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Bacterial smear test of drainage fluid after pancreaticoduodenectomy can predict postoperative pancreatic fistula

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ABSTRACT

Objectives: It is widely accepted that postoperative pancreatic fistula (POPF) accompanied by bacterial infection results in a worse outcome than POPF alone. However, few studies evaluating predictive indicators of POPF have focused on bacterial infection.
Methods: A consecutive 100 patients who underwent pancreaticoduodenectomy at our institute for periampullary disease were enrolled. POPF was assessed according to the International Study Group of Pancreatic Fistula consensus guidelines; grades B and C were defined as clinically relevant POPF (CR-POPF). The patients' characteristics, perioperative surgical factors, and laboratory data including the results of culture and smear testing performed using drainage fluid on postoperative days (PODs) 1 and 3 were analyzed.
Results: The overall incidence of CR-POPF was 25%. Univariate analyses revealed that the factors associated with CR-POPF were male sex, soft pancreas, MPD diameter, higher serum C-reactive protein concentration and white blood cell count on POD 3, higher amylase concentration in drainage fluid, and culture and/or smear positivity of drainage fluid. Multivariate analysis newly revealed that the smear positivity of drainage fluid on POD 3 was the independent risk factors for CR-POPF ($p = 0.027$).
Conclusions: Smear positivity of drainage fluid on POD 3 after pancreaticoduodenectomy may be a new predictor of CR-POPF.

Introduction

Pancreaticoduodenectomy (PD) is the standard operation performed for pancreatic head and periampullary tumors. The perioperative mortality rate of PD has been declining, and is currently relatively low [1,2]. However, PD is still a complex, high-risk, surgical procedure, with an overall postoperative complication rate of over 60% [3]. The most common and important complication after PD is postoperative pancreatic fistula (POPF), which can cause death due to intra-abdominal hemorrhage or sepsis [4,5].

Some predictive indicators and scoring systems have been suggested to predict the development of POPF [6–8]. It is widely accepted that POPF accompanied by a bacterial infection may potentially result in a worse outcome than POPF alone; however, the mechanism has not been elucidated in detail. Although a few studies reported an association between POPF and bacterial infection [9–11], there is currently no evidence available regarding the reliability of using bacterial information as an early predictive indicator of POPF.

The present study evaluated the association between the results of bacterial smear testing and culture of postoperative abdominal drainage fluid and the development of POPF, and assessed the predictive value of bacterial information as a predictive marker for POPF after PD.

Methods

Patients

From January 2013 to May 2018, 100 consecutive patients underwent PD at Tottori University Hospital (Yonago, Japan) for periampullary disease arising from the head of the pancreas, the distal

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common bile duct, and the duodenum; these 100 patients were included in the current retrospective study. The study protocol was approved by the ethical review board of Tottori University (June 3rd, 2018. approval number: 17A135).

Parameters

The clinicopathological parameters and laboratory data of all patients were extracted from the electronic medical records. The patients' characteristics (age, sex, body mass index, cancer type, preoperative serum albumin concentration, presence of preoperative biliary infection and preoperative biliary drainage, soft pancreas, and main pancreatic duct [MPD] diameter), and surgical and postoperative parameters (operation time, blood loss volume, postoperative complications, C-reactive protein [CRP] and white blood cell count [WBC] on postoperative days [PODs] 1 and 3, drainage fluid amylase concentration on PODs 1 and 3, and results of a smear test and culture of drainage fluid collected on PODs 1 and 3) were analyzed. The amylase concentration of the drainage fluid was defined as the maximum value obtained from several drains.

Surgical procedure and perioperative management

All patients underwent subtotal stomach-preserving PD or pylorus-preserving PD by open laparotomy. All operations were performed at Tottori University Hospital by surgeons with substantial expertise in the hepato-biliary-pancreatic field. Broad-spectrum β-lactam penicillin antibiotics (e.g. piperacillin) were administered within 30 min before skin incision, and then every 3 h intraoperatively. The prophylactic postoperative antibiotics were used until POD 1, which were generally the same antibiotics as those used intraoperatively. Perioperative somatostatin analogs were not used routinely.

Reconstruction after PD was done using the modified Child's method. After surgical resection, end-to-side hepaticojejunal anastomosis was performed using monolayer sutures. Subsequently, end-to-side pancreatojejunostomy was performed using the duct-to-mucosa anastomosis method. Polyvinyl chloride plastic tube cut to a length of 5–6 cm was used as a lost stent after the anastomosis.

