Impact of residual in situ carcinoma on postoperative survival in 125 patients with extrahepatic bile duct carcinoma

Yoshitsugu Nakanishi, M.D.[1, 2, 4]; Satoshi Kondo, M.D.[1]; Yoh Zen, M.D.[4]; Atsuya Yonemori, M.D.[1,2]; Kanako Kubota, M.D.[2]; Hiroshi Kawakami, M.D.[3]; Eiichi Tanaka, M.D.[1]; Satoshi Hirano, M.D.[1]; Tomoo Itoh, M.D.[2]; and Yasuni Nakanuma, M.D.[4]

[1)]Department of Surgical Oncology, Hokkaido University Graduate School of Medicine, Sapporo; [2)]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo; [3)]Department of Gastroenterology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-Ku, Sapporo 060-8638; [4)]Department of Human Pathology, Kanazawa University Graduate School of Medicine, 13-1 Takaramachi, Kanazawa 920-8640, Japan

Corresponding author: Yoshitsugu Nakanishi, M.D.
Department of Surgical Oncology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan
Tel: 81-11-706-7714; Fax: 81-11-706-7158; E-mail: y.nakanishi@mac.com

Short title: Impact of remnant in situ carcinoma

Running title: Impact of remnant in situ carcinoma of extrahepatic cholangiocarcinoma

Key words: carcinoma in situ; superficial spread; extrahepatic bile duct carcinoma; prognosis

ABSTRACT

Purpose: The aim of this study was to determine the impact of the presence of carcinoma in situ at the bile duct stump on postoperative survival in patients who underwent resection of extrahepatic bile duct carcinoma.

Methods: The patients with resected extrahepatic bile duct carcinoma were divided into three groups according to resected margin status: no evidence of residual carcinoma (Negative group, n=96); carcinoma in situ at the bile duct stump (CIS group, n=10); and invasive carcinoma at any surgical margin (Invasive group, n=19). Cause-specific survival for these groups was compared statistically.

Results: Surgical margin status was identified as a prognostic factor on univariate analysis (p=0.005) and was an independent prognostic factor on multivariate analysis (p=0.018). The CIS group displayed significantly better survival than the Invasive group (p=0.006), and the survival was comparable to that for the Negative group (p=0.533). Two of three patients in the CIS group with local recurrence died >5 years after surgical resection.

Conclusions: Patients with positive ductal margins of carcinoma in situ of the extrahepatic bile duct do not appear to show different survival after resection compared to patients with negative margins, but remnant carcinoma in situ is likely to develop late local recurrence.

INTRODUCTION

Many studies have reported positive surgical margins as an important predictor of poor prognosis in patients with extrahepatic bile duct carcinoma.[1-5] However, several reports have found that prognosis for patients with a positive bile duct stump does not differ significantly from that of carcinoma-negative patients, and some reports have even described long-term survival of patients with a positive surgical bile duct stump.[6,7]

In most studies dealing with the postoperative prognosis of extrahepatic bile duct carcinoma patients, status of the surgical bile duct stump has been histologically classified into only two categories: negative or positive. However, histologically positive surgical bile duct stumps can be further subclassified into two subtypes: invasive carcinoma and carcinoma in situ. Extrahepatic bile duct carcinoma is sometimes associated with more than high-grade atypical intraepithelial cells that are continuously adjacent to the main tumour, representing what is called the superficial spread of extrahepatic bile duct carcinoma.[8-12] However, to the best of our knowledge, only three reports have described the impact of remnant carcinoma in situ at the bile duct stump on postoperative survival,[13-15] and the effects of remnant carcinoma on postoperative course remain unclear.

In this study, status of the surgical margins and postoperative course were evaluated in 125 patients with extrahepatic bile duct carcinoma to elucidate the influences of remnant carcinoma in situ on postoperative survival.

MATERIALS AND METHODS

Extrahepatic bile duct carcinoma patients

First, patients in whom the epithelium at the bile duct stump was exfoliated and the nearest epithelium represented carcinoma in situ were excluded from this study, since such patients were impossible to classify by surgical margin status. As a result, the population for this study comprised a total of 125 patients (100 men, 25 women) who underwent surgical resection for invasive extrahepatic bile duct carcinoma in the Second Department of Surgery, Hokkaido University Hospital between December 1989 and January 2007. Tumours arising from the ampulla of Vater and cystic duct were not included in this study.

