Original Scientific Article

Impact of Preoperative Occult-bacterial Translocation on Surgical Site Infections in Patients Undergoing Pancreatoduodenectomy

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Precis (46 words, 350 characters)

Occult-bacterial translocation (O-BT), defined as the condition in which microorganisms are detected in blood by a highly sensitive method, was investigated. Preoperative blood samples were contaminated in 21% of patients undergoing pancreatoduodenectomy. Preoperative O-BT status was one of the independent risk factors for surgical site infections.

Abstract

BACKGROUND: Occult-bacterial translocation (O-BT) has been reported as the condition in which microorganisms are detected in blood or lymph node by a highly sensitive method. However, the clinical impact of preoperative O-BT on postoperative complications is unclear.

STUDY DESIGN: A prospective observational study with patients undergoing pancreatoduodenectomy for periampullary diseases was conducted. Blood samples were collected immediately after the induction of anesthesia. The status of O-BT was investigated using bacterium-specific ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR). The impact of O-BT on surgical site infections (SSIs) was analysed.

RESULTS: A total of 155 patients were included. The positive rate in preoperative blood samples detected by RT-qPCR was significantly higher than that obtained by the culture method (32 of 155 vs 4 of 155, $p<0.001$). Preoperative blood samples were contaminated with 1.0 to 19.2 bacterial cells/mL in positive patients, and 30 of the 41 detected microorganisms were obligate anaerobes. No differences in preoperative factors were observed between patients with positive and negative RT-qPCR results. The incidence of any SSI was significantly higher in patients with contaminated preoperative blood (≥1.2 bacterial cells/mL) than in other patients (14 of 27 vs 35 of 128, $p=0.013$). Multivariable analysis indicated that contaminated preoperative blood was identified as one of the independent risk factors for SSIs (odds ratio 2.71, 95% CI 1.04 to 7.24, $p=0.041$).

CONCLUSIONS: O-BT, predominantly with obligate anaerobes, was commonly observed in preoperative blood samples. In addition to the previously known risk factors, O-BT may be one of the risk factors for SSIs following pancreatoduodenectomy.

Keywords: occult-bacterial translocation, surgical site infection, pancreatoduodenectomy

Abbreviations and Acronyms

O-BT	= occult-bacterial translocation
PD	= pancreatoduodenectomy
PDAC	= pancreatic ductal adenocarcinoma
POPF	= postoperative pancreatic fistula
PPPD	= pylorus-preserving pancreatoduodenectomy
ROC	= receiver operating characteristic
RT-qPCR	= reverse transcriptase–quantitative polymerase chain reaction
SSI	= surgical site infection
SSPPD	= subtotal stomach-preserving pancreatoduodenectomy

INTRODUCTION

Recently, remarkable advances in surgical techniques have reduced the mortality rate after pancreatoduodenectomy (PD) to less than 3% in most centers[1]. Despite this substantial decline in the operative mortality over the past two decades, PD remains a challenging procedure with major complication rates of 20 to 40%, even in high-volume centers[2]. Preventing surgical site infections (SSIs), including pancreatic fistula, which is the most commonly observed organ/space SSI after PD, is critical for reducing postoperative morbidities and shortening the postoperative hospital stay.

In previous studies[3-7], the authors' institution employed a highly sensitive quantitative detection system for the intestinal bacteria using 16S or 23S ribosomal RNA (rRNA)-targeted reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR)[8, 9] in patients undergoing gastrointestinal surgeries. Interestingly, bacterial translocation from the dysregulated intestinal microenvironment to mesenteric lymph nodes or blood samples was frequently observed in highly invasive surgeries, such as esophagectomy and major hepatectomy with extrahepatic bile duct resection[3-6]. This condition should be called "occult-bacterial translocation (O-BT)" because it did not fulfil the criteria of sepsis in most cases[10]. Surprisingly, when the preoperative blood samples were investigated, approximately 20% were contaminated with microorganisms[4-6]. However, the association between O-BT in preoperative blood samples and postoperative complications is still unclear.

The aim of this study was to investigate the impact of preoperative O-BT on postoperative infectious complications. For that purpose, we conducted a prospective observational study with patients who were scheduled to undergo PD and employed a bacterial detection system using RT-qPCR[8, 9]. This RT-qPCR method enables the detection of obligate anaerobes, which are difficult to isolate by conventional culture methods, as well as facultative anaerobes and aerobes with extremely high sensitivity. The infectious status of preoperative blood and the association between preoperative O-BT and postoperative outcomes were investigated.

