TABLE 2 Patient characteristics of initially unresectable locally advanced biliary tract cancer

Patient no.	Age (years)	Sex	Diagnosis	Reasons for unresectability	Biliary drainage	Chemotherapy	Downsizing (RECIST)	Operation	Status	Survival (months)
1	60	F	ICC	Hepatic vein invasion	No	GEM	Yes (SD)	Yes	Alive	66
2	60	M	ICC	Hepatic vein invasion	No	GEM	Yes (PR)	Yes	Alive	44
3	67	M	ICC	Arterial invasion	Yes	GEM	Yes (SD)	Yes	Dead	10
4	72	M	ICC	Insufficient remnant liver volume	No	GEM	Yes (PR)	Yes	Alive	13
5	57	F	GBC	Arterial invasion	Yes	GEM	Yes (SD)	Yes	Alive	42
6	57	F	GBC	Arterial invasion	Yes	GEM	Yes (PR)	Yes	Dead	18
7	57	F	GBC	Arterial invasion Portal vein invasion	No	GEM	Yes (SD)	Yes	Dead	19
8	61	M	GBC	Arterial invasion	No	GEM	Yes (SD)	Yes	Dead	8
9	57	M	ICC	Arterial invasion	No	GEM	No (PD)	No	Dead	11
10	77	F	ICC	Arterial invasion Portal vein invasion	No	GEM → S-1	No (SD)	No	Alive	13
11	84	F	ICC	Arterial invasion Portal vein invasion	No	GEM	No (SD)	No	Dead	6
12	75	M	ECC	Arterial invasion Broad biliary infiltration	Yes	GEM → S-1	Yes (SD)	No	Dead	30
13	69	F	ECC	Arterial invasion Portal vein invasion	Yes	GEM	No (SD)	No	Alive	27
14	65	M	ECC	Arterial invasion	Yes	GEM	No (PD)	No	Dead	5
15	66	M	ECC	Arterial invasion Portal vein invasion	Yes	GEM → S-1	No (PD)	No	Dead	6
16	81	M	ECC	Arterial invasion	Yes	GEM → S-1	No (SD)	No	Dead	11
17	59	F	ECC	Arterial invasion Portal vein invasion	Yes	GEM	No (PD)	No	Dead	4
18	63	M	ECC	Arterial invasion	Yes	GEM	No (PD)	No	Dead	8
19	65	F	ECC	Arterial invasion	Yes	GEM → S-1	No (SD)	No	Dead	19
20	49	M	GBC	Arterial invasion Broad biliary infiltration	Yes	GEM	No (PD)	No	Dead	9
21	71	M	GBC	Broad biliary infiltration Insufficient remnant liver volume	Yes	GEM	No (PD)	No	Dead	4
22	64	F	GBC	Arterial invasion Portal vein invasion	Yes	GEM	No (PD)	No	Dead	5

ICC intrahepatic cholangiocarcinoma, *GBC* gallbladder carcinoma, *ECC* extrahepatic cholangiocarcinoma, *GEM* gemcitabine, *S-1* tegafur–gimeracil–oteracil, *PR* partial response, *SD* stable disease, *PD* progressive disease

those unable to undergo surgery ($P = 0.032$) (Fig. 2a). The survival rate at 5 years is 40.8% in 249 patients with initially resectable BTC. Similar survival rate was obtained in patients with initially unresectable locally advanced BTC (survival rate at 5 years of 45.0%) after downsizing chemotherapy and subsequent surgical resection (Fig. 2b)

DISCUSSION

Since 1999, when no effective chemotherapy existed for BTC, there have been numerous clinical trials of gemcitabine for unresectable advanced BTC, with comparatively good results reported.[5–7] According to these reports, the response rate ranges 10–60%, with a median survival of 5–14 months. Such dramatic advances in chemotherapy in recent years mean that improved response rates and longer survival periods can be anticipated even in cancers formerly regarded as untreatable in patients with BTC.

In 2004, Adam et al. administered chemotherapy for initially unresectable colorectal cancer liver metastases and performed hepatectomy on patients in whom major response was obtained, reporting good results, with a 5 years survival rate of 33%.[13] Numerous reports of the results of hepatectomy after downsizing for colorectal

TABLE 3 Surgical procedure and histopathologic features of resected patients with biliary tract cancer after downsizing chemotherapy

Patient no.	Diagnosis	Operation methods	Vascular resection	Operation time (min)	Blood loss (g)	T	N	Stage	R
1	ICC	Left trisectionectomy, BDR	IVC	580	1,285	4	1	IVA	0
2	ICC	Left trisectionectomy, BDR	IVC	519	8,900	4	0	IVA	0
3	ICC	Left hemihepatectomy with caudate lobectomy, BDR	IVC, PV	637	1,150	4	1	IVA	1
4	ICC	Left hemihepatectomy with caudate lobectomy	–	590	3,115	4	0	IVA	0
5	GBC	Right hemihepatectomy with caudate lobectomy, BDR	–	400	490	4	1	IVA	0
6	GBC	Right hemihepatectomy with caudate lobectomy, BDR	PV	439	1,080	4	1	IVA	1
7	GBC	Central inferior hepatectomy (S4a + S5), BDR	PV, RHA	404	240	4	1	IVA	1
8	GBC	Central inferior hepatectomy (S4a + S5), BDR	–	499	2,080	4	1	IVA	1

BDR bile duct resection, *IVC* inferior vena cava, *PV* portal vein, *RHA* right hepatic artery

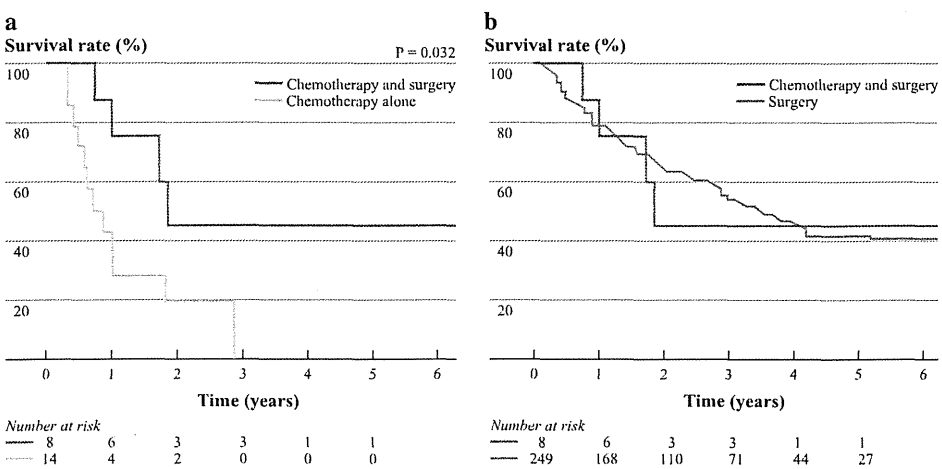

FIG. 2 Survival curves after the induction of chemotherapy for initially unresectable locally advanced BTC. **a** The *black line* represents patients in whom surgical resection was possible after downsizing. The *gray line* represents patients in whom resection was not possible. Median survival time (MST) in patients with surgical resection after downsizing chemotherapy and chemotherapy alone without surgical resection was 19.3 and 7.5 months, respectively; $P = 0.032$ (log rank test). **b** The *solid line* represents patients in whom surgical resection was possible after downsizing. The *dotted* line represents the patients with initially resectable BTC. The survival rate at 5 years did not differ between the patients with initially resectable BTC and initially unresectable locally advanced BTC after surgical resection after downsizing chemotherapy

cancer metastases subsequently appeared from many institutions. According to these reports, the downsized rate at which initially unresectable tumors became resectable was 11–36%.[14–18]

Reported resection rates for initially unresectable pancreatic cancer after downsizing chemotherapy range 8.3–64.2%.[19] The postulated reason for this wide range of resection rates might be due to the different definitions of unresectability at different institutions. The National Comprehensive Cancer Network (NCCN) has produced guidelines for pancreatic cancer with definitions of unresectability and borderline resectability. Although the current NCCN guidelines indicate clear criteria for resectability, there is still scope for debate on the indications of borderline resectability and unresectability.[20] In addition, even when radical resection is performed, surgical resection of pancreatic cancer at high-volume centers tends to comprise active surgical resection including combined vascular resection, and postoperative rates of complications and mortality are also low, with reports accordingly stating that surgical treatment should be carried out at such specialist facilities.[21,22] There are currently

no definitions for unresectability or borderline resectability in BTC, with completely different indications for resection used by different institutions and practitioners. Proactive expansion of surgical indications in high-volume centers lead to curative resection for advanced BTC with vascular invasions and extensive biliary infiltration. Several authors have described combined vascular resection for advanced BTC and have advocated an aggressive surgical strategy. Thus, the reason for unresectability in this paper was defined as local vascular invasion to be unable to reconstruct, extensive infiltration of the bile duct to be unable to achieve a curative resection, and extensive hepatic invasion to be unable to excise due to insufficient liver volume even after portal vein embolization.

In general, securing a clear margin after surgical resection is regarded as an important prognostic factor. Curative resection (R0) is based on the concept of our surgical treatment. We have previously reported that the aggressive combined vascular resection to achieve a curative resection bring about the beneficial effects on the prognosis in the patients with advanced BTC.[23] On the other hands, a number of recent reports have stated that because of advances in multidisciplinary treatment, even resection with microscopic residual tumor at the resected margin (R1) now does not always have the affect on the prognosis or risk of recurrence compared with R0 surgery in colorectal liver metastases and pancreas cancer.[12,13,24] In present study, all patients who underwent R0 surgical resection are currently alive with a mean follow-up of 41 months. Thus, R0 surgical resection should be performed in patients with initially unresectable locally advance BTC even after downsizing chemotherapy. We have compared the survival time after surgical resection between initially unresectable locally advanced BTC and initially resectable BTC. Similar survival rate was obtained between two groups. This result indicated that surgical resection after downsizing chemotherapy might be beneficial to the initially unresectable locally advanced BTC.

