び臨床性能試験計画案について、医薬品・医療器総合機構の医療機器・体外診断用医薬品対面助言を実施し、試験を行う。データマネージャー、モニタリングを附属病院先端医療開発センターにより実施する。

（株）キュービクスは、改良型高精度膵癌RNA 測定診断薬「膵癌血液 mRNA 測定検査」の体外診断用医薬品に必要な安定性や品質などを保証し、体外診断用医薬品の承認をめざす。これまでの臨床研究で既に整備されている金沢大学を含む北陸地方の関連施設による多施設共同臨床性能試験を実施する。

（倫理面への配慮）

臨床性能試験では、ヘルシンキ宣言に基づく倫理的原則に留意し、医薬品、医療機器等の品質、有効性及び安全性の確保等に関する法律及び GCP、試験実施計画書を遵守して実施する計画書を作成する。被験者の登録及び症例報告書における被験者の特定は、被験者識別コードで行うとともに、試験の実施に係わる原資料の直接閲覧、医学雑誌への発表、規制当局等への資料提出等において、本試験に係わる者は被験者の秘密を保全する。実施前に、治験に準じ院内受託研究審査委員会に計画についての審査をうけ、承認を得て実施する。

C. 研究結果

平成 26 年 11 月 18 日に、「膵臓癌診断キットの研究開発に関する全体会議」において、RNA 測定診断薬「消化器がんマイクロアレイ血液検査」から膵癌高精度判定血液 RNA 測定診断薬「膵癌血液 mRNA 測定検査」への改良開発、体外診断薬と実施すべき臨床性能試験計画案についての医薬品・医療器総合機構の医療機器・体外診断用医薬品対面助言面談、臨床性能試験実施、承認申請の工程を策定した。工程に従い、医薬品・医療機器総合機構の医療機器・体外診断用医薬品に関する対面助言準備面談を平成 27 年 3 月 19 日に実施予定である。

体外診断薬の臨床性能試験実施、体外診断用医薬品承認のため必要な、プロジェクトマネジメント、生物統計、データマネジメントについて、名古屋大学医学部附属病院の先端医療・臨床研究支援センターが運営する中部先端医療開発円環コンソーシアムの支援が、同理事会にて承認された。また、医薬品開発業務受託機関を選定した。

D. 考察

開発する「膵癌血液mRNA測定検査」の体外診断用医薬品薬「承認を目的とする臨床性能試験への準備が予定どおり進捗した。

E. 結論

開発中の「膵癌血液 mRNA 測定検査」の臨床性能試験へのプロジェクトマネジメントを含め、試験実施体制を整備し、開発工程を策定した。次年度に、体外診断薬「膵癌血液 mRNA 測定検査」、臨床性能試験計画案について医薬品・医療機器総合機構の対面助言を実施し、試験開始予定である。

F. 研究発表

1. 論文発表

本業務項目関連にはなし。

2. 学会発表

4) 酒井佳夫、金子周一、和田隆志. 消化

器癌における末梢血液細胞の遺伝子発現変化に反映される生体のがん反応解析と癌診断法開発. 一般演題「最新検査・毒性検査・その他」第61回日本臨床検査医学会学術集会. 平成26年11月25日　福岡国際会議場、福岡.

G. 知的所有権の出願・取得状況

1. 特許取得

「遺伝子発現プロファイルによる消化器癌、胃癌、大腸癌、膵臓がんおよび胆道癌の検出」（特許第 4953334 号）

2. 実用新案登録

なし

3. その他

特記事項なし

III. 学会等発表実績

様式第１９

学　会　等　発　表　実　績

委託業務題目「ＲＮＡ測定による膵癌血液診断法の実用化研究」
機関名　国立大学法人金沢大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
消化器癌における末梢血液細胞の遺伝子発現変化に反映される生体のがん反応解析と癌診断法開発、口頭	酒井佳夫、金子周一、和田隆志	第61回日本臨床検査医学会学術集会、福岡国際会議場、福岡	2014年11月25日	国内
切除可能膵頭部癌に対するリンパ行性進展・局所進展からみた治療アルゴリズム、口頭（シンポジウム）	北川裕久、田島秀浩、中川原寿俊、牧野　勇、宮下知治、寺川裕史、正司政寿、中沼伸一、林　泰寛、高村博之、太田哲生	第45回日本膵臓学会大会、北九州市	2014年7月11日	国内
BilIN と PanIN　の分子病理学的異常に関する比較検討、ポスター発表	佐藤保則、原田憲一、佐々木素子、中沼安二	第50回日本肝臓学会総会、東京	2014年5月29日～30日	国内
血小板の制御による膵癌転移治療の新戦略～癌転移に対する血小板の役割とその制御～、口頭（シンポジウム）	宮下知治、田島秀浩、中沼伸一、酒井清祥、木下淳、牧野　勇、中村慶史、林　泰寛、尾山勝信、中川原寿俊、高村博之、二宮　致、北川裕久、伏田幸夫、太田哲生	第23回日本がん転移学会学術集会・総会、金沢市	2014年7月	国内
当科におけるUICC規約膵体部癌に対する治療成績、口頭（シンポジウム）	田島秀浩、北川裕久、太田哲生	第12回日本消化器外科学会大会、神戸市	2014年10月	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Histological characterization of biliary intraepithelial neoplasia with respect to pancreatic intraepithelial neoplasia.	Sato Y, Harada K, Sasaki M, Nakanuma Y.	International Journal of Hepatology Volume 2014 (2014), Article ID 678260, 7 pages http://dx.doi.org/10.1155/2014/678260	April 10, 2014.	国外
En bloc vascular resection for the treatment of borderline resectable pancreatic head carcinoma.	Kitagawa H, Tajima H, Nakagawara H, Makino I, Miyashita T, Shoji M, Nakanuma S, Hayashi H, Takamura H, Ohta T.	Mol Clin Oncol. 2014 May;2(3):369-374. Epub 2014 Feb 27.	May, 2014.	国外
A modification of radical antegrade modular pancreatosplenectomy for adenocarcinoma of the left pancreas: Significance of En Bloc resection including the anterior renal fascia.	Kitagawa H, Tajima H, Nakagawara H, Makino I, Miyashita T, terakawa H, Nakanuma S, Hayashi H, Takamura H, Ohta T.	World J Surg. 2014 Sep;38(9):2448-54. doi: 10.1007/s00268-014-2572-5.	Sep., 2014	国外

Ⅳ．研究成果の刊行物・別刷

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Volume 2014, Article ID 678260, 7 pages
http://dx.doi.org/10.1155/2014/678260

Hindawi

Research Article

Histological Characterization of Biliary Intraepithelial Neoplasia with respect to Pancreatic Intraepithelial Neoplasia

Yasunori Sato,[1] Kenichi Harada,[1] Motoko Sasaki,[1] and Yasuni Nakanuma[1,2]

[1] *Department of Human Pathology, Kanazawa University Graduate School of Medicine, 13-1 Takara-machi, Kanazawa 920-8640, Japan*
[2] *Department of Pathology, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan*

Correspondence should be addressed to Yasuni Nakanuma; nakanuma@staff.kanazawa-u.ac.jp

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Biliary intraepithelial neoplasia (BilIN) is a precursor lesion of hilar/perihilar and extrahepatic cholangiocarcinoma. BilIN represents the process of multistep cholangiocarcinogenesis and is the biliary counterpart of pancreatic intraepithelial neoplasia (PanIN). This study was performed to clarify the histological characteristics of BilIN in relation to PanIN. Using paraffin-embedded tissue sections of surgically resected specimens of cholangiocarcinoma associated with BilIN and pancreatic ductal adenocarcinoma associated with PanIN, immunohistochemical staining was performed using primary antibodies against MUC1, MUC2, MUC5AC, cyclin D1, p21, p53, and S100P. For mucin staining, Alcian blue pH 2.5 was used. Most of the molecules examined here showed similar expression patterns in BilIN and PanIN, in which their expression tended to increase along with the increase in atypia of the epithelial lesions. Significant differences were observed in the increase in mucin production and the expression of S100P in PanIN-1 and the expression of p53 in PanIN-3, when compared with those in BilIN of a corresponding grade. These results suggest that cholangiocarcinoma and pancreatic ductal adenocarcinoma share, at least in part, a common carcinogenic process and further confirm that BilIN can be regarded as the biliary counterpart of PanIN.

