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Original Article

Histologic determination of primary site of perihilar cholangiocarcinoma based on microscopic tumor invasion of the vasculo-biliary sheaths

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Abstract: This study aimed to histologically characterize microscopic tumor invasion of the vasculo-biliary sheaths in perihilar cholangiocarcinoma so as to determine the primary tumor site and to clarify clinicopathologic differences according to the primary site. A retrospective analysis was conducted of 52 patients who underwent resection for perihilar cholangiocarcinoma involving both the hepatic hilus and the liver, verified histologically. Histologic features of the vasculo-biliary sheath invasion were evaluated by double staining with hematoxylin-eosin to assess general morphology and Victoria Blue to detect the elastic fibers of the vasculo-biliary sheaths. The perihilar cholangiocarcinomas were classified into extrahepatic-type (34 patients), featuring an extrahepatic component involving the liver, and intrahepatic-type (18 patients), featuring an intrahepatic component involving the hepatic hilus. Hemihepatectomy with extrahepatic bile duct resection was the most common surgical procedure. Tumor size ($p = 0.002$), pN classification ($p = 0.005$), and pM classification ($p = 0.023$) were significant independent prognostic factors. The primary site was not significantly associated with survival after resection ($p = 0.214$), as patients with extrahepatic-type tumors had a cumulative 5-year survival rate of 32%, compared with 28% for patients with intrahepatic-type tumors. Double staining with hematoxylin-eosin and Victoria Blue permits histologic discrimination between tumors of extrahepatic and intrahepatic origin, and thereby determination of the primary tumor site in clinical cases of perihilar cholangiocarcinoma. Combining extrahepatic-type and intrahepatic-type tumors under the term perihilar cholangiocarcinoma is valid clinically, as these tumors show comparable surgical outcomes with similar clinical management.

Keywords: Perihilar cholangiocarcinoma, hilar cholangiocarcinoma, intrahepatic cholangiocarcinoma, vasculo-biliary sheaths, surgical resection, prognosis

Introduction

Cholangiocarcinoma can develop anywhere along the biliary tract, from proximal peripheral intrahepatic ducts to the distal intraduodenal bile duct [1]. In the 7th edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual [1], extrahepatic cholangiocarcinoma is divided into perihilar and distal subgroups, with perihilar cholangiocarcinoma defined anatomically as a tumor located in the extrahepatic biliary tree extending from either the right or left hepatic duct to the origin of the cystic duct. DeOliveira et al [2] recently proposed a definition of perihilar cholangiocarcinoma that includes tumors above the junction of the cystic duct up to and including the second biliary branches of the right and left bile ducts. However, as the boundary between the extrahepatic and intrahepatic bile ducts is unclear, perihilar cholangiocarcinoma potentially includes two types of tumor: an extrahepatic-type tumor arising from the large hilar bile duct and an intrahepatic-type tumor with an intrahepatic component involving the hepatic hilus as suggested by Nagino [3]. Thus, these two types of tumor are usually grouped together clinically under the term perihilar cholangiocar-

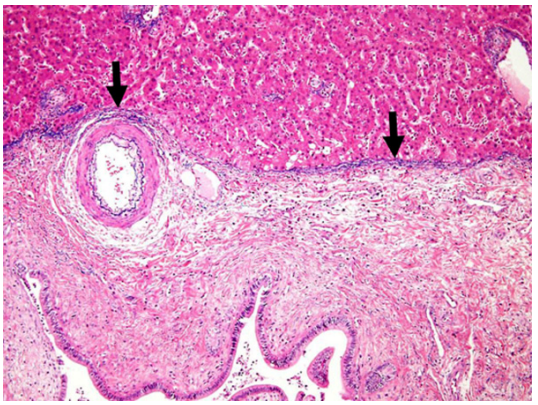

Figure 1. Boundary between the hepatic hilus and liver parenchyma. Arrows indicate elastic fibers, which correspond to the elastic fibers of the vasculo-biliary sheath (hilar plate and Laennec's capsule) between the hepatic hilus and liver parenchyma. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 100).

cinoma [2, 4-9]. However, no histologic consensus has been established to discriminate between extrahepatic-type tumors with hepatic involvement and intrahepatic-type tumors involving the hepatic hilus.

Thus, depending on the anatomic site of tumor origin, perihilar cholangiocarcinomas can be potentially classified into extrahepatic- or intrahepatic-type tumors. To this end, Sano et al [8] briefly mentioned using the elastica stain to delineate the elastic fibers of the hepatic hilum and the intrahepatic Glisson's capsule in difficult cases to determine tumor origin, estimated tumor domination, and whether it was inside or outside the hilar plate. Couinaud [10-12] proposed the concept of the vasculo-biliary sheath, which includes Walaeus's sheath described in 1640, Glisson's sheath described in 1642, Laennec's capsule described in 1803, and the hilar plate. We hypothesized that microscopic invasion of the vasculo-biliary sheath could serve to discriminate between an extrahepatic-type tumor with hepatic involvement and an intrahepatic-type tumor involving the hepatic hilus. To address this issue, we undertook a retrospective study using double staining with hematoxylin-eosin and Victoria Blue, which stains elastic fibers, to histologically examine the vasculo-biliary sheaths. Our aim was to elucidate those features of the sheath invasion that could serve to both discriminate between extrahepatic-type tumors with hepatic involvement and intrahepatic-type tumors involving the hepatic hilus, and clarify the clinicopathologic differences between the two types of tumor.

