, several oral microbes were reported to be more abundant and possible predictors and risk factors for survival in pancreatic cancer (Table 6)[[27-29](#_ENREF_27),[39](#_ENREF_39),[40](#_ENREF_40)]. In addition, antibodies against *Porphyromonas gingivalis* can serve as a risk factor for pancreatic cancer onset[[27](#_ENREF_27" \o "Fan, 2016 #27)].

Although the salivary microbiome may be useful for the medical care of patients with pancreatic cancer, it is influenced by differences in oral hygiene, mastication, and swallowing among individuals[[27](#_ENREF_27),[41](#_ENREF_41),[42](#_ENREF_42)]. In contrast, the duodenal microbiota is more relevant to the pancreas and bile duct than is the salivary microbiota. Therefore, the duodenal microbiota was hypothesized to directly reflect the dysbiosis associated with PB Ca, and in fact, the results of this study reveal that the duodenal microbiota might be beneficial for screening PB Ca. The microbiota around the pancreas and biliary duct have been reported. Microbes of the duodenal mucosa, bile juice, cancer tissue, and cyst fluid of IPMN in pancreaticobiliary tumor patients have been investigated (Table 6)[[30](#_ENREF_30" \o "Michaud, 2007 #30),[43-47](#_ENREF_43)]. However, the methods used to analyze the microbiota around the pancreas and bile duct in these reports were more invasive than duodenal juice sampling.

The mechanism by which microbes lead to PB Ca remains unknown. The etiology with respect to the salivary microbiota has been considered in past reports. *P. gingivalis* can interrupt signaling pathways by modulating receptor expression and cytokine secretion to evade the host’s immune system[[48-52](#_ENREF_48" \o "Duncan, 2004 #41)]. Moreover, *P. gingivalis* activates the Toll-like receptor signaling pathway[[53](#_ENREF_53),[54](#_ENREF_54)], which has been reported to be related to pancreatic carcinogenesis[[55](#_ENREF_55),[56](#_ENREF_56)]. In other reports, oral bacteria were found outside of the oral cavity in the gastrointestinal tract. Immune responses against these bacteria can cause inflammation and carcinogenesis in the pancreas. Lipopolysaccharide has also been reported to drive pancreatic carcinogenesis by blocking the MyD88-dependent, Toll-like receptor 4 and MyD88-independent pathways[[57](#_ENREF_57" \o "Bracci, 2017 #50)]. In another report, the mechanism was described as follows. Bacterial ligands detected by Toll-like receptors cause a Th1/Th2/Th17 imbalance in the tumor microenvironment, promoting tumorigenesis in combination with *Kras* mutation. In the duodenum, microbes may reach the pancreatic duct or biliary duct through the Vater papilla. In the pancreas or bile duct, pattern recognition receptors (such as Toll-like receptors) are stimulated by the pathogenic molecular patterns of bacterial ligands and induce lower levels of immune suppression, leading to the development of PB Ca[[58](#_ENREF_58" \o "Sethi, 2019 #1604)]. These results from past reports suggest that some type of immune system response is the link between the duodenal microbiota and PB Ca.

On the one hand, the relationship between *Clostridium* cluster XVIII and carcinogenesis has not been reported, even though *Clostridium* cluster XVIII is reported to have the potential to enhance regulatory T (Treg) cells[[59](#_ENREF_59" \o "Atarashi, 2013 #1814)]. Many Treg cells exist in tumor tissue and prevent the immune response to tumors. Therefore, Tregs contribute to tumor progression and poor prognosis[[60-64](#_ENREF_60" \o "Bazewicz, 2019 #1827)]. Thus, *Clostridium* cluster XVIII may increase in response to cancer and activate Tregs. Alternatively, *Clostridium* cluster XVIII may activate Tregs, with oncogenesis advancing.

This report has some limitations. First, this study was small and performed at a single institution. However, based on the data from *Clostridium* cluster XVIII, the average value of the malignant group was 4.5%, and that of the benign group was 1.5%. Total thirty patients were needed to achieve an a error of 5% and a β value of 0.2. When *Clostridium* cluster XVIII was the main outcome, the minimum necessary sample size was secured. Although this is the first report to describe the relationship between the duodenal juice microbiota and PB Ca, the diseases in the malignant group were not uniform. If subgroup analyses of pancreatic diseases were performed, the abundance of some duodenal microbes would be significantly different between the benign and malignant groups (Table 7). We hope that a future study with a larger number of patients will confirm our results for both pancreatic cancer and biliary cancer. Second, healthy control subjects were not enrolled in this study. However, as esophagogastroduodenoscopy under sedation is rarely performed in healthy patients, this limitation was unavoidable in the study design. Third, T-RFLP was applied. Investigations into the duodenal microbiota have been limited because duodenal juice cannot be collected in large volumes (less than 0.5 mL is typically collected). However, the measurement of the duodenal microbiota was demonstrated to be possible. Follow-up studies using next-generation sequencing are warranted[[65](#_ENREF_65" \o "Qin, 2010 #1573)]. Fourth, examining the duodenal microbiota requires a somewhat invasive technique. In the future, the development of serum antibody testing for the duodenal microbiota should be pursued.

