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ORIGINAL SCIENTIFIC REPORT

Intraoperative Body Fluid Amylase as a Novel Indicator of Postgastrectomy Pancreatic Fistula

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Abstract

Background Although many methods to prevent the development of postoperative pancreatic fistula (POPF) after gastrectomy have been reported, POPF can only be identified after it has occurred. Various therapeutic measures could be taken if signs of POPF could be detected intraoperatively.

Methods We conducted a prospective study in which we attempted to predict POPF by measuring the intraoperative amylase concentration in the peripancreatic body fluid. To collect the body fluid, three sponges were placed around the pancreas at lymph node station Nos. 6, 8, and 11 during lymphadenectomy. The amylase concentration was measured in the body fluid squeezed from the sponges. We investigated whether the intraoperative body fluid amylase concentration (IBAC) was associated with POPF formation.

Results In total, 109 patients were enrolled from February 2016 to March 2018, and we analyzed 81 eligible patients. Clavien–Dindo grade $\geq$ II POPF occurred in eight patients (9%). The IBAC was significantly higher in sponges No. 6 ($P = 0.044$) and No. 8 ($P = 0.007$). The incidence of POPF was predicted by using an IBAC cutoff value for No. 6 (1047 IU/L; sensitivity 87.5%; specificity 65.0%; positive likelihood ratio 2.5) and No. 8 (400 IU/L; sensitivity 87.5%; specificity 68.5%; positive likelihood value 2.8), respectively. The IBAC in sponge No. 11 tended to be higher ($P = 0.054$).

Conclusions By measuring the IBAC, surgeons might predict POPF easily and noninvasively during surgery. This method is one of the most effective ways to predict POPF intraoperatively.

Introduction

Postoperative pancreatic fistula (POPF) formation, along with anastomotic leakage and intra-abdominal abscess formation, is one of the most severe postoperative complications after gastrectomy for gastric cancer. Once severe POPF has occurred, the duration of hospitalization and medical costs will increase.

Many surgeons have long experienced challenges in the management of POPF, making extensive efforts to determine the best way to prevent or predict this complication [1–11]. Previous studies on this topic are divided into two types. One type involves precautionary measures such as anatomical findings on computed tomography (CT) or intraoperative manipulations [1–6]. The other type involves examination of postoperative predictive factors using the patient's postoperative laboratory data [7–11]. Although precautionary methods are certainly necessary, prevention of POPF using such measures is impossible. Even if

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surgeons consider that the surgery had been perfectly performed, many patients still develop POPF. Therefore, it is essential to predict POPF as soon as possible. However, the results of previous studies have not been particularly useful because when using postoperative data, POPF has already occurred or the drain might not have detected pancreatic fluid leakage. These late actions are the reason previous methods have not been able to prevent POPF adequately.

Against this background, we considered the intraoperative detection of potential signs of POPF. No reports have described how to predict POPF during surgery. The purpose of this study was to identify intraoperative signs of POPF and thus predict the risk of POPF intraoperatively.

Materials and methods

Study design

This prospective single-center observational study was approved by the Tokyo Medical Dental University hospital review board (M2015-570) before initiation, and all patients provided written informed consent. We used a receiver operating characteristic (ROC) curve analysis to investigate the relationship between the intraoperative body fluid amylase concentration (IBAC) and the development of POPF. To calculate the sample size, we set the following parameters: area under the curve (AUC) = 0.8, power = 0.8, and significance level = 0.05. The incidence of POPF was 7% based on recent surgical results in our hospital [12, 13]; therefore, the ratio of non-POPF/POPF was set at 13:1. As a result, the suitable sample size was calculated to be 100 patients. The sample size was calculated using Easy R (EZR) software [14].

Eligibility criteria

The eligibility criteria for this study were gastric cancer for which distal gastrectomy, proximal gastrectomy, or total gastrectomy with D1 + or higher lymph node dissection was indicated based on the Japanese Gastric Cancer Treatment Guidelines 2014 (version 4) [15]; the ability to undergo anesthesia with an Eastern Cooperative Oncology Group performance status of 0–2 [16]; an age of 20–90 years; and provision of written informed consent. The exclusion criteria were a requirement for pancreatectomy for treatment of pancreatic invasion; a Clavien–Dindo grade $\geq$ III complications unrelated to POPF; and inadequacy for patient participation in the study as determined by the investigators.