Tissue preparation

All surgically resected specimens and intraoperative frozen sections of bile duct stumps, if performed, were fixed in 10% buffered formalin. Serial sections at 3- to 6-mm intervals were prepared from the entire area of the extrahepatic bile duct.

Classification of surgical margins

In this study, patients were classified into three groups according to resected margin status:

Negative group (n=96); CIS group (n=10); and Invasive group (n=19). The CIS group comprised cases with carcinoma in situ at the bile duct stump, but no invasive carcinoma at any surgical margins. We defined carcinoma in situ as intraepithelial atypical cells showing cellular atypia corresponding to biliary intraepithelial neoplasia-3 (BilIN-3).[16] In all 10 cases classified into the CIS group, carcinoma in situ displayed continuity from the main tumour to the bile duct stump on serial surgical sections. The Invasive group comprised patients with invasive carcinoma at the surgical margins, including not only the bile duct stumps, but also the radial margins. Numbers of patients with invasive carcinoma at the bile duct stump, radial margin, and both bile duct and radial margin were 5, 8, and 6, respectively. Patients with both of carcinoma in situ at the bile duct stump and invasive carcinoma at the surgical margins were classified into the Invasive group. Surgical procedures for the three groups are shown in ***Table 1***.

Comparison of clinicopathological features

To identify differences among the three groups in clinicopathological features, various factors were compared, including age, sex, location of main tumour, gross type of main tumour, predominant histological differentiated grade of main tumour, depth of invasion of main tumour (pT), lymph node metastasis (pN), distant metastasis (pM), hepatic invasion, pancreatic invasion, venous vessel invasion, lymphatic vessel invasion, perineural invasion, and adjuvant therapy. Locations of main tumours were classified as “proximal site” or “distal site”, defined as the extra- and intra-pancreatic bile duct, respectively. Gross types of main

tumour were classified as “localising type” or “infiltrating type”. “Localising type” was defined as the presence of protrusion into the bile duct lumen (for example, papillary or nodular appearance), while “infiltrating type” was defined as having an almost flat and sclerosing appearance. Predominant histological differentiation grade was classified as either well-differentiated (G1) or other (non-G1). International Union Against Cancer (UICC) criteria were used to classify pTNM.[17] Adjuvant therapy was not examined basically at our department. However, it was done by the judgement of the individual attending physicians at the follow-up hospitals.

Patient follow-up after surgical resection

The survival status of all patients was determined. All surviving patients were surveyed about their present condition by their follow-up medical support institutions. For non-surviving patients, causes of death were determined. In addition, for patients with recurrence, sites of initial recurrence were determined based on follow-up computed tomography (CT), magnetic resonance imaging (MRI), bone scintigraphy or autopsy findings, where available.

Survival analysis

Cause-specific survival curves for the three groups were determined using the Kaplan-Meier method, and differences among the three groups were examined statistically. Deaths with recurrence were treated as failure cases and deaths due to complications associated with the

operation or other disease without evidence of recurrence were treated as censored cases. To elucidate the effects of surgical margin status on postoperative survival, variables were compared on univariate analysis. In addition, multivariate analysis was performed to identify factors independently associated with post-resectional survival.

Statistical analysis

Variables were compared using Pearson's χ^2 test. Distributions of values were compared using the Mann-Whitney U test. Cause-specific survival curves were calculated using the Kaplan-Meier method, and the log-rank test was used to determine significant differences in survival. The Cox proportional hazards model was used for multivariate analysis. In this model, stepwise selection was used for variable selection with entry and removal limits of $p<0.1$ and $p>0.15$, respectively. All tests were two-sided and values of $p<0.05$ were considered statistically significant.

RESULTS

Comparison of clinicopathological features

The results of comparisons for clinicopathological features among the three groups according to resected margin status are shown in ***Table 2***. The CIS group predominantly showed less depth of invasion, absence of lymph nodes metastases, and localising type of gross

appearance of the main tumour compared with the Negative and Invasive groups (p=0.009, p=0.031, and p=0.075, respectively).