1. Introduction

Cholangiocarcinoma that arises under conditions of chronic biliary diseases such as hepatolithiasis often undergoes the multistep carcinogenesis process [1]. Biliary intraepithelial neoplasia (BilIN) is known as a premalignant lesion of cholangiocarcinoma that represents the multistep cholangiocarcinogenesis [2]. The classification is applicable to flat atypical epithelial lesions in the intrahepatic large bile ducts and the extrahepatic bile ducts, and it is also applied to lesions in the gallbladder according to the current World Health Organization (WHO) classification for tumors of the digestive system [3].

BilIN is a concept that is proposed based on the morphological resemblance to pancreatic intraepithelial neoplasia (PanIN). Similar to PanIN, BilIN is classified into three grades according to the degree of cytological and architectural atypia: BilIN-1 (low-grade lesions), BilIN-2 (intermediate-grade lesions), and BilIN-3 (high-grade lesions, carcinoma in situ). Using the BilIN classification, there is increasing evidence that molecular and genetic alterations accumulate during the progression of BilIN to cholangiocarcinoma [4–7].

Since the biliary tract and pancreas share a common developmental process as well as morphological characteristics as duct systems, it is plausible that some biliary and pancreatic diseases show similar pathological features and biological behaviors [8]. Indeed, our recent comparative analysis showed that hilar cholangiocarcinoma and ductal adenocarcinoma of the pancreas share many clinicopathological features [9]. In addition, we showed that intraductal papillary neoplasm of the bile duct (IPNB) and intraductal papillary mucinous neoplasm (IPMN) of the pancreas, as well as mucinous cystic neoplasm (MCN) of the biliary tract and pancreas, exhibit similar immunohistochemical phenotypes, suggesting a common carcinogenic process of the tumors [10], where all these tumors were classified as premalignant lesions according to the current WHO classification.

Table 1: Primary antibodies used for immunohistochemical analysis.

Antigen	Clone	Company	Dilution	Antigen retrieval
MUC1	DF3	Toray Fuji Bionics (Tokyo, Japan)	1:50	MW
MUC2	Ccp58	Novocastra (Newcastle, UK)	1:100	MW
MUC5AC	CLH2	Novocastra	1:200	MW
Cytokeratin 20	Ks 20.8	DakoCytomation (Glostrup, Denmark)	1:50	MW
Cyclin D1	SP4	Nichirei (Tokyo, Japan)	Prediluted	MW*
p21	EPR3993	Abcam (Cambridge, UK)	1:100	MW
p53	DO-7	DakoCytomation	1:100	MW
S100P	EPR6143	Abcam	1:100	MW

MW: microwaving in 10 nmol/L citrate buffer (pH 6.0) for 20 minutes; MW*: microwaving in tris-ethylenediaminetetraacetic acid buffer (pH 9.0) for 20 minutes.

As far as the histological characteristics of BilIN and PanIN are concerned, previous studies have examined their features individually, and detailed data on comparative analysis of BilIN and PanIN are lacking. This study was therefore conducted to clarify the histological characteristics of BilIN with respect to PanIN.

2. Materials and Methods

2.1. Tissue Preparation. Hepatolithiatic livers associated with perihilar cholangiocarcinoma were used as a model of multistep cholangiocarcinogenesis. A total of 25 hepatolithiatic livers with cholangiocarcinoma and a total of 22 pancreatic specimens with pancreatic ductal adenocarcinoma were retrieved from the files of our laboratory and affiliated hospitals. The patients were selected during the period between 1997 and 2007. All cases were surgically resected, and all liver and pancreatic specimens were histologically accompanied by BilIN and PanIN, respectively. In all cases of cholangiocarcinoma, the main part of the tumor was located in hilar or perihilar region of the liver, and they appeared to arise from the intrahepatic large bile ducts or the right or left hepatic bile duct. Most cholangiocarcinoma cases showed macroscopic features of mass-forming type and/or intraductal growth type. Foci of BilIN were microscopically located in the intrahepatic large bile ducts and the hepatic bile ducts, and they were not observed in the septal and interlobular bile ducts. The mean age and sex distribution (male-female ratio) of the patients were 62 years and 11:14 for the liver specimens and 68 years and 12:10 for the pancreatic specimens, respectively. The samples were fixed in 10% neutral formalin and embedded in paraffin. Then, 4-μm-thick paraffin-embedded sections were prepared. One representative section from each case was used.

2.2. Histochemistry and Immunohistochemistry. Alcian blue (at pH 2.5) was used for mucin staining. Immunostaining was performed using the sections with the primary antibodies listed in Table 1. After the blocking of endogenous peroxidase, the sections were incubated in protein block solution (DakoCytomation, Glostrup, Denmark). They were then incubated overnight at 4°C with each of the primary antibodies. Their sources, optimal dilution, and antigen retrieval methods are shown in Table 1. They were treated with secondary antibodies conjugated to a peroxidase-labeled polymer using the HISTOFINE system (Nichirei, Tokyo, Japan). Color development was performed using 3,3′-diaminobenzidine tetrahydrochloride, and the sections were lightly counterstained with hematoxylin. Negative controls consisted of substitution of the primary antibodies with nonimmune serum and were consistently negative.

2.3. Histological Assessment. Semiquantitative analysis of the stained sections was performed. Staining intensity was evaluated in a high-power field for the neoplastic and nonneoplastic epithelia of the bile ducts and pancreatic ducts. From the sections of 25 liver specimens and 22 pancreatic specimens, foci of interest were selected. The number of foci examined was as follows: nonneoplastic large bile duct, 14; BilIN-1, 17; BilIN-2/3, 24, invasive carcinoma (cholangiocarcinoma), 50; nonneoplastic pancreatic duct, 13; PanIN-1, 22; PanIN-2/3, 15; invasive carcinoma (pancreatic ductal adenocarcinoma), 44.

For mucin staining with Alcian blue (pH 2.5), the signal intensity in the cytoplasm and/or on the luminal surface of the epithelial cells was evaluated using the following grading system: 1+ (mild), 2+ (moderate), and 3+ (marked). The cytoplasmic and/or luminal immunostaining of MUC1 and the cytoplasmic immunostaining of MUC2 and MUC5AC were graded as follows: 0 (negative), 1+ (mild to moderate), and 2+ (marked). For evaluation of the nuclear staining of cyclin D1, p21, p53, and S100P, the percentage of positive nuclei to the total number of nuclei of the epithelial cells was calculated, and it was graded as follows: 0 (negative), 1+ (not exceeding 10%), and 2+ (more than 10%). For p53 nuclear staining, only the proportion of intensely positive nuclei was scored.

2.4. Statistics. Statistical significance was determined using the Mann-Whitney U-test. A P value less than 0.05 was accepted as the level of statistical significance.