**CONCLUSION**

In conclusion, the duodenal microbiota may contribute to PB Ca screening.

**ARTICLE HIGHLIGHTS**

***Research background***

Pancreaticobiliary cancer (PB Ca) is a lethal disease; however, there are currently no appropriate diagnostic and prognostic markers. Recently, the human microbiota was reported to be a causative factor, diagnostic marker, and prognostic marker for gastrointestinal malignant diseases.

***Research motivation***

The oral and fecal microbiota have been reported to be useful diagnostic markers for gastrointestinal cancer. The duodenum is located closer to the pancreas and bile duct than the oral cavity and colon. Therefore, we hypothesized that assessment of the duodenal microbiota might improve the diagnostic accuracy for PB Ca.

***Research objectives***

To investigate the diagnostic accuracy of duodenal microbiota evaluation for PB Ca.

***Research methods***

Thirty-four PB Ca and benign pancreaticobiliary disease patients were recruited for this study, and their duodenal juice was aseptically collected by endoscopy. The duodenal microbiota was analyzed, and the relative abundances of species in the duodenal microbiota were compared between PB Ca patients and benign pancreaticobiliary disease patients. The PB Ca diagnosability was compared between a conventional tumor marker and species in the duodenal microbiota with significantly different abundances in PB Ca patients *vs* benign pancreaticobiliary disease patients.

***Research results***

The abundances of cancer antigen 19-9 (CA19-9), *Bifidobacterium, Clostridium* cluster XVIII, and *Prevotella* were significantly different between PB Ca patients and benign pancreaticobiliary disease patients. The diagnostic capacity of *Clostridium* cluster XVIII was the highest among the four markers (CA19-9, *Bifidobacterium, Clostridium* cluster XVIII, and *Prevotella*). The combined assessment of *Clostridium* cluster XVIII and CA19-9 Levels was useful for PB Ca diagnosis.

***Research conclusions***

It was possible to investigate the microbiota of duodenal juice. Duodenal microbiota evaluation may contribute to the diagnosis of PB Ca.

***Research perspectives***

In the future, novel diagnostic and prognostic markers and treatments could be developed by investigating the relationship between the duodenal microbiota and PB Ca.

**ACKNOWLEDGEMENTS**

We thank all the staff at the Department of Gastroenterology of Fukushima Medical University, the Department of Endoscopy of Fukushima Medical University Hospital, and the Gastroenterology Ward of Fukushima Medical University Hospital. We also thank TechnoSuruga Laboratory for T-RFLP.

**REFERENCES**

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]

2 **Geer RJ**, Brennan MF. Prognostic indicators for survival after resection of pancreatic adenocarcinoma. *Am J Surg* 1993; **165**: 68-72; discussion 72-3 [PMID: 8380315 DOI: 10.1016/s0002-9610(05)80406-4]

3 **Picozzi VJ**, Oh SY, Edwards A, Mandelson MT, Dorer R, Rocha FG, Alseidi A, Biehl T, Traverso LW, Helton WS, Kozarek RA. Five-Year Actual Overall Survival in Resected Pancreatic Cancer: A Contemporary Single-Institution Experience from a Multidisciplinary Perspective. *Ann Surg Oncol* 2017; **24**: 1722-1730 [PMID: 28054192 DOI: 10.1245/s10434-016-5716-z]

4 **White RJ**, Hasan S, Monga D, Finley G, Islam M, Schiffman S, Williams HK, Kulkarni A, Thakkar S, Kirichenko AV, Wegner RE. Time to Adjuvant Systemic Therapy Following Pancreatic Cancer Resection and Effect on Outcome. *Pancreas* 2019; **48**: 1086-1091 [PMID: 31404024 DOI: 10.1097/MPA.0000000000001373]

5 **Uchida N**, Kamada H, Ono M, Aritomo Y, Masaki T, Nakatsu T, Kuriyama S. How many cytological examinations should be performed for the diagnosis of malignant biliary stricture *via* an endoscopic nasobiliary drainage tube? *J Gastroenterol Hepatol* 2008; **23**: 1501-1504 [PMID: 18028351 DOI: 10.1111/j.1440-1746.2007.05214.x]

6 **Rösch T**, Hofrichter K, Frimberger E, Meining A, Born P, Weigert N, Allescher HD, Classen M, Barbur M, Schenck U, Werner M. ERCP or EUS for tissue diagnosis of biliary strictures? A prospective comparative study. *Gastrointest Endosc* 2004; **60**: 390-396 [PMID: 15332029 DOI: 10.1016/s0016-5107(04)01732-8]