Survival after surgical resection

The 125 patients included 31 patients alive without recurrence (median survival, 42 months; range, 16-148 months), 7 patients alive with recurrence (median survival, 31 months; range, 25-53 months), 70 patients who died with recurrence (median survival, 20 months; range, 6-75 months), and 17 patients who died of other disease without recurrence (median survival, 4 months; range, 0.2-170 months), including two deaths within 30 days after surgery (1.6%). The number of patients with recurrence was 56 (58%) in the Negative group, 5 (50%) in the CIS group, and 16 (84%) in the Invasive group. All deaths with recurrence occurred ≤5 years after surgery, excluding two of three patients with local recurrence in the CIS group.

Cause-specific survival curves

Survival curves for the three groups are shown in ***Figure 1***. Median cause-specific survival times calculated using the Kaplan-Meier method were 38 months (range, 0.2-170 months) in the Negative group, 51 months (range, 17-75 months) in the CIS group, and 17 months (range, 2-53 months) in the Invasive group. The Negative group displayed significantly better survival compared to the Invasive group (p=0.004), but not compared to the CIS group (p=0.533). In addition, a significant difference in survival was apparent between the Invasive

and CIS groups (p=0.006).

In the CIS group, the incidences of pT1 or pT2, absence of lymph node metastasis, and localizing type of gross appearance of the main tumour were significant high. Therefore, we also examined the difference between the CIS and the Negative groups in survival in each subgroup classified by these factors to evaluate the effect of carcinoma in situ at ductal margin. As a result, the CIS group did not display difference compared to the Negative group in pT1 or pT2, absence lymph node metastasis, and localizing type of gross appearance of the main tumour subgroups (p=0.941, p=0.748, and p=0.714, respectively).

Uni- and multivariate survival analyses of clinicopathological features

Impacts of clinicopathological variables on cause-specific survival in all 125 patients are shown in ***Table 3***. Predominant histological differentiation of main tumour, lymph node metastasis, distant metastasis (all sites of which were para-aorta lymph nodes), venous vessel invasion, and surgical margin status were identified as prognostic factors on univariate analysis (p=0.010, p<0.001, p<0.001, p<0.001, and p=0.005, respectively). On multivariate analysis, predominant histological differentiation of main tumour, distant metastasis, venous vessel invasion, and surgical margin status represented independent prognostic factors (p=0.027, p=0.003, p<0.001, and p=0.018, respectively) (***Table 4***).

Sites of recurrence

Initial recurrence sites in 71 patients with recurrence and detailed data for 10 patients with carcinoma in situ at the bile duct stump are shown in ***Tables 5*** and ***6***, respectively. Sites were not determined for six patients because records were unavailable from follow-up hospitals. The most frequent sites of initial recurrence were local for the CIS (60%) and Invasive groups (44%), and in the liver (52%) for the Negative group. In the comparison of recurrence patterns (local vs. liver vs. other sites) among the three groups, recurrences in the CIS group tended to more frequently present as local recurrence compared to those in the Negative group (p=0.002, Pearson's χ^2 test).

In the CIS group, disease-free periods and postoperative survival periods for all three patients with local sites of initial recurrence were 29, 53 and 66 months, and 34, 75 and 70 months, respectively. Median disease-free period was 53 months for the three patients with initial local recurrence in the CIS group and 13 months (range, 4-38 months) for the 12 patients in the Invasive and Negative groups (p=0.014, Mann-Whitney U test). Median survival time until death was 70 months for the three CIS patients, compared to 17 months (range, 5-53 months) for the 12 patients in the Invasive and Negative groups (p=0.014, Mann-Whitney U test) (***Figure 2***).

DISCUSSION

This study evaluated the impact of resected margin status, particularly as remnant carcinoma in situ, on post-resectional survival in patients with extrahepatic bile duct carcinoma. The result clarified that: 1) surgical margin status of remnant invasive carcinoma represents an independent prognosis factor; 2) survival for patients with remnant carcinoma in situ does not differ compared to survival for patients without remnant carcinoma; 3) patients with remnant carcinoma in situ or invasive carcinoma experience local recurrence more frequently than patients without remnant carcinoma; and 4) remnant carcinoma in situ is likely to cause local recurrence later in the postoperative phase compared to remnant invasive carcinoma.

