Perineural Invasion and Preoperative Serum CA19-9 as Predictors of Survival in Biliary Tract Cancer

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Abstract. *Background: Billiary tract cancer requires invasive surgical procedures for cure, and the risk factors related to patient prognosis remain controversial. Patients and Methods: Out of the 111 patients who underwent resection of extrahepatic biliary tract tumors from 1986 to 2010, the records for 88 with both ampullary and extrahepatic bile duct cancer, which included all information for evaluation of the clinicopathological factors, were employed in a multivariate analysis. Results: On univariate analysis, significant prognostic factors of poor survival unrelated to TNM factors were preoperative biliary drainage, high preoperative CA19-9 value, high preoperative CEA value, lymphatic invasion, perineural invasion, macroscopic growth pattern, histology, operative procedures (surgery), tumor persistence, high postoperative CA19-9 value, and postoperative chemotherapy. On multivariate analysis, perineural invasion (p=0.025) was the only prognostic factor independent of stage, for survival of patients with biliary tract cancer including ampullary cancer. When ampullary cancer was excluded, both perineural invasion and preoperative CA19-9 were the remaining prognostic factors independent of stage. The combination of both factors can very accurately identify long-term and short-term survivors of biliary tract cancer. Conclusion: The present study, to our knowledge, for the first time shows that both perineural invasion and preoperative CA19-9 are important prognostic factors in biliary tract cancer, and this would be beneficial for clinical clarification of the optimal strategies for this type of cancer.*

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Key Words: Biliary tract cancer, perineural invasion, CA19-9, CEA.

Recent studies have shown an increased global incidence of biliary tract cancer (1). Surgical resection is the only method expected to cure this type of cancer. This aggressive malignancy is largely incurable (2), but a combination of improved staging, active biliary stenting, safe but extensive surgery to obtain negative margins, and newer techniques for chemoradiotherapy have improved outcomes for patients with this type of disease (3, 4). Biliary tract inflammation is a well-defined risk factor for biliary tract cancer (5, 6).

The primary tumor and tumor node metastasis (TNM) stage were found to be independent predictors of survival in biliary tract cancer (7-9). However, there is variation in multiple stages and loci at presentation, so that there are differences in the ablative operation procedure (10, 11) and adjuvant therapy (12-14). Therefore, follow-up for recurrence after surgical excision, as well as surveillance are important factors, but there are few definitive data regarding the biological traits of biliary tract cancer.

A practical clinical approach is based on criteria related to prognosis, yet various candidate prognostic factors independent of staging have been proposed. Many previous multivariate studies have suggested that perineural invasion (15), pancreatic invasion (15), respectability (16), postoperative adjuvant chemotherapy (17), lymphatic invasion (18), portal invasion (18), tumor differentiation (19), and preoperative carbohydrate antigen 19-9 (CA19-9) value (20) were candidate independent prognostic factors. However, individual studies have identified several different clinical factors, because they did not comprehensively include all such critical factors simultaneously in a multivariate study.

In the present study, all such candidate factors were analyzed in order to validate the clinical significance of independent prognostic factors in biliary tract cancer.

Patients and Methods

A total of 88 patients were registered for clinicopathological and prognostic analysis, out of the 111 patients who underwent surgical removal of tumors of the extrahepatic bile duct, including a portion of the ampullary region, at Kitasato University Hospital from 1986

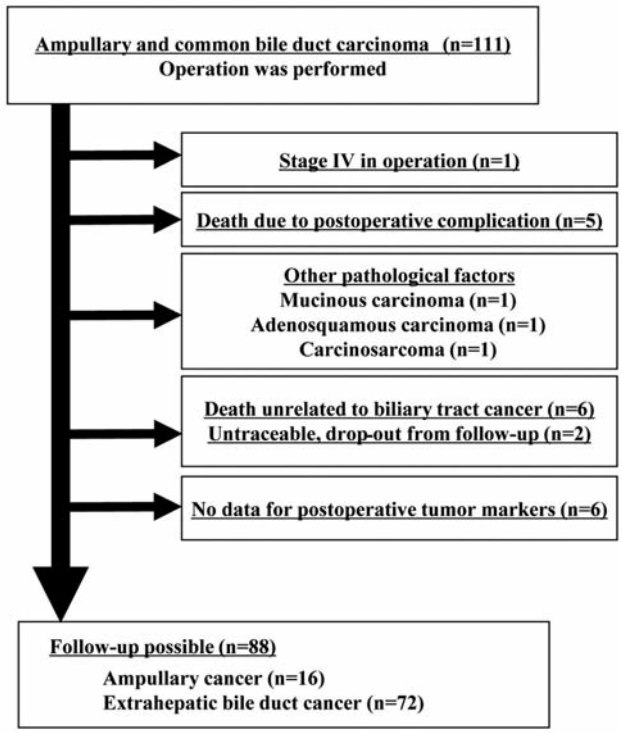

Figure 1. *Patient selection flowchart.*

to 2010. The patient selection flowchart is shown in Figure 1. The papilla of Vater of the ampullary region occurs at the outlet of the bile duct, and resectability of ampullary cancer is considered superior to that of other bile duct cancers (21, 22), but in actually resected cases, both ampullary cancer and other bile duct cancers have a similar prognosis, if the stage is the same in our data. Thus, the present study initially included ampullary cancer, as well as pure extrahepatic biliary tract cancers. Patients with tumors with distant metastases were initially excluded (Figure 1).