3. Results and Discussion

Morphological appearances such as loss of nuclear polarity, increased nucleus-to-cytoplasm ratio, nuclear hyperchromasia, and architectural atypia were basically similar between

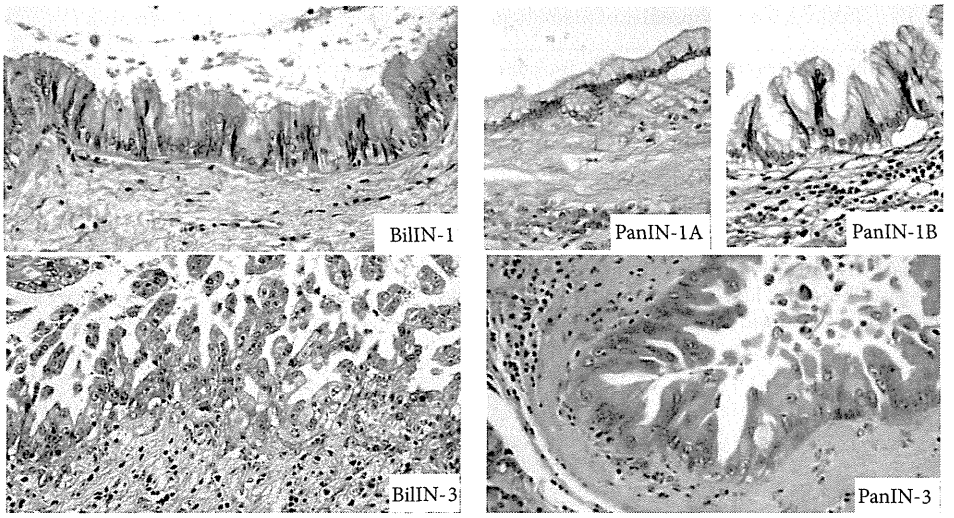

FIGURE 1: Histology of biliary intraepithelial neoplasia (BilIN) and pancreatic intraepithelial neoplasia (PanIN). Representative images of BilIN-1 and BilIN-3 and PanIN-1A, PanIN-1B, and PanIN-3 are shown. Hematoxylin and eosin staining. Original magnifications, ×400.

the corresponding grades of BilIN and PanIN, which were observed in sections stained with hematoxylin and eosin (Figure 1).

Mucin staining with Alcian blue (pH 2.5) showed that both BilIN and PanIN frequently had cytoplasmic and/or luminal surface mucin (Figure 2). According to the grade of BilIN and PanIN, PanIN-1 tended to have more abundant cytoplasmic mucin than BilIN-1, and the results of semiquantitative analysis confirmed this tendency (Figure 3). The abundant mucin expression in PanIN-1 is consistent with the definition of PanIN-1 in which the lesion is composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin [11].

The immunohistochemical expression of MUC1 was increased along with the increase in the grade of BilIN and PanIN, and no significant difference in its expression status was observed between BilIN and PanIN (Figures 2 and 3). Similarly, the expression of MUC5AC was frequently observed in all grades of both BilIN and PanIN (Figures 2 and 3). The results of the expression status of MUC1 and MUC5AC in BilIN were almost identical to those in our previous report [4].

Focal immunohistochemical expression of MUC2 was observed in several foci of BilIN, whereas MUC2 positivity was exceptional in PanIN (Figures 2 and 3). Although the expression of CK20 was typically negative in both BilIN and PanIN in this study (data not shown), BilIN is not infrequently associated with metaplastic change of intestinal type, while intestinal-type PanIN is generally not found [12, 13]. These observations may explain the focal MUC2 expression in BilIN rather than in PanIN.

The results of immunostaining of MUC1, MUC2, MUC5AC, and CK20 for BilIN and PanIN in this study are summarized in Table 2. For comparison, the results of our previous comparative analysis that examined the immunohistochemical characteristics of IPNB, IPMN of the pancreas, hepatic MCN, and pancreatic MCN [10] are also shown in Table 2. It is noteworthy that all of these premalignant lesions show similar immunoprofiles to each other between the biliary tract and pancreas, supporting the concept that BilIN, IPNB, and hepatic MCN are biliary counterparts of PanIN, IPMN, and pancreatic MCN, respectively.

As for the expression of cell cycle-related molecules, the immunohistochemical expression of cyclin D1 and p21 was absent or focal in nonneoplastic epithelium of the bile ducts and the pancreatic ducts. They were occasionally observed in BilIN-1 and PanIN-1 and more frequently in BilIN-2/3 and PanIN-2/3 (Figures 2 and 3), in which the frequency of the expression of cyclin D1 and p21 in BilIN in this study was comparable to that in our previous report [5]. Semiquantitative analysis showed that there was no significant difference in their expression status between BilIN and PanIN.

The expression of p53 was not observed in nonneoplastic epithelium of the bile ducts and the pancreatic ducts, as well as in BilIN-1/2 and PanIN-1/2. By contrast, BilIN-3 and PanIN-3 occasionally showed the expression of p53, and its frequency was significantly higher in PanIN-3 than in BilIN-3 (Figures 2 and 3). Because the process of carcinogenesis is often complicated by inflammatory changes in the biliary tract, the molecular alterations may be more complex in BilIN due to cholangitis than those seen in PanIN, where the influence of inflammation is usually insignificant in the development of pancreatic cancer. In fact, our recent study showed that the detection rate of *KRAS* mutation in BilIN was not as high as that seen in PanIN [6]. Therefore, it is predicted that factors other than genetic alterations may also affect the process of the development of BilIN and cholangiocarcinoma.

S100P is a molecule that is highly expressed by perihilar and extrahepatic cholangiocarcinoma as well as pancreatic ductal adenocarcinoma [9, 14]. In this study, the expression of S100P was frequently observed in both BilIN and PanIN of all grades (Figure 2). Semiquantitative analysis showed that its

Table 2: Immunoprofiles of premalignant lesions of the biliary tract and pancreas.

	Intraepithelial neoplasia		Intraductal papillary neoplasm		Mucinous cystic neoplasm	
	BilIN	PanIN	IPNB	IPMN	Hepatic MCN	Pancreatic MCN
MUC1	+	+	+	+	+	+
MUC2	+	−	+	+	−	−
MUC5AC	++	++	++	++	++	++
CK20	−	−	+	+	−	−

The results of comparative analysis for biliary and pancreatic neoplasms in the present study and our previous report (10) are summarized. −: likely absent; +: occasionally present; ++: usually present. BilIN: biliary intraepithelial neoplasia; CK: cytokeratin; IPMN: intraductal papillary mucinous neoplasm; IPNB: intraductal papillary neoplasm of the bile duct; MCN: mucinous cystic neoplasm; PanIN: pancreatic intraepithelial neoplasia.