Whether remnant carcinoma in situ at the bile duct stump develops into invasive carcinoma has been unclear. In this study, only 10 patients showed carcinoma in situ at the bile duct stump. However, in patients with recurrence, the frequency of local recurrence in the CIS group was 60%, representing a significant difference from the Negative group (p=0.002). We therefore believe that carcinoma in situ has the potential to progress to invasive carcinoma.

In extrahepatic bile duct carcinoma, major recurrence sites include the liver, lymph nodes, and local sites. Selection of the presence of venous vessel invasion, para-aortic lymph node metastasis, and surgical margin status as independent prognostic factors by multivariate analysis, as seen in the present study, is thus unsurprising. Patients with remnant invasive carcinoma at the resected margins had worse survival compared to those without remnant carcinoma, as previously reported,[1-4] but those with remnant carcinoma in situ did not. Two potential reasons for this phenomenon are as follows.

First, the biological nature of main tumours with extensive superficial spread, which is likely to be responsible for remnant carcinoma in situ at the bile duct stump, tends to be less malignant compared to that of conventional cholangiocarcinoma. In our previous study[11], main tumours of extrahepatic bile duct carcinoma with extensive superficial spread showed a tendency of shallower invasion, localising-type gross appearance, and good histological differentiation compared to conventional cholangiocarcinoma. These findings of cholangiocarcinoma with superficial spread have been reported in other two institutions.[12,15] This suggests that the majority of superficial spread arises from main tumours with a low invasive nature. In addition, Igami et al. reported that venous invasion, lymph node metastasis, and distant metastasis were less frequent in cholangiocarcinoma with superficial spread than in cholangiocarcinoma without superficial spread.[12] Likewise, in the present study, the CIS group tended to show shallower invasion, localising-type gross appearance of main tumour, and absence of lymph nodes metastasis compared to tumours in the Negative and Invasive groups. These factors have been reported as better prognostic factors in several studies dealing with extrahepatic bile duct carcinoma.[7, 12, 18-20]

Second, remnant carcinoma in situ is likely to develop into invasive carcinoma in the late phase after surgery. In this study, the CIS group did not display difference in survival compared to the Negative group even in pT1/2, absence of lymph node metastasis, and localising-type of main tumour subgroups. However, all deaths due to recurrence occurred ≤5 years after surgery, excluding the two cases with local recurrence in the CIS group. Wakai et

al. also reported that two of four patients with remnant carcinoma in situ died of local recurrence >5 years after surgery.[14] We have previously reported two patients in whom progression from remnant carcinoma in situ to recurrent invasive carcinoma required 9 and 12 years after the first surgery at another institutes. [8,9] Ojima et al. reported that the period until anastomotic recurrence after surgical resection in patients with invasive carcinoma at bile duct resection margin was significantly shorter than that in patients with carcinoma in situ at the bile duct resection margin.[15] The less malignant nature of main tumours and slower growth of remnant carcinoma in situ thus seems responsible for the lack of difference in postoperative survival between the CIS and Negative groups.

Our previous study clarified that tumours accompanying extensive superficial spread are pathologically characterised by a localising-type gross appearance.[11] In addition, areas of carcinoma in situ showed low papillary growth histologically, corresponding to the granular mucosal surface on gross appearance. Some reports have suggested that area of superficial spread could be observed as a fine granular or papillary appearance on cholangioscopy.[21,22] Since the granular mucosal surface is thought to be difficult to detect on routine imaging modalities such as abdominal ultrasonography, computed tomography, and cholangiography, particularly in patients with localising-type tumour, cholangioscopy and/or endoscopic biopsy may help determine the extent of carcinoma in situ.

Despite the slower growth, remnant carcinoma in situ has the potential to develop into lethal invasive carcinoma. Carcinoma in situ thus should be completely resected to achieve

long-term survival. When carcinoma in situ spreads too widely, however, extended surgery such as hepatopancreatoduodenectomy may be required. For patients with poor general condition or high operative risk, limited resection of the main invasive cancer with carcinoma in situ remaining at the ductal stumps, as an alternative procedure, might bring considerable survival benefits.