Recently, the possibilities of gemcitabine-based multidrug regimens have been investigated, with good results reported for the coadministration of gemcitabine with cisplatin.[25,26] In the United Kingdom, good results were obtained in a randomized phase III trial (ABC-02) of gemcitabine with cisplatin, with a 81.4% tumor control rate in the group given both drugs compared with 71.8% for the single drug.[27] Also in Japan, a randomized, controlled trial of combined therapy with gemcitabine and cisplatin for unresectable BTC compared with gemcitabine alone is underway as a joint study with other institutions. This study was also demonstrating similarly favorable results.[28] The establishment of gemcitabine and cisplatin combined therapy as a standard therapy for unresectable BTC is therefore anticipated. In future the introduction of gemcitabine and cisplatin combination therapy showed evaluated for the effect on preoperation neoadjuvant chemotherapy.

In this series, there was no patient with ECC judged to be resectable after chemotherapy. All patients with ECC underwent biliary drainage, and the tube provoked inflammation in the bile duct and surrounding tissues, making it difficult to reach an accurate diagnosis of the extent of tumor progression by means of CT. This meant that vascular invasion and bile duct invasion may have been overestimated on CT performed after tube insertion, making it difficult to select resectable patients. A new modality of assessment should be required to allow the use of downstaging chemotherapy response in BTC especially in ECC after biliary stent insertion. BTC also encompasses various diseases of ICC, ECC, and GBC with different biological characteristics, and these may also differ in terms of the effectiveness of chemotherapy and prognosis. For this reason, separate study and analyzes might be required for ICC, ECC, and GBC. However, although individual facilities treat limited numbers of BTC patients, large-scale clinical trials, such as multicenter studies, should be done for obtaining the appropriate results.

There are no clear criteria concerning the period for which chemotherapy should be administered preoperatively or the appropriate time for surgery in patients whose disease becomes resectable after chemotherapy. It has been reported that few patients who have developed resistance to a first-line regimen for colorectal cancer liver metastases can undergo resection after switching to a second-line regimen. Although there are few second-line chemotherapy regimens available for BTC, surgical resection might have to be performed immediately when it is determined to be possible.[29] Furthermore, attention must also be paid to chemotherapy-induced liver toxicity because major hepatic resection is usually required for radical surgical resection for advanced BTC. There have been no reports, however, of increased liver toxicity or postoperative complications when gemcitabine is used as preoperative chemotherapy. In our series, there were no cases of liver damage caused by long-term administration of gemcitabine. However, further care should be required when gemcitabine is provided as part of multidrug therapies with agents such as cisplatin.

In conclusion, in this study, preoperative chemotherapy enabled the downsizing of initially unresectable locally advanced BTC, with surgical resection made possible in a certain proportion of patients. Proactive resection can also be anticipated in these patients to bring about an improved survival. Downsizing chemotherapy should be proactively carried out as a new multidisciplinary treatment strategy for the treatment of initially unresectable locally advanced BTC for the aim of expanding the surgical indication.

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Geminin Expression in Pancreatic Neuroendocrine Tumors

Possible New Marker of Malignancy

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Objectives: We evaluated geminin labeling index (LI) as a new prognostic indicator of pancreatic neuroendocrine tumor.

Methods: Twenty-seven patients who underwent surgery were retrospectively referred. Labeling indices for geminin and Ki-67 were calculated and compared with clinicopathologic factors. Then, the concordance of positivity between 2 LIs was evaluated using the color difference quotation.

Results: The median (range) of LIs for geminin and Ki-67 was 1.0% (0.05%–14.9%) and 1.5% (0.02%–8.8%), respectively. When the high LI was defined as more than 2.0% according to the receiver operating characteristic curves determining the metastasis, both geminin LI (hazard ratio [HR], 31.382; 95% confidence interval [CI], 3.177–309.99; $P = 0.003$) and Ki-67 LI (HR, 6.182; 95% CI, 1.221–31.298; $P = 0.028$) were significant risk factors of recurrence in the univariate analysis. The Kaplan-Meier curves consistently exhibited the superiority of geminin LI (log rank, $P < 0.001$) to Ki-67 LI (log rank, $P = 0.041$) in predicting the disease-free survival. In the color difference quotation, the median ΔE of geminin stain (16.12; range, 5.8–41.9) was significantly larger than that of Ki-67 stain (13.17; range, 3.4–37.9).

Conclusions: The geminin LI was suggested to be more closely correlated with outcome and had more consistent positivity than the Ki-67 LI.

Key Words: geminin, neuroendocrine tumor, color difference quotation, prognostic factor, Ki-67

(*Pancreas* 2012;41: 512–517)

The annual incidence of pancreatic neuroendocrine tumor (PNET) is about 0.32 cases per population of 100,000 in the United States and 2.23 cases per population of 100,000 in Japan. Pancreatic neuroendocrine tumors are thought to represent 1%–2% of all pancreatic neoplasms. The apparent incidence and prevalence of PNET have increased substantially during the last 30 years, probably because of the rapid progress of innovative diagnostic techniques.[1] The best treatment for PNET is curative surgical resection, which has a disease-free survival rate of 82% after surgery.[2] Pancreatic neuroendocrine tumors have a wide spectrum of clinical presentations. Therefore, multiple studies have attempted to develop staging and grading systems to better define prognosis.[2–5] The 2000 World Health Organization (WHO) classification system used both stage-related criteria (size and presence of metastases) and grade-related criteria (mitotic rate, perineural invasion, angioinvasion, and Ki-67 proliferative index) to predict outcome. Though this approach included most well-accepted pathologic prognostic factors, the multiple grading parameters made it difficult to reproduce grades reliably among pathologists and institutions, and this grading system has since been replaced by the current WHO classification.[6] Immunohistochemistry for Ki-67 protein is commonly used to evaluate the proliferative activity of tumor cells, and numerous studies have shown that the labeling index (LI) of the Ki-67 protein is correlated with the clinical outcome of patients with a variety of malignant tumors, including PNET.[2,3,7–10] The Ki-67 protein is detected during all active cell cycle phases (G_1, synthesis, G_2, and mitosis and cytokinesis) but not in resting (G_0) cells, although its function remains uncertain.[11,12] Although histologic grade-based estimations of prognosis are extremely useful for interpreting biopsy samples, additional reliable markers are needed.

Geminin, a negative regulator of DNA replication, has recently been described as a novel marker of malignant potential.[13] During the G_1 phase, DNA replication is initiated by the recruitment of the origin recognition complex, composed of cell division cycle-6 and Cdt1, to specific points of replication origin in the genome; this recruitment, in turn, loads the minichromosome maintenance (Mcm) complex, which is composed of Mcm-2 to Mcm-7.[14] Geminin is specifically expressed during the S, G_2, and early M phases and interacts with Cdt1 to prevent the loading of the Mcm complex to points of origin that have already been initiated, thus ensuring a single replication per 1 cell cycle.[15,16] Geminin expression has been widely observed in various malignant neoplasms, and the number of geminin-positive cells is reportedly proportional to the cell proliferation index, as measured using Ki-67 expression.[13] High levels of geminin expression are reportedly correlated with a poorer clinical outcome in breast cancer,[17] renal cell carcinoma,[18] prostatic adenocarcinoma,[19] salivary gland carcinomas,[20] lung cancer,[21] and gastric hyperplasia.[22] However, the prognostic significance of geminin expression in PNET remains unknown.

The purpose of this study was to determine whether geminin expression defines the aggressiveness of PNET and to compare the clinical and diagnostic use of the geminin LI with that of the Ki-67 LI.

MATERIALS AND METHODS

Patients

Between 1994 and 2010, a total of 27 consecutive patients underwent primary surgical treatment for PNET at our institution. The medical records and surgical specimens of these patients were retrospectively examined in the present study.

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Received for publication February 2, 2011; accepted September 23, 2011.

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Dr Aizawa states no source of financial support and no disclosure of funding received for this work.

The authors declare no conflict of interest.

Patients with recurrent tumors were excluded. Follow-up clinical information was obtained from the patients' medical records. The follow-up time was measured from the date of surgery until disease-caused death or the end of the follow-up period.

Clinicopathologic Parameters

The grading and staging of each tumor were performed according to the WHO classification, the cancer staging manual of the American Joint Committee on Cancer (AJCC), and the classification proposed by the European Neuroendocrine Tumor Society (ENETS).[23,24]

The prognostic values of the following clinicopathologic parameters were examined in the present study: tumor diameter (<2 vs ≥2 cm), lymphatic or blood vessel infiltration (absent vs present), perineural invasion (absent vs present), serosal or retroperitoneal invasion (absent vs present), tumor extension beyond the pancreas (absent vs present), mitotic index per 10 high-power fields (10 HPFs) (<2 vs ≥2), regional and distant metastasis (absent vs present), and pathological stage (AJCC stage ≥ IIA vs AJCC stage ≤ I, ENETS stage ≥ IIb vs ENETS stage ≤ IIa).

Histologic Examination and Immunohistochemistry

Surgical specimens were fixed in 10% formalin and embedded in paraffin. Two pathologists (M.A. and M.K.), who were unaware of the clinical data, reviewed all the hematoxylin-and-eosin–stained sections and reclassified and graded the specimens according to the histologic parameters.

Serial 4-μm sections were used for immunohistochemical staining. Deparaffinized and rehydrated sections were immersed in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity. Heat-induced antigen retrieval was performed for 20 minutes at 95°C with a 10-mM citrate buffer (pH 6.0). After the slides had cooled at room temperature for 1 hour, they were exposed to 2% normal swine serum in phosphate-buffered saline for 30 minutes, then allowed to react overnight at 4°C with the following mouse monoclonal antibodies: antihuman geminin (diluted 1:40, clone EM6; Novocastra, Newcastle, United Kingdom) or antihuman Ki-67 (diluted 1:100, clone MIB-1; Dako, Glostrup, Denmark). After washing with phosphate-buffered saline 3 times, the sections were reacted with EnVision plus (Dako) for 30 minutes at room temperature. The peroxidase reaction products were developed with 3,3′-diaminobenzidine, and the sections were counterstained with hematoxylin.