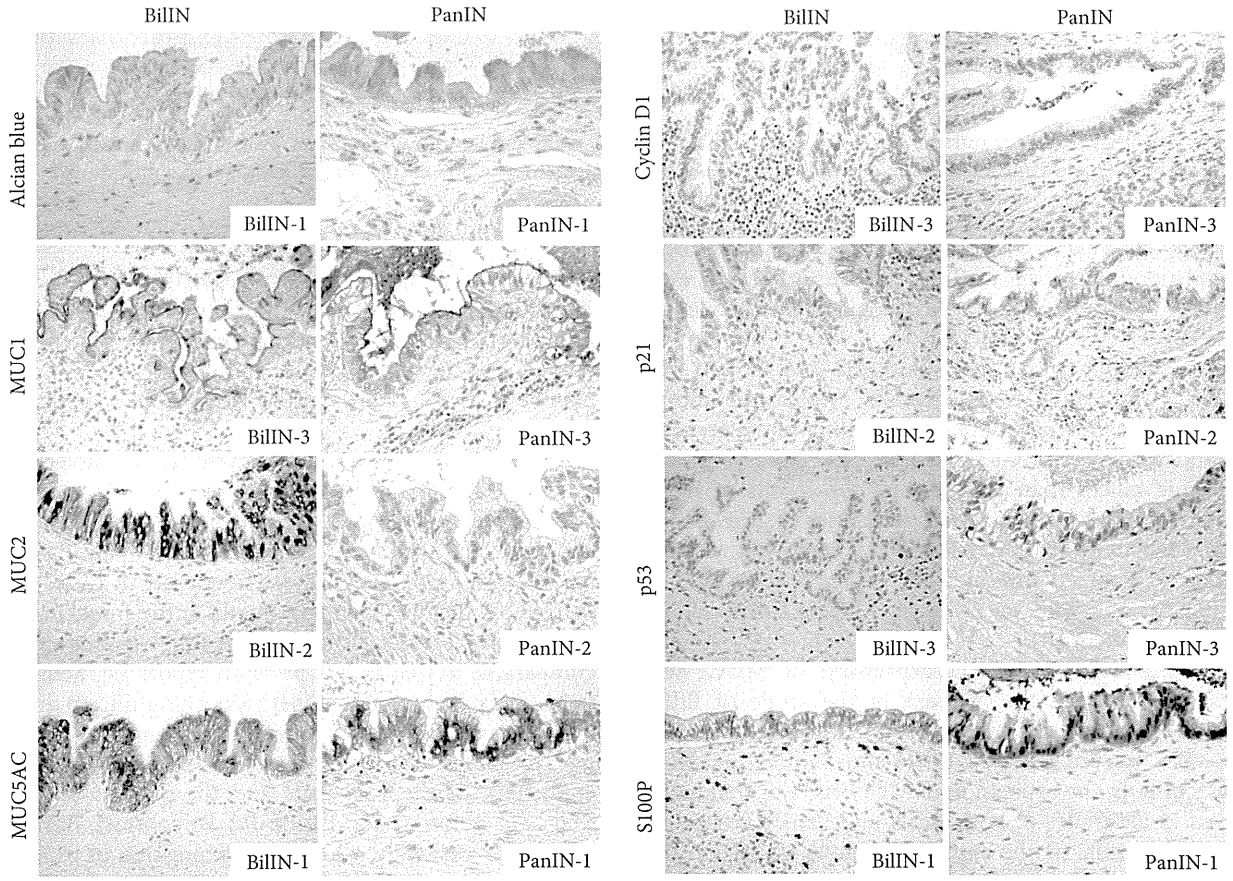

Figure 2: Representative images of histochemical and immunohistochemical staining. The results of mucin staining with Alcian blue (pH 2.5) and immunostaining of MUC1, MUC2, MUC5AC, cyclin D1, p21, p53, and S100P for biliary intraepithelial neoplasia (BilIN) and pancreatic intraepithelial neoplasia (PanIN) are shown. Original magnifications, ×400.

expression was significantly high in PanIN-1 compared with that in BilIN-1, although both BilIN-1 and PanIN-1 exhibited a high frequency of S100P expression (Figure 3).

Most of the molecules examined in this study showed similar expression patterns in BilIN and PanIN. There were significant differences in the increase in mucin production and the expression of S100P in PanIN-1 and the expression of p53 in PanIN-3, when compared with those in BilIN of corresponding grade.

The immunohistochemical expression of MUC1, cyclin D1, p21, p53, and S100P tended to be increased in invasive foci of cholangiocarcinoma and pancreatic ductal adenocarcinoma when compared to those in BilIN-2/3 and PanIN-2/3, respectively (Figure 3). These results were consistent with the concept of multistep carcinogenesis.

4. Conclusions

BilIN and PanIN showed similar histological and immunohistochemical features with several exceptions. These results suggest that cholangiocarcinoma and pancreatic ductal adenocarcinoma share, at least in part, a common carcinogenic

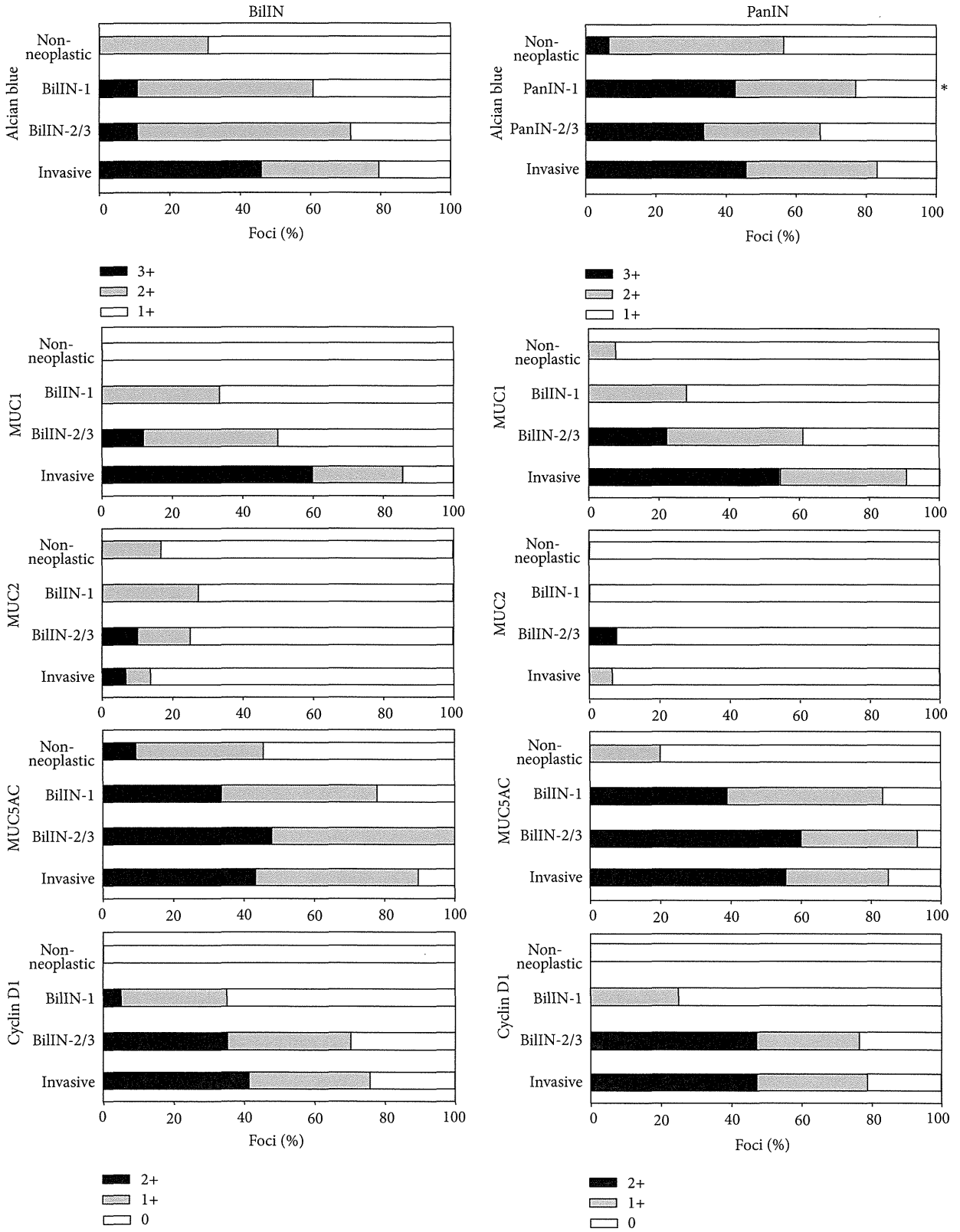

FIGURE 3: Continued.