REFERENCES

1. Kayahara M, Nagakawa T, Ohta T, Kitagawa H, Tajima H, Miwa K. Role of nodal involvement and the periductal soft-tissue margin in middle and distal bile duct cancer. Ann Surg 1999; 229: 765-83.

2. Burke EC, Jarnagin WR, Hochwald SN, Pisters PW, Fong Y, Blumgart LH. Hilar Cholangiocarcinoma: patterns of spread, the importance of hepatic resection for curative operation, and a presurgical clinical staging system. Ann Surg 1998; 228: 385-94.

3. Kurosaki I, Tsukada K, Watanabe H, Hatakeyama K. Prognostic determinants in extrahepatic bile duct cancer. Hepatogastroenterol 1998; 45: 905-9.

4. DeOliveira ML, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg 2007；245: 755-62.

5. Kondo S, Takada T, Miyazaki M, Miyakawa S, Tsukada K, Nagino M, et al. Guidelines for the management of biliary tract and ampullary carcinomas: surgical treatment. J Hepatobiliary Pancrat Surg 2008; 15: 41-54.

6. Jarnagin WR, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, et al. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. Ann Surg 2001; 234: 507-19.

7. Bhuiya MR, Nimura Y, Kamiya J, Kondo S, Nagino M, Hayakawa N. Clinicopathologic factors influencing survival of patients with bile duct carcinoma: multivariate statistical analysis. World J Surg 1993; 17: 653-7.

8. Nakanishi Y, Kondo S, Hirano S, Ambo Y, Tanaka E, Morikawa T, et al. Recurrence of mucosal carcinoma of the bile duct, with superficial flat spread, 12 years after operation. J Hepatobiliary Pancreat Surg 2006; 13: 355-8.

9. Sasaki T, Kondo S, Ambo Y, Hirano S, Sichinohe T, Kaga K, et al. Local recurrence at hepaticojejunostomy 9 years after resection of bile duct cancer with superficial flat spread. J Hepatobiliary Pancreat Surg 2006; 13: 458-62.

10. Sakamoto E, Nimura Y, Hayakawa N, Kamiya J, Kondo S, Nagino M, et al. The pattern of infiltration at the proximal border of hilar bile duct carcinoma: a histologic analysis of 62 resected cases. Ann Surg 1998; 227: 405-11.

11. Nakanishi Y, Zen Y, Kawakami H, Kubota K, Itoh T, Hirano S, et al. Extrahepatic bile duct carcinoma with extensive intraepithelial spread: a clinicopathological study of 21 cases. Mod Pathol 2008; 21: 807-16.

12. Igami T, Nagino M, Oda K, Nishino H, Ebata T, Yokoyama Y, et al. Clinicopathological study of cholangiocarcinoma with superficial spread. Ann Surg 2009; 249: 296-302.

13. Sasaki R, Takeda Y, Funato O, Nitta H, Kawamura H, Uesugi N, et al. Significance of Ductal Margin Status in Patients Undergoing Surgical Resection for Extrahepatic Cholangiocarcinoma. World J Surg 2007; 31: 1788-96.

14. Wakai T, Shirai Y, Moroda T, Yokoyama N, Hatakeyama K. Impact of ductal resection margin status on long-term survival in patients undergoing resection for extrahepatic cholangiocarcinoma. Cancer 2005; 103: 1210-6.

15. Ojima H, Kanai Y, Iwasaki M, Hiraoka N, Shimada K, Sano T, et al. Intraductal carcinoma component as a favourable prognostic factor in biliary tract carcinoma. Cancer Sci

2009; 100: 62-70.

16. Zen Y, Adsay NV, Bardadin K, Colombari R, Ferrell L, Haga H, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol 2007; 20: 701-9.

17. Sobin LH, Wittekind C. International Union Against Cancer (UICC). TNM Classification of Malignant Tumors, 6th ed. New York: Wiley-Liss; 2002, p.87-9.