The LI of each marker was calculated by manually counting the number of brown-stained tumor cell nuclei among the total number of tumor cells in the most highly immunoreactive area at a magnification of 400-fold, with the aid of an eyepiece grid (5 × 5 squares). Indices were expressed as a percentage value corresponding to the number of positive cells among approximately 2000 tumor cells.

Evaluation of Color Difference Quotation

Immunohistochemically stained full-face sections from each case with geminin and Ki-67 overexpression were digitized using the Slide Path and the NanoZoomer Digital Pathology System (Hamamatsu, Welwyn Garden City, United Kingdom). Approximately 7 minutes was required to scan a slide at a resolution of 40×. Subsequently, 400-fold magnified images from highly immunoreactive areas were exported for analysis.

The image analysis was performed using Photoshop (version 7; Adobe Systems, San Jose, CA). First, the image mode was converted from RGB to Lab color mode. Two hundred fifty representative positive cells were selected in each of 4 cases with high geminin expression levels (LI ≥ 2.0%) and 7 cases with high Ki-67 expression levels (LI ≥ 2.0%). All the cells with a recognizable brown stain were measured. The L*a*b* value of the nuclear areas was outputted as a range from 0 to 255. The values represented a Lab color space composed of 3 axes in a spherical form: L^*, a^*, and b^*. The L^* axis was associated with the lightness of the color, whereas the a^* and b^* axes were associated with the red-green scale and the yellow-blue scale, respectively. After the conversion of the scale to the CIE LAB color system, the L^* value was described as a decimal scale from 0 to 100, and the a^* and b^* values ranged from −128 to 127. The difference between the average values for a positive cell and an adjacent negative cell was calculated as ΔL^*, Δa^*, and Δb^*. Then, the color difference ΔE was estimated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. ΔE was then compared between the geminin-stained and Ki-67–stained sections.

Statistical Analysis

The Spearman rank correlation test was used to determine associations between continuous variables. Receiver operating characteristic curves were plotted to calculate the sensitivity, specificity, positive predictive value, and negative predictive value for the presence of metastasis. The cutoff values for the geminin LI and the Ki-67 LI were chosen so as to obtain the best combination of predictive values. A univariate analysis using the Cox proportional hazards model was applied to estimate the associations of clinicopathologic factors, including the immunohistochemical results, with the disease-free survival period. Survival curves were drawn using the Kaplan-Meier method, and the differences were analyzed using a log-rank test. Differences in nonparametric data were estimated using the Mann-Whitney U test. All P values < 0.05 were considered statistically significant. The statistical analyses were performed using Dr. SPSS II for Windows (SPSS Japan, Tokyo, Japan).

RESULTS

Demographic Characteristics and Tumor-Related Factors

Among the 27 patients, the median age at the time of diagnosis was 56 years; 14 patients (51.9%) were men, and 13 patients (48.1%) were women. In all the patients, a curative resection was performed, followed by a histologic assessment of the tumor grading. The tumor-related factors are summarized in Table 1. The tumor was located in the pancreatic head in 11 cases (40.7%), in the body in 12 cases (44.4%), and in the tail in 4 cases (14.8%). Only 1 case of functional PNET (insulinoma) was included. The median and range of the maximum tumor diameter were 26 mm and 8 to 92 mm, respectively. The diameter was 2 cm or larger in 11 cases (40.7%). Local invasion was observed in 2 cases (7.4%). One of these cases exhibited an obstruction of the inferior common bile duct, and another case presented with invasion to both the splenic artery and vein. Lymph node metastasis was encountered in 10 cases (37.0%), and a solitary liver metastasis was resected in 1 case (3.7%). With respect to mitosis, fewer than 2 mitoses per 10 HPFs were present in 19 cases (70.4%), from 2 to 10 mitoses per 10 HPFs were encountered in 8 cases (29.6%), and none of the cases had more than 20 mitoses per 10 HPFs.

The tumor grade classifications according to the WHO system and tumor staging according to the AJCC or ENETS criteria are shown in Table 1. Nineteen cases (70.4%) were classified as grade 1 neuroendocrine tumor (NET), and 8 cases

TABLE 1. Patient Demographics and Tumor-Related Factors for 27 Patients

Characteristics		
Age, y	Median	56
	Range	31–76
Sex, n (%)	Men	14 (51.9)
	Women	13 (48.1)
Location, n (%)	Ph	11 (40.7)
	Pb	12 (44.4)
	Pt	4 (14.8)
Functional tumor, n (%)		1 (3.7)
Maximum diameter, mm	Median	26
	Range	8–92
Diameter, n (%)	<2 cm	16 (59.3)
	≥2 cm	11 (40.7)
Local invasion, n (%)		2 (7.4)
Metastasis, n (%)	—	17 (63.0)
	Lymph node	10 (37.0)
	Liver	1 (3.7)
Mitosis, n (%)	0–1 per 10 HPFs	19 (70.4)
	2–20 per 10 HPFs	8 (29.6)
	>20 per 10 HPFs	0 (0.0)
WHO grading, n (%)	NET G1	19 (70.4)
	NET G2	8 (29.6)
	NEC	0 (0.0)
AJCC stage, n (%)	IA	8 (29.7)
	IB	9 (33.3)
	IIA	0 (0.0)
	IIB	9 (33.3)
	III	0 (0.0)
	IV	1 (3.7)
ENETS stage, n (%)	I	8 (29.7)
	IIa	7 (25.9)
	IIb	2 (7.4)
	IIIa	0 (0.0)
	IIIb	9 (33.3)
	IV	1 (3.7)

NET G1 indicates grade 1 NET; NET G2, grade 2 NET; Pb, pancreatic body; Ph, pancreatic head; Pt, pancreatic tail.

(29.6%) were classified as grade 2. According to the AJCC staging, 17 cases (63.0%), 9 cases (33.3%), 0 cases (0%), and 1 case (3.7%) were classified as stages I, II, III, and IV, respectively. According to the classification proposed by ENETS, 8 cases (29.7%), 9 cases (33.3%), 9 cases (33.3%), and 1 case (3.7%) were classified as stages I, II, III, and IV, respectively.

The median and range of the observation period were 1704 days and 37 to 4206 days, respectively. Three patients died, one of whom had a tumor-related death; the other 2 patients had treatment-related deaths. Recurrence after surgery was observed in 6 patients (22.2%).

Geminin and Ki-67 Expression

The immunohistochemical analysis examined the expressions of geminin and Ki-67 protein in all the cases (Fig. 1). Immunoreactivity was observed exclusively in the nuclei of the tumor cells. Geminin was also immunoreactive in the perichromosomal cytoplasm of mitotic cells in a few cases. The median LIs for geminin and Ki-67 were 1.0% and 1.5%, respectively. The geminin LI was slightly but significantly lower than that of Ki-67. Figure 2 shows the positive correlation between the geminin LI and the Ki-67 LI (Spearman $R_s = 0.757$, $P < 0.001$).

The receiver operating characteristic curves for the geminin LI, the Ki-67 LI, and the mitosis count (all of which were continuous variables), which were used to predict the presence of metastatic lesions, are shown in Figure 3. The curves for the 2 LIs were similar. The area under the curve was calculated to be 0.829 (95% confidence interval [CI], 0.660–0.999) for the geminin LI, 0.776 (95% CI, 0.598–0.955) for the Ki-67 LI, and 0.594 (95% CI, 0.362–0.826) for the mitosis count. The geminin LI seemed to have a slightly superior ability to predict metastasis, compared with the Ki-67 LI. The sensitivity, specificity, positive predictive value, and negative predictive value of a geminin LI greater than 2.0% (n = 4) and of a Ki-67 LI greater than 2.0% (n = 7) for determining metastasis were 33.3%, 90.5%, 50.0%, and 82.6% and 50.0%, 80.0%, 42.9%, and 85.0%, respectively. We defined high-geminin expression cases as those with a geminin LI greater than 2% because this cutoff had the best discriminatory power for the predictive values. According to the current WHO classification, a Ki-67 LI of 2.0% can be used to discriminate G1 tumors, and this cutoff also had the best discriminatory power for the predictive values in the present analysis. Thus, we regarded a Ki-67 LI greater than 2.0% as indicating a high Ki-67 expression level.

Correlations of Geminin and Ki-67 LIs With Prognosis

Because there was only 1 tumor-related death in this series, we examined the predictive values of each LI for the disease-free survival period after surgery. The results of a univariate Cox regression analysis are shown in Table 2. A mitosis count of 2 or more per 10 HPFs (hazard ratio [HR], 10.204; 95% CI, 1.684–61.834; $P = 0.012$), a local invasion (HR, 18.762; 95%

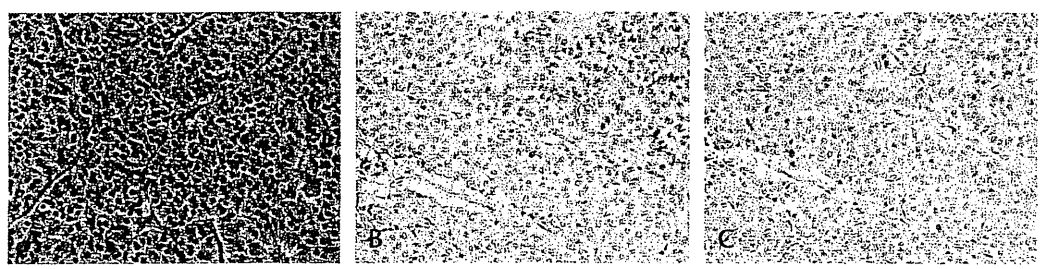


FIGURE 1. Representative photomicrograph of a PNET specimen. Hematoxylin and eosin staining (A) shows a typical trabecular arrangement of uniform tumor cells. The cells have eosinophilic cytoplasm and centrally located, round nuclei. Immunohistochemical staining for geminin (B) and Ki-67 (C) shows brown-stained tumor cell nuclei. The number of geminin-positive cells was smaller than the number of Ki-67–positive cells in most cases (original magnification ×400).