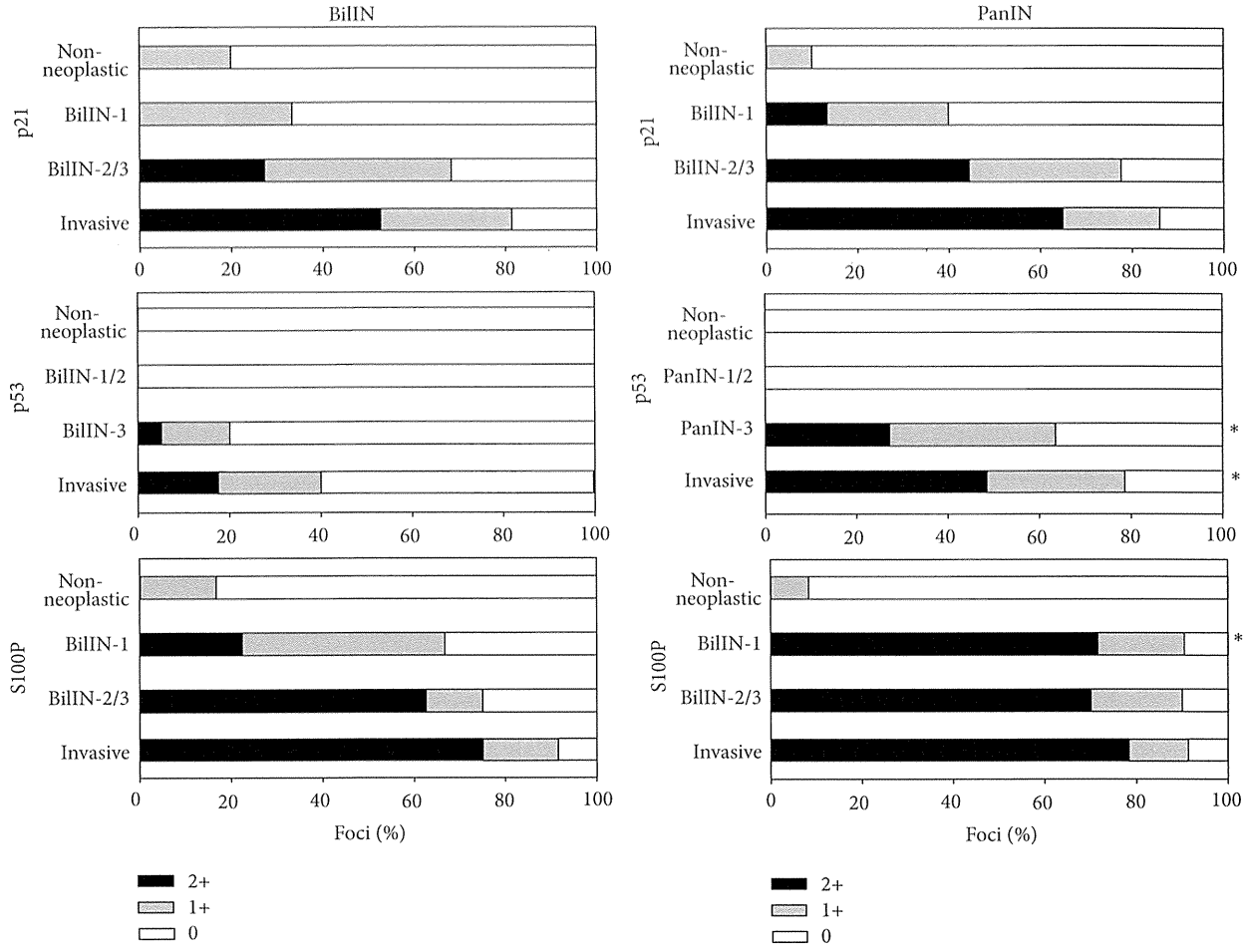

FIGURE 3: Semiquantitative analysis of the results of histochemical and immunohistochemical staining. The analysis was performed as described in Section 2 for the lesions of nonneoplastic epithelium of the bile ducts and the pancreatic ducts, biliary intraepithelial neoplasia (BilIN), pancreatic intraepithelial neoplasia (PanIN), and invasive carcinoma. $^{*}P < 0.05$ versus the results of BilIN of corresponding histological grade or cholangiocarcinoma.

process and further confirm that BilIN can be regarded as the biliary counterpart of PanIN.

Abbreviations

BilIN: Biliary intraepithelial neoplasia
CK: Cytokeratin
IPMN: Intraductal papillary mucinous neoplasm
IPNB: Intraductal papillary neoplasm of the bile duct
MCN: Mucinous cystic neoplasm
PanIN: Pancreatic intraepithelial neoplasia
WHO: World Health Organization.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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En bloc vascular resection for the treatment of borderline resectable pancreatic head carcinoma

HIROHISA KITAGAWA[1], HIDEHIRO TAJIMA[1], HISATOSHI NAKAGAWARA[1], ISAMU MAKINO[1], TOMOHARU MIYASHITA[1], MASATOSHI SHOJI[1], SHINICHI NAKANUMA[1], NORIHIRO HAYASHI[1], HIROYUKI TAKAMURA[1], TETSUO OHTA[1] and HIROSHI OHTAKE[2]

Departments of [1]Gastroenterologic Surgery and [2]Cardiovascular Surgery, Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa 920-8641, Japan

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Abstract. Borderline resectable (BR) pancreatic head carcinoma (PhC) is an advanced disease, presenting with infiltration of major vessels. Major vascular resection (VR), particularly arterial resection, to achieve microscopic no residual tumor (R0) is a controversial approach, due to the potential complications. In this study, we aimed to clarify the benefit of en bloc R0 resection with VR for PhC by retrospectively evaluating 78 PhC patients who underwent pancreatoduodenectomy at our institute. The patients were divided into 4 groups as follows: R, resectable (n=20); BR-V, BR involving the superior mesenteric vein or portal vein (PV) (n=28); BR-SMA, BR involving the superior mesenteric artery (n=21); and BR-HA, BR involving the hepatic artery (n=9). In total, 65 patients underwent VR, with 63, 21 and 9 patients undergoing PV, SMA and HA resection, respectively. The R0 rates were as follows: R group, 85%; BR-V, 82%; BR-SMA, 71%; and BR-HA, 33%. The median survival time and 5-year survival rate for R0 resection were 31 months and 25% in the R group, 22 months and 28% in the BR-V group, 17 months and 27% in the BR-SMA group and 10 months and 0% in the BR-HA group, respectively. The prognosis was comparable among the BR-V, BR-SMA and R groups, but was significantly poorer in the BR-HA group. In total, 5 patients (6.4%) died perioperatively (4 from postoperative hemorrhage and 1 from suffocation due to failure of expectoration, without pneumonia or asthma). Of the 4 patients who succumbed to hemorrhage, 3 had undergone arterial resection. Therefore, en bloc resection with major VR for R0 may be suitable for BR-V and BR-SMA PhC patients.