All 88 patients had complete information available for the 29 clinicopathological factors (Table I). The clinicopathological factors included six preoperative factors, nine TNM stage-determining pathological factors, eight pathological factors unrelated to TNM factors, and six treatment factors. The six preoperative factors were sex, age, preoperative jaundice, biliary drainage method, preoperative serum carcinoembryonic antigen value (pre CEA), and preoperative CA19-9 value (preCA19-9). The nine stage-determining factors, including stage itself, were the 6th Union for International Cancer Control (UICC) T-factor, hepatic infiltration (Hinf), gallbladder infiltration (Ginf), pancreatic invasion, duodenal infiltration (Du), portal venous invasion (PVI), arterial system invasion (AI), lymph node metastasis (the 6th UICC N), and 6th UICC stage. The eight non-stage-determining pathological factors were tumor location, lymphatic permeation (LY), vascular permeation (VP), perineural invasion (PNI), mode of histological infiltration (INF), volume of stromal connective tissue (stroma factor), macroscopic growth pattern, and histology (differentiation). The six treatment factors were operative procedure, residual tumor (R factor), postoperative CEA value (postCEA), postoperative CA19-9 value (postCA19-9), postoperative jaundice, and postoperative therapy. Prognostic analysis was performed for 5-year disease-specific survival (DSS).

The involved regions in the 88 patients were the *porta hepatis* bile duct (n=5), superior bile duct (n=3), central part bile duct (n=25), lower part bile duct (n=39), and papilla of Vater (n=16). Macroscopically, the growth pattern of extrahepatic bile duct cancer excluding the papilla type was subdivided into three types: (papillary, nodular, and flat), and that of papilla carcinoma was classified into tumor, mixed and ulcer type according to the General Rules for Surgical and Pathological Studies on Cancer of the Billiary Tract The 5th Edition (23). The operative methods included pancreatico-duodenectomy (PD) for 15 patients and pylorus-preserving PD for 59 patients. Other operative procedures for the remaining 15 patients included bile duct excision for 10 patients and hepatectomy for five, two of which also had concomitant PD. The distributions of the 6th UICC stage were 8, 5, 3, 14, 30, and 28 patients in stage 0, IA, IB, IIA, IIB, and III, respectively.

A total of 64/88 patients (72.3%) who underwent surgical resection received postoperative chemotherapy, and of these, two received concomitant radiotherapy. Postoperative chemotherapy was given to 3/8 stage 0 cases, 3/5 stage IA, all three stage IB, 10/14 stage IIA, 24/30 stage IIB, and 22/28 stage III cases. The adjuvant regimens consisted largely of 5-fluorouracil (5-FU)-based chemotherapy; (5-FU-only (n=4), 5-FU/cisplatin (n=4)); tegafur/uracil (UFT)-based chemotherapy ((UFT-only (n=16), UFT/mitomycin-C (n=2), UFT/radiotherapy (n=1)); or tegafur/gimeracil/oteracil potassium (S-1) (S-1-only (n=8) and S-1/radiotherapy (n=1)) as oral therapy, and others included mitomycin-C (n=2), doxifluridine (n=2), minocycline (n=1), and gemcitabine (gemcitabine-only (n=9), gemcitabine/5-FU/cisplatin (n=3), gemcitabine/S-1 (n=11), as venous infusion and as oral therapy.

Statistical analysis. The Kaplan Meier method was used to evaluate 5-year DSS, and differences in survival rates were assessed by the log-rank test. DSS was measured from the date of operation to the date of cancer death. The variables that had prognostic potential in the univariate analysis ($p<0.05$) were entered into the multivariate logistic analysis. A value of $p<0.05$ was considered significant. All statistical analyses were carried out with the SAS software package JMP version 9.0 (SAS Institute, Cary, NC USA).

Results

Identification of univariate prognostic factors in cancer of the extrahepatic biliary tract and ampullary regions. In order to determine the prognosis of biliary tract cancer, 88 patients with 72 extrahepatic biliary tract carcinomas and 16 ampullary carcinomas who underwent surgical resection at the Kitasato University Hospital were initially examined. The clinical and pathological characteristics of the 88 patients in this study (62 men and 26 women) are summarized in Table I. Their mean age was 65.3 years (range=38-84 years). Of the six preoperative factors, the factors conferring poor prognosis were biliary drainage procedures ($p=0.048$), high pre-CA19-9 ($p=0.0038$), and high pre-CEA ($p=0.0032$). Of the nine TNM pathologic of factors (6th UICC edition), the poor prognostic factors were high T-factor ($p=0.015$), Ginf ($p=0.0004$), PVI ($p<0.0001$), lymph node metastasis ($p=0.021$), and the higher

Table I. *Univariate analysis of clinicopathological factors affecting survival rate (ampullary and extrahepatic bile duct carcinoma).*