7 **Foutch PG**, Kerr DM, Harlan JR, Kummet TD. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. *Am J Gastroenterol* 1991; **86**: 577-580 [PMID: 2028947 DOI: 10.3109/00365529109043642]

8 **Kubota Y**, Takaoka M, Tani K, Ogura M, Kin H, Fujimura K, Mizuno T, Inoue K. Endoscopic transpapillary biopsy for diagnosis of patients with pancreaticobiliary ductal strictures. *Am J Gastroenterol* 1993; **88**: 1700-1704 [PMID: 8213710 DOI: 10.1109/TCSI.2006.876416]

9 **Lee JG**, Leung JW, Baillie J, Layfield LJ, Cotton PB. Benign, dysplastic, or malignant--making sense of endoscopic bile duct brush cytology: results in 149 consecutive patients. *Am J Gastroenterol* 1995; **90**: 722-726 [PMID: 7733076]

10 **Ponchon T**, Gagnon P, Berger F, Labadie M, Liaras A, Chavaillon A, Bory R. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. *Gastrointest Endosc* 1995; **42**: 565-572 [PMID: 8674929 DOI: 10.1016/s0016-5107(95)70012-9]

11 **Pugliese V**, Conio M, Nicolò G, Saccomanno S, Gatteschi B. Endoscopic retrograde forceps biopsy and brush cytology of biliary strictures: a prospective study. *Gastrointest Endosc* 1995; **42**: 520-526 [PMID: 8674921 DOI: 10.1016/s0016-5107(95)70004-8]

12 **Howell DA**, Parsons WG, Jones MA, Bosco JJ, Hanson BL. Complete tissue sampling of biliary strictures at ERCP using a new device. *Gastrointest Endosc* 1996; **43**: 498-502 [PMID: 8726766 DOI: 10.1016/s0016-5107(96)70294-8]

13 **Sugiyama M**, Atomi Y, Wada N, Kuroda A, Muto T. Endoscopic transpapillary bile duct biopsy without sphincterotomy for diagnosing biliary strictures: a prospective comparative study with bile and brush cytology. *Am J Gastroenterol* 1996; **91**: 465-467 [PMID: 8633492]

14 **Mansfield JC**, Griffin SM, Wadehra V, Matthewson K. A prospective evaluation of cytology from biliary strictures. *Gut* 1997; **40**: 671-677 [PMID: 9203949 DOI: 10.1136/gut.40.5.671]

15 **Schoefl R**, Haefner M, Wrba F, Pfeffel F, Stain C, Poetzi R, Gangl A. Forceps biopsy and brush cytology during endoscopic retrograde cholangiopancreatography for the diagnosis of biliary stenoses. *Scand J Gastroenterol* 1997; **32**: 363-368 [PMID: 9140159 DOI: 10.3109/00365529709007685]

16 **Glasbrenner B**, Ardan M, Boeck W, Preclik G, Möller P, Adler G. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. *Endoscopy* 1999; **31**: 712-717 [PMID: 10604612 DOI: 10.1055/s-1999-73]

17 **Jailwala J**, Fogel EL, Sherman S, Gottlieb K, Flueckiger J, Bucksot LG, Lehman GA. Triple-tissue sampling at ERCP in malignant biliary obstruction. *Gastrointest Endosc* 2000; **51**: 383-390 [PMID: 10744806 DOI: 10.1016/s0016-5107(00)70435-4]

18 **Macken E**, Drijkoningen M, Van Aken E, Van Steenbergen W. Brush cytology of ductal strictures during ERCP. *Acta Gastroenterol Belg* 2000; **63**: 254-259 [PMID: 11189981 DOI: 10.1007/s002610000019]

19 **de Bellis M**, Sherman S, Fogel EL, Cramer H, Chappo J, McHenry L Jr, Watkins JL, Lehman GA. Tissue sampling at ERCP in suspected malignant biliary strictures (Part 2). *Gastrointest Endosc* 2002; **56**: 720-730 [PMID: 12397282 DOI: 10.1067/mge.2002.129219]

20 **Khan SA**, Davidson BR, Goldin R, Pereira SP, Rosenberg WM, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Thursz MR, Wasan H; British Society of Gastroenterology. Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 2002; **51 Suppl 6**: VI1-VI9 [PMID: 12376491 DOI: 10.1136/gut.51.suppl\_6.vi1]

21 **Kostic AD**, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. *Genome Res* 2012; **22**: 292-298 [PMID: 22009990 DOI: 10.1101/gr.126573.111]

22 **Yamamura K**, Baba Y, Nakagawa S, Mima K, Miyake K, Nakamura K, Sawayama H, Kinoshita K, Ishimoto T, Iwatsuki M, Sakamoto Y, Yamashita Y, Yoshida N, Watanabe M, Baba H. Human Microbiome Fusobacterium Nucleatum in Esophageal Cancer Tissue Is Associated with Prognosis. *Clin Cancer Res* 2016; **22**: 5574-5581 [PMID: 27769987 DOI: 10.1158/1078-0432.CCR-16-1786]