Materials and methods

Patients

From January 1988 to December 2011, a total of 164 consecutive Japanese patients with cholangiocarcinoma underwent a radical resection with curative intent at the Niigata University Medical and Dental Hospital. Six of these patients had an intraductal growth-type tumor and were excluded from this study. Patients with peripheral-type cholangiocarcinoma arising from the intrahepatic small bile ducts [13] or extrahepatic cholangiocarcinoma without hepatic involvement, and those who underwent an extrahepatic bile duct resection alone or a minor hepatectomy, were also excluded from this study. In the present study, peripheral-type cholangiocarcinoma was defined as a tumor occupying the hepatic periphery [13, 14], mainly located in the left lateral segments of the liver (intrahepatic biliary tributaries on the left side of the umbilical portion of the left portal vein) or mainly located in the right side of the liver parenchyma (intrahepatic biliary tributaries above the junction of the right anterior and posterior sectoral bile ducts). We identified 52 patients who underwent resection for perihilar cholangiocarcinoma involving both the hepatic hilus and the liver, verified histologically from the hospital database. Thus, the final number of patients in this retrospective study was 52, comprising 36 men and 16 women with a median age of 67 years (range, 35-80 years). The study protocol, which conformed to the ethics guidelines of the 1975 Declaration of Helsinki, was approved by the Institutional Review Board of Niigata University Medical and Dental Hospital.

Surgical resection procedures

Surgical resection procedures depended on the location of the primary tumor. Metastatic lesions were indicated for resection if considered resectable and if the patients had acceptable operative risk. Terminology regarding hepatectomy procedures followed the Brisbane 2000 Terminology of Liver Anatomy and Resections [15]. Major hepatectomy (resection

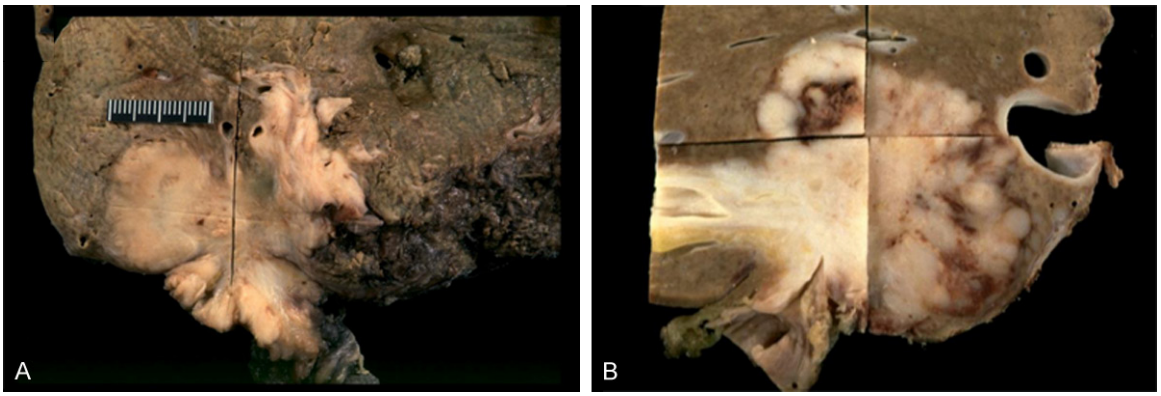

Figure 2. Macroscopic appearance of perihilar cholangiocarcinoma. A. Extrahepatic-type tumor with hepatic involvement. B. Intrahepatic-type tumor involving the hepatic hilus.

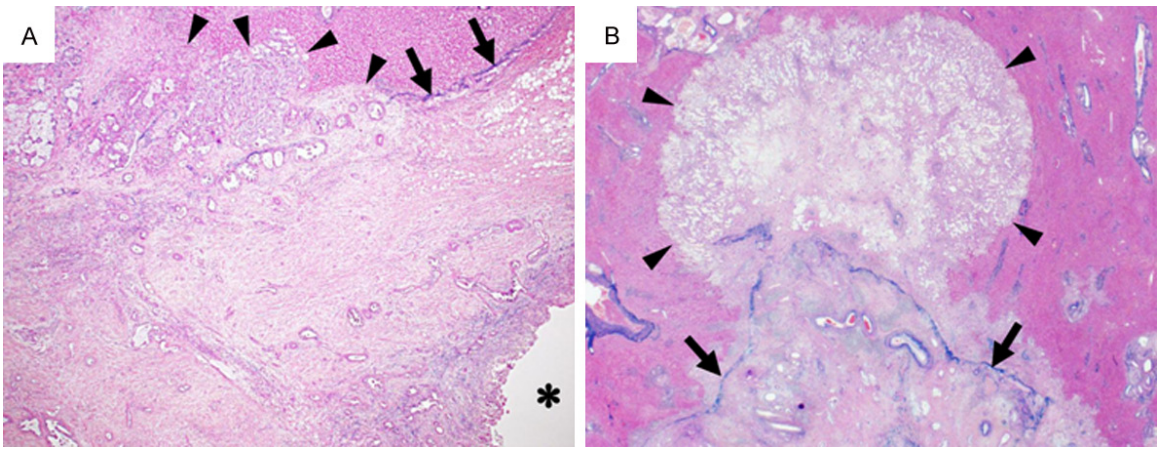

Figure 3. Cells from the extrahepatic component of the tumor involving the liver in an extrahepatic-type tumor. A. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 20). Arrows indicate elastic fibers of the vasculo-biliary sheath. Arrowheads indicate cells from the extrahepatic component of the tumor invading through the elastic fibers of the vasculo-biliary sheath. *Indicates the lumen of a large hilar bile duct. B. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 10). Arrows indicate elastic fibers of the vasculo-biliary sheath. Arrowheads indicate cells from the extrahepatic component of the tumor invading through the elastic fibers of the vasculo-biliary sheath.