Factor category	Variables	No. of patients	Univariate analysis 5-year survival rate (%)	*p*-Value
Preoperative factor	Gender			
	Male/female	62/26	46.0/55.8	0.27+
	Age, years			
	<65, ≥65	37/51	63.6/34.7	0.14+
	Preoperative jaundice*			
	Absence/presence	46/42	45.8/53.7	0.34+
	Biliary drainage			
	ENBD•ERBD/PTCD•PTGBD/no drainage	50/25/13	51.6/33.9/69.2	0.048
	High value of preoperative serum CA19-9			
	Yes/no	32/56	74.0/35.0	0.0038
	High value of preoperative serum CEA			
	Yes/no	85/3	50.5/33.3	0.0032
Pathological factor	T-factor			
According to the sixth UICC TNM	Tis/T1/T2/T3/T4	9/10/9/32/28/	100.0/56.0/54.7/44.6/32.6	0.015
	Hepatic infiltration			
	Absence/presence	84/4	51.5/0.0	0.113+
	Gallbladder infiltration			
	Absence/presence	80/8	53.6/0.0	0.0004
	Pancreatic infiltration			
	Absence/presence	38/50	53.5/46.0	0.39+
	Duodenal side infiltration			
	Absence/presence	56/32	47.8/51.5	0.94+
	Portal venous invasion			
	Absence/presence	82/6	53.4/0.0	<0.0001
	Arterial system invasion			
	Absence/presence	87/1	48.5/100.0	0.39+
	Lymph node metastasis			
	Absence/presence	41/47	60.9/40.0	0.021
	UICC Stage			
	0/IA/IB/IIA/IIB/III	8/5/3/14/30/28	8/40.0/66.7/60.0/50.6/32.6	0.019
Pathological factor	Location			
(non-TNM factors)	A/Bi/Bm/Bs/Bp	16/39/25/3/5	79.3/52.4/32.9/0.0/50.0	0.11+
	Lymphatic permeation			
	Absence/presence	19/69	81.5/42.9	0.013
	Vascular permeation			
	Absence/presence	25/63	58.9/45.3	0.25+
	Perineural invasion			
	Absence/presence	31/57	84.7/29.2	<0.0001
	Mode of histological infiltration			
	Alpha/beta/gamma	6/61/21	66.7/50.7/38.5	0.24+
	Volume of stromal connective tissue			
	Medullary/intermediate/scirrhous	12/65/11	50.0/52.0/30.7	0.37+
	Macroscopic growth pattern			
	(Bile duct) Papillary/nodular/flat	12/26/34	12.7/49.6/43.7	0.034
	(Ampullary) Tumor/mixture/ulcer	8/7/1	83.3/100.0/100.0	
	Histology			
	Tub1/Tub2/Tub3/Pap	38/26/9/15	62.2/41.7/18.8/49.9	0.041
Treatment factor	Operative procedures			
(postoperative factor)	PD•PpPD /other	74/14	57.7/8.5	0.0020
	Resection status			
	R0-R1/2	53/35	57.9/35.8	0.033
	High levels of postoperative serum CA19-9			
	Yes/no	68/20	57.5/20.3	0.012
	High levels of postoperative serum CEA			
	Yes/no	86/2	47.8/100.0	0.23+
	Postoperative jaundice			
	Yes/no	85/3	48.4/66.7	0.68+
	Postoperative therapy			
	Gemcitabine/others/no therapy	23/42/23	52.7/37.0/61.7	0.033

*Preoperative jaundice was defined according to the state after drainage treatment. +Not significant.

Table II. *Multivariate prognostic analysis in biliary tract cancer (ampullary and extrahepatic bile duct carcinoma).*

Clinicopathological factor	Category	RR	95% CI	*p*-Value
UICC Stage (6th edition)	0	Reference		0.036
	IA	14.6		
	IB	16.1		
	IIA	3.32		
	IIB	9.25		
	III	15.9		
Perineural invasion	Absence	Reference		0.007
	Presence	2.2	1.22-4.41	
High value of preoperative serum CA19-9	Absence	Reference		0.070*
	Presence	1.67	0.96-3.08	
Lymphatic permeation	Absence	Reference		0.47*
	Presence	1.58	0.47-6.01	
Biliary drainage	No drainage	Reference		0.81*
	ERBD/ENBD	0.92	0.48-1.77	
	PTCD/PTGBD	1.22	0.65-2.43	
Histology	Tub1	Reference		0.27*
	Tub2	1.33	0.67-2.63	
	Tub3	1.59	0.58-4.13	
	Pap	0.77	0.32-1.70	
Resection status	R0	Reference		0.24*
	R1/2	0.74	0.45-1.22	
High value of postoperative serum CA19-9	Absence	Reference		0.21*
	Presence	1.31	0.82-2.14	
Postoperative therapy	No therapy	Reference		0.087
	Gemcitabine	0.64	0.29-1.36	
	others	1.76	0.83-2.25	

*Not significant.

UICC stage (p=0.019). Out of the eight non-TNM pathological factors, the significant prognostic factors were LY (p=0.013), PNI (p<0.0001), macroscopic growth (p=0.034), and histological diagnosis (p=0.041). Finally, out of the six treatment factors, poor prognostic factors were operative procedures (p=0.0020), R1/2 (p=0.033), high postCA19-9 (p=0.012), and postoperative therapy (p=0.033).

Multivariate prognostic analysis of 88 biliary tract cancers including ampullary cancer. Significant prognostic factors that were not attributed to UICC staging factors were entered into a multivariate analysis. PreCEA and macroscopic findings were excluded from the multivariate analysis, because few patients had a high preCEA value (n=3), and macroscopic findings were defined separately for extrahepatic biliary duct cancer and ampullary cancer. Operative procedures were also excluded from the multivariate analysis, because surgery (other than PD) was not appropriate as a reference due to heterogeneous patient distribution (with good prognosis and poor prognosis assumed). A multivariate proportional hazards model was applied to the remaining nine factors. PNI was the only prognostic factor (p=0.007) independent of the 6th UICC stage (p=0.036) (Table II). The Kaplan Meier curve according to PNI status for the 88 cases is shown in Figure 2a.

Univariate prognostic analysis restricted to the 72 cases of extrahepatic biliary tract cancer. In order to determine the prognosis of pure extrahepatic biliary tract cancer excluding ampullary cancer, the analysis was then restricted to the 72 cases of extrahepatic biliary tract cancer. The clinical and pathological characteristics of these cases are summarized in Table III. Their mean age was 65.3 years (range=38-84 years). Of the six preoperative factors, the only significant factor was high preCA19-9 value (p=0.034). Of the nine TNM pathological factors, the significant factors were Ginf (p=0.0020), PVI (p<0.0001), and lymph node metastasis (p=0.011); stage was not a significant prognostic factor. Out of the eight non-TNM pathological factors, the only significant one was PNI (p=0.0037). Finally, of the six treatment factors, operative procedures were significant (p=0.013).