METHODS

Patients with periampullary disease scheduled to undergo PD at Nagoya University Hospital between August 2011 and August 2015 were eligible to participate in this prospective observational study. Written informed consent for participation was obtained from each patient as required by the Human Research Review Committee of Nagoya University Hospital (approval number; 2020-0290). Clinical and pathological data were collected from the prospectively maintained pancreatic resection database. The biliary bacterial contamination data in this study cohort have been reported previously for different research purposes[7].

When patients were diagnosed with borderline resectable pancreatic ductal adenocarcinoma (PDAC) abutting major arteries according to the latest version of the National Comprehensive Cancer Network guidelines, they received neoadjuvant therapy. In most of the patients, the regimen consisted of radiation therapy (50.4 gray in 28 fractions) combined with oral S-1 (TS-1®; Taiho Pharmaceutical, Tokyo, Japan)[11]. The remaining patients received gemcitabine plus S-1. Patients who received neoadjuvant therapy underwent surgery at least 3 weeks after the last administration of chemotherapy. Biliary drainage was performed in patients whose total bilirubin level was greater than 2 mg/dL based on the institutional criteria[12]. The method of endoscopic biliary drainage was determined at the discretion of the endoscopists at Nagoya University Hospital or affiliated hospitals. Patients who received biliary drainage were scheduled for surgery after jaundice and any signs of inflammation disappeared.

Surgical procedure

Most patients underwent subtotal stomach-preserving PD (SSPPD), which involves the resection of the pyloric ring with preservation of greater than 95% of the stomach, but conventional PD with distal gastrectomy or pylorus-preserving PD (PPPD) was occasionally performed. Portal vein resection was performed in combination with a standard pancreatectomy in patients with possible or definitive tumor invasion. Reconstruction was performed using a modified Child method in PD and SSPPD or the Traverso method in PPPD, which involved an end-to-side pancreaticojejunostomy and an end-to-side choledochojejunostomy. In all patients, pancreaticojejunostomy was performed via anastomosis of the pancreatic duct to all layers of the jejunal wall with anastomosis of the pancreatic parenchyma to the jejunal seromuscular layer after resection[2]. An end-to-side antecolic gastrojejunostomy in PD and SSPPD or a duodenojejunostomy in PPPD was performed by hand suturing in a two-layer fashion. For external stent drainage, a 4 Fr to 6 Fr polyvinyl catheter was inserted into the pancreatic duct in patients with a non-dilated duct (3 mm or less) and was not inserted in patients with a dilated duct (larger than 3 mm) according to a previous report[13]. Before the abdomen was closed, the intra-abdominal area was washed with 5000 to 10 000 mL of saline to prevent surgical site infection. Then, 19 Fr BLAKE silicone drains were routinely placed at the ventral and dorsal sides of the pancreaticojejunostomy and were connected to continuous suction reservoirs for fluid collection. The drains were replaced periodically to prevent contamination of the drainage system.

Postoperative management

Postoperative octreotide was not routinely administered. Broad-spectrum antibiotics, including 2nd- to 4th-generation cephalosporin, fluoroquinolone or ampicillin/sulbactam, were prophylactically administered preoperatively and for 3 days after surgery. Postoperative pancreatic fistula (POPF) was diagnosed and graded in accordance with the International Study Group in Pancreatic Surgery definition classification[14], and grade B pancreatic fistula (fistula requiring persistent drainage for >3 weeks or a clinically relevant change in management of POPF) or grade C pancreatic fistula (fistula leading to organ failure or requiring reoperation) was regarded as clinically significant. Abdominal drains were removed on postoperative day 4 to 7 in patients without POPF.

Sample collection

To assess the presence of O-BT by RT-qPCR, preoperative blood samples (1 mL) were collected into a test tube containing 2 mL of RNAprotect™ Bacterial Reagent (Qiagen, Hilden, Germany) under sterile conditions immediately after the induction of anesthesia and before administration of prophylactic antibiotics. It had been confirmed that false positives by RT-qPCR were extremely rare[8, 9]. The samples were held at room temperature for 5 min before storage at −80°C. Blood samples were also collected for two sets of conventional culture methods. For each set of blood cultures, 10 mL of blood was drawn under sterile conditions and then immediately inoculated into separate culture bottles (Organon Teknika, Durham, North Carolina, USA) for aerobic and anaerobic cultures. Blood samples were incubated until bacterial growth was detected or for 7 days. Bacteraemia was diagnosed when the results of two sets of culture bottles matched.