of ≥ 3 Couinaud segments) indicated formal hemihepatectomy or more extensive resection [16]. Indications for combined major hepatectomy and pancreaticoduodenectomy for perihilar cholangiocarcinoma included hepatic involvement plus extensive ductal involvement, hepatic involvement plus direct invasion of the pancreas or duodenum, or hepatic involvement plus bulky peripancreatic lymph node metastases. Intraoperative ultrasonography was employed in all patients. All 52 patients also underwent dissection of the regional lymph nodes. The regional lymph nodes of perihilar bile ducts were classified according to the AJCC Cancer Staging Manual (7th edition, 2010) [1].

Patient follow-up after resection

Patients undergoing a surgical resection were followed regularly in outpatient clinics every 3-6 months, with a median follow-up period of 90 months (range, 1-285 months). At the time of disease status assessment, 34 patients had died of tumor recurrence and 9 patients had died of other causes with no evidence of tumor recurrence. The remaining 9 patients were alive with no evidence of disease.

Pathologic evaluation

Resected specimens were submitted to the Department of Surgical Pathology in our hospi-

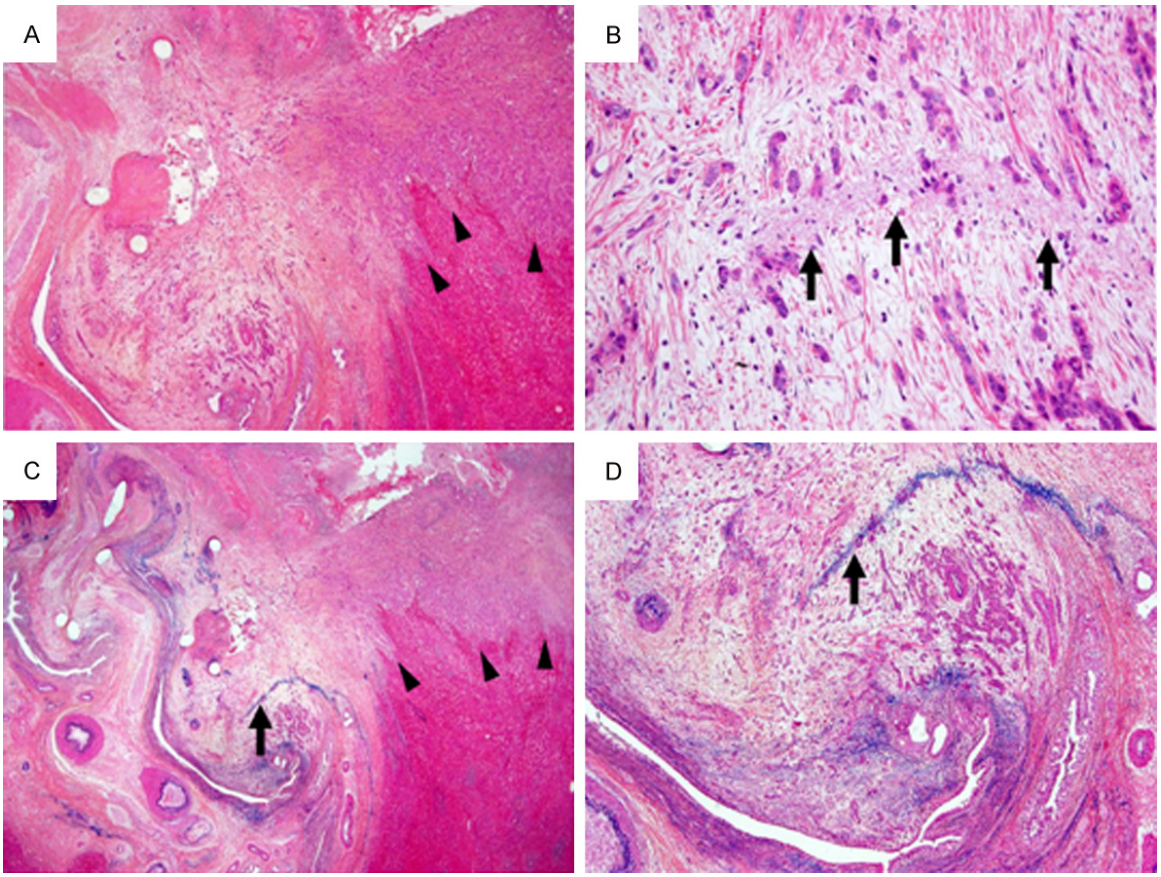

Figure 4. Cells from the intrahepatic component of the tumor involving the hepatic hilus in an intrahepatic-type tumor. A. Hematoxylin-eosin staining (original magnification × 20). Cells from the intrahepatic component of the tumor (arrowheads) involving the hepatic hilus. B. Hematoxylin-eosin staining (original magnification × 100). Arrows indicate elastic fibers of the vasculo-biliary sheath. C. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 12.5). Cells from the intrahepatic component of the tumor (arrowheads) invade the hepatic hilus, resulting in a bending of elastic fibers (arrow) towards the hepatic hilus. D. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 40). Arrow indicates a bend of elastic fibers in the vasculo-biliary sheath.

