Original Article

Effectiveness of additional resection of the invasive cancer-positive proximal bile duct margin in cases of hilar cholangiocarcinoma

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Background: The survival benefits of additional resection of the positive proximal ductal margin (PM) in hilar cholangiocarcinoma (HCCA) remains controversial. This retrospective study investigated the effectiveness of additional resection of the invasive cancer PM under different levels of preoperative carbohydrate antigen 19-9 (CA19-9).

Methods: Patients who underwent hepatectomy for HCCA from 2000 to 2017 were analyzed. Surgical variables, resection margin status, length of the PM (LPM), prognostic factors, and survival were evaluated.

Results: A total of 228 patients were enrolled: 175 PM(–) without additional resection patients (group A), 21 PM(–) after additional resection (group B), 16 PM(+) without additional resection (group C), and 16 PM(+) after additional resection (group D). The median survival of group B (20.99 months) was similar to that of group A (23.00 months; P=0.16), and both were significantly better than those of group C (11.60 months) and D (9.50 months), especially when preoperative CA19-9>150 U/mL (P<0.05). The survival of patients with an LPM >10 mm was significantly better compared with those with an LPM ≤10 mm, especially when preoperative CA19-9 was >150 U/mL (P<0.05). Only in the LPM >10 mm group, the survival of group B was comparable with that of group A (P>0.05).

Conclusions: HCCA patients could get a survival benefit from a negative PM resulting from additional resection. Survival could be comparable with that of negative PM without additional resection among HCCA patients. An LPM >10 mm is possibly more associated with better survival compared with whether additional resection of the positive PM is performed under different levels of preoperative CA19-9.

Keywords: Klatskin tumor; margins of excision; bile ducts; antigens, neoplasm; carbohydrate antigen 19-9 (CA19-9)

Submitted Nov 07, 2017. Accepted for publication Mar 19, 2018.
doi: 10.21037/hbsn.2018.03.14
View this article at: http://dx.doi.org/10.21037/hbsn.2018.03.14

Introduction

Hilar cholangiocarcinoma (HCCA), or Klatskin tumor, is an advanced tumor at or near the confluence of the right and left hepatic duct. Radical surgical resection (extrahepatic bile duct resection, hepatectomy with *en-bloc* total caudate lobe resection, and regional lymphadenectomy along with (+/–) vascular resection and reconstruction) is the only way to get long-term survival and potential cure (1,2). A tumor-free resection margin (R0) is a critical factor for survival and is the only factor that can be modified surgically (2). The remaining microscopic invasive carcinoma at the ductal resection margin leads a poor survival for the

patients (3,4). Contrastingly, cases with complete resection have 5-year survival rates of 25–40% (5). Therefore, R0 resection could improve surgical outcomes for patients with HCCA. Microscopic tumor spread along the bile ducts beyond the gross tumor border is a characteristic feature of this tumor, inducing unlooked-for tumor infiltration of the resection margin (2). Therefore, a wide enough R0 margins are required for curative resection. More extensive resection is recommended to achieve R0 resection when a positive proximal bile duct resection margin [PM(+)] is demonstrated on intraoperative frozen section (FS) analysis. However, this is often difficult because of invasion onto critical vessels and adjacent liver parenchyma, and additional resection of the liver parenchyma often increases the morbidity and mortality. It is not technically possible sometime.

Serum carbohydrate antigen 19-9 (CA19-9) has been used extensively in clinical treatment process of HCCA. Additional resection of the PM(+) might be restricted only in patients with low levels of CA19-9 and no distant metastasis (6). Previously, we reported that preoperative CA 19-9 levels predict resectability, survival, and early recurrence in patients with resectable HCCA (7,8). Thus, tumor markers also partially represent the degree of malignancy of the tumor.

Studies of intraoperative additional resection of the PM(+) HCCA produced conflicting conclusions (1,6,9-13). Some descripted that additional resection achieved a significant survival benefit; whereas others did not (1,9,10). Thus, this study designed to investigate the effectiveness of additional resection of PM(+) compared with negative proximal bile ductal margin without additional resection, considering the level of the preoperative CA19-9.

Methods

Patients

Five hundred and twenty-seven patients diagnosed with HCCA at West China Hospital, Sichuan University between June 2000 and January 2017 were identified from a retrospectively collected database. Patients who have undergone curative resection of histologically-proven HCCA were included. Patients with preoperative serum CA19-9 levels <5 U/mL (CA19-9 non-secretory), and cholangitis were excluded. Only patient who had undergone the extrahepatic bile duct resection plus hepatectomy and regional lymphadenectomy were enrolled. Informed consent was obtained from all patients for surgical treatment. Data collection and analysis were performed according to the ethical standards of the Helsinki Declaration.