23 **Ahn J**, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, Goedert JJ, Hayes RB, Yang L. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Inst* 2013; **105**: 1907-1911 [PMID: 24316595 DOI: 10.1093/jnci/djt300]

24 **Mai V**, Morris JG Jr. Need for prospective cohort studies to establish human gut microbiome contributions to disease risk. *J Natl Cancer Inst* 2013; **105**: 1850-1851 [PMID: 24316594 DOI: 10.1093/jnci/djt349]

25 **Krishnan S**, Eslick GD. Streptococcus bovis infection and colorectal neoplasia: a meta-analysis. *Colorectal Dis* 2014; **16**: 672-680 [PMID: 24824513 DOI: 10.1111/codi.12662]

26 **Komiya Y**, Shimomura Y, Higurashi T, Sugi Y, Arimoto J, Umezawa S, Uchiyama S, Matsumoto M, Nakajima A. Patients with colorectal cancer have identical strains of *Fusobacterium nucleatum* in their colorectal cancer and oral cavity. *Gut* 2019; **68**: 1335-1337 [PMID: 29934439 DOI: 10.1136/gutjnl-2018-316661]

27 **Fan X**, Alekseyenko AV, Wu J, Peters BA, Jacobs EJ, Gapstur SM, Purdue MP, Abnet CC, Stolzenberg-Solomon R, Miller G, Ravel J, Hayes RB, Ahn J. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. *Gut* 2018; **67**: 120-127 [PMID: 27742762 DOI: 10.1136/gutjnl-2016-312580]

28 **Farrell JJ**, Zhang L, Zhou H, Chia D, Elashoff D, Akin D, Paster BJ, Joshipura K, Wong DT. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut* 2012; **61**: 582-588 [PMID: 21994333 DOI: 10.1136/gutjnl-2011-300784]

29 **Torres PJ**, Fletcher EM, Gibbons SM, Bouvet M, Doran KS, Kelley ST. Characterization of the salivary microbiome in patients with pancreatic cancer. *PeerJ* 2015; **3**: e1373 [PMID: 26587342 DOI: 10.7717/peerj.1373]

30 **Michaud DS**, Joshipura K, Giovannucci E, Fuchs CS. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. *J Natl Cancer Inst* 2007; **99**: 171-175 [PMID: 17228001 DOI: 10.1093/jnci/djk021]

31 **Michaud DS**, Izard J, Wilhelm-Benartzi CS, You DH, Grote VA, Tjønneland A, Dahm CC, Overvad K, Jenab M, Fedirko V, Boutron-Ruault MC, Clavel-Chapelon F, Racine A, Kaaks R, Boeing H, Foerster J, Trichopoulou A, Lagiou P, Trichopoulos D, Sacerdote C, Sieri S, Palli D, Tumino R, Panico S, Siersema PD, Peeters PH, Lund E, Barricarte A, Huerta JM, Molina-Montes E, Dorronsoro M, Quirós JR, Duell EJ, Ye W, Sund M, Lindkvist B, Johansen D, Khaw KT, Wareham N, Travis RC, Vineis P, Bueno-de-Mesquita HB, Riboli E. Plasma antibodies to oral bacteria and risk of pancreatic cancer in a large European prospective cohort study. *Gut* 2013; **62**: 1764-1770 [PMID: 22990306 DOI: 10.1136/gutjnl-2012-303006]

32 **Tanaka M**, Fernández-Del Castillo C, Kamisawa T, Jang JY, Levy P, Ohtsuka T, Salvia R, Shimizu Y, Tada M, Wolfgang CL. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatology* 2017; **17**: 738-753 [PMID: 28735806 DOI: 10.1016/j.pan.2017.07.007]

33 **Takahashi S**, Tomita J, Nishioka K, Hisada T, Nishijima M. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One* 2014; **9**: e105592 [PMID: 25144201 DOI: 10.1371/journal.pone.0105592]

34 **Nagashima K**, Hisada T, Sato M, Mochizuki J. Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. *Appl Environ Microbiol* 2003; **69**: 1251-1262 [PMID: 12571054 DOI: 10.1128/AEM.69.2.1251-1262.2003]

35 **Nagashima K,** Mochizuki J, Hisada T, Suzuki S, Shimomura K. Phylogenetic analysis of 16S rRNA gene sequences from human fecal microbiota and improved utility of T-RFLP profiling. *Biosci Microflora* 2006; **25**: 99-107 [DOI: 10.12938/bifidus.25.99]

36 **Ley RE**, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023 [PMID: 17183309 DOI: 10.1038/4441022a]