Multivariate prognostic analysis of the 72 cases of extrahepatic biliary tract cancer. The identified univariate prognostic factors not ascribed to UICC stage were then entered into a multivariate analysis. The factors significantly (p<0.05) associated with DSS in a univariate manner were preCA19-9, PNI, and postoperative therapy. A multivariate proportional hazards model was applied to these three

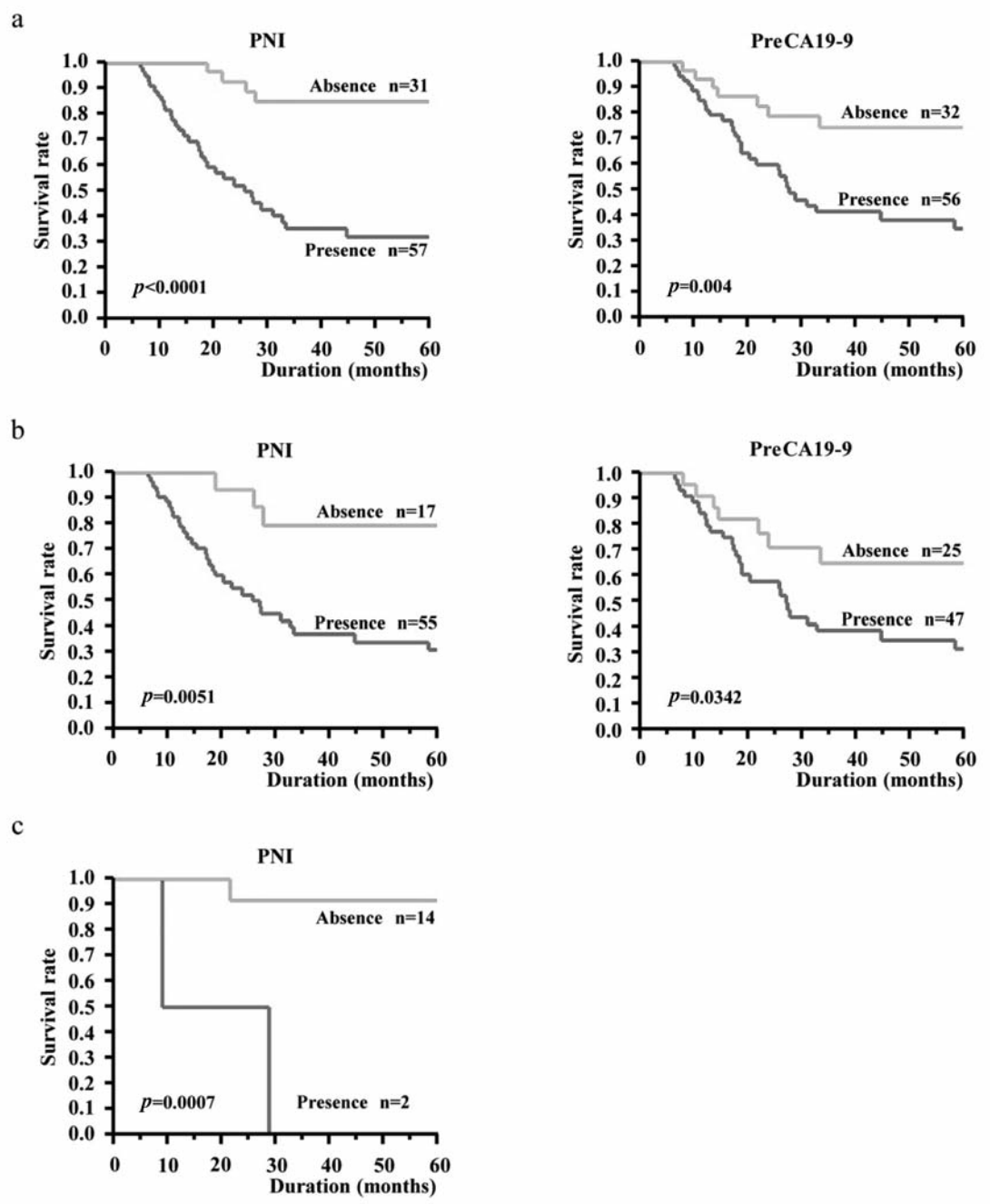

Figure 2. *Five-year disease-specific survival of patients with perineural invasion (PNI) and high preoperative serum CA19-9 value (preCA19-9). a: Patients with ampullary and extrahepatic bile duct cancer. b: Patients with extrahepatic bile duct cancer-only. c: Patients with ampullary cancer.*

factors, as well as stage. Both high preCA19-9 value (p=0.047) and PNI (p=0.019) were the only significant prognostic factors independent of the 6th UICC stage (Table IV). Kaplan Meier curves according to PNI and preCA19-9 value in the 72 cases are shown in Figure 2b. The prognostic curve according to preCA19-9 value in biliary tract cancer, including ampullary cancer, is shown in Figure 2a.