Preoperative evaluation and surgery

We previously described our standard management of HCCA (7,8,14). Multi-detector row spiral computed tomography (MDCT), and magnetic resonance imaging (MRI) were used to evaluate the location, extent, and staging of the tumor. Preoperative biliary drainage was used for preoperative optimization of the liver, using endoscopic retrograde cholangiopancreatography (ERCP) (n=46) and percutaneous transhepatic cholangiodrainage (PTCD) (n=128). The optimal cut-off value for preoperative CA19-9 was set at 150 U/L which was reported by our previously study (8). Unresectable patients were identified as those with: (I) advanced bile duct infiltration that precluded intact tumor removal; (II) invasion of major vascular systems, such as bilateral portal vein involvement, which hampers vascular reconstruction; (III) lymph nodes metastases beyond the hepatoduodenal ligament; (IV) unilateral hepatic lobe atrophy with invasion of the contralateral portal vein or hepatic artery; (V) unilateral hepatic lobe atrophy with invasion of the contralateral secondary biliary radicles; (VI) unilateral secondary biliary radicles involvement with invasion of the contralateral portal vein or hepatic artery; and (VII) pathologically confirmed HCCA with evidence of distant metastases (7). The volumes of liver to be resected were calculated using serial MDCT images. Selective preoperative portal vein embolization was indicated to decrease the risk of postoperative hepatic failure in patients whose residual liver parenchymal volume was less than 40% of the total liver volume. The preoperative serum CA19-9 level (pr-CA) was assayed immediately before surgery because it can be affected by cholestasis or cholangitis.

Pathological evaluation and measurement of the length of proximal ductal margin (PM)

Proximal bile ducts were transected above the level of the gross tumor and regarded as the specimen margin (*Figure 1*) (9). The specimen margin was not submitted for FS analysis. A separate cut-off of the proximal bile duct (ductal margin) above the initial specimen margin (toward the liver remnant) was then transected and submitted for FS analysis (*Figure 1*). When the PM was positive for invasive