Correspondence to: Professor Hirohisa Kitagawa, Department of Gastroenterologic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara machi, Kanazawa, Ishikawa 920-8641, Japan
E-mail: kitagawa@staff.kanazawa-u.ac.jp

Key words: borderline resectable, pancreatic head carcinoma, en bloc arterial resection, meso-pancreatoduodenum, superior mesenteric artery

Introduction

Borderline resectable (BR) pancreatic adenocarcinoma is an advanced disease and conventional resection has been proven to be inadequate for improving patient prognosis. The criteria of the resectability status are defined by the National Comprehensive Cancer Network guidelines as tumor infiltration into nearby major vessels (1). A combination of vascular resection (VR) is required to achieve no microscopic residual tumor (R0) resection for BR pancreatic head carcinoma (PhC). The principle underlying our surgical strategy for resectable (R) PhC is total excision of the lymphatic basin of the pancreatic head, which is termed meso-pancreatoduodenum (meso-pd). For BR PhC, additional venous and/or arterial resection may be required for R0 resection. In the present study, 78 patients with PhC were evaluated, including 65 patients who underwent VR and were consecutively treated at our institute between 2002 and 2012, in order to clarify the benefit of the en bloc VR technique for R0 resection of BR PhC.

Patients and methods

Diagnostic procedures and staging. The PhCs were classified as follows: R; BR-V, BR involving the superior mesenteric vein (SMV) or portal vein (PV); BR-SMA, BR involving the superior mesenteric artery; and BR-HA, BR involving the hepatic artery. The classification was performed on the basis of the extent of the cancer nest, which was determined by multi-detector row computed tomography (MDCT). The extent of nerve plexus (PLX) invasion was determined by either the coarse reticular pattern or the mass and strand pattern connected to the main lesion of the carcinoma (2). Abutment or near abutment of the SMV/PV, SMA or HA by the cancer nest was considered an indication for en bloc resection of these vessels.

The resected specimens were serially sliced into 5-mm stepwise sections along the axial plane. The tumor stage and grade were classified according to the 7th edition of the tumor-node-metastasis classification system of the International

Union against Cancer (UICC) (3). Tumor-node-metastasis staging was performed in accordance with the UICC/American Joint Commission on Cancer staging system (4), which corresponds to the histopathological reporting of pancreatic cancer of the Royal College of Pathologists (5). Margin positivity was defined as tumor clearance of <1 mm.

This retrospective study was approved by the appropriate Institutional Review Board, and informed consent was obtained from each patient.

Surgical procedures. The basic and standard protocol for the treatment of PhC was total meso-pd resection, en bloc resection of the pancreatic head and the lymphatic basin. The lower dissection limit of the mesentery was above the third duodenal portion and the posterior dissection plane included the anterior renal fascia. The PLX surrounding the SMA was not included in the meso-pd. VR was optional, depending on the extent of tumor infiltration. All the SMV/PV resections were performed using the sleeve resection technique. The preferred reconstruction technique following segmental resection was primary end-to-end anastomosis; however, interpositioning of the autologous venous graft from the external iliac vein was completed to provide a tension-free anastomosis, when necessary. Following venous confluence resection, the splenic vein stump was closed and the inferior mesenteric vein was preserved, if possible. SMA resection was performed in 21 cases, from its origin until the infiltration-free portion (6). In the first 17 cases, we performed interpositioning of the autologous venous graft of the saphenous vein for reconstruction with a tension-free, end-to-end anastomosis. For the following 4 cases, we performed a direct anastomosis of the aorta inferior to the origin of the inferior mesenteric artery, using an autologous venous graft of the saphenous vein, via side-to-end anastomosis for the proximal site and end-to-side anastomosis for the distal site. Prior to SMV/PV or SMA resection and reconstruction, occlusion of the SMA was repeated 3 times to induce ischemic preconditioning in the mesentery. HA resection was performed in 7 cases. End-to-end reconstruction was performed in 5 cases to restore the arterial blood supply to the liver, whereas in the remaining 2 cases it was unnecessary. An autologous venous graft of the saphenous vein was used for reconstruction in 1 case. Vascular reconstruction following SMA or HA resection was performed using a 2-step method. Arterial reconstruction and reperfusion were performed, followed by SMV/PV reconstruction. The specimen was mobilized prior to VR, resulting in en bloc resection that included the involved vessel as the last step of the surgical procedure.

In-hospital parameters. The following patient parameters were routinely assessed, included in an online prospective database and analyzed: Perioperative morbidity, particularly surgical complications (occurrence of postpancreatectomy hemorrhage; thrombosis of the PV, SMV, SMA or HA in patients undergoing VR; abdominal or liver abscess formation and duodenal ulcer) and perioperative mortality, defined as in-hospital mortality or death within the first month following discharge from the hospital.

Follow-up. The routine postoperative evaluation included a regularly scheduled physical examination, measurement of carcinoembryonic antigen and carbohydrate antigen 19-9 levels and imaging studies with MDCT every 3 months.

Statistical analysis. The associations between categorical variables were assessed using the Fisher's exact test or the χ^2 test. The Kaplan-Meier method was used to estimate survival probability at 24 and 60 months after surgery. The differences between patient groups with respect to survival were assessed using log-rank tests. $P<0.05$ was considered to indicate a statistically significant difference. SPSS software for Windows®, version 13 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Procedures and perioperative patient characteristics. The patient characteristics, surgical procedures and perioperative outcomes of the entire study cohort are summarized in Table I. Of the 78 patients who underwent pancreatoduodenectomy for PhC, 20 patients had R PhC, 28 had BR-V PhC, 21 had BR-SMA PhC and 9 had BR-HA PhC. Of the 20 patients with R PhC, 10 underwent SMV/PV resection. Of the 28 patients with BR-V PhC, 25 underwent SMV/PV resection and 3 underwent synchronous resection of the SMA. In the BR-SMA group, all 21 patients underwent SMV/PV resection, with synchronous resection of the SMA in 17 patients. In the BR-HA PhC group, all 9 patients underwent SMV/PV resection, with 7 patients undergoing synchronous resection of the HA and 1 patient undergoing resection of the SMA. Total pancreatectomy was performed in the remaining 2 BR-HA PhC patients who exhibited extensive involvement of the splenic artery beyond the bifurcation of the common hepatic and splenic arteries.

Intraoperative parameters, morbidity and mortality. The operative time was significantly longer in patients with BR-V, BR-SMA and BR-HA PhC, compared to that in patients with R PhC ($P<0.001$) and the intraoperative blood loss was significantly greater for BR-SMA and BR-HA PhC compared to that for R and BR-V PhC ($P<0.001$). Overall, 6 patients experienced postoperative hemorrhage. In the BR-V PhC group, postoperative hemorrhage occurred in 2 patients, 1 due to failure of the anastomosis of the SMA and the other due to rupture of the ligated stump of the right gastric artery. Both hemorrhages were induced by abdominal abscess without pancreatic fistula, and the latter was fatal. In the BR-SMA PhC group, postoperative hemorrhage occurred in 3 patients, 1 due to rupture of a pseudo-aneurysm induced by a pancreatic fistula, 1 due to rupture of an old aortic aneurysm induced by an abdominal abscess and 1 due to failure of the SMA anastomosis induced by an abdominal abscess. The resulting hemorrhage in the former 2 patients was fatal. In the BR-HA PhC group, postoperative hemorrhage occurred at the HA anastomosis site in 1 patient with severe arterial sclerosis. Although hemostasis was achieved, the patient succumbed to rapid recurrence of liver and lung metastases. Overall, there were 5 cases (6.4%) of perioperative mortality, with 4 deaths due to postoperative hemorrhage and 1 due to suffocation by failure of expectoration, without pneumonia or asthma.

Table I. Characteristics of the study population (n=78).