The antecolic gastrojejunostomy was constructed in a standard two-layer fashion, typically with Braun anastomosis performed approximately 10 cm distally, followed by intra-abdominal washing with 7–10 L of normal saline.

Finally, three surgical silicone drains (6 mm) were used to drain fluid from the surgical field. Two of the drains were positioned around the pancreatojejunostomies (superior and inferior sides), and one was positioned around the hepaticojejunal anastomosis. The three drains were each connected to a closed-drainage bag, and the discharge from each drain was managed separately.

After POD 3, the drains were removed as soon as the physicians judged that there was no concern about POPF. In patients with suspicion of developing POPF from clinical findings (leukocytosis, fever, signs of peritonitis, localized fluid collection and others), we routinely used antibiotics and replaced drains in early stage in order to prevent retrograde infection and drainage failure by drain occlusion. These patients with such alteration in the management of postoperative pathway were classified into grade B POPF.

The CR-POPF infectious pathogen strains confirmed with blood culture, drainage fluid collected via abdominocentesis or drain culture after POD 5, if identified. Nasogastric tubes were used in all patients to detect acute postoperative intraluminal bleeding, and were normally removed on POD 1.

Culture and smear testing of the drain discharge fluid

Drainage fluid was collected from each of the three drains on PODs 1 and 3. The samples were analyzed via the smear test and culture in our laboratory at Tottori University Hospital. Clinical technologists routinely performed the smear test using a Gram stain for each sample. In cases where bacteria was detected, microscopic examination at 1000 × magnification was performed to obtain a semiquantitative bacterial measurement for each sample (1 + indicated < 1 cell/field; 2 + indicated 1–5 cells/field; 3 + indicated 6–30 cells/field; 4 + indicated > 30 cells/field). The bacteria were judged as gram-positive or gram-negative, and morphological observations were performed. Drainage fluid samples were also cultured on agar medium using standard procedures, and the results were assessed after 48 h. Semiquantitative scoring was determined by the four-quadrant method, and was classified as follows: 0 indicated no growth; 1 + indicated growth in 1/4 of the dish; 2 + indicated growth in 1/2 of the dish; 3 + indicated growth in 3/4 of the dish; 4 + indicated growth over the whole dish. Smear and culture results on PODs 1 and 3 did not affected the treatment strategies including initiation and selection of antibiotics.

Definitions of complications

POPF was assessed in accordance with the International Study Group of Pancreatic Fistula consensus guidelines [12]. Grade B POPF required a change in the postoperative management; drains were either left in place for 3 weeks or repositioned through endoscopic or percutaneous procedures. Grade C POPF required reoperation or led to single or multiple organ failure and/or mortality attributable to the pancreatic fistula. The cases with a serum amylase concentration of more than three times the upper limit of normal were defined as having a biochemical leak (BL), which was previously defined as grade A POPF. We classified both grades B and C POPF as clinically relevant POPF (CR-POPF).

Other postoperative complications that occurred within 30 days were evaluated using the Clavien-Dindo (CD) classification system [13].

Statistical analysis

Continuous variables were reported as means and standard deviations, while categorical (dichotomous) data were reported as proportions (%).

Univariate analyses were performed using Fisher's exact test for dichotomous variables, and Mann-Whitney *U* test for continuous variables. Logistic regression analysis was used to calculate the odds ratios for multivariate analysis. All statistical analyses were performed using the statistical software SPSS v. 23.0 statistical software (IBM Corporation, Armonk, New York, USA), and P values less than 0.05 were considered statistically significant.

Results

Patient characteristics, intraoperative findings and indications for PD are summarized in Table 1. Grades B and C POPF were observed in 25 of 100 (25%) patients; this cohort was defined as the CR-POPF group. The remaining 75 patients (including those with no POPF and BL) were classified as the no CR-POPF group. The mean age, body mass index, preoperative serum albumin, presence of preoperative biliary infection and biliary drainage, operation time, and blood loss did not significantly differ between the two groups. Compared with the no CR-POPF group, the CR-POPF group contained a significantly greater proportion of males ($P = 0.011$) and patients with a soft pancreas ($P < 0.001$). The MPD diameter was

Table 1
Patient characteristics and intraoperative findings.