tal for histologic evaluation. In 1982, our institution established uniform standards for the final histopathologic description of hepato-biliary tumors and the described liver analysis technique was standard for the 24 years of the current study. Extrahepatic bile duct tumors have traditionally been separated into perihilar (or proximal), middle, and distal subgroups. In the 7th edition of the AJCC Cancer Staging Manual [1], extrahepatic cholangiocarcinoma is divided into perihilar and distal subgroups, in which perihilar cholangiocarcinomas are defined anatomically as tumors located in the extrahepatic biliary tree proximal to the origin of the cystic duct. They may extend proximally into the right hepatic duct, the left hepatic duct, or both. Therefore, histopathologic findings in the current study were described according to the AJCC Cancer Staging Manual for perihilar bile duct cancer [1]. The extent of the primary tumor was determined by examining multiple sections (median, 22 sections; range, 12-44 sections) of the entire lesion in each resected specimen. Histologic grade was determined by the area of the tumor having the highest grade [1]. Residual tumor status was assessed clinically and histologically according to the AJCC Cancer Staging Manual [1].

Histologic determination of the primary site based on microscopic tumor invasion of the vasculo-biliary sheaths

Paraffin-embedded blocks (median, 4; range, 2-9) of the perihilar region including the vasculo-biliary sheaths from each resected specimen were used for double staining with hematoxylin-eosin and Victoria Blue (Victoria Blue staining

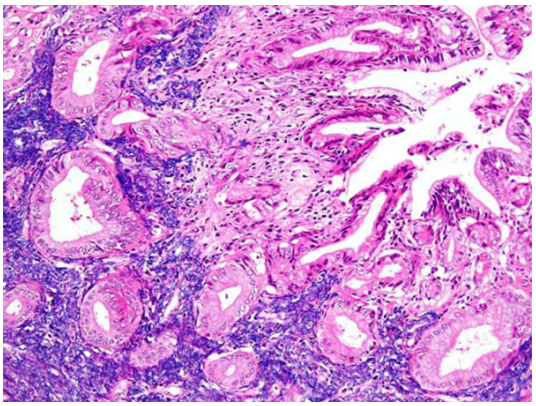

Figure 5. Intramucosal part of carcinoma over invasive carcinoma. Double staining with hematoxylin-eosin and Victoria Blue (original magnification × 200).

was used to detect elastic fibers [17]). Two serial 3-μm sections were recut and prepared from each block: one for hematoxylin-eosin staining and one for double staining with hematoxylin-eosin and Victoria Blue. Two independent pathologists blinded to the clinical details assessed each section.

Elastic fibers (**Figure 1**) of the vasculo-biliary sheaths (hilar plate and Laennec's capsule) between the hepatic hilus and liver parenchyma were detected using the double staining regimen, and the primary site of perihilar cholangiocarcinoma was determined based on the direction of microscopic tumor invasion. Perihilar cholangiocarcinomas involving both the hepatic hilus and the liver were classified into two types: an extrahepatic-type tumor involving the liver (**Figure 2A**) or an intrahepatic-type tumor involving the hepatic hilus (**Figure 2B**). Extrahepatic-type tumors were defined as tumors arising from the large hilar bile duct, with an extrahepatic component involving the liver through the vasculo-biliary sheaths (**Figure 3**). Intrahepatic-type tumors were defined as tumors arising from the intrahepatic bile ducts, with an intrahepatic component involving the hepatic hilus through the vasculo-biliary sheaths (**Figure 4**).

The mucosa adjacent to invasive carcinomas of the bile duct often shows carcinoma in situ [18-20], which has the histologic features of carcinoma without evidence of invasion into the lamina propria [18], whereas the intramucosal part of carcinoma (**Figure 5**) is often seen over invasive carcinoma. The existence of intramucosal part of carcinoma was also evaluated according to the primary tumor site, based on microscopic tumor invasion in the elastic fibers of the vasculo-biliary sheaths.

Prognostic factors

To elucidate factors influencing long-term survival of patients after surgical resection, 21 variables (**Table 1**) together with the primary tumor site (extrahepatic-type vs. intrahepatic-type) were tested in all 52 patients. The cutoff level for patient age (65 years) was based on the respective median values, whereas the size of the primary tumor (cutoff level, 3 cm) was determined according to the new staging system for perihilar cholangiocarcinoma proposed by DeOliveira et al [2]. The cutoff levels for preoperative serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were determined according to the relevant reference ranges (≤ 5 ng/mL and ≤ 37 U/mL, respectively).