Clinical significance of PNI and preCA19-9 value in biliary tract cancer. In the present study, PNI was found to be a reproducibly, independent prognostic factor in biliary tract cancer, irrespective of the inclusion of ampullary cancer. Although ampullary carcinomas were few in number (n=16), two patients with PNI were included, and they had a dismal prognosis (Figure 2c, p=0.0007). Ampullary cancer included

Table III. *Univariate analysis of clinicopathological factors affecting survival rate (extrahepatic bile duct carcinoma).*

Factor category	Variable	No. of patients	Univariate analysis 5-year survival rate (%)	*p*-Value
Preoperative factor	Gender			
	Male/female	52/20	38.8/49.3	0.32+
	Age, years			
	<65/≥65	30/42	58.8/25.4	0.064+
	Preoperative jaundice*			
	Absence/presence	40/32	42.3/41.5	0.75+
	Biliary drainage			
	ENBD•ERBD/PTCD•PTGBD/no drainage	40/22/10	45.5/29.1/60.0	0.13+
	High value of preoperative serum CA19-9			
	Absence/presence	25/47	64.5/31.4	0.034
	High value of preoperative serum CEA			
	Absence/presence	70/2	42.7/50.0	0.18
Pathological factor	T factor			
According to the sixth UICC TNM	Tis/T1/T2/T3/T4	5/10/6/26/25	100.0/56.0/0.0/37.1/32.8	0.083+
	Hepatic infiltration			
	Absence/presence	68/4	44.6/0.0	0.20+
	Gall bladder infiltration			
	Absence/presence	64/8	46.9/0.0	0.0020
	Pancreatic infiltration			
	Absence/presence	31/41	39.9/43.6	0.89+
	Duodenal side infiltration			
	Absence/presence	52/20	45.6/41.5	0.54+
	Portal venous invasion			
	Absence/presence	67/5	48.3/0.0	<0.0001
	Arterial system invasion			
	Absence/presence	71/1	43.6/100.0	0.35+
	Lymph node metastasis			
	Absence/presence	36/36	58.4/31.1	0.011
	UICC Stage			
	0/IA/IB/IIA/IIB/III	9/5/3/14/30/27	100.0/40.0/50.0/66.7/36.5/29.8	0.097+
Pathological factor	Location			
(non-TNM factor)	Bi/Bm/Bs/Bp	39/25/3/5	52.4/39.5/0.0/50.0	0.26+
	Lymphatic permeation			
	Absence/presence	14/58	70.0/40.6	0.051+
	Vascular permeation			
	Absence/presence	21/51	49.3/43.1	0.36+
	Perineural invasion			
	Absence/presence	17/55	79.0/33.8	0.0037
	Mode of histological infiltration			
	Alpha/beta/gamma	3/49/20	33.3/47.0/40.6	0.89+
	Volume of stromal connective tissue			
	Medullary/intermediate/scirrhous	8/53/11	28.6/50.5/30.7	0.60+
	Macroscopic growth pattern			
	(Bile duct) Papillary/nodular/flat	12/26/34	14.6/56.9/43.7	0.13+
	Histology			
	Tub1/Tub2/Tub3/Pap	31/22/9/10	59.6/42.3/18.8/35.0	0.16+
Treatment factor	Operative procedure			
(postoperative factor)	PD •PpPD/others	58/14	51.6/8.5	0.013
	Resection status			
	R0 - R1/2	39/33	49.6/38.8	0.42+
	High levels of postoperative serum CA19-9			
	Yes/no	55/17	50.1/29.1	0.081+
	High levels of postoperative serum CEA			
	Yes/no	69/3	42.6/100.0	0.18+
	Postoperative jaundice			
	Yes/no	69/3	43.3/66.7	0.54+
	Postoperative therapy			
	Gemcitabine/others/no therapy	18/37/17	47.3/30.5/53.9	0.037

*Preoperative jaundice was defined according to the state after drainage treatment. +Not significant.

Table IV. *Multivariate prognostic analysis in biliary tract cancer restricted to extrahepatic bile duct carcinoma.*

Clinicopathological factor	Category	RR	95% CI	*p*-Value
UICC Stage (6th edition)	0	Reference		0.069*
	IA	7.20		
	IB	17.7		
	IIA	4.75		
	IIB	10.2		
	III	13.3		
High value of preoperative serum CA19-9	Absence	1.51	1.01-2.44	0.047
	Presence			
Perineural invasion	Absence	1.92	1.10-4.03	0.019
	Presence			
Postoperative therapy	No therapy	Reference		0.065*
	Gemcitabine	0.62	0.29-1.21	
	Other	1.72	1.07-2.88	

+Not significant.

fewer patients with PNI (2/16) than extrahepatic biliary tract cancer (17/72; 23.6%), but the difference between the two types of cancer was not significant.

Out of the 88 biliary tract carcinomas, positive rates of PNI were examined in each stage (Figure 3a). Interestingly, PNI was infrequently found in stage 0 (12.5%), but the PNI-positive rate increased abruptly in stage I (IA+IB) and was constant through stage I to III. These findings suggest that PNI is not a prognostic factor as stage progresses, but it rather represents an aggressive biological trait. Interestingly, the preCA19-9 value also had a similar pattern (Figure 3a).

If restricted to the 72 cases of extrahepatic biliary tract cancer, the rates of PNI in each stage were also compared (Figure 3b). Again, PNI was infrequently found in stage 0 (20%), but the PNI-positive rate increased abruptly in stage I (IA+IB) and was constant through stage I to III. The preCA19-9 value had also a similar pattern in extrahepatic biliary tract cancer (Figure 3b).

Utility of PNI and preCA19-9 values in combination, for survival prediction in extrahepatic biliary tract cancer. When PNI and preCA19-9 values were combined to predict survival of patients with biliary tract cancer, irrespective of inclusion of ampullary cancer, prognosis was clearly predicted (Figure 4a and 4b). Cases negative for both survived five years, while those positive for both had a dismal prognosis. Patients were then sub-classified according to stage (stage 0-IIA, IIB, and III) in extrahepatic bile duct cancer, and the prognostic stratification is shown in Figure 4c-e, respectively. Using both factors, both patients with a dismal prognosis and long-term survivors were predicted. Representable histology of extrahepatic billiary tract cancer positive and negative for PNI are shown in Figure 5a and b, respectively.