Variables		overall (n = 100)	no CR-POPF(n = 75)	CR-POPF(n = 25)	P value
Age (years)		69.6 ± 12.1	69.5 ± 12.8	69.8 ± 10	0.72
Sex; male		58; 58%	38; 50.7%	20; 80%	0.011*
BMI (kg/m2)		22.1 ± 2.8	21.8 ± 2.7	23 ± 2.8	0.055
Preoperative albumin (g/dL)		3.9 ± 0.6	3.9 ± 0.6	3.9 ± 0.5	0.975
Preoperative biliary infection		24; 24%	15; 20%	9; 36%	0.114
Preoperative biliary drainage		41; 41%	27; 36%	14; 56%	0.101
Disease	ductal adenocarcinoma	37; 37%	35; 46.7%	2; 8%	
	bile duct cancer	20; 20%	12; 16%	8; 32%	
	adenocarcinoma of the papilla	18; 18%	9; 12%	9; 36%	
	IPMN	13; 13%	11; 14.7%	2; 8%	
	SPN	2; 2%	1; 1.3%	1; 4%	
	Others	10; 10%	7; 9.3%	3; 12%	
Soft pancreas		62; 62%	38; 50.7%	24; 96%	<0.001*
MPD diameter (mm)		4.6 ± 2.4	4.9 ± 2.5	3.7 ± 1.9	0.015*
Operation time (min)		543 ± 97	534 ± 93	569 ± 105	0.161
Blood loss (g)		536 ± 361	531 ± 348	548 ± 405	0.744

BMI, body mass index; CR-POPF, clinically relevant postoperative pancreatic fistula.
MPD, main pancreatic duct; IPMN, intraductal papillary mucinous neoplasm; SPN, solid pseudopapillary neoplasm.
*, Statistically significant in univariate analysis.
Values are expressed as the mean ± standard deviation unless stated otherwise.

significantly smaller in the CR-POPF group than in the no CR-POPF group (P = 0.015). The incidence of pancreatic cancer accounted for 11% (7/62) in 62 patients with soft pancreas, whereas it accounted for 74% (28/38) in 38 patients without soft pancreas (P < 0.001, not shown in Table 1).

The complications within 30 days postoperatively are summarized in Table 2. All patients discharged without mortality in this study.

Table 3 shows the associations between the laboratory data of blood and postoperative drainage fluid and the incidence of CR-POPF. The CR-POPF group had significantly greater drainage fluid amylase concentration, culture-positivity, and smear test-positivity than the no CR-POPF group on both PODs 1 and 3. The results of smear and culture test on same day did not always correspond with each other; six patients had positivity of both smear and culture on POD 3, whereas four patients had both of them on POD 1.

The CRP concentration and WBC on POD 3 were also significantly greater in the CR-POPF group than in the no CR-POPF group.

We evaluated the validities of drainage fluid tests using the positive predictive value (PPV) and likelihood ratio (LR) for CR-POPF prognosis (Table 4). The smear test on POD 3 had a good PPV (0.89) and the best LR (24) of the available data; the LR for the culture test on POD 1 was not applicable because of a lack of cases that were "test negative and disease negative".

Based on the univariate analyses and the evaluation of the accuracy of the tests performed on the drainage fluid, a multivariate analysis was performed for CR-POPF occurrence; the independent variables used in this multivariate analysis were male sex, presence of a soft pancreas, MPD diameter, drainage fluid amylase concentration on POD 1, the CRP and WBC on POD 3 and smear test result on POD 3. MPD diameter and drainage fluid amylase concentration were converted into dichotomous variables in accordance with the cutoff values used in previous reports: the cutoff for MPD diameter was 5 mm [6], and that for drainage fluid amylase concentration was 5000 IU/L on POD 1 [8]. CRP concentration and WBC on POD 3 were divided by the respective median values (12.23 mg/dL for CRP, and $8.75 \times 10^3/\mu L$ for WBC) Multivariate analysis revealed that the independent risk factors for CR-POPF were drainage fluid amylase concentration (≧ 5000 IU/L) on POD 1, male sex, WBC on POD3 (≧ $8.75 \times 10^3/\mu L$) and smear test-positivity on POD 3 (Table 5).