Statistical analysis

Medical records and survival data were obtained for all 52 patients. Categorical variables were compared by Fisher's exact test or Pearson's χ^2 test. The causes of death were determined from the medical records. The follow-up period was defined as the interval from the resection to the last follow-up. The Kaplan-Meier method was used to estimate the cumulative incidences of cancer-specific survival and differences in these incidences were evaluated by the log-rank test. The Cox proportional hazards regression model was used to identify factors that were independently associated with survival. In this model, a stepwise selection was used for variable selection with entry and removal limits of $p < 0.05$ and $p > 0.1$, respectively. The stability of this model was confirmed using a step-backward and step-forward fitting procedure. The variables identified as having an independent influence on survival were identical using both procedures. All statistical evaluations were performed using the PASW Statistics 17 software package (SPSS Japan, Tokyo, Japan). All tests were two-sided and a p value < 0.05 was considered to indicate statistical significance.

Results

Surgical resection procedures

All 52 patients underwent a major hepatectomy with extrahepatic bile duct resection (**Table 1**).

Table 1. Clinicopathologic characteristics of patients according to the primary site of perihilar cholangiocarcinoma

Variable	No. of patients		p value
	Extrahepatic-type (n = 34)	Intrahepatic-type (n = 18)	
Age (≤ 65/> 65 years)	15/19	9/9	0.774
Gender (M/F)	23/11	13/5	> 0.999
Preoperative serum CEA level (≤ 5/> 5 ng/mL)	25/9	8/10	0.068
Preoperative CA19-9 level (≤ 37/> 37 U/mL)	11/23	3/15	0.329
Preoperative jaundice (absent/present)	7/27	16/2	< 0.001
Bismuth classification (I/II/III/IV)	3/1/20/10	0/0/12/6	0.610
Type of hepatectomy (hemihepatectomy/trisectionectomy)	33/1	15/3	0.114
Hepatectomy side (left-sided/right-sided)	17/17	14/4	0.076
Pancreaticoduodenectomy (absent/present)	26/8	18/0	0.039
Portal vein resection (absent/present)	29/5	10/8	0.040
Hepatic artery resection (absent/present)	33/1	16/2	0.272
Tumor size (≤ 3/> 3 cm)	10/24	4/14	0.746
pT classification (pT1-pT2/pT3-pT4)*	18/16	2/16	0.006
pN classification (pN0/pN1/pN2)*	16/9/9	9/6/3	0.806
pM classification (pM0/pM1)*	34/0	13/5	0.003
Histologic grade (G1/G2/G3)*	2/17/15	3/6/9	0.361
Lymphatic vessel invasion (absent/present)*	12/22	2/16	0.100
Vascular invasion (absent/present)*	18/16	2/16	0.006
Perineural invasion (absent/present)*	5/29	2/16	> 0.999
Residual tumor status (R0/R1)*	29/5	10/8	0.040
Adjuvant treatment (absent/present)	20/14	5/13	0.044

*According to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual [1]; Abbreviations: CEA (carcinoembryonic antigen); CA19-9 (carbohydrate antigen 19-9); pT classification (pathologic primary tumor classification); pN classification (pathologic lymph node metastasis classification); pM classification (pathologic distant metastasis classification); G1 (well differentiated); G2 (moderately differentiated); G3 (poorly differentiated); R0 (no residual tumor); R1 (microscopic residual tumor).

Table 2. Intramucosal part of carcinoma over invasive carcinoma according to the primary site of perihilar cholangiocarcinoma

Intramucosal part of carcinoma	No. of patients		p value
	Extrahepatic-type (n = 34)	Intrahepatic-type (n = 18)	
Extrahepatic site (absent/present)	16/18	18/0	< 0.001
Intrahepatic site (absent/present)	34/0	4/14	< 0.001

Hepatectomy procedures included hemihepatectomy in 48 patients and trisectionectomy in 4 patients. Of the 52 patients, combined major hepatectomy and pancreaticoduodenectomy was performed in 8 patients who had extrahepatic-type tumor with extensive ductal involvement. Pancreaticoduodenectomy procedures included the Whipple procedure in 3 patients and a pylorus-preserving pancreaticoduodenectomy in 5 patients. Fourteen patients also underwent a combined resection of contiguous tissues as follows: the portal vein (n = 13), hepatic artery (n = 3), transverse colon (n = 1), omentum (n = 1), and inferior vena cava (n = 1). In the 8 patients undergoing a Whipple or pylorus-preserving pancreaticoduodenectomy, the right portion of the superior mesenteric node group was also dissected.

Adjuvant treatment after surgical resection was administered at the discretion of the individual surgeon. Twenty-eight patients received adjuvant treatment consisting of oral or intravenous administration of 5-fluorouracil (8 patients) or intravenous administration of gemcitabine (18 patients). The remaining 2 patients with posi-