Surgeons

Twelve surgeons, including fellows, performed the operations. In some cases, the operator changed during the surgery.

Procedure for measuring IBAC

We used Securea^{TM} surgical sponges (Hogy Medical Co., Ltd., Tokyo, Japan) to absorb body fluid and push down the pancreas. We placed these sponges in three locations and removed them after finishing each lymphadenectomy. One sponge was placed beside the pancreas head after taking down the mesocolon, and the sponge was removed after finishing No. 6 lymphadenectomy. The other two sponges were placed under the caudate lobe of the liver and the rolled-up stomach before touching the suprapancreatic area, and these sponges were removed after finishing No. 8 and 11 lymphadenectomies (Fig. 1). The sponges were squeezed immediately upon removal, and the IBAC was measured.

Drained body fluid amylase concentration after surgery

At the end of each operation, we placed a drain behind an esophagojejunal or gastrojejunal anastomosis. The drained body fluid amylase concentration was measured on postoperative days (POD) 1 and 3.

Definition and diagnosis of POPF

We defined POPF in two ways. (a) We used the definition established by the International Study Group on Pancreatic Fistula (ISGPF) [17]. (b) We determined clinical POPF by the presence of peripancreatic fluid on a CT scan with abdominal pain and fever. POPF grading was performed according to the Clavien–Dindo classification [18]. A Clavien–Dindo grade $\geq$ II POPF was regarded as significant in the present study.

Statistical analysis

Data are presented as median [interquartile range]. Continuous variables were assessed for significance using the Mann–Whitney test. Categorical data were evaluated using the Chi-square test. An ROC curve was created to identify the best cutoff value of the IBAC for assessing the risk of POPF. Diagnostic reliability was considered using the positive likelihood ratio (LR +), which are not affected by the incidence of the POPF.

All analyses were performed using JMP Ver. 13.2 software (SAS Institute Inc., Cary, NC, USA), and

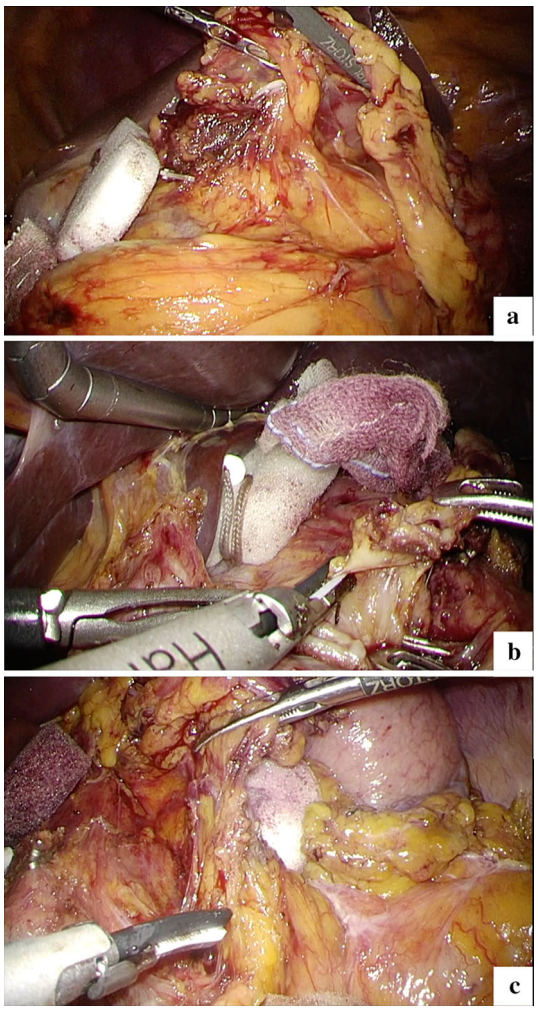

Fig. 1 Sponge placement **a** Placement beside the pancreas head after taking down the mesocolon (No. 6). **b** Placement under the caudate lobe of the liver (No. 8). **c** Placement under the rolled-up stomach (No. 11)

differences were considered statistically significant at $P < 0.05$.