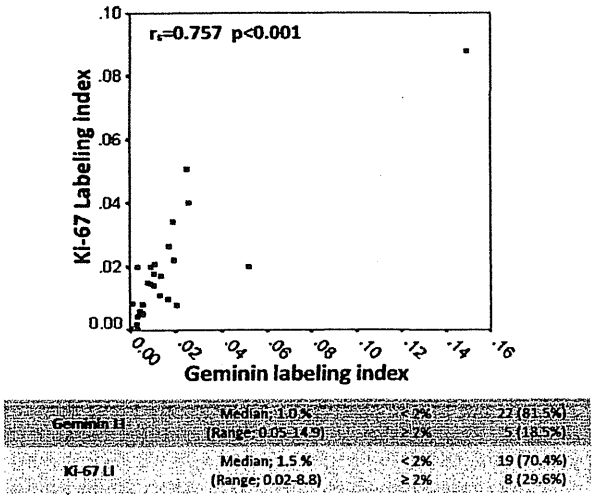


FIGURE 2. Scatterplot of the geminin LI and the Ki-67 LI (top) shows a positive correlation between the 2 LIs (Spearman rank correlation coefficient; $r_s = 0.757$; $P < 0.001$). The geminin expression level was lower than the Ki-67 expression level (bottom).

CI, 1.163–302.6; $P = 0.039$), a metastasis (HR, 10.469; 95% CI, 1.103–102.77; $P = 0.041$), a Ki-67 LI greater than 2.0% (HR, 6.182; 95% CI, 1.221–31.298; $P = 0.028$), a geminin LI greater than 2.0% (HR, 13.709; 95% CI, 1.919–97.739; $P = 0.009$), an AJCC stage of IIA or greater (HR, 8.758; 95% CI, 1.483–51.716; $P = 0.017$), and an ENETS stage of IIb or greater (HR, 16.793; 95% CI, 1.834–153.738; $P = 0.013$) were significantly correlated with recurrence. A multivariate Cox regression analysis revealed that none of these factors were independent prognostic factors. The Kaplan-Meier curves consistently exhibited a more significant relationship with the disease-free survival period after surgery for geminin (log rank, $P < 0.001$) than for Ki-67 (log rank, $P = 0.012$) (Fig. 4).

Concordance of Positivity Between Geminin and Ki-67 Stains

The immunoreactions were quantified using the CIE LAB color system. The color difference quotation was used to evaluate the positivity of the 2 stains. The color difference, ΔE, and a geminin-stained image are shown in Figure 5. The ΔE values

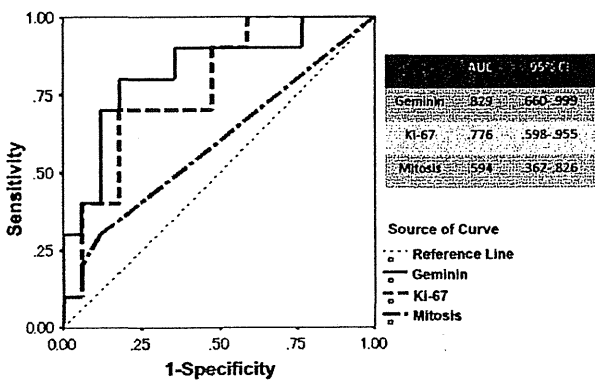


FIGURE 3. Receiver operating characteristic curves comparing the predictive value of the geminin LI to that of the Ki-67 LI or the mitosis count for determining the presence of metastasis.

TABLE 2. Univariate Cox Regression Analysis of Risk of Recurrence After Surgery

Variables	HR	95% CI	*P*
Diameter ≥2 cm	1.209	0.221–6.627	0.827
Mitosis ≥2 per 10 HPFs	10.204	1.684–61.834	0.012
v(+) or ly(+)	0.813	0.114–5.807	0.813
pn(+)	3.615	0.375–34.837	0.266
s(+) or rp(+)	2.068	0.411–10.4	0.378
Local invasion (+)	18.762	1.163–302.6	0.039
Metastasis (+)	10.469	1.103–102.77	0.041
Ki-67 LI >2.0%	6.182	1.221–31.298	0.028
Geminin LI >2.0%	13.709	1.919–97.739	0.009
WHO grade G2	2772.5	0.000–95.889 × 10^7	0.429
AJCC stage ≥IIA	8.758	1.483–51.716	0.017
ENETS stage ≥IIb	16.793	1.834–153.74	0.013

Local invasion indicates (+), presence of local invasion; ly(+), presence of lymphatic invasion; metastasis (+), presence of metastasis; pn(+), presence of peri-neural invasion; rp(+), presence of retroperitoneal invasion; s(+), presence of serosal invasion; v(+), presence of venous invasion.

corresponded with the optical intensity of the positive cells. The same consistency was observed for the images with Ki-67 staining (data was not shown). The distributions of ΔE in the geminin and Ki-67 staining images are shown in Figure 6. $\Delta E = 0$ signified no color difference from negative cells, and the left side of the histogram's distribution indicates the number of cells with equivocal positivity. A larger ΔE reflects a greater color disparity between the positive and negative cells. The medians (ranges) of the ΔE values for geminin and Ki-67 staining were

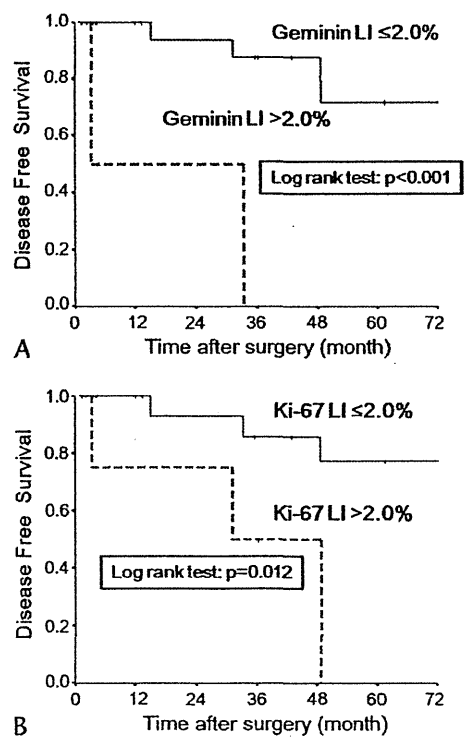
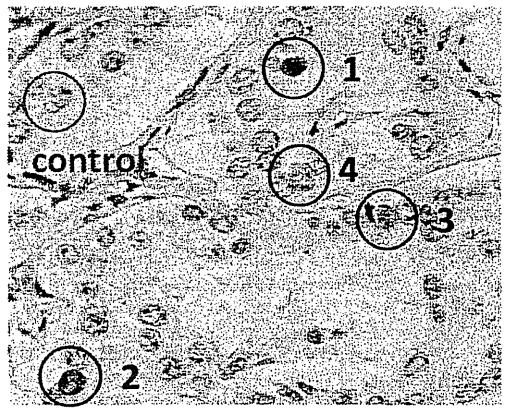


FIGURE 4. Disease-free survival period after surgery according to the geminin LI (A) and the Ki-67 LI (B).



#	L* value	a* value	b* value	ΔE
1	65.17	-1.22	15.30	26.22
2	67.90	7.30	11.63	21.87
3	77.14	5.71	5.74	11.10
4	82.30	3.29	-0.71	2.51
Ctr	83.94	2.82	2.55	

FIGURE 5. The color difference ΔE in geminin stain is shown. ΔE values were calculated from the difference of L*a*b* values between positive cells (in numbered red circles) and a negative cell (in green circle).

16.12 (5.8–41.8) and 13.17 (3.4–37.9), respectively. The ΔE for the geminin stain was significantly larger than that for the Ki-67 stain ($P < 0.001$).

DISCUSSION

The criteria used to predict the outcome of patients with PNET has been simplified in the 2010 WHO classification.[6] Pancreatic neuroendocrine tumors are divided into well-differentiated NETs and poorly differentiated neuroendocrine carcinoma (NEC). The definition of NEC is the presence of more than 20 mitoses per 10 HPFs. Neuroendocrine tumors were further subcategorized as low-grade NET (G1), characterized by the presence of 0 to 1 mitoses and a Ki-67 LI of 0% to 2%, and intermediate-grade NET (G2), characterized as 2 to 20 mitoses per 10 HPFs and a Ki-67 LI of 3% to 20%. Actually, immunohistochemical staining for Ki-67 has been the most reliable modality for assessing the proliferative activity.[2,3,7] In addition, staging has been noted to be an independent prognostic indicator, and the AJCC staging manual and the staging classification proposed by the ENETS are thought to be useful for predicting the prognosis of patients with PNET. In the present study, 19 and 8 cases were classified as G1 and G2, respectively. No cases of NEC were seen, consistent with the presence of only 1 tumor-related death. Regarding recurrence after radical resection, this grading system is not a reliable prognostic factor (Table 2). Unlike the WHO grading, however, both the AJCC and ENETS stagings are significantly correlated with recurrence; similarly, the superiority of these stagings to anticipate disease-free survival has been previously reported.[25] The present analysis suggested that local spread beyond the pancreas might be a key event.