18. Mizumoto R, Ogura Y, Kusuda T. Definition and diagnosis of early cancer of the biliary tract. Hepatogastroenterol 1993; 40: 69-77.

19. Su CH, Tsay SH, Wu CC, Shyr YM, King KL, Lee CH, et al. Factors influencing postoperative morbidity, mortality, and survival after resection for hilar cholangiocarcinoma. Ann Surg 1996; 223: 384-94.

20.Miyakawa S, Ishihara S, Horiguchi A, Takada T, Miyazaki M, Nagakawa T. Biliary tract cancer treatment: 5,584 results from the biliary tract cancer statistics registry from 1998 to 2004 in Japan. J Hepatobiliary Pancreat Surg 2009; 16: 1-7.

21. Sato M, Inoue H, Ogawa S, Ohashi S, Maetani I, Igarashi Y, et al. Limitations of percutaneous transhepatic cholangioscopy for the diagnosis of the intramural extension of bile duct carcinoma. Endoscopy 1998; 30: 281–8.

22. Nimura Y, Kamiya J. Cholangioscopy. Endoscopy 1998; 30: 182–8.

FIGURE LEGENDS

Figure 1: Cause-specific survival curves for the three groups. Significant differences are apparent between the CIS and Invasive groups, and between the Negative and Invasive groups (p=0.006 and p=0.004, respectively). However, no significant difference in survival is seen between the CIS and Negative groups (p=0.533).

Figure 2: Distributions of postoperative survival time for patients with local recurrence. A significant difference was identified between CIS and Negative/Invasive groups (p=0.014, Mann-Whitney U test).

Table 1: Surgical procedures performed

	Margin status		
	Negative	CIS	Invasive
Extrahepatic bile duct resection	6	5	7
Caudate lobectomy	2	0	0
PD*	30	3	4
Right hepatectomy	28	0	2
Right hepatic trisectionectomy	4	1	0
Left hepatectomy	14	1	6
Left hepatic trisectionectomy	1	0	0
Central bi-sectionectomy	1	0	0
Right hepatectomy + PD	10	0	0
Total	96	10	19

*PD, pancreatoduodenectomy

Table 2: Comparison of clinicopathological features among the three groups

Variable	*Group by status* Negative n=96	CIS n=10	Invasive n=19	p
Age (years)				0.711
<70	46	6	10	
>=70	50	4	9	
Sex				0.593
male	75	9	16	
female	21	1	3	
Adjuvant therapy				0.957
absence	80	8	16	
presence	16	2	3	
Location				0.091
proximal	76	8	19	
distal	20	2	0	
Gross type				0.075
localising	42	8	8	
infiltrating	54	2	11	
Predominant histological differentiation				0.090
G1	29	6	3	
non-G1	67	4	16	
Depth of invasion				0.009
pT1 or T2	42	9	6	
pT3 or T4	54	1	13	
Lymph node metastasis				0.031
absence	55	8	6	
presence	41	2	13	
Distant metastasis				0.017
absence	94	10	16	
presence	2	0	3	
Hepatic invasion				0.257
absence	76	10	16	
presence	20	0	3	
Pancreatic invasion				0.548
absence	72	9	17	
presence	24	1	2	
Lympatic vessel invasion				0.688
absence	31	3	8	
presence	65	7	11	
Venous vessel invasion				0.252
absence	44	2	7	
presence	52	8	12	
Perineural invasion				0.641
absence	13	2	4	
presence	83	8	15	

Location: Proximal, extrapancreatic bile duct; Distal, intrapancreatic bile duct. Predominant histological type: G1, well-differentiated. Depth of invasion: classication by UICC.