Results

We enrolled 109 patients from February 2016 to March 2018. We excluded 16 patients who met the exclusion criteria (two with pancreatectomies, two anastomotic leakage, one reoperation by port-site hernia, one aneurysm perforation, and ten inadequate cases) and 12 patients in whom the IBAC could not be measured because of a shortage of body fluid. In ten inadequate cases patients who underwent interventions that affected the surgical outcome, such as the drain that cannot be placed in the correct position or use of fibrin glue, were included. As a result, we analyzed 81 patients (Fig. 2).

Patient characteristics

The patient characteristics and surgical results are shown in Table 1. The clinical stages were determined according to the Japanese Classification of Gastric Cancer, 3rd English edition [19]. All reconstructions after distal gastrectomy were performed by Roux en Y reconstruction. No patients underwent splenectomy along with total gastrectomy. We found no significant differences in any parameters between the POPF and No POPF groups except for the postoperative hospital stay. A Clavien–Dindo grade $\geq$ II POPF occurred in eight patients (9%) (Table 2). Four patients met the POPF definition established by the ISGPF. The other four patients had abdominal pain and fever. Additionally, CT scans revealed peripancreatic fluid retention (Fig. 3).

IBAC

The IBAC and the drained body fluid amylase concentration data are shown in Table 3. Sixty-eight of all 81 patients underwent IBAC measurement in each of the 3 sponge locations, and 13 patients who underwent proximal gastrectomy underwent IBAC measurement at lymph node station Nos. 8 and 11. The IBAC was significantly different between the POPF and No POPF groups at No. 6 (2365 vs. 833 IU/L, respectively; $P = 0.044$) and No. 8 (607 vs. 222 IU/L, respectively; $P = 0.007$). No significant difference was observed at No. 11. However, the IBAC tended to be higher in the POPF than No POPF group (816 vs. 392 IU/L, respectively; $P = 0.054$). The drained body fluid amylase concentration was not significantly different between both groups on POD1.

Figure 4 shows the ROC curve of the IBAC at the No. 6 and No. 8 locations. The cutoff values for predicting POPF were 1047 IU/L (sensitivity, 87.5%; specificity, 65.0%; AUC = 0.72; positive predictive value, 23%; negative predictive value 98%; LR + , 2.5) and 400 IU/L (sensitivity, 87.5%; specificity, 68.5%; AUC = 0.79; positive predictive value, 21%; negative predictive value 97%; LR + , 2.8), respectively.

Discussion

Many methods with which to predict POPF using postoperative laboratory data have been described. Some researchers used the drained body fluid amylase concentration [8, 11] or C-reactive protein concentration [8, 10]. One study used a predictive scoring system with risk factors such as age, drained amylase concentration, body mass index, and other parameters [7, 8, 10]. Although early findings of abnormalities and a quick response would lead to the prevention of severe POPF, the postoperative period

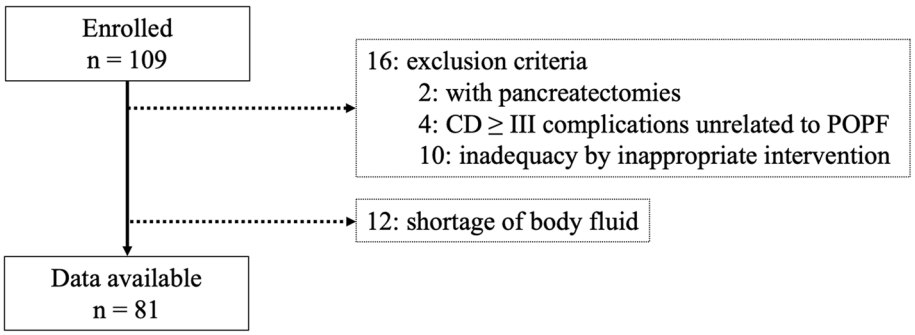

Fig. 2 Study flowchart. CD Clavien–Dindo grade; POPF postoperative pancreatic fistula

might already be too late for effective therapy, or the drain might not be sufficient. With these methods, surgeons can only identify POPF after it has occurred, and their treatment options are thus limited. However, our breakthrough method can intraoperatively detect pancreatic fluid leakage and determine whether this leakage will cause POPF. Moreover, an important finding in this study is that we could presume where the POPF would most likely occur.