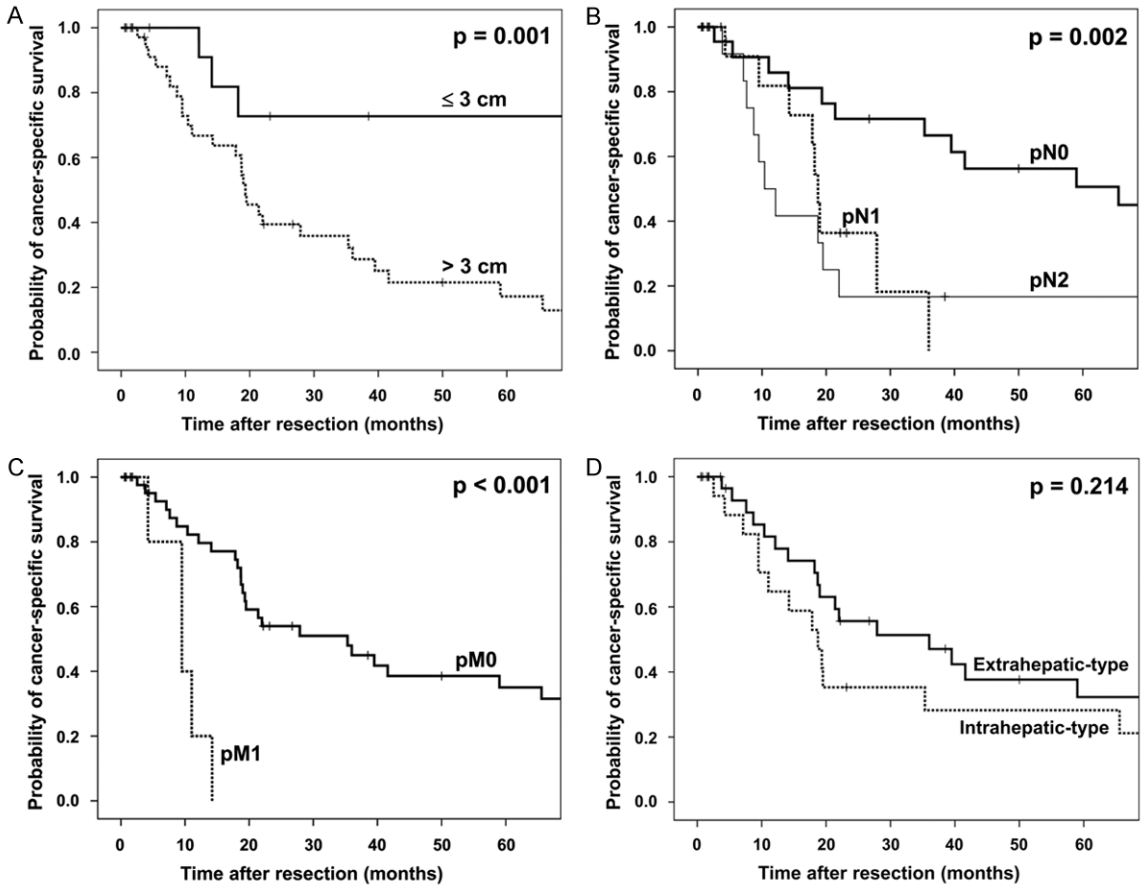

Figure 6. Kaplan-Meier cancer-specific survival estimates in 52 patients undergoing resection for perihilar cholangiocarcinoma. A. Tumor size ≤ 3 cm vs. tumor size > 3 cm (p = 0.001). B. pN0 vs. pN1 vs. pN2 (p = 0.002). C. pM0 vs. pM1 (p < 0.001). D. Extrahepatic-type tumor with hepatic involvement vs. intrahepatic-type tumor involving the hepatic hilus (p = 0.214). Abbreviations: pN (pathologic lymph node metastasis); pM (pathologic distant metastasis).

tive ductal resection margins received adjuvant local radiotherapy with (1 patient) or without (1 patient) systemic chemotherapy.

Pathologic evaluation of resected specimens

The median tumor size was 4.0 cm (range, 1.0-9.0 cm). Adenocarcinoma was identified as the primary tumor in all 52 patients. Thirty-nine patients had no residual tumor (R0), whereas microscopic residual tumor (R1) was found in the remaining thirteen patients. Residual tumor was found in the intrahepatic and/or extrahepatic ductal stump (10 patients), hepatectomy margins (2 patients), and around the preserved hepatic arteries (1 patient). Twenty-seven patients (52%) had pathologic lymph node metastasis (pN). Fifteen patients had pN1 disease and 12 had pN2 disease. Five patients had pathologically significant distant metastasis (pM) including local peritoneal seeding (n = 3) and ipsilateral liver metastasis (n = 2). Eleven patients had Stage II disease, 9 had Stage IIIA disease, 12 had Stage IIIB disease, 4 had Stage IVA disease, and 16 had Stage IVB disease.

Histologic determination of the primary site based on microscopic tumor invasion of the vasculo-biliary sheaths

Tumors were classified as having an extrahepatic component involving the liver (**Figure 3**) or having an intrahepatic component involving the hepatic hilus through the vasculo-biliary sheaths (**Figure 4**), based on the double staining with hematoxylin-eosin and Victoria Blue. Thirty-four patients had extrahepatic-type tumors with hepatic involvement and eighteen had intrahepatic-type tumors involving the

Table 3. Independent factors significantly influencing long-term survival after resection

Variable	No. of patients	Univariate analysis			Multivariate analysis	
		5-year survival rate (%)	MST (months)	p value	Hazard ratio (95% CI)	p value
Tumor size				0.001		
≤ 3 cm	14	73	NA		1.000	
> 3 cm	38	17	19		6.442 (1.992-20.831)	0.002
pN classification*				0.002		0.005
pN0	25	51	66		1.000	
pN1	15	0	19		1.162 (0.301-4.492)	0.828
pN2	12	17	10		4.956 (1.743-14.088)	0.003
pM classification*				< 0.001		
pM0	47	35	35		1.000	
pM1	5	0	10		4.027 (1.217-13.325)	0.023

*According to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual [1]; Abbreviations: MST (median survival time); CI (confidence interval); NA (not available); pN classification (pathologic lymph node metastasis classification); pM classification (pathologic distant metastasis classification).

hepatic hilus. The elastic fibers of the vasculobiliary sheaths remained even in the areas of invasive carcinoma (**Figure 3**) and were bent toward invasive areas of the hepatic hilus (**Figure 4**).