Detection of microorganisms by RT-qPCR in blood samples

Microorganisms in each sample were detected by 16S or 23S rRNA-targeted RT-qPCR using Yakult Intestinal Flora-SCAN analysis system (YIF-SCAN®) as previously described[8, 9]. In brief, RT-qPCR was performed to detect representative bacteria associated with postoperative infectious complications and considered pathogenic based on previous studies[15-17]. The examined microorganisms included facultative anaerobes (family *Enterobacteriaceae*, genus *Enterococcus*, genus *Streptococcus*, and genus *Staphylococcus*), aerobes (genus *Pseudomonas*) and obligate anaerobes (*Clostridium coccoides* group, *Clostridium leptum* subgroup, *Bacteroides fragilis* group, genus *Bifidobacterium*, *Atopobium* cluster, genus *Prevotella*, *Clostridium difficile*, and *Clostridium perfringens*). Samples of preoperative blood collected at the authors' institution were sent to the Yakult Central Institute for analysis. The patient's information was unknown to the technician performing the analysis. RNA was extracted using the method described elsewhere[8]. A standard curve was generated with the RT-qPCR data (using the threshold cycle value, the cycle number when the threshold fluorescence was reached) for the dilution series of the standard strains described elsewhere. For determination of the microorganisms present in the samples, three serial dilutions of an extracted RNA sample were used for RT-qPCR; the threshold cycle values in the linear range of the assay were applied to the standard curve generated in the same experiment to obtain the corresponding bacterial cell count in each nucleic acid sample and thus converted to the number of microorganisms per sample. The result was considered positive when the quantitative result was one or more bacterial cells per 1 mL blood sample. In quantitative analysis, the number of microorganisms was assigned to 0.5 bacterial cells/mL in samples below the detection limit. The assay sensitivity and reliability were demonstrated in previous studies[8, 9].

Evaluated clinical factors

The following clinical factors were analysed in the present study: age; sex; presence or absence of preoperative biliary drainage; blood examination results, including C-reactive protein and procalcitonin; body mass index; operative procedure; pancreatic texture; diameter of the main pancreatic duct; operative time; intraoperative blood loss volume; intraoperative blood transfusion; SSIs defined by the Centers for Disease Control and Prevention; and postoperative complications defined by the Clavien-Dindo classification[18, 19].

Statistical analysis

All quantitative variables were reported as medians with interquartile ranges. Qualitative variables were compared by Pearson's χ^2 test, or by Fisher's exact test when $n<10$; quantitative variables were compared by the Mann–Whitney U test. The McNemar test was used for paired nominal data. Univariable and multivariable analyses were performed to detect risk factors for SSIs using the logistic regression model. In the multivariable analyses, preoperative and intraoperative factors that had significant associations with SSIs in this study were selected in the model. The cut-off levels for quantitative RT-qPCR values and other factors were determined to maximize the difference in predicting any SSI by receiver operating characteristic (ROC) curves. Statistical analysis was performed using JMP Pro 13 software (SAS Institute Inc., Cary, NC) and GraphPad Prism8 (GraphPad Software, San Diego, CA). Values of $p<0.05$ were considered statistically significant.

RESULTS

Patient characteristics

A total of 173 patients who were scheduled to undergo PD were enrolled. Among them, blood samples were collected from 172 patients, excluding one patient due to patient refusal (Fig. 1). During surgery, 17 patients were excluded due to unresectability (n=15) or necessity of total pancreatectomy (n=2). In total, 155 patients were included and analysed. Eighty patients whose initial total bilirubin level was greater than 2 mg/dL underwent preoperative biliary drainage. More than half of the patients (n=81) had pancreatic ductal adenocarcinoma, followed by 25 patients with intraductal papillary mucinous neoplasms and 15 patients with peripapillary carcinomas. Twenty-six patients with PDAC had received neoadjuvant therapy.

Microorganisms in preoperatively collected blood samples

The positive rate of microorganisms in preoperative blood samples detected by RT-qPCR was significantly higher than that obtained by the culture method (32 of 155 [20.6%] vs 4 of 155 [2.6%], p<0.001). Of note, preoperative blood samples were contaminated with 1.0 to 19.2 bacterial cells/mL in 32 positive patients (Fig. 2).