Before planning this study, we thought there were many POPF that could not be detected. This "undetected" means that a body fluid could not be collected at a proper position or by an improper position. Undetected POPF might have been regarded as some intra-abdominal abscess. Therefore, we added the definition (b) for the diagnosis of POPF. In order to reduce undetected POPF, it was important that our study could reveal not only to detect during surgery but also where it occurred. Although ISGPF criteria have also been commonly used for predicting POPF in gastrectomy [1, 5, 7], sometimes POPF might not be caught by the drain amylase concentration. In pancreatectomy, the drain is put the pancreas stump. And in gastrectomy, many surgeons place the drain in the suprapancreatic area, as we did [1, 3, 7, 8]. Of course, drains are not always effective. However, the area which has the possibility of leakage of pancreatic juice is much larger in gastrectomy. We thought this was one of the reasons that the POPF was sometimes not detected by the drain amylase concentration. Our data show that four patients (50%) did not meet the ISGPF definition and still developed POPF. Furthermore, in two of four patients, fluid retention existed around pancreas head far from a drain position. The number of patients who were not detected POPF was more than we expected. Although we have attempted previously described methods, the drained body fluid amylase concentration cannot be used in such cases of ineffective drainage [8, 11]. Other techniques, such as measurement of the postoperative C-reactive protein concentration or the use of a predictive scoring system, have also been shown to be ineffective in predicting these cases appropriately [7, 8, 10]. From these assessments, our study is the only way to reduce undetected POPF.

Our study proved that we could know if POPF occurs, and where it occurs during surgery. Therefore, we can procedure intraoperative measures to prevent the development of severe POPF including undetected one. One effective method might be using medical materials to repair the injured pancreas. Some reports have described the intraoperative prevention of pancreatic fistula using fibrin glue sealant and polyethylene glycolic acid felt [20, 21]. Although this method is useful and might be used by many surgeons, it is not practical to perform in all cases because of its high cost. Based on our study, however, it will be possible to intervene only in appropriate cases before finishing the surgery. Thus, the incidence of POPF can be reduced to a much greater degree. We excluded six cases by using fibrin glue. Despite the pancreas injured seriously, they did not develop POPF. We are planning a new study which uses these techniques combined with our method. The time required for measuring the IBAC is 30–60 min in our hospital. We submit specimens as soon as they are obtained and can determine the IBAC while performing the reconstruction.

Since the incidence of POPF was low, the positive predictive values of this study were not high. So, we used a likelihood ratio for assessing the value of IBAC. A likelihood ratio of greater than 1 indicates the result is associated with the disease. LR + of IBAC for Nos. 6 and 8 was 2.5 and 2.8, respectively. These data showed the clinical applicability of our study. Surgeons should be aware of an important factor when collecting body fluid. Depending on the particular institute, a certain amount of fluid is necessary to measure the amylase concentration. However, the least amount of a body fluid which could measure the IBAC was 0.5 ml in our hospital. To our knowledge, it was easy to collect 0.5 ml if the pancreas had been injured. In cases involving smooth completion of the lymphadenectomy without pancreatic injury, it may be difficult to collect an adequate amount of body fluid, especially at lymph node station No. 6. When body fluid cannot be collected,