Characteristic data from smear-positive patients on POD 3 are shown in Table 6. All nine smear-positive results were from drains positioned around the pancreatojejunostomies. The results of seven cases (78%) were semiquantitatively classified as 1+. The infectious bacterial pathogens were isolated from blood culture or drainage fluid collected via abdominocentesis or from the drain after POD 5 in six patients. Particularly, the infectious bacterial pathogens identified after POD5 from five of these six patients were consistent with the gram stain results of the smear test, whereas the results of preoperative culture from biliary drainage fluid or blood had no connection with the pathogens.

Table 2
Postoperative complications after PD.

Complications after operation		n = 100	(%)
Overall	CD ≧ 2	37	(37)
	CD ≧ 3	27	(27)
Pancreatic fistula	BL	20	(20)
	Grade B	24	(24)
	Grade C	1	(1)
Intra abdominal abcess		4	(4)
Diarrhea		4	(4)
Bile leak		3	(3)
Delayed gastric emptying		3	(3)
Gastrointestinal bleeding		2	(2)
Ascites		2	(2)
Bile ductitis		2	(2)
Wound infection		1	(1)
Pneumonia		1	(1)
Lymphorrhea		1	(1)
Pancreatitis		1	(1)
Brain infarction		1	(1)
Mortality		0	(0)

Some data overlapped. PD, pancreaticoduodenectomy.
BL, biochemical leak; CD, Clavien-Dindo grade.

Discussion

Despite the improvements in the PD procedure and perioperative treatment in recent decades, POPF is still the most important complication after PD. POPF can cause mortality or delay the initiation of adjuvant chemotherapy. Furthermore, POPF increases medical costs and the duration of postoperative hospitalization [14,15]. It has recently been suggested that POPF may be prevented by the performance of pancreatico-enteric anastomosis methods such as pancreaticogastrostomy [16] or invagination via

Table 3
Postoperative data.

Variables		overall (n = 100)	no CR-POPF(n = 75)	CR-POPF(n = 25)	P value
CRP (mg/dL)	1POD	7.5 ± 2.6	7.5 ± 2.7	7.4 ± 2.6	0.76
	3POD	14.8 ± 7.9	12.6 ± 6.6	21.4 ± 7.7	<0.001*
WBC (×10ˆ3/μL)	1POD	9.9 ± 3.4	9.7 ± 3.2	10.8 ± 4	0.28
	3POD	9.3 ± 3.4	8.9 ± 3.1	10.5 ± 3.8	0.0078*
drain amylase (IU/L)	1POD	11446 ± 22804	5003 ± 10078	30775 ± 36236	<0.001*
	3POD	1094 ± 4283	302 ± 420	3469 ± 8204	<0.001*
culture positive cases	1POD	5; 5%	0; 0%	5; 20%	<0.001*
	3POD	11; 11%	3; 4%	8; 32%	<0.001*
smear positive cases	1POD	7; 7%	2; 2.7%	5; 20%	0.0101*
	3POD	9; 9%	1; 1.3%	8; 32%	<0.001*
CD2≦complication except for POPF	yes	18; 18%	15; 20%	3; 12%	0.55
	no	82; 82%	60; 80%	22; 88%	

CRP, C-reactive protein; CD, Clavien-Dindo grade; POD, postoperative day.
WBC, white blood cell count; * Statistically significant in univariate analysis.
Values are given as the mean ± standard deviation or n (%).

Table 4
Accuracy of drainage fluid culture and smear test for detecting CR-POPF.

		Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI	LR	95% CI
culture positive	1POD	0.2	0.068–0.41	1	0.93–1	1	0.36–1	0.79	0.69–0.87	N/A	N/A
	3POD	0.32	0.15–0.54	0.96	0.89–0.99	0.73	0.39–0.94	0.81	0.71–0.89	8	2.3–28
smear positive	1POD	0.2	0.068–0.41	0.97	0.91–1	0.71	0.29–0.96	0.79	0.69–0.86	7.5	1.6–36
	3POD	0.32	0.15–0.54	0.99	0.93–1	0.89	0.52–1	0.81	0.72–0.89	24	3.2–183

CI, confidence interval; CR-POPF, clinically relevant postoperative pancreatic fistula.
POD, postoperative day; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; N/A, not applicable.

Table 5
Independent prognostic factors for CR-POPF.