In terms of the individual microorganisms, only four types of microorganisms were isolated by the culture method, including the genus *Streptococcus*, the genus *Klebsiella*, the genus *Propionibacterium*, and the genus *Fusobacterium (*Fig. 3*)*. On the other hand, a total of 41 microorganisms were detected by RT-qPCR. Importantly, 30 of the 41(73%) microorganisms detected by the RT-qPCR method were obligate anaerobes, which were never isolated by the conventional culture method except for the genus *Fusobacterium* from one patient. The *Clostridium leptum* subgroup (n=16), the *Clostridium coccoides* group (n=9), and the *Atopobium* cluster (n=4), which are obligate anaerobes, were predominant, followed by the genus *Staphylococcus* (n=4), which is a facultative anaerobe. The *Clostridium difficile* and *Clostridium perfringens* were not detected in preoperative blood samples.

Demographics of patients with positive or negative microorganisms in blood samples

Preoperative clinical characteristics, including neoadjuvant therapy and factors associated with inflammation, such as preoperative biliary drainage, preoperative cholangitis, preoperative white blood cells, neutrophils, c-reactive protein, and procalcitonin, showed no difference between patients with positive and negative microorganisms in blood samples (Table 1).

Risk factors for SSIs

Overall, 49 patients (32%) had at least one SSI, including nine patients with superficial incisional SSI, one patient with deep incisional SSI, and 44 patients with organ/space SSI (Table 2). Among 44 patients with organ/space SSI, 24 patients had POPF. Among the preoperative and intraoperative factors, soft pancreas (p=0.004), portal vein resection (p=0.019), operative time (p=0.001), and blood loss (p=0.002) were significantly associated with the presence of any SSI. When the quantitative RT-qPCR result was dichotomized based on ROC curve analysis, the optimized cut-off level was determined to be 1.2 bacterial cells/mL. Of note, the incidence of any SSI was significantly higher in patients with contaminated preoperative blood (≥1.2 bacterial cells/mL) than in other patients (14 of 27 [51.9%] vs 35 of 128 [27.3%], p=0.013).

To further investigate the risk factors for SSIs, univariable and multivariable analyses including multiple preoperative and intraoperative factors as well as the preoperative blood contamination status determined by RT-qPCR were performed. In univariable analyses for any SSI, soft pancreas, absence of portal vein resection, longer operative time (≥380 min), greater intraoperative blood loss (≥500 mL), and contaminated preoperative blood (≥1.2 bacterial cells/mL) were significantly associated with any SSI (Table 3). Multivariable analysis including possible risk factors for any SSI indicated that contaminated preoperative blood was identified as one of the independent risk factors for any SSI (odds ratio 2.71, 95% CI 1.04 to 7.24, p=0.041) (Table 3).

DISCUSSION

Technical progress in recent decades has allowed reliable gut microbiome analysis using RT-qPCR or next-generation sequencing targeting bacterial 16S ribosomal RNA or DNA, even in blood samples[20]. In this study, a highly sensitive and quantitative assay based on RT-qPCR, which enables to detect most of the microorganisms belonging to the intestinal microbiota, revealed a detection rate of microorganisms in approximately 20% of preoperative blood samples before therapeutic intervention. The actual number of microorganisms detected in preoperative blood samples was less than 20 per 1 mL of blood, and more than two-thirds of the microorganisms were obligate anaerobes. This level of bacterial contamination can be called O-BT because it usually does not accompany symptoms related to sepsis. The detection of O-BT is almost impossible using the conventional culture method because the number of microorganisms is too small and the detection of obligate anaerobes is difficult. In previous studies[4-6], the impact of O-BT in preoperative blood samples on patients undergoing esophagectomy or PD was investigated, and the detection rate of microorganisms was similar to the present study (approximately 20%). However, the impact of microorganism detection on postoperative complications was not clarified in previous studies, probably due to the limited number of patients. This study recruited a sufficient number of patients and showed that patients with contaminated preoperative blood developed SSIs approximately twice as often as those without. To the best of our knowledge, this is the first report that showed an association between the detection of a small number of bacteria in preoperatively collected blood and the postoperative incidence of SSIs in patients undergoing PD.

When preoperative factors, including preoperative biliary drainage and cholangitis, were analysed, no significant association was found between patients with and without O-BT. The results suggested that O-BT may not originate from the contaminated biliary tract but may originate from a dysregulated intestinal microenvironment. Increased gut permeability, or leaky gut, has been shown in a mouse model of cancer cachexia[21]. Having cancer or other diseases as well as surgical stress may induce a leaky condition of the gut mucosal epithelium, and this condition may induce O-BT. In fact, microorganisms detected in the blood samples were predominantly obligate anaerobes, which constitute more than 99% of the intestinal microbiota.