37 **Yatsunenko T**, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. *Nature* 2012; **486**: 222-227 [PMID: 22699611 DOI: 10.1038/nature11053]

38 **Kanda Y**. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452-458 [PMID: 23208313 DOI: 10.1038/bmt.2012.244]

39 **Olson SH**, Satagopan J, Xu Y, Ling L, Leong S, Orlow I, Saldia A, Li P, Nunes P, Madonia V, Allen PJ, O'Reilly E, Pamer E, Kurtz RC. The oral microbiota in patients with pancreatic cancer, patients with IPMNs, and controls: a pilot study. *Cancer Causes Control* 2017; **28**: 959-969 [PMID: 28762074 DOI: 10.1007/s10552-017-0933-8]

40 **Lu H**, Ren Z, Li A, Li J, Xu S, Zhang H, Jiang J, Yang J, Luo Q, Zhou K, Zheng S, Li L. Tongue coating microbiome data distinguish patients with pancreatic head cancer from healthy controls. *J Oral Microbiol* 2019; **11**: 1563409 [PMID: 30728915 DOI: 10.1080/20002297.2018.1563409]

41 **Lockhart PB**, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. Bacteremia associated with toothbrushing and dental extraction. *Circulation* 2008; **117**: 3118-3125 [PMID: 18541739 DOI: 10.1161/CIRCULATIONAHA.107.758524]

42 **Crasta K**, Daly CG, Mitchell D, Curtis B, Stewart D, Heitz-Mayfield LJ. Bacteraemia due to dental flossing. *J Clin Periodontol* 2009; **36**: 323-332 [PMID: 19426179 DOI: 10.1111/j.1600-051X.2008.01372.x]

43 **Mitsuhashi K**, Nosho K, Sukawa Y, Matsunaga Y, Ito M, Kurihara H, Kanno S, Igarashi H, Naito T, Adachi Y, Tachibana M, Tanuma T, Maguchi H, Shinohara T, Hasegawa T, Imamura M, Kimura Y, Hirata K, Maruyama R, Suzuki H, Imai K, Yamamoto H, Shinomura Y. Association of Fusobacterium species in pancreatic cancer tissues with molecular features and prognosis. *Oncotarget* 2015; **6**: 7209-7220 [PMID: 25797243 DOI: 10.18632/oncotarget.3109]

44 **Riquelme E**, Zhang Y, Zhang L, Montiel M, Zoltan M, Dong W, Quesada P, Sahin I, Chandra V, San Lucas A, Scheet P, Xu H, Hanash SM, Feng L, Burks JK, Do KA, Peterson CB, Nejman D, Tzeng CD, Kim MP, Sears CL, Ajami N, Petrosino J, Wood LD, Maitra A, Straussman R, Katz M, White JR, Jenq R, Wargo J, McAllister F. Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes. *Cell* 2019; **178**: 795-806.e12 [PMID: 31398337 DOI: 10.1016/j.cell.2019.07.008]

45 **Di Carlo P**, Serra N, D'Arpa F, Agrusa A, Gulotta G, Fasciana T, Rodolico V, Giammanco A, Sergi C. The microbiota of the bilio-pancreatic system: a cohort, STROBE-compliant study. *Infect Drug Resist* 2019; **12**: 1513-1527 [PMID: 31354308 DOI: 10.2147/IDR.S200378]

46 **Mei QX**, Huang CL, Luo SZ, Zhang XM, Zeng Y, Lu YY. Characterization of the duodenal bacterial microbiota in patients with pancreatic head cancer vs. healthy controls. *Pancreatology* 2018; **18**: 438-445 [PMID: 29653723 DOI: 10.1016/j.pan.2018.03.005]

47 **Gaiser RA**, Halimi A, Alkharaan H, Lu L, Davanian H, Healy K, Hugerth LW, Ateeb Z, Valente R, Fernández Moro C, Del Chiaro M, Sällberg Chen M. Enrichment of oral microbiota in early cystic precursors to invasive pancreatic cancer. *Gut* 2019; **68**: 2186-2194 [PMID: 30872392 DOI: 10.1136/gutjnl-2018-317458]

48 **Duncan L**, Yoshioka M, Chandad F, Grenier D. Loss of lipopolysaccharide receptor CD14 from the surface of human macrophage-like cells mediated by Porphyromonas gingivalis outer membrane vesicles. *Microb Pathog* 2004; **36**: 319-325 [PMID: 15120158 DOI: 10.1016/j.micpath.2004.02.004]

49 **Stathopoulou PG**, Benakanakere MR, Galicia JC, Kinane DF. The host cytokine response to Porphyromonas gingivalis is modified by gingipains. *Oral Microbiol Immunol* 2009; **24**: 11-17 [PMID: 19121064 DOI: 10.1111/j.1399-302X.2008.00467.x]