Characteristics	Resectable n=20	BR-V n=28	BR-SMA n=21	BR-HA n=9	P-value
Gender (M/F)	(13/7)	(14/14)	(15/6)	(7/2)	0.316
Age, years (range)	66 (52-77)	64 (44-78)	60 (38-78)	65 (53-79)	0.295
Operative time, min (range)	648 (422-811)	750 (528-1,015)	850 (690-1,045)	829 (580-1,110)	<0.001
PPPD/PD	6/14	6/22	4/17	1/8[a]	<0.001
Vascular resection					
SMV/PV	10	25	21	9	
SMA	0	3	17	1	
HA	0	0	0	7	
Blood loss, ml (range)	662 (115-1,840)	883 (210-3,510)	2768 (250-8,880)	2981 (1,170-5,640)	<0.001
Surgical morbidity (major)	4 (20%)	3 (11%)	8 (38%)	3 (33%)	0.126
Hemorrhage	0	2	3	1	
Pancreatic fistula (grade B,C)	3	2	2	0	
PV thrombosis	0	0	1	0	
Arterial thrombosis	0	0	0	0	
Abdominal abscess	0	2	2	1	
Liver abscess	0	0	0	1	
Duodenal ulcer	0	0	1	0	
Perioperative mortality	0	1	2	2	0.120

[a]Two patients with total pancreatectomy were included. PD, pancreatoduodenectomy; PPPD, pylorus-preserving PD; SMV, superior mesenteric vein; PV, portal vein; SMA, superior mesenteric artery; HA, hepatic artery; BR, borderline resectable; BR-V, BR involving the SMV or PV.

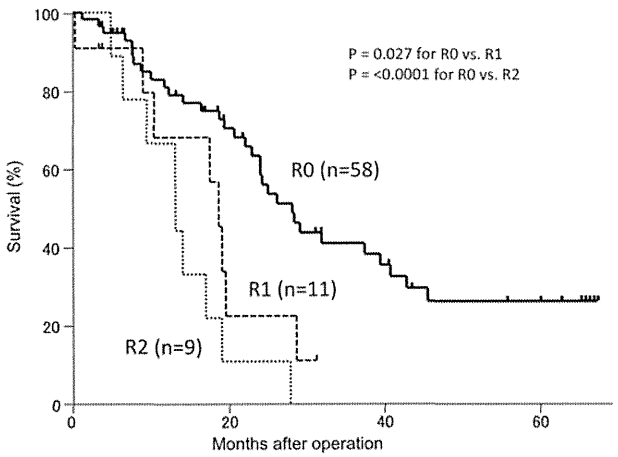

Figure 1. Kaplan-Meier survival curves for 78 patients with pancreatic ductal adenocarcinoma grouped according to their resectability status. The differences were statistically significant (log-rank test). R, resectable; R0, no residual tumor; R1, microscopic residual tumor; R2, macroscopic residual tumor.

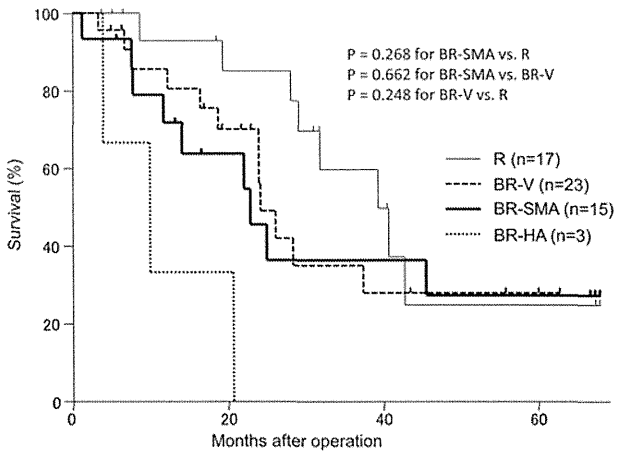

Figure 2. Kaplan-Meier survival curves for 58 patients who achieved R0 resection according to the vascular infiltrations (logrank test). R, resectable; BR, borderline resectable; SMA, superior mesenteric artery; HA, hepatic artery; BR-V, BR involving the superior mesenteric or portal vein.

Histopathology. The histopathological results of the patients are summarized in Table II. All the patients had histopathologically confirmed pancreatic ductal adenocarcinoma. The microscopic R0 rates were 85% (17/20), 82% (23/28), 71% (15/21) and 33% (3/9) in the R, BR-V, BR-SMA and BR-HA PhC groups, respectively. Vascular infiltration was defined as tumor clearance of <1 mm. The histopathological analysis of the BR-SMA or BR-HA PhC groups revealed evidence of SMA or HA infiltration in 20 (95%) and 9 (100%) patients, respectively (Table II).

Survival. The median survival time (MST) and the 5-year survival rate were 22 months and 26% for the R0 patients, respectively (Fig. 1). No patients with microscopic residual tumor (R1) or macroscopic residual tumor (R2) remained alive at 3 years postoperatively. For the R0 cases, the MSTs and 5-year survival rates were 31 months and 25% for the R PhC group, 22 months and 28% for the BR-V PhC group and 17 months and 27% for the BR-SMA PhC group, respectively (Fig. 2), with no statistically significant difference among these

Table II. Histopathology.

Tumor characteristics and resectability	R (n=20)	BR-V (n=28)	BR-SMA (n=21)	BR-HA (n=9)	P-value
T stage					<0.001
T1	3	2	0	0	
T2	0	0	0	0	
T3	17	25	11	2	
T4	0	1	10	7	
N stage					0.069
N0	9	6	6	0	
N1	11	22	15	9	
Grade					0.651
G1	6	5	4	1	
G2	12	18	16	6	
G3	2	5	1	2	
Resectability status (%)					0.062
R0	17 (85)	23 (82)	15 (71)	3 (33)	
R1	2 (10)	3 (11)	2 (10)	2 (22)	
R2	1 (5)	2 (7)	4 (19)	4 (45)	
Vascular infiltration[a]					
SMV/PV	2	17	19	9	
SMA	0	0	20	1	
HA	0	1	0	9	

[a]Vascular infiltration positivity was defined as tumor clearance of <1 mm. R, resectable; R0, no residual microscopic tumor; R1, residual microscopic tumor; R2, residual macroscopic tumor; SMV, superior mesenteric vein; PV, portal vein; SMA, superior mesenteric artery; HA, hepatic artery; BR, borderline resectable; BR-V, BR involving the SMV or PV.

groups. Overall, 7 patients remained alive at 5 years postoperatively (2 patients in the R PhC group, 2 patients in the BR-V PhC group and 3 patients in the BR-SMA PhC group).

Discussion

Surgical resection is the only potentially curative approach for the management of PhC. Our strategy for surgical extirpation of PhC comprised total meso-pd resection, as a primary lymphatic basin resection, and VR for R0 resection margins, when necessary. In selected patients with arterial involvement, arterial en bloc resection for PhC may result in an overall survival comparable to that obtained with standard resection for R PhC and improved compared to that obtained with palliative bypass for BR PhC (7,8). In the present study, the prognoses of the BR-V and BR-SMA PhC groups were comparable to that of the R PhC group; however, the BR-HA PhC group had a significantly worse prognosis. For BR-HA PhC, it was difficult to perform R0 resection and hepatic recurrences developed within 1 year postoperatively in 6 of the 9 cases.