Why does O-BT before surgery have an impact on the incidence of SSI? This association may be partially explained by a gut-derived remote infection reported as the “Trojan horse hypothesis”[22]. Based on the results of animal experiments, Krezalek *et al*[22] suggested that certain microorganisms can travel from the gut to the wound by their capture by immune cells, similar to Trojan horses, and cause SSI. Although it has never been shown in humans, this hypothesis supports the observations in the present study. Another possible explanation for the association between O-BT and SSIs is an immune deficiency in patients with O-BT. The gut microbiota plays important roles, such as strengthening gut integrity, harvesting energy, protecting against pathogens and regulating host immunity[20]. In patients with O-BT, who may have leaky gut, microbial imbalance (dysbiosis) can lead to an immune deficiency; thus, these patients are more likely to develop SSIs after surgery[23]. With regard to the intraoperative factors, portal vein clamping during portal vein resection could increase gut permeability. However, unexpectedly, portal vein resection was significantly associated with low incidence of any SSI. We previously reported that the intermittent Pringle maneuver was unlikely to induce intraoperative O-BT in portal blood and postoperative complications in patients who underwent major hepatobiliary resection with cholangiojejunostomy[24]. Although, the situation in this study was different from the current study, the results of two studies indicated that the clamping of the portal vein wound not necessarily lead to O-BT associated with leaky gut. The underlying mechanism between the mutual association between O-BT and SSIs should be further investigated in a future study.

It is necessary to consider how to prevent SSIs associated with O-BT. One plausible measure is the administration of prophylactic antibiotics based on the results of RT-qPCR. Prophylactic antibiotics are empirically selected based on past studies that reported the microorganisms that caused postoperative infectious complications[25, 26]. However, almost all these studies were based on the results of the conventional culture method, and pathogenic obligate anaerobes were not generally considered. The results of this study using RT-qPCR revealed the importance of recognizing obligate anaerobes in patients undergoing PD and may lead to a paradigm shift in antibiotic use. Although RT-qPCR does not indicate the sensitivity of bacteria to specific antibiotics at the present time, it can determine the presence of drug-resistance genes in bacteria when specific primers are used[27]. The sample analysis by RT-qPCR finishes within 6 hours and this method features high sensitivity and cost effectiveness compared with other technologies such as next-generation sequencing; thus, examination of O-BT can be applied to perioperative management, although further studies with more sophisticated RT-qPCR methods are needed. Another measure is the administration of synbiotics (a combination of prebiotics and probiotics) before surgery. A preventive role of synbiotics on postoperative infectious complications has been shown in several studies[15, 16, 28]. It should also be noted that synbiotic treatment reduced O-BT in preoperative blood samples in a previous study[6]. Another study also showed that synbiotic treatment increased faecal organic acid concentrations and improved the intestinal microenvironment[29]. Synbiotics may function as intestine stabilizers and thus may contribute to preventing O-BT.

Several limitations of the present study should be acknowledged. First, although the RT-qPCR method is highly sensitive, the identified microorganisms are limited by the primer sets. There is still room to optimize the method for detecting pathogenic microorganisms in blood samples. Second, this study was not designed as a randomized interventional study; therefore, it is difficult to eliminate the effects of prophylactic antibiotics, which were selected at the surgeon's discretion, on the results of SSIs. The effects of type of used prophylactic antibiotics and the duration of antibiotics administration on the incidence of SSIs are also unknown. However, at least in this study, there was no association between the incidence of SSIs and the types of used antibiotics and the duration of antibiotic administration (data not shown). Third, it is uncertain whether a specific group of microorganisms played a role as a pathogen for SSIs in this study because the samples from SSIs were not evaluated by RT-qPCR. However, recent studies have reported that any kind of microorganism constituting the gut microbiome could be a potential pathogen for SSIs in particular conditions, such as surgical stress[30]. Fourth, the findings in this study are intriguing but exploratory due to the limited sample size. Thus, further studies are needed.

CONCLUSIONS

PD remains a challenging procedure with high major complication rates[2]. This study is the first to demonstrate an impact of O-BT in preoperative blood samples detected by RT-qPCR on surgical outcomes in patients undergoing PD. O-BT, predominantly with obligate anaerobes, was commonly observed in preoperative blood samples. In addition to the previously known risk factors, O-BT may be one of the risk factors for SSIs following PD. The results of this study may open a new path for the selection of exact antibiotics that targets possible pathogenic bacteria of SSIs. This study also encourages future studies for the preventive strategy of SSIs associated with O-BT using symbiotic treatment that targets gut immunity because O-BT presumably originates from the intestinal microbiota[10]. Although our findings were exploratory, the clinical significance of O-BT in PD or other surgical procedures should be further investigated to support the limitations of conventional culture methods.