Table 1 Patient characteristics and surgical results

Variables	All patients $n = 81$	POPF $n = 8$	No POPF $n = 73$	P value
Age, years	72 [68–78]	63 [53–76]	73 [69–78]	0.077
Male/Female	49/32	6/2	43/30	0.469
BMI, kg/m^2	22.2 [20.0–24.2]	23.7 [22.3–25.3]	22.1 [19.8–24.2]	0.143
Comorbidities				1.000
Yes	67 (83)	7 (88)	60 (82)	
No	14 (17)	1 (12)	13 (18)	
ASAPS				0.632
1	3 (4)	0 (0)	3 (4)	
2	67 (84)	8 (100)	59 (82)	
3	9 (11)	0 (0)	9 (13)	
4	1 (1)	0 (0)	1 (1)	
Tumor location				0.507
U	17 (21)	1 (13)	16 (22)	
M	38 (47)	3 (37)	35 (48)	
L	26 (32)	4 (50)	22 (30)	
JCGC stage				0.837
cStage I	51 (63)	4 (50)	47 (64)	
cStage II	15 (19)	2 (25)	13 (18)	
cStage III	14 (17)	2 (25)	12 (16)	
cStage IV	1 (1)	0 (0)	1 (1)	
Surgical technique				0.587
Open	10 (12)	0 (0)	10 (14)	
Laparoscopic	71 (88)	8 (100)	63 (86)	
Resection type				0.369
Distal	55 (68)	7 (88)	48 (66)	
Proximal	13 (16)	0 (0)	13 (18)	
Total	13 (16)	1 (12)	12 (16)	
Nodal dissection				0.302
D1+	52 (64)	4 (50)	48 (66)	
D2	29 (36)	4 (50)	25 (24)	
Operative time, min	345 [286–392]	371 [333–443]	339 [283–388]	0.203
Blood loss, ml	92 [31–220]	81 [41–197]	99 [30–233]	0.734
Retrieved lymph nodes	34 [26–47]	39 [30–52]	33 [25–46]	0.281
Postoperative hospital stay, days	7 [6–8]	11 [7–16]	7 [6–8]	0.006

Data are presented as n (%) or median [interquartile range]

POPF postoperative pancreatic fistula, *BMI* body mass index, *ASAPS* American Society of Anesthesiologists physical status classification, *JCGC* Japanese classification of gastric carcinoma

the pancreas is not injured or the severe POPF will not be developed. In the present study, the IBAC could not be measured in 12 patients for these reasons. Although these patients did not develop a POPF, this large number was hard to anticipate initially, and this is one of the reasons for the decreased number of analyzed patients. The number of analyzed patients was less than the scheduled sample size. Thus, the AUCs were somewhat lower than the design.

POPF is a severe postoperative complication after gastrectomy, and no effective precautionary measures are available. However, measuring the IBAC can predict POPF intraoperatively and make the chance to take precautionary measures during surgery. We can prevent POPF and intra-abdominal abscess formation by acting earlier than ever.

The limitations of our study are as follows. First, this was a single-hospital study. Although our data are

Table 2 Cases of POPF

Case	CD	Amylase concentration (IU/L)				ISGPF criteria	Area of fluid retention on CT
		No. 6	No. 8	No. 11	POD 3		
1	II	2702	400	616	261	–	Around the pancreas head
2	II	1047	954	312	109	–	Around the pancreas head
3	II	1298	421	300	334	–	Around the pancreas head and body
4	II	133	249	1393	28	–	Around the pancreas head and body
5	II	3975	1195	1015	4840	Met	Around the pancreas body
6	II	13,125	20,190	1759	2123	Met	Around the pancreas head and body
7	II	2028	417	3352	6470	Met	Around the pancreas head
8	IIIa	3786	793	456	681	Met	Around the pancreas head

POPF postoperative pancreatic fistula, *CD* Clavien–Dindo classification, *POD* postoperative day, *ISGPF* international study group of pancreatic fistula, *CT* computed tomography