50 **Singh A**, Wyant T, Anaya-Bergman C, Aduse-Opoku J, Brunner J, Laine ML, Curtis MA, Lewis JP. The capsule of Porphyromonas gingivalis leads to a reduction in the host inflammatory response, evasion of phagocytosis, and increase in virulence. *Infect Immun* 2011; **79**: 4533-4542 [PMID: 21911459 DOI: 10.1128/IAI.05016-11]

51 **Taxman DJ**, Swanson KV, Broglie PM, Wen H, Holley-Guthrie E, Huang MT, Callaway JB, Eitas TK, Duncan JA, Ting JP. Porphyromonas gingivalis mediates inflammasome repression in polymicrobial cultures through a novel mechanism involving reduced endocytosis. *J Biol Chem* 2012; **287**: 32791-32799 [PMID: 22843689 DOI: 10.1074/jbc.M112.401737]

52 **Palm E**, Khalaf H, Bengtsson T. Porphyromonas gingivalis downregulates the immune response of fibroblasts. *BMC Microbiol* 2013; **13**: 155 [PMID: 23841502 DOI: 10.1186/1471-2180-13-155]

53 **Hayashi C**, Papadopoulos G, Gudino CV, Weinberg EO, Barth KR, Madrigal AG, Chen Y, Ning H, LaValley M, Gibson FC 3rd, Hamilton JA, Genco CA. Protective role for TLR4 signaling in atherosclerosis progression as revealed by infection with a common oral pathogen. *J Immunol* 2012; **189**: 3681-3688 [PMID: 22956579 DOI: 10.4049/jimmunol.1201541]

54 **Zambirinis CP**, Levie E, Nguy S, Avanzi A, Barilla R, Xu Y, Seifert L, Daley D, Greco SH, Deutsch M, Jonnadula S, Torres-Hernandez A, Tippens D, Pushalkar S, Eisenthal A, Saxena D, Ahn J, Hajdu C, Engle DD, Tuveson D, Miller G. TLR9 Ligation in pancreatic stellate cells promotes tumorigenesis. *J Exp Med* 2015; **212**: 2077-2094 [PMID: 26481685 DOI: 10.1084/jem.20142162]

55 **Zhang JJ**, Wu HS, Wang L, Tian Y, Zhang JH, Wu HL. Expression and significance of TLR4 and HIF-1alpha in pancreatic ductal adenocarcinoma. *World J Gastroenterol* 2010; **16**: 2881-2888 [PMID: 20556833 DOI: 10.3748/wjg.v16.i23.2881]

56 **Ochi A**, Nguyen AH, Bedrosian AS, Mushlin HM, Zarbakhsh S, Barilla R, Zambirinis CP, Fallon NC, Rehman A, Pylayeva-Gupta Y, Badar S, Hajdu CH, Frey AB, Bar-Sagi D, Miller G. MyD88 inhibition amplifies dendritic cell capacity to promote pancreatic carcinogenesis *via* Th2 cells. *J Exp Med* 2012; **209**: 1671-1687 [PMID: 22908323 DOI: 10.1084/jem.20111706]

57 **Bracci PM**. Oral Health and the Oral Microbiome in Pancreatic Cancer: An Overview of Epidemiological Studies. *Cancer J* 2017; **23**: 310-314 [PMID: 29189325 DOI: 10.1097/PPO.0000000000000287]

58 **Sethi V**, Vitiello GA, Saxena D, Miller G, Dudeja V. The Role of the Microbiome in Immunologic Development and its Implication For Pancreatic Cancer Immunotherapy. *Gastroenterology* 2019; **156**: 2097-2115.e2 [PMID: 30768986 DOI: 10.1053/j.gastro.2018.12.045]

59 **Atarashi K**, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**: 232-236 [PMID: 23842501 DOI: 10.1038/nature12331]

60 **Bazewicz CG**, Dinavahi SS, Schell TD, Robertson GP. Aldehyde dehydrogenase in regulatory T-cell development, immunity and cancer. *Immunology* 2019; **156**: 47-55 [PMID: 30387499 DOI: 10.1111/imm.13016]

61 **Fridman WH**, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012; **12**: 298-306 [PMID: 22419253 DOI: 10.1038/nrc3245]

62 **Protti MP**, De Monte L, Di Lullo G. Tumor antigen-specific CD4+ T cells in cancer immunity: from antigen identification to tumor prognosis and development of therapeutic strategies. *Tissue Antigens* 2014; **83**: 237-246 [PMID: 24641502 DOI: 10.1111/tan.12329]

63 **Saito T**, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, Maeda Y, Hamaguchi M, Ohkura N, Sato E, Nagase H, Nishimura J, Yamamoto H, Takiguchi S, Tanoue T, Suda W, Morita H, Hattori M, Honda K, Mori M, Doki Y, Sakaguchi S. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nat Med* 2016; **22**: 679-684 [PMID: 27111280 DOI: 10.1038/nm.4086]

64 **Tanaka A**, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* 2017; **27**: 109-118 [PMID: 27995907 DOI: 10.1038/cr.2016.151]

65 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

**Footnotes**

**Institutional review board statement:** This study was approved by the Institutional Review Board of Fukushima Medical University (Approval No. 2451).