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Figure legends

Figure 1. Study flow chart.

TP, total pancreatectomy.

Figure 2. Number of bacteria detected by RT-qPCR.

RT-qPCR, ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction.

Figure 3. Number of individual microorganisms detected by both culture and RT-qPCR methods.

Other obligate anaerobes detected by the culture method consist of *Fusobacterium,* and other facultative anaerobes consist of *Propionibacterium*.

Atopobium, *Atopobium* cluster; *Bacteroides*, *Bacteroides fragilis* group; *C. coccoides*, *Clostridium coccoides* group; *C. leptum*, *Clostridium leptum* subgroup; RT-qPCR, ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction.

Table 1 Preoperative Clinical Characteristics of Patients with Positive or Negative RT-qPCR in Preoperative Blood.

Variable	RT-qPCR in preoperative blood		p Value
	Negative (n=123)	Positive (n=32)	
Age, y, median (IQR)	67 (62-72)	69 (61-74)	0.330
Sex, n (%)			0.418
Male	75 (61)	22 (69)	
Female	48 (39)	10 (31)	
Body mass index, kg/m^2, median (IQR)	21.0 (19.2-22.8)	20.8 (19.2-24.2)	0.532
Preoperative biliary drainage, n (%)	66 (54)	14 (44)	0.318
Diabetes mellitus, n (%)	41 (33)	9 (28)	0.674
Preoperative cholangitis, n (%)	16 (13)	4 (13)	1.000
Preoperative pancreatitis, n (%)	13 (11)	3 (9)	1.000
Neoadjuvant therapy, n (%)	24 (20)	2 (6)	0.109
Diagnosis, n (%)			0.505
PDAC	68 (55)	13 (40)	
IPMN	17 (14)	8 (25)	
Peripapillary carcinoma	12 (10)	3 (9)	
Bile duct cancer	8 (7)	4 (13)	
PNET	8 (7)	2 (6)	
Others	10 (8)	2 (6)	
Preoperative albumin, g/dL, median (IQR)	3.8 (3.5-4.1)	4.0 (3.5-4.2)	0.295
Preoperative white blood cell, μ/L, median (IQR)	3750 (3000-4625)	3750 (3200-4850)	0.637
Preoperative neutrophil, μ/L, median (IQR)	2400 (1900-3100)	2600 (1900-3450)	0.428
Preoperative c-reactive protein, mg/dL, median (IQR)	0.12 (0.05-0.29)	0.10 (0.05-0.25)	0.727
Preoperative procalcitonin, ng/mL, median (IQR)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.226

IPMN, intraductal papillary mucinous neoplasm; IQR, interquartile range; PDAC, pancreatic ductal adenocarcinoma; PNET, pancreatic neuroendocrine tumor; RT-qPCR, ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction.

Table 2 Preoperative and Intraoperative Clinical Characteristics of Patients with and Without Any SSI.

Variable	Any SSI		p Value
	Absence (n=106)	Presence (n=49)	
Age, y, median (IQR)	67 (62-73)	68 (61-72)	0.893
Sex, n (%)	62:44	35:14	0.122
Male			
Female			
Body mass index, kg/m², median (IQR)	20.6 (19.1-22.8)	21.6 (19.2-24.0)	0.182
Preoperative biliary drainage, n (%)	55 (52)	25 (51)	0.920
Diabetes mellitus, n (%)	31 (29)	19 (39)	0.238
Preoperative cholangitis, n (%)	14 (13)	6 (12)	1.000
Preoperative pancreatitis, n (%)	11 (10)	5 (10)	0.974
Neoadjuvant therapy, n (%)	21 (20)	5 (10)	0.169
Diagnosis, n (%)			0.481
PDAC	61 (58)	20 (41)	
IPMN	14 (13)	11 (22)	
Peripapillary carcinoma	10 (9)	5 (10)	
Bile duct cancer	7 (7)	5 (10)	
PNET	6 (6)	4 (8)	
Others	8 (8)	4 (8)	
Preoperative albumin, g/dL, median (IQR)	3.9 (3.5-4.1)	3.9 (3.5-4.3)	0.245
Preoperative white blood cell, μ/L, median (IQR)	3800 (3050-4750)	3700 (2950-4700)	0.715
Preoperative neutrophil, μ/L, median (IQR)	2400 (1900-3150)	2300 (1850-3250)	0.498
Preoperative c-reactive protein, mg/dL, median (IQR)	0.13 (0.05-0.32)	0.09 (0.06-0.21)	0.283
Preoperative procalcitonin, ng/mL, median (IQR)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.205
MPD diameter, mm, median (IQR)	4.0 (3.0-5.0)	3.2 (2.0-4.2)	0.250
Soft pancreas, n (%)	45 (42)	33 (67)	0.004
Operative method, n (%)			0.282
SSPPD	99 (93)	44 (90)	
PD	5 (5)	5 (10)	
PPPD	2 (2)	0 (0)	
Portal vein resection, n (%)	42 (40)	10 (20)	0.019
Operative time, min, median (IQR)	423 (356-483)	464 (408-562)	0.001
Blood loss, mL, median (IQR)	540 (320-958)	874 (528-1301)	0.002
Blood transfusion, n (%)	22 (21)	8 (16)	0.663