The usefulness of geminin staining to predict the outcome of several neoplasms has been demonstrated using retrospective analyses.[17–22] The present study also indicated that geminin expression was a more useful indicator of disease-free survival than not only Ki-67 expression but also AJCC and ENETS staging (Table 2). Geminin expression is specifically limited during the S, G2, and early M phases, and it probably reflects the proliferative activity more precisely than these other factors. Indeed, the number of positive tumor cells for geminin was significantly smaller than that for Ki-67. Although the survival analysis using Kaplan-Meier curves suggested that the geminin LI was more associated with the prognosis than the Ki-67 LI (Fig. 4), the present study has a limitation to evaluate the prognosis in accordance with the small number of cases. Further analyses of a larger population is needed to determine the prognostic use of the geminin LI. Moreover, the mechanism by which geminin expression contributes to the aggressiveness of neoplasms remains unknown. The inhibition of Cdt1 by geminin has been regarded as a pivotal event in the licensing of DNA replication, so an increase in Cdt1 inhibition biologically results in cell cycle arrest. This discrepancy between geminin expression and cell proliferation remains to be explained. The predictive superiority of the geminin LI to the Ki-67 LI in the present analysis may depend on some aspect of the malignant potential other than the proliferative activity.

In addition, the immunoreactivity of geminin staining in each tumor cell was relatively clear, whereas weak positivity for Ki-67 staining was observed in some tumor cells (Fig. 1). Thus, fewer intraobserver and interobserver differences between pathologists or institutions can be expected using the geminin LI. Actually, the difficulty in grading PNETs has been attributed to the need for concordance, along with the lower frequencies of proliferative marker positivity in PNETs. In the present study, we performed a color difference quotation analysis using the CIE LAB color system. Several color analyses have reported that the

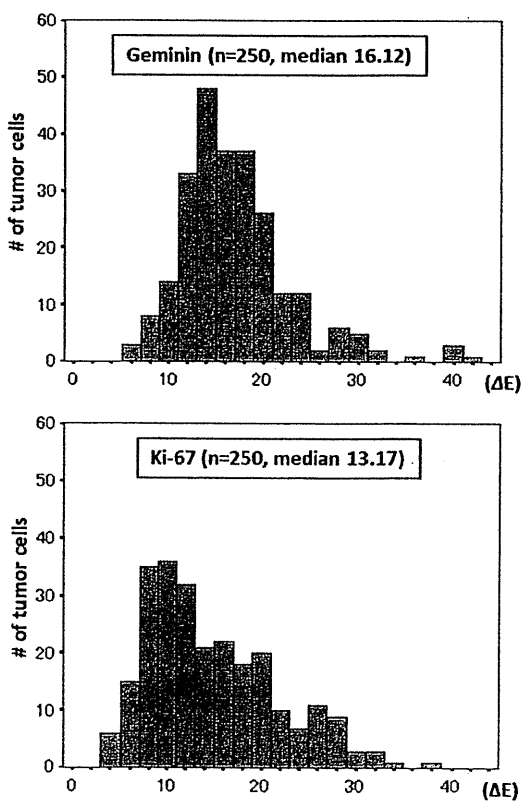


FIGURE 6. The distribution of each ΔE in geminin and Ki-67 stain is shown. The difference of each ΔE was evaluated as statistically significant ($P < 0.001$) using the Mann-Whitney U test.

color parameters of the CIE LAB color system are closely related to the psychophysical characteristics of color perception.[26–28] This analysis was the first application of the CIE LAB color system for the quantification of immunohistochemical positivity. As shown in Figure 5, a precise correspondence between ΔE and the optical color intensities was observed. Furthermore, the ΔE for geminin staining was larger than that for Ki-67 staining. These results suggest that a greater concordance was achieved using the geminin LI rather than the Ki-67 LI. The use of the color difference quotation enabled subjective optical intensities to be measured as absolute values, and no inconsistencies with regard to determining positivity were encountered. Thus, the CIE LAB color system may be a promising tool for making objective histopathologic assessments.

Pancreatic neuroendocrine tumor constitutes a heterogeneous group of rare neoplasms. Recent advances in abdominal imaging techniques have increased the detection of incidental nonfunctional PNET. In particular, endoscopic ultrasound and endoscopic ultrasound–guided fine needle aspiration biopsy procedures have drastically improved diagnostic accuracy.[29] Nowadays, minimally invasive surgery is usually recommended as a pancreas-preserving maneuver.[30] Therefore, accurate estimates of the malignant potential before surgery are becoming increasingly important for optimal patient management. Despite the importance of such estimations, pretreatment evaluations remain difficult. Only microscopic observations are acceptable for tumor grading and staging because PNET can exhibit heterogeneous biological behavior even within the same tumor. In the present study, a heterogeneous expression level was observed throughout the tumor for both geminin and Ki-67 staining. The use of geminin expression for the assessment of biopsy samples or aspirated specimens was not evaluated in the present study. Thus, the establishment of a preoperative classification based on geminin expression will require further research.

In conclusion, the geminin expression level in PNETs was correlated with the disease-free survival period after curative resection. The geminin LI may be more useful than the Ki-67 LI for predicting postoperative outcome.

ACKNOWLEDGMENT

The authors thank Dr Hiroshi Yamaguchi and Dr Toshio Ohmori, Imaging Technology Center, FUJIFILM Corp, Minamiashigara, Japan, for their technical advice.

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J Hepatobiliary Pancreat Sci (2012) 19:249–258
DOI 10.1007/s00534-011-0416-3

ORIGINAL ARTICLE

Compliance with and effects of preoperative immunonutrition in patients undergoing pancreaticoduodenectomy

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Published online: 11 June 2011

Abstract
Background/purpose This study was conducted to ascertain the feasibility and effectiveness of preoperative enteral immunonutrition using an immune-enhanced formula (Impact) in patients undergoing pancreaticoduodenectomy.
Methods Twenty-five patients undergoing an elective pancreaticoduodenectomy were asked to ingest Impact for 5 days (750 mL/day) prior to surgery in addition to their normal diets. We retrospectively compared the early postoperative outcomes of the Impact group ($n = 18$), which consisted of patients who fully complied with the study protocol, and a control group ($n = 13$), which consisted of patients who had not ingested Impact prior to surgery.
Results Overall, 82.6% of the patients complied with the preoperative oral ingestion of Impact; all but four patients tolerated a daily intake of 750 mL. While the clinical backgrounds of the Impact and control groups were not significantly different, the frequency of incisional wound infection was lower (0 vs. 30.8%, $p = 0.012$) and the change in systemic severity as evaluated using the acute physiology and chronic health evaluation (APACHE)-II scoring system was milder ($p = 0.033$) in the Impact group than in the control group.
Conclusion The preoperative oral ingestion of Impact was well tolerated and appeared to be effective for preventing incisional wound infection and reducing the response to surgical stress in patients undergoing a pancreaticoduodenectomy.

Keywords Immunonutrition · Pancreaticoduodenectomy · Surgical site infection · Nutrition

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Introduction

In recent years, pancreaticoduodenectomy (PD) has gained acceptance as an appropriate surgical procedure for selected patients with diseases of the pancreas head and periampullary region. Improvements in surgical techniques and accumulating experience have reduced the complication rate after PD. The postoperative mortality rates after PD are typically 5% or less at major surgical centers [1, 2], although the morbidity rates remain high, ranging from 10 to 50% [3–5]. Thus, postoperative morbidity after PD remains problematic and can lead to delays in the postoperative resumption of adequate oral food intake. Even in series with relatively good rates of postoperative morbidity, about 10% of the patients develop wound infections [1, 3–6]. However, the morbidity rate increases considerably if other complications, such as pancreatic fistula or delayed gastric emptying, are included [7]. Bacteria from the gut, especially Enterococci and *Escherichia coli* [8], translocate into the mesenteric lymph nodes or blood, where they cause the majority of the observed infections. Several conditions before, during, or after surgery can facilitate this bacterial translocation, including a reduction in postoperative intestinal motility, jaundice, the use of antibiotics resulting in small bowel bacterial overgrowth [9], the loss

of mucosal barrier function caused by malnutrition, manipulation of the bowel, and parenteral nutrition [10].

Recently, enteral immune-enhancing formulas supplemented with arginine, omega-3 fatty acids, and ribonucleic acid (RNA) have been suggested to improve the immune response and wound healing in postoperative patients [11, 12]. Arginine, which is classified as a semi-essential amino acid for catabolism, serves as a substrate for the urea cycle and the production of nitric oxide during protein synthesis. Arginine is known to promote T cells and to have a direct enhancing effect on their activities [13], enhancing the phagocytosis of neutrophils. Arginine also reduces the production of inflammatory mediators, such as interleukin (IL)-1beta, tumor necrosis factor alpha (TNF-α), and IL-6 at the site of tissue injury and is capable of enhancing cellular immunity in rat septic models [14]. Finally, arginine accelerates tissue growth after infection [15]. Omega-3 fatty acids compete with omega-6 fatty acids for cyclo-oxygenase metabolism at the cell membrane and for the production of eicosapentanoic acid (EPA). In addition, omega-3 fatty acids increase the production of some prostaglandins (PGs) and leukotrienes, reducing the proinflammatory potential, and inhibit the production of some other PGs (PGE2) and leukotrienes, reducing the cytotoxicity of macrophages, lymphocytes, and natural killer (NK) cells [11]. Supplementation with agents rich in omega-3 fatty acids also decreases prostacyclin and thromboxane (TX)-A2 synthesis and increases the antiaggregatory substance TXA3 [16]. Omega-3 fatty acids and EPA are believed to inhibit excessive inflammatory responses but not to be immunosuppressive. The intravenous administration of omega-3 fatty acids significantly reduced the production of proinflammatory cytokines in a recent clinical trial in patients with sepsis [17]. RNA supplementation is necessary for the proliferation of immune cells or cells involved in wound healing [18].