**Conflict-of-interest statement:** The authors declare no competing interests.

**Data sharing statement:** The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Corresponding Author's Membership in Professional Societies:** Fukushima Medical University, No. 03727100.

**Peer-review started:** March 22, 2021

**First decision:** July 3, 2021

**Article in press:**

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Japan

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Yao D **S-Editor:** Ma YJ **L-Editor: P-Editor:**

**Figure Legends**



**Figure 1 Analysis of the duodenal microbiota.** A, B: *Bifidobacterium* levels were significantly higher in the malignant group than in the benign group; A, C: *Clostridium* cluster XVIII levels were significantly higher in the malignant group than in the benign group; A, D: *Prevotella* levels were significantly higher in the benign group than in the malignant group. B: benign group; M: malignant group.



**Figure 2 Comparison of the ability of microbiome components and cancer antigen 19-9 Levels to diagnose pancreaticobiliary cancer.** The area under the receiver operating characteristic curve of *Clostridium* cluster XVIII was the highest among the three microbes and cancer antigen 19-9 Levels. CA19-9: Cancer antigen 19-9; AUC: The area under the curve.

**Table 1 Final patient diagnoses**

|  |  |
| --- | --- |
| **Benign group****(*n* = 22)** | **Malignant group****(*n* = 12)** |
| Chronic pancreatitis | 6 | Pancreatic cancer, stage (I/II/III/IV) | 9 (2/5/1/1/) |
| IPMN | 5 | Biliary ductal cancer, stage (I/II/III/IV) | 3 (1/2/0/0) |
| GB ADM | 3 |  |  |
| Autoimmune pancreatitis | 3 |  |  |
| CBD stricture of unknown origin | 2 |  |  |
| Serous cystic neoplasm | 2 |  |  |
| CBD stone | 1 |  |  |

IPMN: Intraductal papillary mucinous neoplasm; GB ADM: Gallbladder adenomyomatosis; CBD: Common bile duct.

**Table 2 Comparison of patient characteristics, tumor markers, and microbiomes**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Benign group****(*n* = 22)** | **Malignant group****(*n* = 12)** | ***P* value** |
| Age, yr (mean ± SD) | 63.0 ± 12.2 | 73.0 ± 8.3 | 0.016 |
| Sex (male/female) | 8/14 | 4/8 | 1.0 |
| Reduction in body weight ≥ 5 kg within 6 mo before duodenal juice sampling, *n* (%) | 1 (4.5) | 2 (16.7) | 0.28 |
| Intake of proton pump inhibitors, *n* (%) | 4 (18.2) | 2 (16.7) | 1.0 |
| CA19-9, U/mL, median (range) | 5.4 (2.0-54.8) | 22.8 (2.0-9893.2) | 0.03 |

CA19-9: Cancer antigen 19-9.

**Table 3 Microbiome comparison**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Benign group****(*n* = 22)** | **Malignant group****(*n* = 12)** | ***P* value** |
| *Bacteroides*, median (range) | 4.3% (0-26.1%) | 5.6% (0-46.4%) | 0.55 |
| *Bifidobacterium*, median (range) | 0 (0-0.5%) | 0.3% (0-4.5%) | < 0.05 |
| *Clostridium* cluster IV, median (range) | 2.9% (0-10.8%) | 3.4% (0-8.8%) | 0.80 |
| *Clostridium* cluster IX, median (range) | 4.7% (0.6%-19.5%) | 4.9% (0-17.8%) | 0.68 |
| *Clostridium* cluster XI, median (range) | 0 (0-0) | 0 (0-0) |  |
| *Clostridium* cluster XVIII, median (range) | 1.3% (0-3.9%) | 3.6% (0.8%-14.9%) | 0.006 |
| *Clostridium* subcluster XIVa, median (range) | 5.1% (0-23.1%) | 6.4% (2.9%-13.7%) | 0.38 |
| Lactobacillales (mean ± SD) | 63.0% ± 19.7% | 62.6% ± 18.3% | 0.95 |
| *Prevotella*, median (range) | 2.2% (0-25.9%) | 0.1% (0-10.9%) | 0.04 |
| Others, median (range) | 4.9% (2.5%-20.4%) | 4.1% (1.5%-6.3%) | 0.14 |

**Table 4 Effects of age on cancer antigen 19-9 levels and the human microbiome**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Age < 69 yr****(*n* = 17)** | **Age ≥ 69 yr****(*n* = 17)** | ***P* value** |
| CA19-9, U/mL, median (range) | 7.1 (2-129.3) | 4.9 (2-9893.2) | 0.77 |
| *Bifidobacterium*, median (range) | 0.3% (0-0.5%) | 0 (0-4.5%) | 0.3 |
| *Clostridium* cluster XVIII, median (range) | 2.6% (0-5.7%) | 1.8% (0-14.9%) | 0.82 |
| *Prevotella*, median (range) | 1.3% (0-18.2%) | 1.9% (0-25.9%) | 0.56 |

CA19-9: Cancer antigen 19-9.