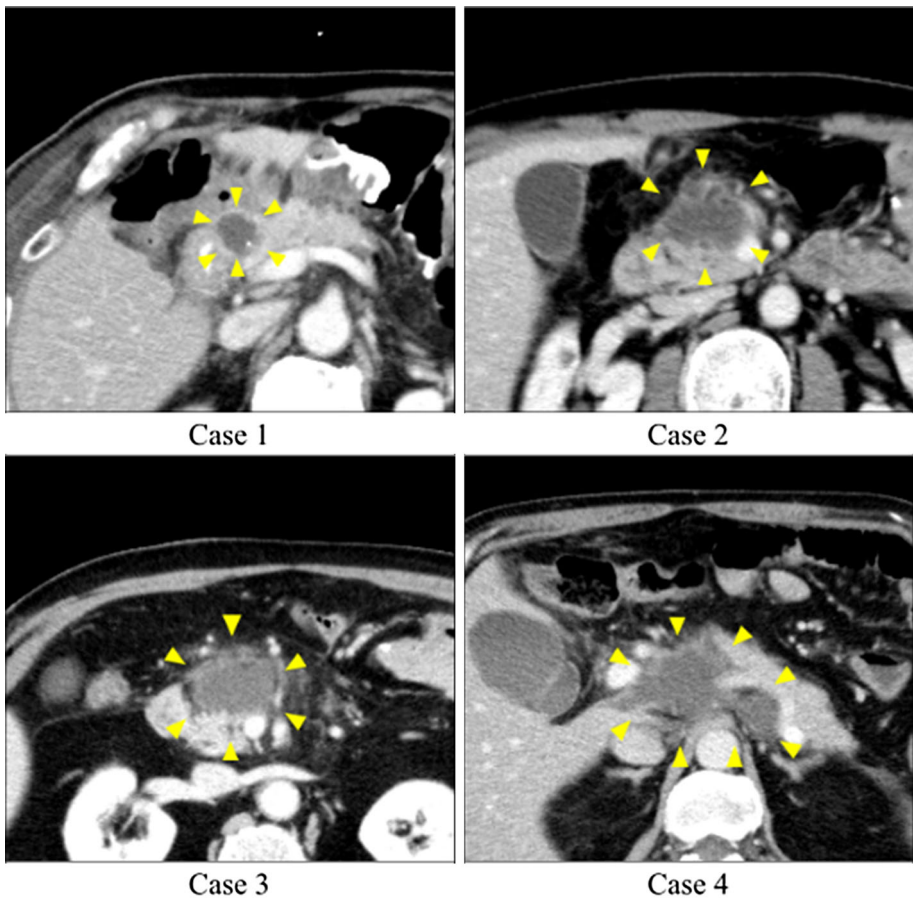

Fig. 3 CT scans showed fluid retention around each pancreas. Arrows show the fluid collections, respectively

valuable, the cutoff value can only be used in our hospital. If a multicenter trial could be conducted and many IBAC samples could be collected, the sensitivity and specificity of the IBAC would increase and the cutoff value could become the universally recommended value. Second, there were variations in the median value of each IBAC. This result might indicate that the sponge locations were not appropriate and might be another reason for the lack of statistical significance of the IBAC at lymph node station No. 11. Therefore, the sponge locations should be reconsidered. Finally, rapid measurement of the IBAC may be

Table 3 IBAC and drained body fluid amylase concentration

Site	All patients $n = 81$	POPF $n = 8$	No POPF $n = 60$	*P* value
No. 6 (IU/L)	1011 [225–3805]	2365 [1110–3928]	833 [181–2027]	0.044
Site	All patients $n = 81$	POPF $n = 8$	No POPF $n = 73$	*P* value
No. 8 (IU/L)	255 [142–516]	607 [404–1135]	222 [133–494]	0.007
No. 11 (IU/L)	426 [212–1078]	816 [348–1668]	392 [202–1026]	0.054
Site	All patients $n = 81$	POPF $n = 8$	No POPF $n = 73$	*P* value
POD 1 (IU/L)	574 [313–1054]	782 [364–2115]	560 [313–985]	0.248
POD 3 (IU/L)	149 [88–365]	508 [147–4161]	138 [84–336]	0.035

Data are presented as median [interquartile range]

IBAC intraoperative body fluid amylase concentration, *POPF* postoperative pancreatic fistula, *POD* postoperative day