		Univariate analysis			Multivariate analysis		
		noCR-POPF (n = 75)	CR-POPF (n = 25)	P value	Odds Ratio	95% CI	P value
MPD diameter	≧5 mm	33	5	0.035*	—		0.61
	<5 mm	42	20				
Drainage fluid amylase concentration on POD 1	≧5000 IU/L	18	22	<0.001*	11.2	1.41–89	0.022*
	<5000 IU/L	57	3				
Soft pancreas	yes	38	24	<0.001*	—		0.36
	no	37	1				
Sex	male	38	20	0.011*	8.71	1.54–49	0.015*
	female	37	5				
CRP on POD 3	≧ 12.23 mg/dL	30	20	<0.001*	—		0.22
	<12.23 mg/dL	45	5				
WBC on POD 3	≧ 8.75 × 10ˆ3/μL	30	20	<0.001*	9.92	1.5–65	0.017*
	<8.75 × 10ˆ3/μL	45	5				
Smear test on POD 3	positive	1	8	<0.001*	22.8	1.4–367	0.027*
	negative	74	17				

CI, confidence interval; CR-POPF, clinically relevant postoperative pancreatic fistula; POD, postoperative day.
CRP, C-reactive protein; MPD, main pancreatic duct; WBC, white blood cell count; *, Statistically significant.

pancreaticojejunostomy [17], and by prophylactic somatostatin analog use (e.g. octreotide [18], and pasireotide [19]); however, the effectiveness of these methods remains controversial [20–22].

To predict the development of POPF, a risk scoring system has been described based on the presence or absence of four factors (small duct, soft pancreas, high-risk pathology, and excessive blood loss) [6,23].

In the current study, patients with soft pancreas consisted of mainly non-pancreatic cancer patients. According to previous studies [24,25], hard pancreatic tissue may reflect the low - exocrine function of the pancreas and tumor involvement/inflammation in pancreas. Adding to the technical difficulty of pancreaticoenteric anastomosis with soft pancreas, the sufficient exocrine function of the pancreas can be a source of POPF.

In 2007, Molinari et al. conducted a prospective study of 137 cases of PD and distal pancreatectomy, and reported that the amylase concentration in drainage fluid on POD 1 after pancreatic surgery is a predictive factor for POPF development [8]; this was followed by other reports that suggested the importance of evaluating the amylase concentration in drainage fluid [26,27].

It is generally well known that POPF is often accompanied by bacterial infection, which can exacerbate the clinical course [11,28,29]. Kawai et al. reported that early removal of postoperative drainage after PD reduces the incidence of intra-abdominal infection and POPF [30]. Yamashita et al. reported that bacteria isolated from drainage fluid in patients with POPF could activate trypsinogen into trypsin [10]. Chymotrypsin is activated from the precursor chymotrypsinogen. Both trypsin and chymotrypsin are endopeptidases from the serine protease family of enzymes, which are the main components of pancreatic juice [32]. It has also been suggested that pancreatic juice can be activated by bacterial phospholipase and lipopolysaccharide [33–35]. Considering these

Table 6
Data from smear-positive patients on POD 3.

Case no.	Age (years)	Sex	CR-POPF	Grade	Diagnosis	Other complications	Smear results	Drain-cultured species of bacteria on POD 3	Infectious bacterial pathogens identified after POD 5	preoperative culture
1	83	M	Yes	C	BDC	—	GPC 1+	***Enterococcus faecium* 1+**	—	*K.pneumoniae (blood)*
2	81	M	Yes	B	ACP	Abcess	GPR 1+	***Corynebacterium* (GPR) spp. 2+**, *Enterobacter aerogenes* (GNR) 2+	***Corynebacterium* (GPR) spp. 2+**, *Enterobacter aerogenes* (GNR) 2+	—
3	69	M	Yes	B	ACP	Bile leak	GNR 1+	—	*Corynebacterium striatum* (GPR) 2+	*Pseudomonas putida (biliary)*
4	66	M	Yes	B	BDC	—	GPC 3+, GNR 4+	***Enterococcus faecalis* (GPC) 3+**, ***Aeromonas caviae* (GNR) 3+**	***Enterococcus faecalis* (GPC) 3+**, ***Aeromonas caviae* (GNR) 2+**	—
5	71	M	Yes	B	BDC	—	GPC 1+	***Enterococcus faecium* (GPC) 3+**	***Enterococcus faecium* (GPC) 3+**	*Enterobactor cloacae (biliary)*
6	81	M	Yes	B	BDC	—	GPC 1+	***Enterococcus faecium* (GPC) 1+**	***Enterococcus faecium* (GPC) 4+**	*Candida albicans (biliary)*
7	65	F	No	BL	IPMN	—	GPC 1+	—	—	—
8	66	M	Yes	B	DA	—	GNR 4+	***Klebsiella oxtoca* (GNR) 4+**	***Klebsiella oxytoca* (GNR) 4+**	—
9	56	M	Yes	B	ACP	—	GPC 1+	—	—	—