Several studies have demonstrated that immune-enhancing formulas may improve the postoperative immune response and reduce inflammatory reactions in various groups of postoperative patients, thereby reducing the incidence of serious infectious complications [12, 19–25]. Thus, the preoperative administration of these formulas in patients undergoing gastrointestinal tract surgery has been recommended [15, 24, 26–29]. In Japan, an enteral diet was introduced for immunonutrition in 2002; however, to the best of our knowledge, the utility of preoperative immunonutrition in patients undergoing PD has yet to be examined. The present study was undertaken to determine whether the preoperative oral intake of an immune-enhancing formula may be suitable for patients undergoing elective PD. Furthermore, we attempted to evaluate the effect of a preoperative immune-enhancing formula containing arginine, omega-3 fatty acids, and RNA (Impact Japanese version; Ajinomoto, Tokyo, Japan) on the early postoperative outcomes of patients, comparing outcomes with a historical control group who had received a normal diet alone.

Patients, materials, and methods

From February 2005 to November 2006, 25 consecutive patients (19 men, 6 women; age range, 48–77 years; median age, 64 years) who were candidates for a curative PD for the resection of a lesion in either the pancreatic head or the periampullary region were prospectively enrolled. The study protocol was reviewed and approved by the institutional review board of our hospital. Consenting patients who did not have malnutrition, bowel obstruction, severe cardiopulmonary complications, diabetes, collagen disease, renal failure, ongoing infection, or immune disorders were enrolled in the study. None of the patients had an immunosuppressive condition preoperatively. Patients were required to sign a written informed consent form once the protocol was explained.

The subjects included 5 patients with pancreatic invasive ductal carcinoma (PIDC), 6 with intraductal papillary mucinous neoplasm (IPMN), 9 with biliary tract cancers [bile duct cancer (BDC) in 6 and carcinoma of the papilla of Vater (VC) in 3], 3 with duodenal carcinoma, and 2 with other diseases (a pancreatic solid and pseudo-papillary neoplasm in 1 and a serous cystic adenoma in 1).

First, patient compliance with the preoperative ingestion of Impact was examined. After hospitalization, the patients were instructed to consume 3 packs/day (750 mL) of Impact Japanese version (Ajinomoto) in addition to their normal diets over a 5-day period immediately before surgery. Regarding the timing of the enteral immunonutrition, studies examining gastrointestinal cancer patients without malnutrition have reported that because a sufficient effect could be achieved with 5 days of preoperative administration, the postoperative administration of Impact was not necessary [26, 30]. In the study by Braga et al. [26], 1000 mL/day of Impact was prescribed to patients without malnutrition, but the actual mean intake was 890 mL. Because the mean body size of Japanese is smaller than that of Westerners, the daily intake of Impact Japanese version was set at 3 packs/day (750 mL/day) in the present study. Impact Japanese version is based on Impact (Novartis Consumer Health, Bern, Switzerland), and has been designed to suit the nutritional needs and flavor preferences of Japanese populations. A total of 750 mL of Impact Japanese version contains 9.6 g of arginine, 2.49 g of omega-3 fatty acids, and 0.96 g of RNA. The kilocalorie/milliliter ratio is 1:1. Regular meals of 1800 or 2000 kcal/day, depending on the patient's body size, were served preoperatively.

The patients were admitted at least 1 week before surgery and underwent mechanical preparation, including the oral intake of 2 L of polyethylene glycol electorolyte lavage solution (Niflec; Ajinomoto). Preoperative cultures were performed using nasal and throat swabs from all the patients to test for methicillin-resistant *Staphylococcus aureus* (MRSA). As a preventative antibiotic, 1 g of cefmetazole sodium (CMZ) (Cefmetazone; Daiichi Sankyo, Tokyo, Japan) was administered intravenously via a drip infusion immediately after the induction of anesthesia. A second dose was given 3 h later, followed by doses every 12 h for 2 days after the surgery. Oral feeding was initiated 5 days after the surgery.

Second, we attempted to evaluate the early postoperative outcome after PD by comparing the Impact group, which consisted of patients who fully complied with the ingestion of Impact for 5 days preoperatively, with a control group, which consisted of patients with a similar clinical background and condition who had undergone the same operative procedure in our hospital in 2004 but who had not ingested an immune-enhanced formula preoperatively. The age, sex, body mass index (BMI), serum albumin level, prognostic nutrition index (PNI) [31], preoperative biliary drainage, operative methods, operation times, and intraoperative blood loss of the two groups were compared. Regarding the postoperative course, the surgical morbidity and mortality and the duration of the hospital stay were investigated. The presence of postoperative complications, such as pancreatic fistula and incisional wound infection, and the infection status were described in the medical records. Incisional wound infection was defined based on the evidence of purulent exudate in the wound and the isolation of pathogenic organisms in culture. Surgical site infection (SSI) was diagnosed according to the Centers for Disease Control (CDC) definitions of SSI [32].

During the perioperative period, laboratory blood tests were performed. The white blood cell (WBC) count and the C-reactive protein (CRP), total protein (TP), serum albumin (Alb), total bilirubin (T-Bil), serum amylase (AMY), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and serum creatinine (Cr) levels were routinely measured at 1, 3, and 7 days after surgery. Changes in body weight (BW), and in the acute physiology and chronic health evaluation (APACHE)II scores [33], and the duration of systemic inflammatory response syndrome (SIRS) in the postoperative course were also investigated. The APACHE-II classification includes twelve physiological measures (temperature, mean arterial pressure, heart rate, respiratory rate, oxygenation, arterial pH, serum sodium, serum potassium, serum creatinine, hematocrit, WBC count, and Glasgow Coma Scale score), age, and the presence of severe chronic health problems. The worst value in each patient was used as the physiological score. This index enables the prediction of perioperative events in patients undergoing various surgical procedures [34–39]. The definition of SIRS was adopted from the report by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [40]. SIRS was defined as the presentation of two or more of the following criteria: (1) temperature >38°C or <36°C; (2) heart rate >90 beats/min; (3) respiration >20/min or $PaCO_2$ <32 mmHg; (4) leukocyte count >12,000/mm^3, <4000/mm^3, or >10% band cells.

Statistical analysis of the data was performed using an unpaired Student's *t*-test, the χ^2 test, and the Mann–Whitney *U*-test. Variations in some parameters over time and comparisons among the two groups were studied using a repeated measure analysis of variance (ANOVA). Data are shown as means (standard deviation). All statistical analyses were performed using StatView-J 5.0 (Abacus Concepts, Berkeley, CA, USA); all two-sided p values <0.05 were considered statistically significant.

Operation procedures

Five staff surgeons performed all the operations. The operative procedure was a standardized substomach-preserving PD. Reconstruction was achieved using a retrocolic jejunal Roux-en-Y limb with an end-to-side pancreaticojejunostomy, an end-to-side hepaticojejunostomy, and a gastrojejunostomy, according to the child procedure. In all patients, a pancreatic stenting tube was placed in the pancreatic duct and fixed with 2 absorbable suture ligations. The main duct was anchored to the adjacent serosa. A 3-0 polypropylene monofilament thread with curved needle was prepared with a straightened needle at each end. The suture was passed from the ventral to the dorsal surface of the pancreas from the cut end and the serosal surface of the jejunum. All end-to-side pancreaticojejunostomies were performed in two layers. The inner layer comprised the opposition of the pancreatic duct and adjacent pancreatic tissue to a small opening in the jejunum (full thickness), which was made by puncturing the tissues with a thick needle connected to the pancreatic stenting tube and utilizing interrupted stitches of 5-0 monofilament polyglyconate. All pancreaticojejunal anastomoses were stented (decompressed) through 6- or 7.5-F polyvinyl chloride tubes, according to the diameter of the main pancreatic duct, and the tubes were guided externally through the jejunal loop. The pancreatic juice was completely drained via the tube, and the tube was removed 3 weeks or more after the surgery.

Hepaticojejunostomy was performed using interrupted polyglyconate sutures. A stenting tube was not inserted through the anastomosis in any of the patients. Penrose drains were routinely placed on the anterior and posterior

Table 1 List of patients with preoperative Impact consumption

Patient	Age (years)	Sex	Disease	Procedure	Duration of oral intake of Impact (days)	Reasons for discontinuation of Impact
1	79	Female	BDC	SSpPD	5	None
2	57	Female	PIDC	SSpPD	5	None
3	58	Male	IPMN	SSpPD	5	None
4	68	Male	VC	SSpPD	5	None
5	77	Male	BDC	SSpPD	5	None
6	68	Male	DC	SSpPD	1	Diarrhea
7	52	Male	BDC	SSpPD	5	None
8	56	Male	IPMN	SSpPD	5	None
9	62	Female	IPMN	SSpPD	5	None
10	77	Male	VC	SSpPD	2	Nausea
11	57	Male	BDC	EBDR	5	None
12	75	Male	BDC	Not resected	3	Diarrhea
13	64	Female	PIDC	SSpPD	5	None
14	48	Male	IPMN	SSpPD	4	Pancreatitis and cholangitis
15	62	Male	IPMN	SSpPD	5	None
16	57	Female	VC	SSpPD	5	None
17	67	Male	DC	SSpPD	5	None
18	59	Male	SPT	SSpPD	5	None
19	64	Male	PIDC	SSpPD	5	None
20	62	Female	DC	SSpPD	5	None
21	72	Male	BDC	SSpPD	5	None
22	67	Male	PIDC	SSpPD	5	None
23	44	Female	SCT	SSpPD	5	None
24	58	Male	IPMN	SSpPD	3	Changed operation date
25	64	Male	PIDC	Not resected (GJB)	1	Changed operation date

BDC bile duct carcinoma, *PIDC* pancreatic invasive ductal carcinoma, *IPMN* intraductal papillary mucinous neoplasm, *VC* papilla of Vater carcinoma, *DC* duodenal carcinoma, *SPT* solid and pseudo-papillary tumor of pancreas, *SCT* serous cystic tumor of pancreas, *SSpPD* substomach-preserving pancreaticoduodenectomy, *EBDR* extra bile duct resection, *GJB* gastrojejunal bypass

surfaces of the pancreaticojejunal anastomosis and the dorsal side of the hepaticojejunostomy.