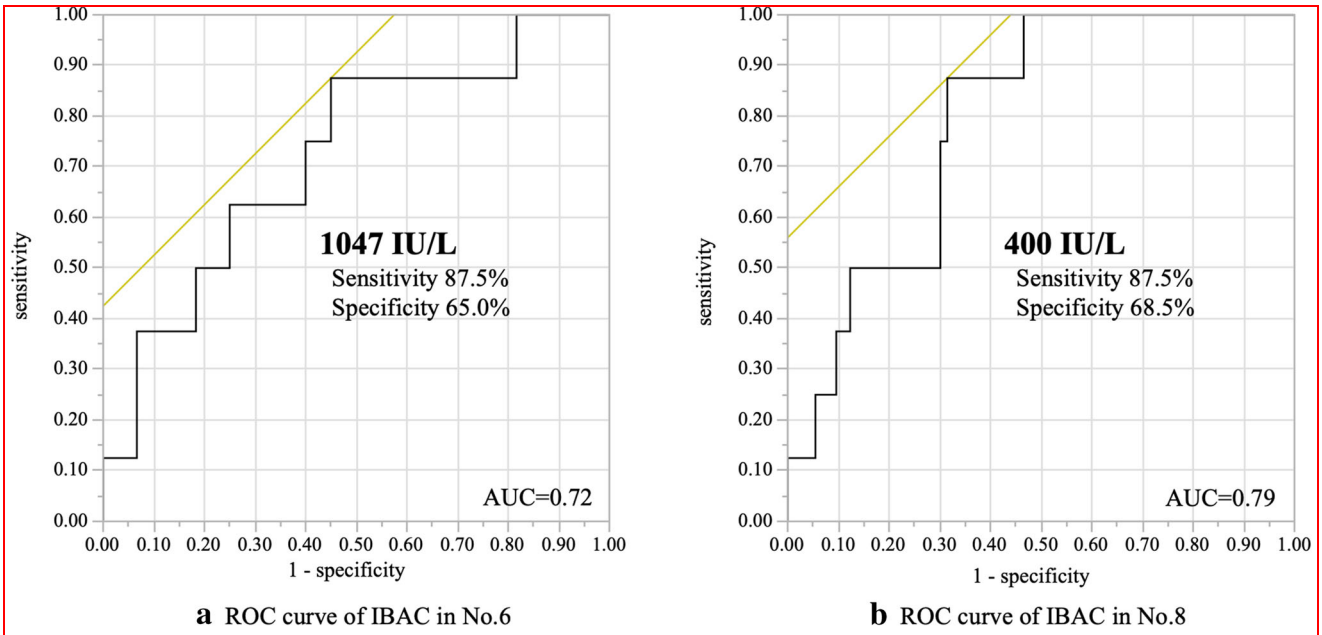

Fig. 4 ROC curve of IBAC in each place. The cutoff values of predicting POPF were 1047 IU/L and 400 IU/L, respectively. ROC receiver operating characteristic; IBAC intraoperative body fluid amylase concentration; AUC area under the curve

difficult in some hospitals. Unfortunately, our method cannot be used in such facilities.

In conclusion, surgeons might easily and noninvasively predict POPF during surgery by measurement of the IBAC. This technique is one of the most effective ways to predict POPF intraoperatively and will lead to reductions in poor patient outcomes and unnecessary medical costs.

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Compliance with ethical standards

Conflict of interest Drs. Toshiro Tanioka, Kazuyuki Kojima, Toshifumi Saito, Emi Kanemoto, Keisuke Okuno, Kentaro Gokita, Kenta Kobayashi, Masatoshi Nakagawa, and Mikito Inokuchi have no conflicts of interest or financial ties to disclose.

Informed consent Informed consent was obtained from all individual participants included in the study.

Human and animal rights This prospective single-center observational study was approved by the Tokyo Medical Dental University hospital review board (M2015-570).