Discussion

In this study, numerous multivariate prognostic factors that have been reported in extrahepatic biliary tract and ampullary cancer were investigated. The sixth UICC T-factor is defined by supposed prognostic factors such as Hinf, Ginf, pancreatic invasion, Du, PVI, and AI, as well as depth of invasion. Prognostic factor candidates unrelated to the sixth UICC TNM stage-related factors were the focus of this analysis, in order to identify novel prognostic factors independent of stage. Of the UICC T-factors, for example, Ginf was the strongest univariate potent prognostic factor, but it was not included in the multivariate analysis. Out of the T-factors, depth of invasion and PVI were also significant univariate prognostic factors, as in previous reports (18, 24), but other factors (Hinf, pancreatic invasion-Panc, Du, AI) did not show prognostic relevance in the present analysis (Table I). This finding is, at least partially, consistent with recent reports, especially in terms of Du (25, 26). Although Du usually occurs after Panc, it is not supposed to be a significant prognostic factor in the presence of Panc (25). On the other hand, Panc was identified as an independent prognostic factor in biliary tract cancer (15, 27-29). The present negative result in terms of Panc involvement in a poor prognosis may be due to the fact that cases with superficial pancreatic invasion showed significantly better survival than those with deep pancreatic invasion ($p<0.001$) (28).

Numerous previous reports with similar concepts actually found that many different prognostic factors were independent of staging factors. Of such reported factors, the most reproducible ones were PNI, tumor margin status, and adjuvant chemotherapy. PNI was identified as an independent prognostic factor in extrahepatic bile duct cancer in eight multivariate analyses (15, 27, 30-35), while a few multivariate studies

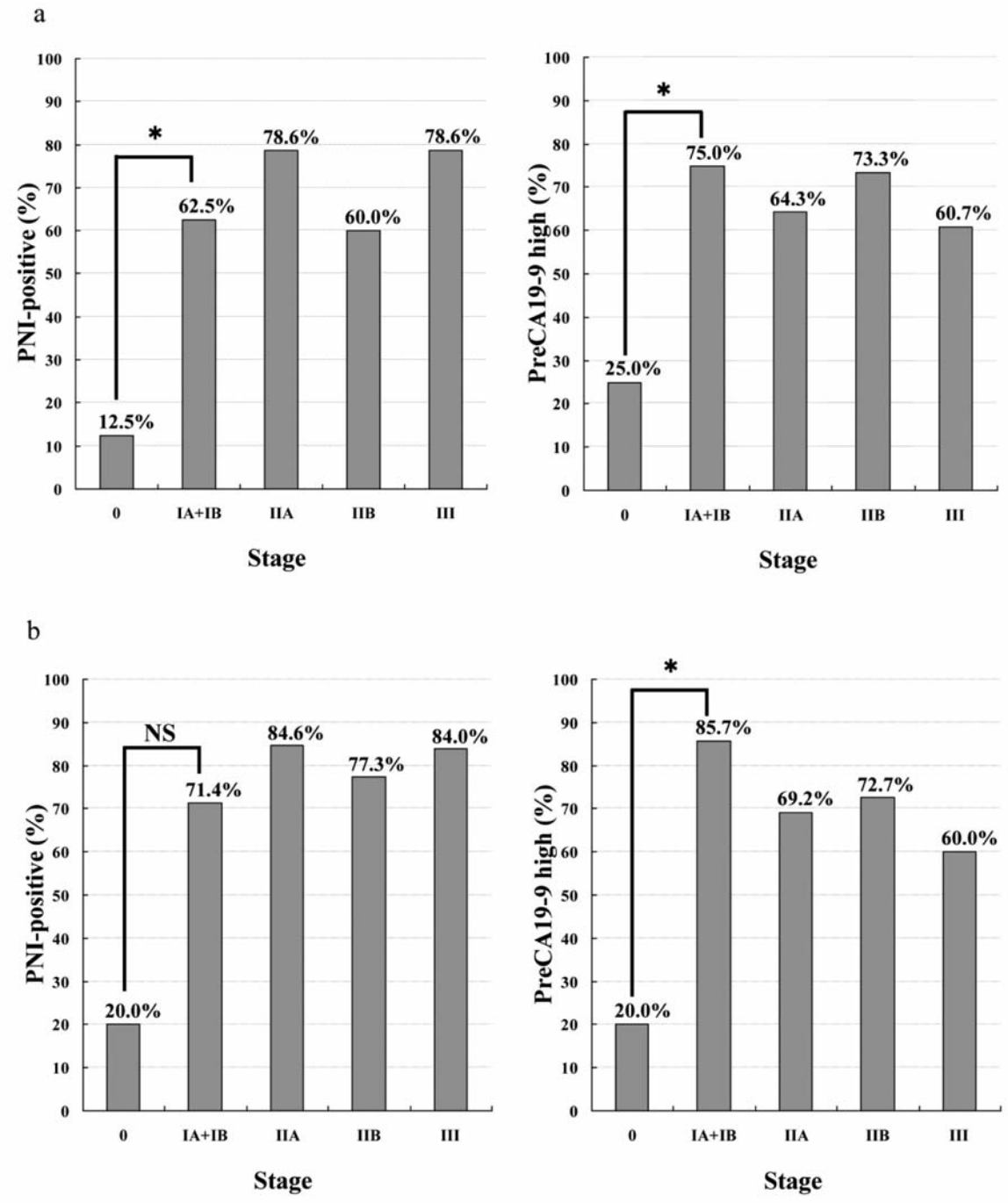

Figure 3. *Positive rates of PNI and preCA19-9 in ampullary and extrahepatic bile duct cancer in each stage. a: Patients with ampullary and extrahepatic bile duct cancer. b: Patients with extrahepatic bile duct cancer-only. PNI- and preCA19-9-positive rates abruptly increase in stage IA+IB and are constant through stages IA to III. The increase in the prevalence or each stage of PNI and high preCA19-9 is the same for the group of all patients and for those with extrahepatic bile duct cancer-only patients. *p<0.05.*