Table 3: Survival analysis by clinicopathological features of 125 cases

Variable	No. of patients n=125	Univariate *p*	Median survival time (mos)
Age (years)		*0.372*	
<70	62		35
≥70	63		31
Sex		*0.612*	
male	100		35
female	25		32
Adjuvant theraphy			
absence	21	0.457	49
presence	104		32
Location		*0.272*	
proximal	103		32
distal	22		70
Gross type		*0.356*	
localising	68		36
infiltrating	57		35
Predominant histological differentiation		*0.010*	
G1	39		53
non-G1	86		28
Depth of invasion		*0.151*	
pT1 or T2	58		39
pT3 or T4	67		23
Lymph node metastasis		*<0.001*	
absence	69		51
presence	56		22
Distant metastasis		*<0.001*	
absence	120		36
presence	5		11
Hepatic invasion		*0.737*	
absence	102		35
presence	23		28
Pancreatic invasion		*0.322*	
absence	96		35
presence	29		22
Lympatic vessel invasion		*0.101*	
absence	42		45
presence	83		30
Venous vessel invasion		< 0.001	
absence	53		*
presence	72		23
Perineural invasion		*0.690*	
absence	19		35
presence	106		34
Resected margin		*0.005*	
Negative	96		38
CIS	10		51
Invasive	19		17

*Predominant histological differentiation: G1, well-differentiated. * less 50 % patient with venous vessel invasion died from recurrence after surgical operation.*

Table 4: Multivariate analysis of survival

Variable		Cause-specific survival rates (%)			Multivariate analysis	
		3-year	5-year	10-year	Relative risk (95%CI)	p
Predominant histological differation						
	G1	72	38	32	1	0.027
	non-G1	37	27	22	1.83 (1.07-3.13)	
Lymph node metastasis						0.115
	absence	65	39	35		
	presence	29	20	13		
Distant metastasis						0.003
	absence	50	32	26	1	
	presence	0	0	0	5.10 (1.74-15)	
Venous vessel invasion						<0.001
	absence	66	50	50	1	
	presence	35	17	-	2.92 (1.71-5.0)	
Rsected margin						0.018
	Negative	50	32	32	1	
	CIS	72	48	0	0.75 (0.48-1.15)	
	Invasive	18	-	-	2.42 (1.34-4.35)	

95% CI: 95% confidence interval

Table 5: Initial recurrence sites in the 3 groups

	Negative	CIS	Invasive
Patients with recurrence	***n=56 (100%)***	***n=5 (100%)***	***n=16 (100%)***
Initial recurrence site			
Local	5 (9%)	3 (60%)	7 (44%)
Liver	29 (52%)	1 (20%)	4 (25%)
Peritoneum	9 (16%)	1 (20%)	0 (0%)
PTBD tube fistula*	3 (5%)	0 (0%)	1 (6%)
Lung	1 (2%)	0 (0%)	0 (0%)
Bone	1 (2%)	0 (0%)	0 (0%)
Lymph nodes	5 (9%)	0 (0%)	0 (0%)
Pancreas	0 (0%)	0 (0%)	1 (6%)
not available	3 (5%)	0 (0%)	3 (19%)

*PTBD, percutaneous transhepatic biliary drainage.

Table 6 : Ten patients with carcinoma in situ at the bile duct stumps

Case	Age (years)	Sex	Predominant histopathological differentiation	pT	pN	Side of bile duct stump involvement of carcinoma in situ	Surgical procedure	Site of initial recurrence	Disease-free periord (mos)	Outcome (mos)
1	76	M	G3	2	0	HM	EBDR	Liver	5	17 Death from recurrence
2	60	M	G2	2	1	Both sides	EBDR			19 Alive without recurrence
3	76	M	G1	1	0	DM	EBDR			23 Death without recurrence (pneumonia, sepsis)
4	79	M	G2	2	0	DM	EBDR			29 Alive without recurrence
5	68	M	G1	2	0	Both sides	EBDR			31 Alive without recurrence
6	55	M	G1	2	0	HM	PD	Anastomic site	29	34 Death from recurrence
7	66	M	G1	2	0	Both sides	LH			46 Alive without recurrence
8	66	F	G1	2	0	Both sides	RHT	Peritoneum	40	51 Death from recurrence
9	72	M	G2	2	1	HM	PD	Anastomic site	66	70 Death from recurrence
10	60	M	G1	3	0	HM	PD	Anastomic site	53	75 Death from recurrence

M: male; F: female; G1: well differentiated; G2: moderately differentiated; G3: poorly differentiated; HM: hepatic side margin; DM: duodenum side margin; EBDR: extrahepatic bile duct resection; PD: pancreatoduodenectomy; LH: left hepatectomy; RHT: right hepatic trisectionectomy.