References

1. Fujita T, Ohta M, Ozaki Y et al (2015) Collateral thermal damage to the pancreas by ultrasonic instruments during lymph node dissection in laparoscopic gastrectomy. Asian J Endosc Surg 8:281–288
2. Migita K, Matsumoto S, Wakatsuki K et al (2016) The anatomical location of the pancreas is associated with the incidence of pancreatic fistula after laparoscopic gastrectomy. Surg Endosc 30:5481–5489
3. Kobayashi N, Shinohara H, Haruta S et al (2016) Process of pancreas head as a risk factor for postoperative pancreatic fistula in laparoscopic gastric cancer surgery. World J Surg 40:2194–2201. https://doi.org/10.1007/s00268-016-3536-8
4. Irino T, Hiki N, Ohashi M et al (2016) The Hit and Away technique: optimal usage of the ultrasonic scalpel in laparoscopic gastrectomy. Surg Endosc 30:245–250
5. Sato Y, Inokuchi M, Otsuki S et al (2017) Risk factor of pancreatic fistula after radical gastrectomy from the viewpoint of fatty pancreas. Dig Surg 34:455–461
6. Kumagai K, Hiki N, Nunobe S et al (2018) Impact of anatomical position of the pancreas on postoperative complications and drain amylase concentrations after laparoscopic distal gastrectomy for gastric cancer. Surg Endosc 32:3846–3854
7. Miyai H, Hara M, Hayakawa T et al (2013) Establishment of a simple predictive scoring system for pancreatic fistula after laparoscopy-assisted gastrectomy. Dig Endosc Off J Jpn Gastroenterol Endosc Soc 25:585–592
8. Kobayashi D, Iwata N, Tanaka C et al (2015) Factors related to occurrence and aggravation of pancreatic fistula after radical gastrectomy for gastric cancer. J Surg Oncol 112:381–386
9. De Sol A, Cirocchi R, Di Patrizi MS et al (2015) The measurement of amylase in drain fluid for the detection of pancreatic fistula after gastric cancer surgery: an interim analysis. World J Surg Oncol 13:65
10. Matsunaga T, Saito H, Murakami Y et al (2017) Serum level of C-reactive protein on postoperative day 3 is a predictive indicator of postoperative pancreatic fistula after laparoscopic gastrectomy for gastric cancer. Asian J Endosc Surg 10:382–387
11. Kamiya S, Hiki N, Kumagai K et al (2018) Two-point measurement of amylase in drainage fluid predicts severe postoperative pancreatic fistula after gastric cancer surgery. Gastric Cancer 21:871–878
12. Inokuchi M, Nakagawa M, Tanioka T et al (2018) Long- and short-term outcomes of laparoscopic gastrectomy versus open gastrectomy in patients with clinically and pathological locally advanced gastric cancer: a propensity-score matching analysis. Surg Endosc 32:735–742
13. Inokuchi M, Kato K, Sugita H et al (2014) Impact of comorbidities on postoperative complications in patients undergoing laparoscopy-assisted gastrectomy for gastric cancer. BMC Surg 14:97
14. Kanda Y (2013) Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transpl 48:452–458
15. Japanese Gastric Cancer A (2017) Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer 20:1–19
16. Oken MM, Creech RH, Tormey DC et al (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655
17. Bassi C, Dervenis C, Butturini G et al (2005) Postoperative pancreatic fistula: an international study group (ISGPF) definition. Surgery 138:8–13
18. Clavien PA, Barkun J, de Oliveira ML et al (2009) The Clavien–Dindo classification of surgical complications: five-year experience. Ann Surg 250:187–196
19. Japanese Gastric Cancer A (2011) Japanese classification of gastric carcinoma: 3rd english edition. Gastric Cancer 14:101–112
20. Hiura Y, Takiguchi S, Yamamoto K et al (2013) Use of fibrin glue sealant with polyglycolic acid sheets to prevent pancreatic fistula formation after laparoscopic-assisted gastrectomy. Surg Today 43:527–533
21. Baiocchi G, Portolani N, Gheza F et al (2011) Collagen-based biological glue after Appleby operation for advanced gastric cancer. World J Gastroenterol 17:4044–4047