IPMN intraductal papillary mucinous neoplasm; IQR, interquartile range; MPD, main pancreatic duct; PD, pancreaticoduodenectomy; PDAC, pancreatic ductal adenocarcinoma; PNET pancreatic neuroendocrine tumor; PPPD, pylorus-preserving pancreaticoduodenectomy; SSI, surgical site infection; SSPPD, subtotal stomach-preserving pancreatoduodenectomy.

Table 3 Univariable and Multivariable Analysis for Possible Risk Factors of Any SSI.

Variable	No. of patients	No. of any SSI	Univariable p Value	Multivariable	
				Odds ratio (95% CI)	p Value
Soft pancreas			0.004	2.80 (1.18-6.86)	0.019
no	77	16			
yes	78	33			
Portal vein resection			0.016	0.31 (0.12-0.82)	0.018
no	103	39			
yes	52	10			
Operative time			<0.001	5.46 (1.79-19.32)	0.003
<380 min	42	5			
≥380 min	113	44			
Blood loss			0.003	2.98 (1.16-8.08)	0.023
<500 mL	61	11			
≥500 mL	94	38			
RT-qPCR in preoperative blood			0.016	2.71 (1.04-7.24)	0.041
<1.2 bacterial cells/mL	128	35			
≥1.2 bacterial cells/mL	27	14			

RT-qPCR, ribosomal RNA-targeted reverse transcriptase–quantitative polymerase chain reaction; SSI, surgical site infection.

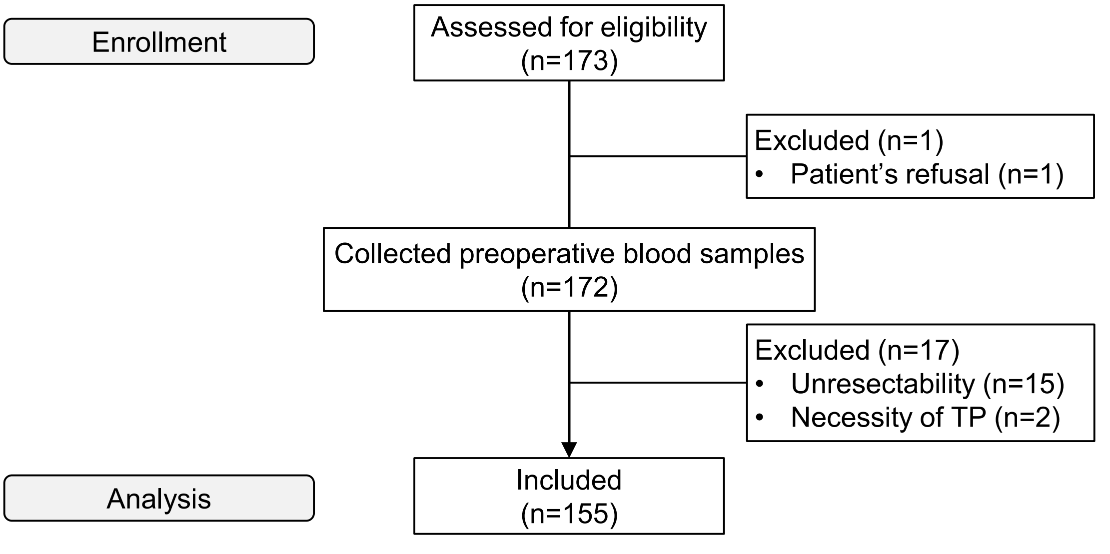
Enrollment
Assessed for eligibility (n=173)
Excluded (n=1)
• Patient's refusal (n=1)
Collected preoperative blood samples (n=172)
Excluded (n=17)
• Unresectability (n=15)
• Necessity of TP (n=2)
Analysis
Included (n=155)