ACP, adenocarcinoma of the papilla; BDC, bile duct cancer; BL, biochemical leak; CR-POPF, clinically relevant postoperative pancreatic fistula.
DA, ductal adenocarcinoma; F, female; GNR, gram-negative rod; GPC, gram-positive coccus; GPR, gram-positive rod; IPMN, intraductal papillary mucinous neoplasm.
M, male; POD, postoperative day; Bacterial species consistent with smear results appear in **bold** type.

previous results together, we hypothesized that bacterial infection develops into CR-POPF, including the progression from BL to CR-POPF. Therefore, detection of the signs of infection may potentially be a new diagnostic strategy for POPF.

Recently, it has reported that a positive bacterial culture of the lavage fluid at the end of the operation [36] and of ascitic fluid collected on POD 1 [9] has adverse impacts on POPF following PD. However, to the best of our knowledge, no study has focused on the results of a smear test of postoperative drainage fluid after PD.

In the current study, we investigated the potential of smear test examination of fluid collected systematically from consecutive 100 patients' postoperative abdominal drains for predicting POPF after PD. The cases that were smear-positive on POD 3 showed a significantly greater incidence of CR-POPF compared with smear-negative cases. In addition, multivariate analysis revealed that smear-positivity on POD 3 was an independent risk factor for the development of POPF, as well as drainage fluid amylase concentration (≧ 5000 IU/L) on POD 1, male sex, WBC (≧ 8.75×10^3/μL) on POD3. Furthermore, a smear test has the great advantages of rapidity and simplicity compared with bacterial culture, which requires 48 h or more to confirm the final result.

In the six patients with bacterial pathogens identified in samples taken after POD 5, enteric bacterial species (*Enterobacter aerogenes*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Klebsiella oxytoca*) were isolated in five patients. After pancreatectomy, drainage fluid cultures often detect enteric bacterial species rather than indigenous bacterial species found on the skin [9,30]; however, it remains unclear whether this is predominantly due to intraoperative contamination in the abdomen or spilling from postoperative anastomotic leakage (major/minor). In the current study, three smear positive patients on POD 3 were not identified with the infectious bacterial pathogen strains in the course of POPF treatment. However, in five patients, the gram stain results of the smear test on POD 3 well reflected the infectious bacterial pathogens identified after POD 5, on the other hand, the results of preoperative culture from biliary drainage fluid or blood could not implicate the pathogens at all. These suggest that the bacteria in drainage fluid on POD 3 has a certain correlation with the bacteria that triggers infection-related development of CR-POPF.

The present study has some limitations as it was a retrospective analysis with a limited number of enrolled patients. Although the usefulness of POD 3 smear test was demonstrated in this study, there were patients with negative POD 3 smear and with CR-POPF. It means that all CR-POPF patients could not be detected with POD 3 smear test positivity (sensitivity; 0.32), nevertheless the smear positivity detected precisely CR-POPF patients (specificity; 0.99).

Also, there was one patient with positive Day 3 smear and without CR-POPF, the reason why the detected bacteria in this patient did not cause CR-POPF development remains unclear. Thus, further improvement in CR-POPF detection with higher accuracy is needed.

However, if the smear test predicts the development of POPF and the presence of pathogenic bacterial species in the early postoperative phase, clinicians can immediately implement careful monitoring, appropriate use of antibiotics, and alternation of the drainage strategy. Especially, initiation of antibiotic therapy targeting the bacteria detected in smear should be verified for smear positive patients on POD 3, as a future strategy of CR-POPF prevention. Moreover, preoperative gastrointestinal microbiome (flora), which is a source of pathogenic bacteria, can be approached for prophylactic treatment using synbiotics.

In conclusion, smear test on POD 3 is an independent predictor of CR-POPF, which might have important implications for POPF management.

To verify the clinical utility of the bacterial smear test, a prospective study with large number of patients is needed in the future.

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