Figure 1
Click here to download high resolution image

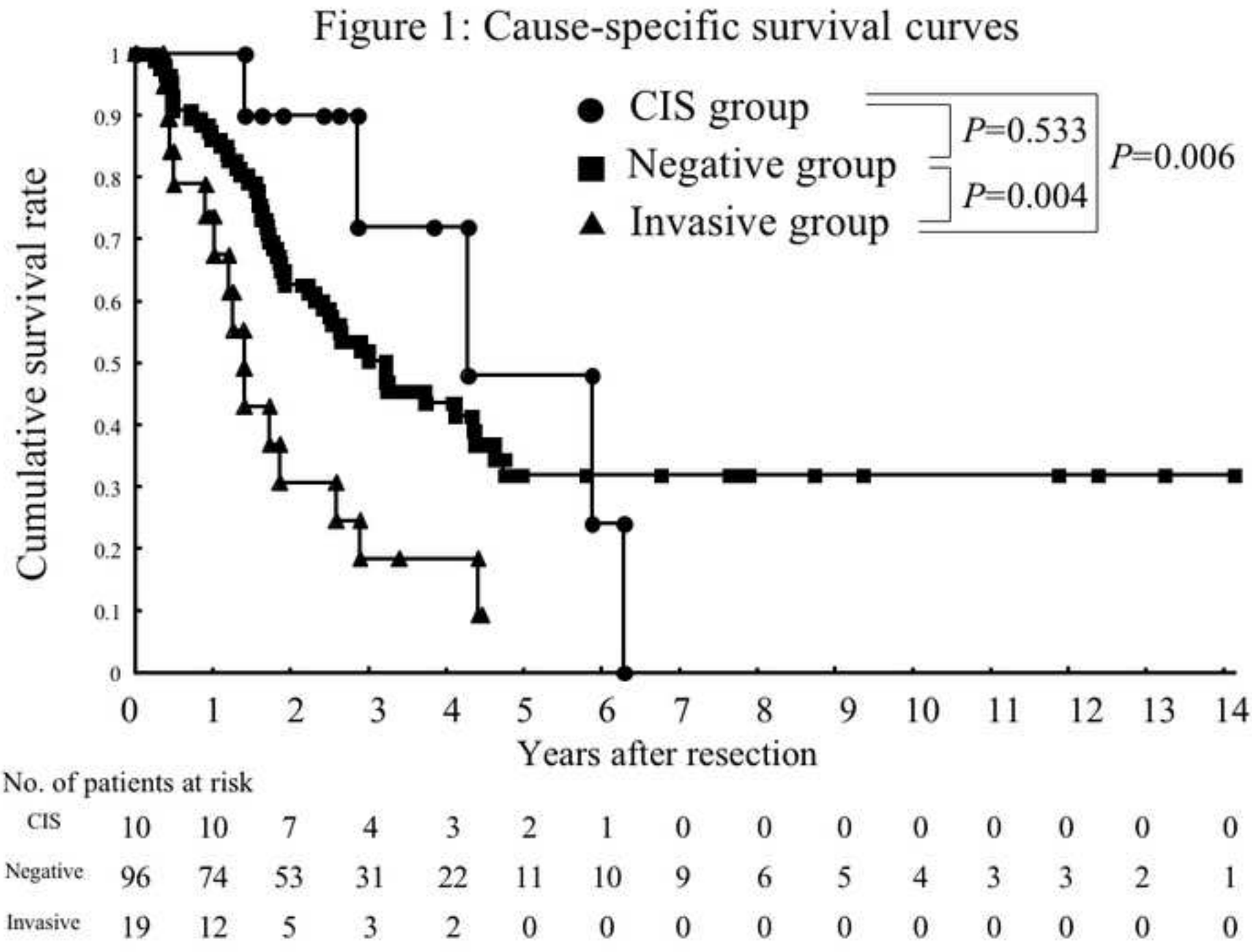

Figure 2
Click here to download high resolution image

Figure 2: Distribution of postoperative survival time for patients with local recurrence