Reconstruction was completed before suturing the abdominal wall. Immediately after the opening of the abdomen, the surgical wound was protected by the placement of a drape. Before closing the abdomen, the abdominal cavity was washed using 3000 mL of warm saline, and the drape was removed. The surgeon and assistant changed gloves, and the abdominal muscle and fascia layers were closed using monofilament absorbable sutures. After washing the skin and subcutaneous fat layer with 500 mL of warm saline, the wound was closed using a skin stapler. Postoperatively, the wound was covered using a transparent protective film and was monitored without sterilization until suture removal.

Results

Compliance with preoperative administration of Impact

The amount of the immunonutrition preparation consumed preoperatively was monitored by the doctor in charge of each patient. A total of 25 patients were enrolled in the study (see Table 1). As the scheduled operation date was moved forward for two patients, these 2 patients had to discontinue Impact consumption. Treatment compliance and other reasons for discontinuation are summarized in Table 2. Nineteen patients (82.6%) fully complied with Impact consumption. The mean period of preoperative oral intake was 4.6 ± 1.1 days. The reasons for the discontinuation of Impact consumption were diarrhea in 2 patients, nausea in 1 patient, and pancreatitis and cholangitis caused by the primary disease in 1 patient. The nausea and diarrhea symptoms occurred 3 days after the start of Impact consumption.

Comparison of early postoperative outcome after PD between the Impact and control groups

Of the 25 patients, 18 were able to complete the Impact consumption protocol. These patients (Impact group) were retrospectively compared with a control group consisting of patients treated at our institution in 2004 who had undergone the same surgical procedure for the treatment of

similar conditions but who had not ingested an immune-enhanced formula preoperatively.

The preoperative and intraoperative clinical background characteristics of the two groups of patients are summarized in Table 3. No significant difference was observed in the total numbers of calories served in the daily hospital meals given for 5 days before surgery and until postoperative day (POD) 7 between the two groups (data not shown). In both the Impact and control groups, peripheral parenteral nutritional infusion was used, without using total parenteral nutrition. Moreover, no differences in age, sex, preoperative nutritional status, operative time, or intraoperative blood loss were observed between the groups.

Table 2 Compliance with oral intake of Impact

Duration of oral intake of immunonutrition (days)	4.6 ± 1.1
No. of patients who completed oral intake	19/23[a] (82.6%)
No. of patients who discontinued treatment	4/23[a] (17.4%)
Reasons for discontinuation	
Diarrhea	2 (8.7%)
Nausea	1 (4.3%)
Pancreatitis and cholangitis caused by primary disease	1 (4.3%)

[a] Not including 2 patients (out of a total of 25 patients in this study) who discontinued preoperative Impact consumption because of changed operation dates

Postoperative SIRS duration and complications

The duration and complications associated with postoperative SIRS in each group are shown in Table 4. The duration of postoperative SIRS and the hospital stay were not significantly different between the groups. The incidences of individual complications were also comparable between the groups. The incidence of incisional wound infection was significantly lower in the Impact group than in the control group (0 vs. 30.8%; $p = 0.012$), but no significant differences in the incidences of other postoperative complications were seen between the groups. The operative mortality rate was 0% for each group.

The effects of immune-enhanced nutrition on laboratory and physical data (WBC count, CRP level, TP, Alb, T-Bil, AMY, GOT, GPT, BUN, Cr, BW, and APACHE-II score) during the perioperative period are shown in Fig. 1. No significant differences in the WBC counts, CRP levels, TP, Alb, T-Bil, AMY, GOT, BUN, and Cr results were seen between the two groups. However, the GPT level was significantly higher in the Impact group (Fig. 1h). While the change in BW during the perioperative period also did

Table 3 Baseline patient characteristics

	Impact ($n = 18$)	Control ($n = 13$)	p
Age (years)	62.6 ± 8.5	65.1 ± 10.0	0.466
Sex (male/female)	11/7	7/6	0.686
BMI	21.9 ± 2.1	22.1 ± 3.2	0.821
Serum albumin (g/dL)	3.9 ± 0.3	3.7 ± 0.5	0.296
PNI	46.5 ± 5.8	43.7 ± 5.0	0.176
Biliary drainage	7	8	0.213
PTBD	5 (71.4%)	5 (62.5%)	0.714
ENBD	2 (28.6%)	3 (27.5%)	
Duration of oral intake of Impact (days)	5	None	None
Resection procedure	SSpPD		
Reconstruction method	Modified child method		
Operation time (min)	329 ± 79	308 ± 88	0.488
Intraoperative blood loss (mL)	921 ± 566	947 ± 654	0.905
Pathological diagnosis			
PIDC	4 (22.2%)	2 (15.4%)	
IPMN			
IPMA	3 (16.7%)	1 (7.7%)	
IPMC	0	1 (7.7%)	
SPT	1 (5.6%)	0	
SCA	1 (5.6%)	0	
BDC	5 (27.8%)	3 (23.1%)	
VC	2 (11.1%)	4 (30.8%)	
DC	2 (11.1%)	2 (15.4%)	

BMI body mass index, *PNI* prognostic nutrition index = (10 × serum albumin) + [0.005 × total lymphocyte count (/mm^3)], *PTBD* percutaneous transhepatic biliary drainage, *ENBD* endoscopic naso-biliary drainage, *SSpPD* substomach-preserving pancreaticoduodenectomy, *PIDC* pancreatic invasive ductal carcinoma, *IPMN* intraductal papillary mucinous neoplasm, *IPMA* intraductal papillary mucinous adenoma, *IPMC* intraductal papillary mucinous carcinoma, *SPT* solid and pseudo-papillary tumor, *SCT* serous cystic adenoma, *BDC* bile duct carcinoma, *VC* papilla of Vater carcinoma, *DC* duodenal carcinoma

Table 4 Early postoperative outcome and complications

	Impact (n = 18)	Control (n = 13)	p
Duration of postoperative SIRS (days)	0.8 ± 1.0	0.9 ± 0.8	0.664
Duration of postoperative hospital stay (days)	29 ± 13	26 ± 12	0.516
Morbidity and mortality			
Pancreatic fistula	12 (66.7%)	8 (61.5%)	0.768
Delayed gastric emptying	2 (11.1%)	1 (5.9%)	0.751
Cholangitis	0	1 (5.9%)	0.232
Wound infection	0	4 (30.8%)	0.012
Perioperative death	0	0	

SIRS systemic inflammatory response syndrome

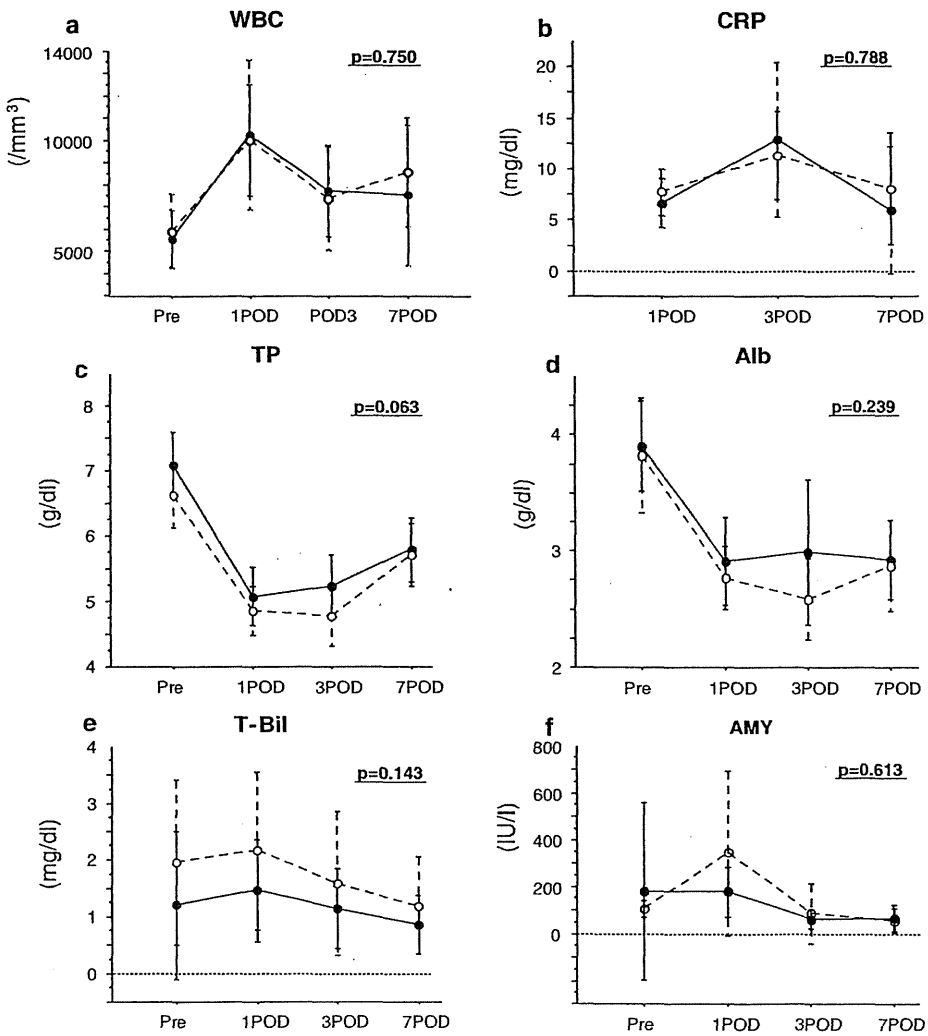
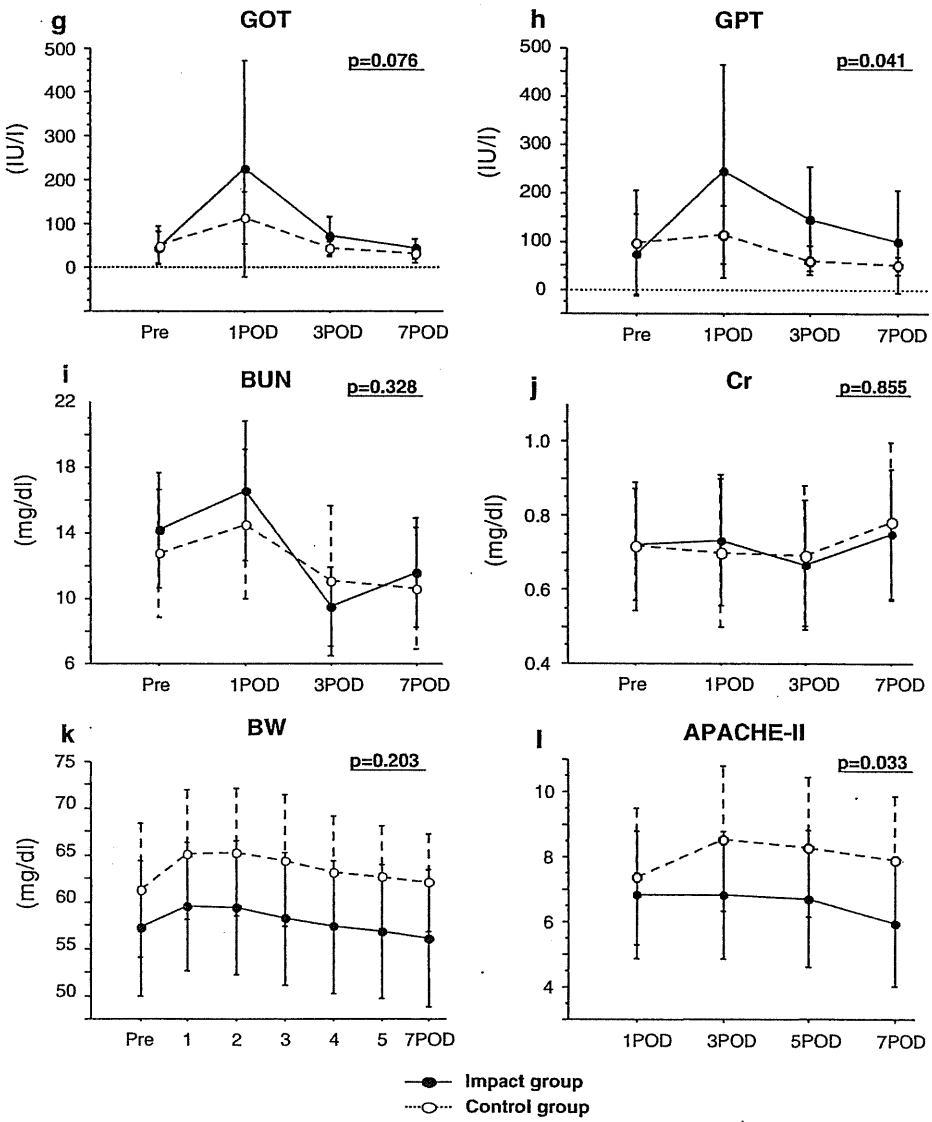