Clinicopathologic characteristics according to the primary site of perihilar cholangiocarcinoma

Comparison of clinicopathologic characteristics between the two groups (**Table 1**) revealed that preoperative jaundice was more frequent in patients with extrahepatic-type tumors ($p < 0.001$). Right or left hemihepatectomy with extrahepatic bile duct resection was the most common surgical procedure in both groups. Portal vein resection was significantly more frequent in patients with intrahepatic-type tumors ($p = 0.040$). Patients with intrahepatic-type tumors had more advanced disease according to the pT classification ($p = 0.006$), pM classification ($p = 0.003$), and vascular invasion ($p = 0.006$), and this group more frequently had microscopic residual tumors ($p = 0.040$) and received adjuvant treatment ($p = 0.044$). The incidences of histologic grade, lymphatic vessel invasion, and perineural invasion were comparable between the groups.

Intramucosal part of carcinoma over invasive carcinoma according to the primary site of perihilar cholangiocarcinoma

Intramucosal part of carcinoma over invasive carcinoma (**Figure 5**) was detected histologically in the resected specimens of 32 patients (**Table 2**). Intramucosal part of carcinoma was detected only at extrahepatic sites in 18 of 34 resected specimens of extrahepatic-type tumor, whereas it was detected at intrahepatic sites in 14 of 18 resected specimens of intrahepatic-type tumor. The presence of intramucosal part of carcinoma was significantly associated with the primary tumor site, based on microscopic tumor invasion of the elastic fibers of the vasculo-biliary sheaths in each type of perihilar cholangiocarcinoma ($p < 0.001$).

Factors influencing long-term survival after resection

The cumulative cancer-specific survival rates after resection in the current patient series were 31% at 5 years and 22% at 10 years (median survival time, 22 months). Univariate analysis revealed that preoperative CA19-9 level ($p = 0.001$), Bismuth classification ($p = 0.013$), type of resection ($p = 0.034$), hepatectomy side ($p = 0.029$), tumor size ($p = 0.001$; **Figure 6A**), pN classification ($p = 0.002$; **Figure 6B**), pM classification ($p < 0.001$; **Figure 6C**), and residual tumor status ($p = 0.012$) were significantly associated with cancer-specific survival. The primary site was not significantly associated with cancer-specific survival after resection ($p = 0.214$), as patients with extrahepatic-type tumors had a median survival time of 36 months with a cumulative 5-year survival rate of 32%, compared with 19 months and 28% for patients with intrahepatic-type tumors (**Figure 6D**). Even in the subgroup of 38 patients

with pM0 disease who underwent R0 resection, survival after resection was not significantly different between the 29 patients with extrahepatic-type tumors (median survival time, 40 months) and the 9 patients with intrahepatic-type tumors (median survival time, 66 months; p = 0.833).

Variables that were significant by univariate analyses were entered into multivariate analyses, which identified tumor size (p = 0.002), pN classification (p = 0.005), and pM classification (p = 0.023) as significant independent variables (**Table 3**).

Discussion

In 1965, Klatskin [4] reported 13 patients who had adenocarcinoma at the hepatic duct confluence within the porta hepatis, although 3 of the 13 patients had a bulky intrahepatic mass involving the hepatic confluence. Since then, Klatskin tumor has been used as a synonym for hilar cholangiocarcinoma. Recently, the term perihilar cholangiocarcinoma has been used clinically for all tumors involving or requiring resection of the hepatic confluence [2, 4-9]. In pathologic terms, perihilar cholangiocarcinoma can be classified into two types of tumor: an extrahepatic-type tumor or an intrahepatic-type tumor [3, 8, 9, 14], whereas in the clinical situation, it is not always possible to identify the primary site of perihilar cholangiocarcinoma (**Figure 2**) [3, 8, 9, 14]. This fact inspired the present study which focused on the histologic features of tumor invasion of the vasculo-biliary sheaths in order to discriminate between extrahepatic-type tumors with hepatic involvement and intrahepatic-type tumors involving the hepatic hilus. To our knowledge, this is the first study to demonstrate that double staining with hematoxylin-eosin and Victoria Blue allows the histologic determination of primary tumor site in clinical cases of perihilar cholangiocarcinoma, by differentiating between tumors of extrahepatic and intrahepatic origin.