eliminated it as an independent prognostic factor (29, 36). The present analysis, again supported the former studies and interestingly, the preCA19-9 value could be an additional independent prognostic factor in extrahepatic biliary cancer. No previous report included both factors in a multivariate study. To the best of our knowledge the present study is first to achieve excellent prognostic stratification with the combination of the independent prognostic factors PNI and preCA19-9

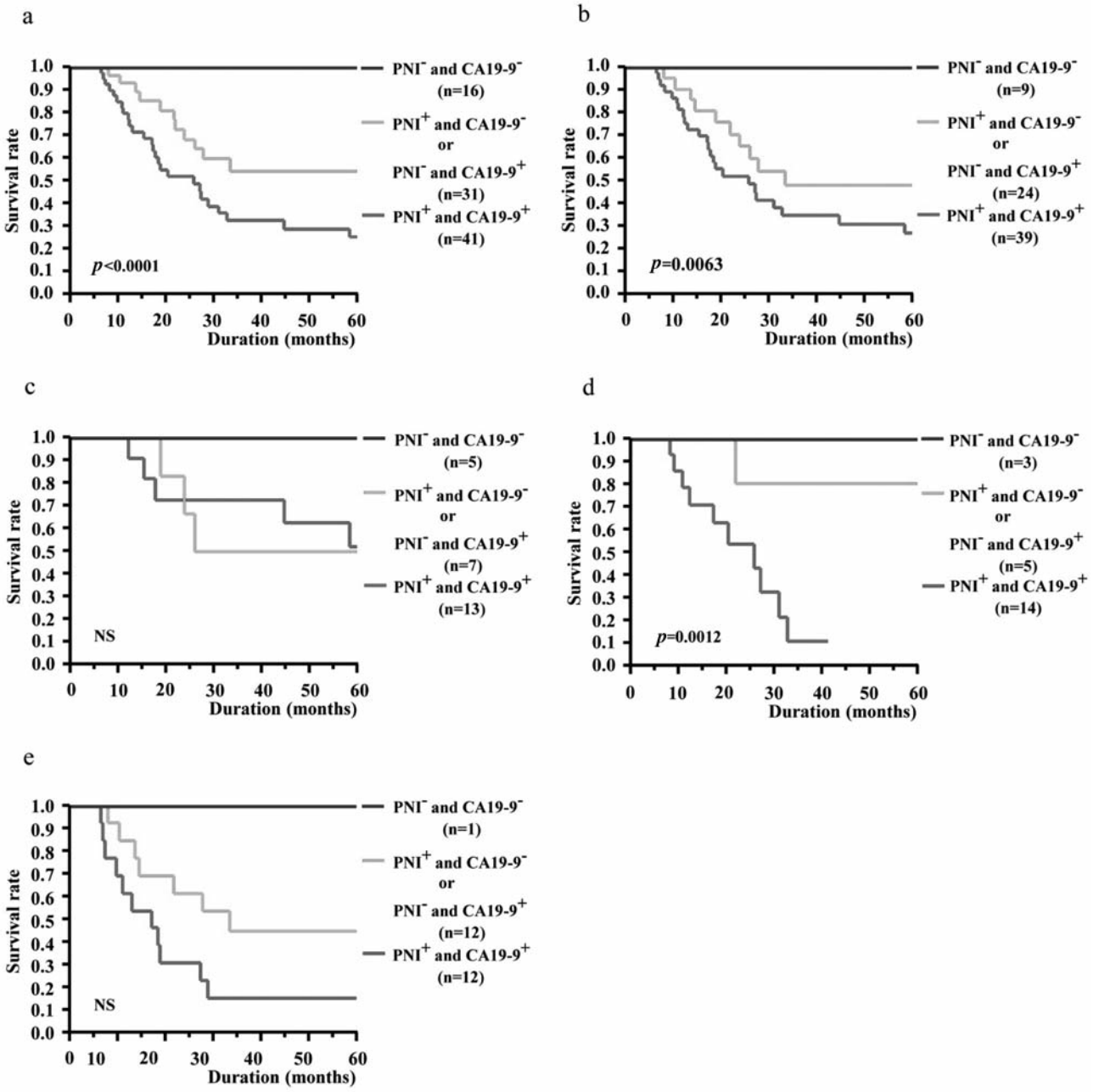

Figure 4. *Combinations of PNI and preCA19-9 were assigned to the stage A, B, and C groups. a: Patients with ampullary and extrahepatic bile duct cancer. b: Patients with extrahepatic bile duct cancer-only. c: Patients with extrahepatic bile duct cancer with UICC stage 0, IA, IB or IIA. d: Patients with extrahepatic bile duct cancer with UICC stage IIB. e: Patients with extrahepatic bile duct cancer with UICC stage III.*

value (Figure 4). Interestingly, the positive rates for both factors were low in pStage 0, but those for both factors were significantly elevated in pStage I and constant through pStage I to III (Figure 3). Patients with tumors negative for both factors showed extraordinarily excellent outcomes, while those with tumors positive for both had a dismal prognosis (Figure 4). Such data would be useful in determining the postoperative surveillance plan. The present data also showed a similar result even in biliary tract cancer including ampullary cancer and in ampullary cancer-alone. Both PNI and pre-CA19-9 value were reported to be independent prognostic factors in ampullary cancer studies (37,38). The present data therefore suggest the clinical utility of PNI and preCA19-9 value in biliary tract cancer including ampullary cancer.