**Table 5 Diagnosis of pancreaticobiliary cancer by the combination of *Clostridium* cluster XVIII and cancer antigen 19-9 levels**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Cutoff value** | **Sensitivity** | **Specificity** |
| CA19-9 | 18.8 U/mL | 66.7% (8/12) | 76.2% (16/211) |
| *Clostridium* cluster XVIII | 3.038% | 58.3% (7/12) | 95.2% (20/211) |
| Combination of *Clostridium* cluster XVIII and CA19-9 |  | 91.7% (11/12) | 71.4% (15/211) |

1CA19-9 data were missing for a patient in the benign group. CA19-9: Cancer antigen 19-9.

**Table 6 Past reports on microbes and pancreaticobiliary cancer**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Disease** | **Ref.** | **Microbes** | **Sample** | **Role** |
| Pancreatic cancer | Michaud *et al*[[30](#_ENREF_30)] | A history of periodontal diseases |  | Risk factor |
|  | Farrell *et al*[[28](#_ENREF_28)] | A combination of *Neisseria elongate* and *Streptococcus mitis* | Oral | Distinguishing from healthy controls |
|  | Torres *et al*[[29](#_ENREF_29)] | Ratio of *Leptotrichia* to *Porphyromonas* | Saliva | Higher in pancreatic cancer patients |
|  | Fan *et al*[[27](#_ENREF_27)] | *Porphyromonas gingivalis* | Oral, antibody | Risk factor |
|  | Olson *et al*[[39](#_ENREF_39)] | Firmicutes | Oral | More abundant |
|  | Lu *et al*[[40](#_ENREF_40)] | *Leptotrichia, Fusobacterium, Rothia, Actinomyces, Corynebacterium, Atopobium, Peptostreptococcus, Catonella, Oribacterium, Filifactor, Campylobacter, Moraxella, Tannerella* | Tongue coating | More prevalent |
|  | Mei *et al*[[46](#_ENREF_46)] | *Acinetobactor, Aquabacterium, Oceanobacillus, Rahnella, Massilia, Delftia, Deinococcus, Sphingobium* | Duodenal mucosa | More abundant |
|  | Mitsuhashi *et al*[[43](#_ENREF_43)] | *Fusobacterium species* | Cancer tissue | Poor prognosis |
|  | Riquelme *et al*[[44](#_ENREF_44)] | *Pseudoxanthomonas, Streptomyces, Saccharopolyspora, Bacillus clausii* | Cancer tissue | Long-term survival |
| Pancreatic and ampullary cancer | Di Calro *et al*[[45](#_ENREF_45)] | *Escherichia coli, Klebsiella pneumoniae* | Bile juice | Predictor for survival |
| IPMN with high-grade dysplasia | Gaiser *et al*[[47](#_ENREF_47)] | *Granulicatella adiacens, Fusobacterium nucleatum* | Cyst fluid | More abundant |

IPMN: Intraductal papillary mucinous neoplasm.

**Table 7 Microbiome comparison in patients with pancreatic disease**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Benign pancreatic diseases****(*n* = 16)** | **Pancreatic cancer****(*n* = 9)** | ***P* value** |
| *Bacteroides*, median (range) | 2.1% (0-26.1%) | 5.8% (0-46.4%) | 0.17 |
| *Bifidobacterium*, median (range) | 0 (0-0.5%) | 0.48% (0-4.5%) | 0.03 |
| *Clostridium* cluster IV, median (range) | 4.0% ± 3.3% | 3.6% ± 2.9% | 0.76 |
| *Clostridium* cluster IX, median (range) | 5.3% ± 3.6% | 6.4% ± 5.2% | 0.57 |
| *Clostridium* cluster XI, median (range) | 0 (0-0) | 0 (0-0) |  |
| *Clostridium* cluster XVIII, median (range) | 1.4% (0-3.9%) | 3.0% (0.8%-14.9%) | 0.04 |
| *Clostridium* subcluster XIVa, median (range) | 3.8% (0-21.4%) | 6.0% (2.9%-13.7%) | 0.32 |
| Lactobacillales, (mean ± SD) | 68.4% ± 19.3% | 59.7% ± 20.4% | 0.3 |
| *Prevotella*, median (range) | 4.2% (2.5%-20.4%) | 4.0% (1.5%-6.3%) | 0.3 |
| Others, median (range) | 2.0% (0-18.3%) | 0.3% (0-11.0%) | 0.3 |