In the surgical management of hepatic malignancy, anatomic resection after intrahepatic portal pedicle ligation at the hepatic hilus (posterior intrahepatic approach), based on first approaching the vasculo-biliary sheaths [10-12, 21-23], has been widely accepted over the past decade [24]. Couinaud [10-12] proposed the concept of the vasculo-biliary sheath including Walaeus's sheath, Glisson's sheath, Laennec's capsule, and the hilar plate, and this concept proved useful for surgical procedures involving hepatic malignancy, as well as for the histologic determination of the primary site in perihilar cholangiocarcinoma. It is not always possible to identify the precise site of tumor origin in the resected specimens of perihilar cholangiocarcinoma (**Figure 2**) or to discriminate between an extrahepatic origin and an intrahepatic origin through routine histologic examination using hematoxylin-eosin staining (**Figure 4A**). In the present study, based on microscopic tumor invasion around the elastic fibers of the vasculo-biliary sheaths, all perihilar cholangiocarcinomas could be divided clearly into extrahepatic-type or intrahepatic-type tumors. The elastic fibers of the vasculo-biliary sheaths remained even in the areas of invasive carcinoma (**Figure 3**) and were bent toward invasive areas of the hepatic hilus (**Figure 4**). These findings suggested that double staining with hematoxylin-eosin and Victoria Blue allows discrimination between an extrahepatic and intrahepatic origin and the histologic determination of the primary site in perihilar cholangiocarcinoma.

The mucosa adjacent to invasive carcinomas of the bile ducts often shows carcinoma in situ [18-20], whereas intramucosal part of carcinoma is often seen over invasive carcinoma (**Figure 5**). In the present study, the existence of intramucosal part of carcinoma over invasive carcinoma was also evaluated according to the primary tumor site, which was determined by microscopic tumor invasion of the elastic fibers of the vasculo-biliary sheaths, revealing that intramucosal part of carcinoma over invasive carcinoma is a critical histologic feature in reflecting the tumor origin.

In the present study, the characteristics of patients with extrahepatic-type tumors and intrahepatic-type tumors showed considerable overlap regarding age, gender, tumor size, histologic grade, lymphatic vessel invasion, and perineural invasion (**Table 1**). Right or left hemihepatectomy with extrahepatic bile duct resection was the most common surgical procedure in both groups (**Table 1**), with combined major hepatectomy and pancreaticoduodenectomy performed in 8 patients who had extrahepatic-type tumors with extensive ductal involvement. An extrahepatic-type tumor arises directly from

the ducts at the confluence, whereas an intrahepatic-type tumor initially arises from a unilateral intrahepatic bile duct and then invades the confluence [9]. Owing to this difference in development, preoperative jaundice was more frequent in patients with extrahepatic-type tumors compared with intrahepatic-type tumors (**Table 1**). As the clinical symptom of jaundice is absent in the majority of patients with intrahepatic-type tumors, this leads to a more advanced primary tumor at the time of detection for intrahepatic-type tumors. Therefore the significant difference in pT and pM classification between extrahepatic-type and intrahepatic-type tumors (**Table 1**), when staged according to the AJCC Cancer Staging Manual (7th edition), is not surprising [1].

In 2011, DeOliveira et al [2] proposed a new staging system for perihilar cholangiocarcinoma to standardize the reporting of this intractable disease, and thus allow comparisons of studies among centers or over time [5, 25-28]. In the proposed new staging system [2], the tumor size is labeled as T1 (< 1 cm), T2 (1-3 cm), or T3 (≥ 3 cm). In the present study, tumor size (cutoff level, 3 cm) was the strongest prognostic factor independently associated with survival after resection (**Table 3**), suggesting that the size of the primary tumor may stratify patients with perihilar cholangiocarcinoma in terms of prognosis after treatment.

Extrahepatic-type tumors and intrahepatic-type tumors are usually grouped together clinically under the term perihilar cholangiocarcinoma [2, 4-9], because they show similar features on cholangiography and require similar surgical management involving resection of the hepatic duct confluence, as found in the present study [3, 9]. However, Sano et al [8] reported that these two types of tumor appear to have different prognoses after hepato-biliary resection in 158 patients with perihilar cholangiocarcinoma. In contrast, Ebata et al [9] advocated that combining both types under the term perihilar cholangiocarcinoma is valid based on their series of 250 patients with perihilar cholangiocarcinoma, as these tumors have comparable biological behavior, with similar clinical management depending on stage and invasion. Thus, there is some controversy as to whether the concept of perihilar cholangiocarcinoma is valid. In the present study, focused on perihilar cholangiocarcinoma involving both the hepatic hilus and the liver, survival after resection was comparable between the two types of tumor (**Figure 6D**). Even in the subgroup of patients with pM0 disease who underwent R0 resection, survival after resection was not significantly different for the two types of tumor. The present study also concluded that the concept of perihilar cholangiocarcinoma including both types of tumor is valid clinically, in terms of comparable surgical outcomes with similar clinical management.

The main limitation of the current study was the retrospective analysis of a small number of patients. To our knowledge, however, this is one of the largest series dealing with the histologic determination of the primary site of perihilar cholangiocarcinoma involving both the hepatic hilus and the liver, and comparing survival data using multivariate analysis. We believe that the limitation did not significantly influence the outcome of the study as the differences between groups were too marked to have resulted from these biases.

In conclusion, determination of the primary site of perihilar cholangiocarcinoma is possible in clinical cases, by using double staining with hematoxylin-eosin and Victoria Blue to discriminate between tumors of extrahepatic and intrahepatic origin. Extrahepatic-type tumors feature an extrahepatic component involving the liver through the vasculo-biliary sheaths, whereas intrahepatic-type tumors feature an intrahepatic component involving the hepatic hilus through the vasculo-biliary sheaths. Combination of extrahepatic-type tumors and intrahepatic-type tumors under the term perihilar cholangiocarcinoma is valid clinically, as these tumors show comparable surgical outcomes with similar clinical management.

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Disclosure of conflict of interest

None.

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