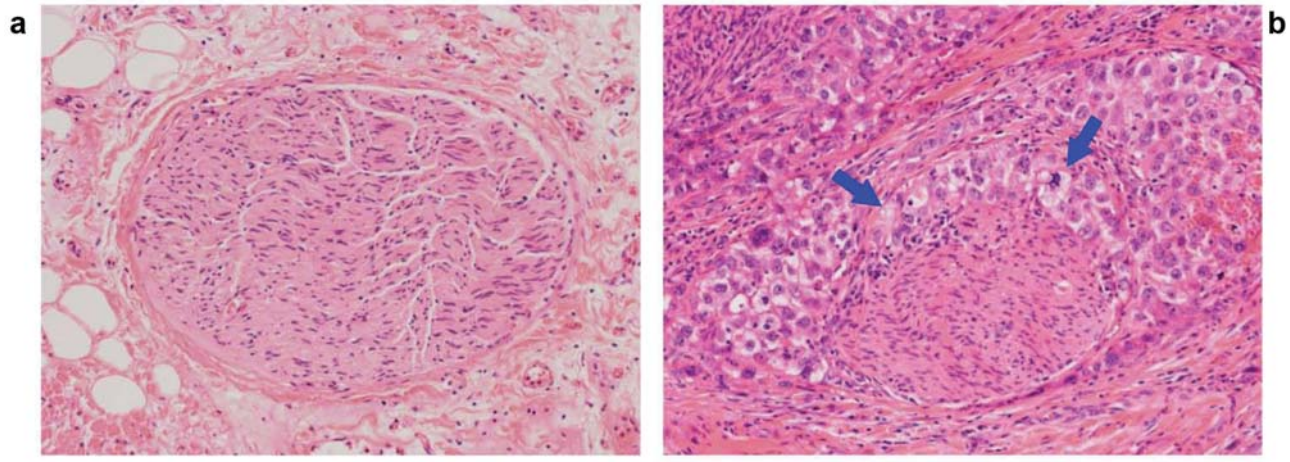

Figure 5. *Perineural invasion (PNI), as demonstrated by hematoxylin and eosin staining is shown in both negative and positive cases. a: Normal gangliocyte with normal perineurium had in a PNI-negative case. b: PNI-positive case showing that cancer cells remarkably invaded the inside of the perineurium.*

The preCA19-9 value itself was associated with poor outcomes in biliary tract cancer, as we have also proposed it to be a critical prognostic factor in stage IV gastric cancer (39), stage IV colorectal cancer (40), and pancreatic cancer (41). These data would allow for the classical hypothesis that cancer cells that express the CA19-9 antigen have enhanced extravasation and metastasis by interaction with E-selectin expressed on the endothelium (42). Recent publications additionally propose alternative emerging mechanisms of serum CA19-9 action, involving systemic dissemination of cancer cells. 1: Mucins expressing CA19-9 were associated with the induction of inflammatory molecules such as interleukin-6 and prostaglandin (PGE-2) in human cancer, and such an inflammatory state is a prerequisite for systemic metastasis (43, 44). Actually, mucins capable of presenting the CA19-9 antigen (45, 46) and the CA19-9 antigen itself (47) were reported to be abundantly expressed in primary biliary tract cancers and had prognostic relevance. 2: P-Selectin, another ligand specific for serum CA19-9, may also be involved in promoting tumor aggregation with platelets, leading to systemic cancer spread (48, 49).

Surgical margin status is one of the most reproducible independent prognostic factors in biliary tract cancer (16-19, 29, 36, 47, 50-55). However, surgical margin status was eliminated after the multivariate analysis in this study. Surgical margin status was usually defined as any margin status (bile duct stump in the hepatic and pancreatic direction and in the vertical direction). However, only one report restricted to the bile duct margin in the hepatic direction, found it to be an independent prognostic factor in biliary tract cancer (36). In contrast to this report, Sakamoto *et al*. (24) proposed that the bile duct margin in the hepatic direction could not be a prognostic indicator; they insisted that surgeons should make efforts to obtain negative radial margins after repeated additional resections. In our current study, the distal (duodenal) and exposure (vertical) margin status was a univariate prognostic factor, but the proximal margin status (bile duct in the duodenal direction) was not. In the present multivariate analysis, the status of both margins was eliminated as an independent prognostic factor (data not shown). The present analysis, thus, suggests that biological aspects representing both PNI-positive and high preCA19-9 values contribute to poor prognosis more than margin status does by itself. Previous studies that mentioned that margin status could be an independent prognostic factor did not include both factors simultaneously. The small contribution of margin status to prognosis might be explained by another concern regarding the quality of the margin status. Margins representing either carcinoma *in situ* or invasive carcinoma were clinically separate, because the specific status of each was different from a prognostic point of view (56-58), and our analysis included both as the same entity together.

Finally, this was a retrospective study with 88 patients that included all possible prognostic marker candidates. PNI and preCA19-9 values were found to be worthy of clinical attention from a prognostic point of view in biliary tract cancer. This information would be helpful to identify patients with recurrence or long-term survivors in an outpatient center and to determine postoperative surveillance and treatment plans in biliary tract cancer, although prospective validation is still needed.

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Received November 6, 2012
Revised November 21, 2012
Accepted November 26, 2012