Achievement of an R0 resection margin status following surgery is essential for the prolonged survival of patients with PhC. Although the demarcation of the dissection line for R0 resection using preoperative imaging is carefully performed, local recurrence due to microscopically positive margins is common, particularly at the SMA (4,9,10). The involvement of the SMA in PhC is termed extrapancreatic PLX invasion and is an indicator of poor prognosis (11-18). The majority of PhCs are scirrhous and are characterized by a fibrous stroma with scattered carcinoma cells. The normal PLX is almost always composed of adipose tissue, with a low computed tomography (CT) number, whereas PLX invasion is fibrous and imaged by MDCT as a coarse reticular pattern or a mass and strand pattern connecting to the main lesion of the carcinoma (2). The extent of the cancer nest is assessed by the fibrous changes connected to the main tumor. Histologically, these fibrous changes consist of desmoplastic tissue with scattered carcinoma cells and have been described as 'peritumoral inflammation' or 'mimicking tumor invasion', according to the low density of the carcinoma cells. To avoid an R1 resection margin during curative surgery, the desmoplastic cancer nest should be resected en bloc, with a macroscopic safety margin of 5 mm. The extent of this safety margin remains controversial, but a microscopic margin of >1 mm on histological examination is recommended (19-25). As preoperative demarcation of the dissection line is assessed by MDCT, which is a crucial decision and must include an adequate safety margin macroscopically. At our institution, VR was defined as abutment or near abutment of the aforementioned vessels by the cancer nest. Therefore, careful review of CT images is crucial in determining the extent of PLX invasion. A window level and width of 40 and 350 HU, respectively, are recommended.

The mesentery is a fan-shaped fold of the peritoneum through which the blood vessels, lymph vessels and nerves of the abdominal visceral organs pass. Therefore, the mesentery corresponds to the initial field of infiltration of carcinoma (26). Our 'meso-pd' concept refers to the mesentery of the pancreatic head and the duodenum, which is a firm and well-vascularized perineural lymphatic layer located dorsal to the pancreas that reaches behind the mesenteric vessels and has been described as the 'mesopancreas' (27). However, the term mesopancreas is insufficient, as this mesentery is common to the pancreatic head and the duodenum. Therefore, we considered the term 'meso-pd' to be more descriptive of this mesentery. The meso-pd is fan-shaped and its trunk is the inferior pancreatoduodenal artery, which is a tributary of the SMA. The meso-pd is a counterpart of the mesocolon and the mesentery, including the meso-pd, rotates between the 6th and 12th week of the prenatal period. The envelope of fibrous sheath or fascia enclosing the meso-pd is invisible (28), since the original fascia is fused and lost during embryonal development. Therefore, a total meso-pd resection was performed with respect to the PLX surrounding the SMA and including the anterior renal fascia. The caudal border of the meso-pd is the lower level of the third duodenal portion, where tiny lymphatic emboli were observed (29).

We determined the manner of lymphatic extension and PLX infiltration of the PhC depending on whether the tumor originated from the embryonic dorsal or ventral pancreatic bud (30,31). Tumors confined to the ventral pancreas extend toward the SMA, whereas tumors confined to the dorsal pancreas extend towards the common HA or hepatoduodenal ligament. If the tumor infiltrates deeply into both areas, the cancer is likely to extend in both directions. Therefore, the meso-pd was considered to be the mesentery of the embryonic ventral pancreas and total meso-pd resection would be essential for PhC confined to the ventral pancreas. We developed an aggressive surgical method termed 'augmented regional pancreatoduodenectomy (ARPD)' in 2002 for the resection of the pancreatic head together with the SMA and SMV/PV for cases of PhC (6). This procedure was performed in 21 patients: 3 with BR-V PhC, 17 with BR-SMA PhC and 1 with BR-HA PhC. The 3 patients with BR-V and the patient with BR-HA were 'nearly BR-SMA cases'; therefore, ARPD was performed. ARPD has theoretical advantages for en bloc and curative resection of carcinomas of the ventral pancreas. By contrast, the mesentery corresponding to the embryonic dorsal pancreas is currently unclear, although it is associated with the HA. Survival following HA resection was poor in our study and our procedure, which focuses on the meso-pd, was shown to be insufficient for the treatment of carcinomas of the dorsal pancreas.

Intraoperative blood loss during ARPD was higher in patients with BR-SMA PhC compared to that in patients with R or BR-V PhC; this difference was most likely due to the improvement in the operative technique with increased experience, with an estimated blood loss of 615±273 ml in the last 4 patients. All the reported deaths occurred in patients who were operated on within the first 3 years. Postoperative hemorrhage was fatal, particularly when induced by a pancreatic fistula or intra-abdominal infection. Failure of the arterial anastomosis occurred in 3 patients, with 1 patient successfully treated by arterial re-anastomosis. The results of the present study indicate that the en bloc resection of the meso-pd with major VR for R0 may be suitable for patients with BR-V PhC and BR-SMA PhC, but not for those with BR-HA PhC.

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A Modification of Radical Antegrade Modular Pancreatosplenectomy for Adenocarcinoma of the Left Pancreas: Significance of *En Bloc* Resection Including the Anterior Renal Fascia

Hirohisa Kitagawa · Hidehiro Tajima · Hisatoshi Nakagawara · Isamu Makino · Tomoharu Miyashita · Hirofumi Terakawa · Shinichi Nakanuma · Hironori Hayashi · Hiroyuki Takamura · Tetsuo Ohta

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Abstract
Background Radical antegrade modular pancreatosplenectomy (RAMPS) has theoretical advantages for curative resection of adenocarcinomas of the left pancreas. The anterior renal fascia is a key structure, and resection planes should run posterior to this fascia. However, it is difficult to delineate this fascia and set a precise dissection plane. We modified RAMPS to achieve such a precise dissection plane with ease.
Methods After clamping the splenic artery, the third duodenal portion was mobilized from the left to the right to locate the inferior vena cava, which was covered by the anterior renal fascia. Here, the anterior renal fascia was incised while approaching the dissection plane. Dissection then continued cephalad, with this plane along the inferior vena cava, and then turned along the left renal vein at the confluence of the left renal vein toward the renal hilum. At this point, dissection continued along the coronal plane to the superior edge of the pancreas.
Results Between July 2007 and December 2012, a total of 24 pancreatic adenocarcinoma patients underwent modified RAMPS. Tumor extension beyond the pancreatic parenchyma (T3) and lymph node metastases was confirmed in 17 and 13 cases, respectively. Histologically clear surgical margins were achieved (R0 resection) in 21 patients (88 %). The 5-year overall survival rate was 53 %. Six patients survived for over 5 years without recurrence.
Conclusions This modification of RAMPS is advantageous for *en bloc* resection while actually including removal of the anterior renal fascia. It is associated with satisfactory survival rates for patients with distal pancreatic carcinomas.

H. Kitagawa (✉) · H. Tajima · H. Nakagawara · I. Makino · T. Miyashita · H. Terakawa · S. Nakanuma · H. Hayashi · H. Takamura · T. Ohta
Department of Gastroenterologic Surgery, Graduate School of Medical Science, Kanazawa University, 13-1 Takara machi, Kanazawa 920-8641, Japan
e-mail: kitagawa@staff.kanazawa-u.ac.jp

Introduction

Surgery for pancreatic adenocarcinoma should principally facilitate the achievement of negative resection margins (R0) and *en bloc* dissection of regional lymph nodes, and much effort has been made for these. Pancreatoduodenectomy for carcinomas of the pancreas head has been modified to achieve sufficient resection margins, especially at the pancreatic posterior and uncinate margins [1, 2]. Distal pancreatectomy is the standard procedure for tumors of the left pancreas. However, conventional distal pancreatectomy for ductal carcinomas has traditionally been associated with unfavorable prognoses. Radical antegrade modular pancreatosplenectomy (RAMPS) was designed by Strasberg et al. [3, 4], and was applied for treating carcinomas of the left pancreas, worldwide. RAMPS facilitates good visibility, dissection of N1 nodes, and tumor isolation following early arterial clamping. However, precisely delineating the anterior renal fascia and achieving a precise dissection plane posterior to the pancreas is difficult. We modified RAMPS to delineate the posterior dissection plane easily and reproducibly. With our method, the left pancreas is resected *en bloc* and wrapped within the anterior renal fascia attached to its posterior surface.