Figure 1

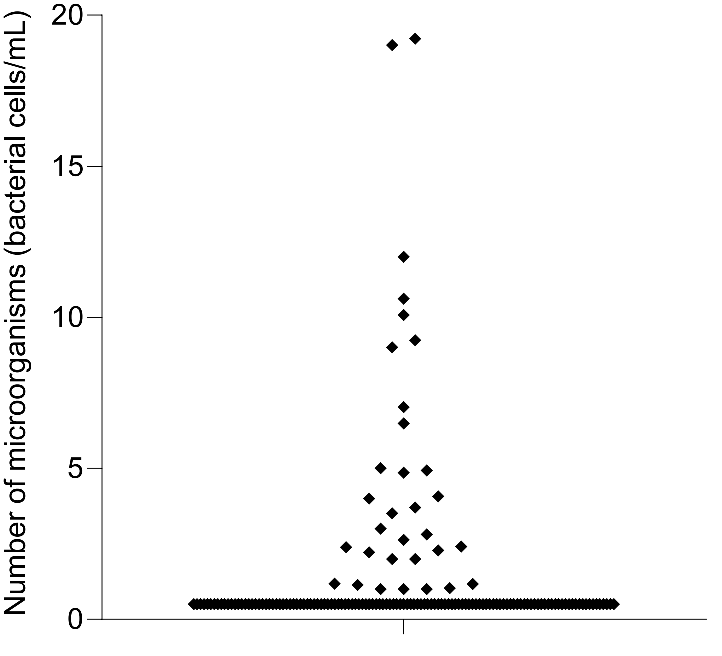

Figure 2

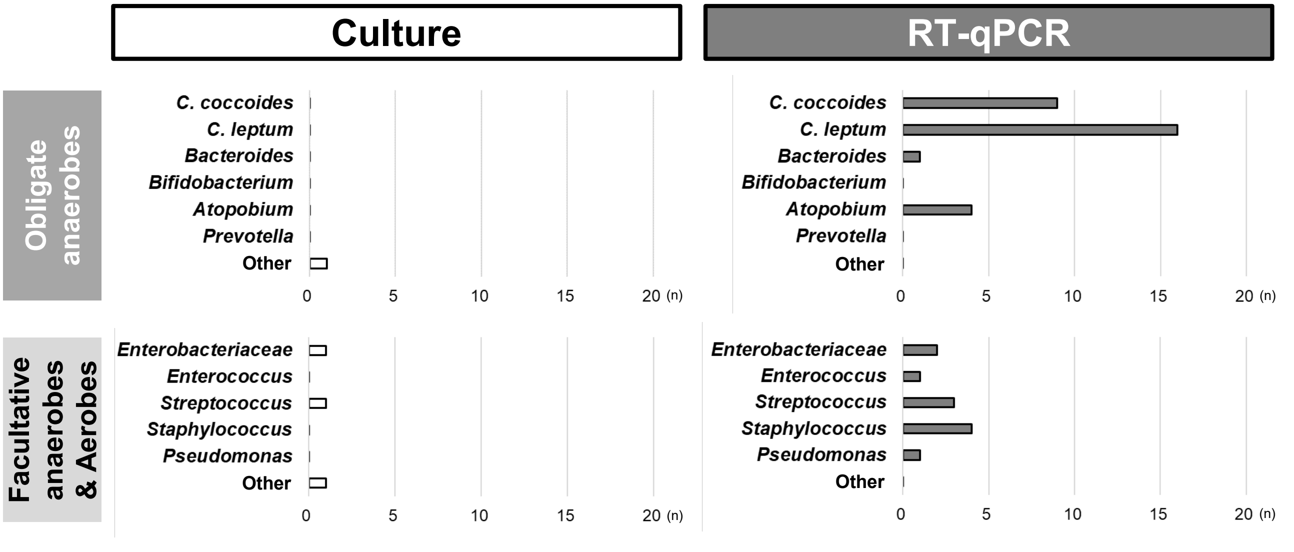
Culture
RT-qPCR
Obligate anaerobes
C. coccoides
C. leptum
Bacteroides
Bifidobacterium
Atopobium
Prevotella
Other
0
5
10
15
20 (n)
Facultative anaerobes & Aerobes
Enterobacteriaceae
Enterococcus
Streptococcus
Staphylococcus
Pseudomonas
Other

Figure 3