Fig. 1 Laboratory blood test results. *Filled circles* Impact group, *open circles* control group. **a** White blood cell count (*WBC*), **b** C-reactive protein (*CRP*), **c** total protein (*TP*), **d** serum albumin (*Alb*), **e** total bilirubin (*T-Bil*), **f** serum amylase (*AMY*), **g** glutamic oxaloacetic transaminase (*GOT*), **h** glutamic pyruvic transaminase (*GPT*), **i** blood urea nitrogen (*BUN*), **j** serum creatinine (*Cr*), **k** body weight (*BW*), **l** acute physiology and chronic health evaluation score II (*APACHE-II*)

not differ significantly between the two groups, the improvements in the gain or loss of BW after surgery showed a better course in the Impact group than in the control group. To evaluate the systemic severity of patients after surgery, we utilized the APACHE-II classification. A high postoperative APACHE-II score predicts an increased

Fig. 1 continued



risk of a complicated postoperative course [33]. The change in the total APACHE-II score after PD was significantly lower in the Impact group than in the control group ($p = 0.033$). Among the factors measured for the APACHE-II scores, the following factors showed significantly lower scores in the Impact group than in the control group: temperature on POD 1 ($p = 0.008$), mean arterial pressure on POD 1 ($p = 0.048$), heart rate on POD 5 ($p = 0.019$) and POD 7 ($p = 0.049$), and hematocrit on POD 7 ($p = 0.006$).

Discussion

Preoperative oral supplementation with Impact (750 mL/day for 5 days) was well tolerated by patients scheduled to undergo PD. The compliance rate was more than 80%, and the duration and dose of Impact used in this study were suitable. This encouraging result suggests that Impact could also be ingested by outpatients prior to elective PD.

In the present series, one patient with IPMN could not tolerate Impact because of pancreatitis and cholangitis. This patient complained of epigastralgia, fever, and jaundice after beginning to consume Impact. The patient's laboratory data showed elevated serum amylase and bilirubin levels. We suspect that this patient's pancreatitis and cholangitis might have originated from an obstruction caused by a mucinous secretion from the primary tumor, because the pancreatitis and cholangitis occurred simultaneously and progressed synchronously. Actually, the elevated serum bilirubin level consisted predominantly of

direct bilirubin. The patient's condition improved immediately after percutaneous transhepatic biliary drainage.

In the second part of this study, we retrospectively compared the outcomes of patients with and without (control group) the preoperative ingestion of an immune-enhanced formula prior to undergoing PD. In patients without hyperbilirubinemia, laboratory data showed that the postoperative GOT and GPT levels were higher in the Impact group than in the control group; in particular, GPT was significantly higher in the Impact group. In a study examining patients with esophageal cancer who ingested Impact immediately before undergoing a transthoracic esophagectomy with lymph node dissection, Takeuchi et al. [24] also reported an immediate postoperative elevation of transaminases. Although the mechanism remains unclear, a preoperative immune-enhanced diet may impose a load on hepatocytes after invasive surgery such as PD. Immune-enhanced formulas have been suggested to possibly cause a high postoperative BUN level as a result of an overload in nitrogen intake [41]. However, in the present series, we did not observe a marked change in the BUN level, and nitrogen overloading did not appear to be excessive.

Regarding the systemic severity of the patients in this study, the APACHE-II score tended to be lower in the Impact group than in the control group. When measured during the immediate postoperative phase, a high APACHE-II score is thought to be linked to mortality, and the APACHE-II score can be regarded as a summary indicator of an individual's response to surgical injury. The patients who received preoperative immunonutrition had a lower systemic severity score, so it appears that Impact consumption might reduce the severity of systemic damage. Several studies have reported that a supplementary diet rich in omega-3 fatty acids is related to a decrease in PGE2, which is a key fever mediator [42–44]. Our results suggest that the preoperative consumption of an immune-enhanced formula may reduce excess postoperative pro-inflammatory cytokine production (such excess production may result in serious complications or lethal multiple organ dysfunctions in patients who have undergone PD). Additional investigations of the detailed changes in some indicators, such as inflammatory cytokines, are needed.

In the present study, incisional wound infection was significantly less frequent in the Impact group than in the control group. SSI including incisional wound infection is a serious complication following surgery, requiring a prolonged hospitalization period, increased medical costs, and decreased patient satisfaction [45, 46]. SSI is primarily caused by surgical procedures, and performing surgery while minimizing the risk of SSI is important. The preoperative oral intake of immune-enhanced formulas, such as Impact, might also be important for preventing incisional wound infection.

The duration of postoperative SIRS and the length of the hospital stay were not significantly different between the two groups in our study. Thus, the effects of the preoperative ingestion of an immune-enhanced formula on the duration of the hospital stay among patients undergoing PD remain unclear. In this study, pancreatic fistula was the most common and important complication, not wound infection. The length of the hospital stay is likely to be affected by the severity of this complication, as it is regarded as a major unfavorable complication after PD. During this study, an end-to-side dunking anastomosis was used for the anastomosis between the pancreatic stump and the jejunum; however, since 2007 (after the completion of the present study), we have adopted a duct-to-mucosa anastomosis with 5-0 absorbable monofilament using a vinyl tube as a lost stent in pancreaticojejunostomy procedures. As a result, the incidence of pancreatic fistula after PD has decreased (data not shown). This concept has also successfully enabled the duration of the hospital stay after PD to be shortened.

To our knowledge, this is the one of few reports to suggest the feasibility and benefit of using an immune-enhanced formula, Impact, as part of the preoperative management of patients scheduled to undergo PD. To date, several groups have reported on immunonutrition in gastrointestinal cancer surgery patients [11, 12, 15, 47]. Most of these reports have demonstrated that patients receiving immunonutrition before and/or after surgery tended to have fewer postoperative complications. Gianotti et al. [22] reported that patients receiving immunonutrition with an enteral formula after PD had a significantly lower incidence of infectious complications than patients in the standard and parenteral groups. Di Carlo et al. [48] also reported similar results for postoperative enteral feeding in patients with pancreatic head cancer. However, no other reports have described patient compliance with preoperative oral intake, or the clinical significance of the preoperative ingestion of immune-enhanced formulas for patients undergoing PD.

In conclusion, a high rate of compliance with the preoperative oral administration of Impact Japanese version (750 mL/day, for 5 days) was observed in Japanese patients without malnutrition who were scheduled to undergo PD. This treatment appeared to be effective for preventing incisional wound infection and reducing systemic severity. To confirm the clinical benefits of preoperative Impact, a randomized control study including the use of a control group receiving a regular diet alone is needed. Of note, the composition of the commercially available Impact in Japan differs slightly from the original Impact used in Western countries, so we approve the

suggestion from Tsujinaka et al. [29] that such a randomized study should be performed exclusively in Japan. In addition, such a study would require a similar quality of operative procedures and perioperative management in both patient groups.

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