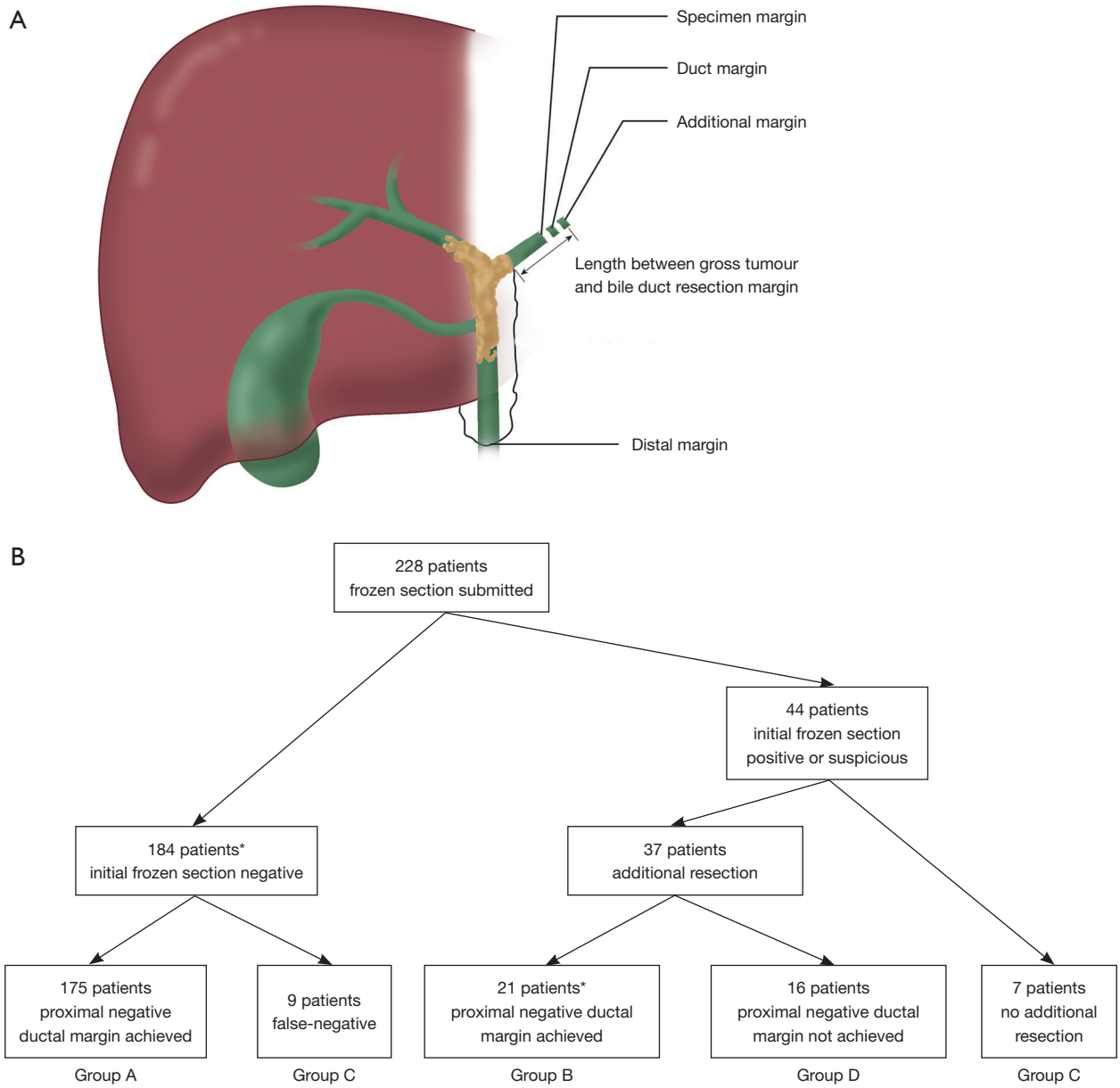

Figure 1 Program of the specimens submitted for histopathologic analysis and the grouping method for patients. (A) The duct margin was submitted for intraoperative frozen section analysis. An additional margin was submitted when a positive duct margin was found at the time of frozen section analysis. The specimen margin was submitted for permanent histopathology only. The distal margin was taken from the distal common bile duct; (B) distribution of patients undergoing radical resection of hilar cholangiocarcinoma based on bile duct margin status, as diagnosed by intraoperative frozen-section assessment and final permanent section pathologic examination. *, indicates that a total of 205 patients were thought to have negative final duct margins with intraoperative frozen section analysis.

carcinoma, additional resection was adopted to achieve a R0 margin, if technically possible. A cut-off from the distal common bile duct margin was also submitted for FS analysis in each case. When the distal ductal margin (DM) was positive, additional resection, including combined pancreaticoduodenectomy, if needed, was performed. All margins submitted for intraoperative FS analysis were subsequently examined histopathologically.

All histological sections were reviewed by two experienced pathologists who were blinded to the clinical

information. The ductal margin status, and the length between the gross tumor and the proximal bile duct resection margin [length of the PM (LPM)] were assessed histologically and calculated based on the permanent FS and formalin-fixed resected specimens (*Figure 1*). Patients were divided into four groups regarding whether intraoperative further resection of the positive PM was performed and the condition of the proximal PM: group A, PM(–) without additional resection; group B, PM(–) with additional resection; group C, final PM(+) without additional resection; and group D, final PM(+) with additional resection. R0 resections were defined as resection without microscopic tumor cells detected in the biliary, vascular, or hepatic parenchymal surgical margins. R1 resections were defined as microscopic tumor deposits in one of the above-mentioned surgical margins. R2 resections were defined as those with a macroscopic tumor left behind in one or more of the surgical margins. Palliative therapy comprised palliative biliary drainage, chemotherapy, or radiotherapy. Nodal status was positive (N1) if one or more of the hilar lymph nodes was infiltrated with tumor cells. N1 comprised 1–3 regional lymph node metastases; and N2 comprised ≥4 regional lymph node metastases. Patients were staged according to the 8th edition of the American Joint Committee on Cancer (AJCC) TNM staging system for HCCA (15).

Follow-up

HCCA is a disastrous malignant disease; therefore, all patients were strictly supervised and followed-up in outpatient clinics. The enrolled patients were assessed every 2–3 months by assessment of liver function, tumor markers, and ultrasonography in the first year after surgery, and thereafter at 3–6 months annually. If recurrence or distant metastasis was suspected, computed tomography or MRI was conducted. The date of the first suspicious radiological finding represented the date of initial disease recurrence.

Statistics

Variables are presented as absolute numbers, percentage or median values, and ranges. Statistical analysis comprised nonparametric tests using the Mann-Whitney U test, Chi-square test, or Fisher's exact test, when appropriate. Univariate analysis of survival probabilities was estimated using the Kaplan-Meier log rank test, from the time of operation to the time of death or the most recent follow-up, excluding postoperative deaths (any deaths occurring within 90 days of surgery or during the same hospital stay, whenever it occurred). All data were updated on July 1, 2017. Factors with P<0.20 in the univariate analysis were subjected to multivariate analysis using the Cox proportional-hazards model, which was also used to evaluate the interactions between the prognostic factors and the LPM on overall survival. Statistical significance was set at P<0.05. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24 (IBM Corporation, Armonk, NY, USA).

Results

Between June 2000 and January 2017, we treated 527 patients with HCCA in our institution, of whom 228 patients were enrolled in the study. Group A: 175 patients, group B: 21 patients, group C: 16 patients, and group D: 16 patients.

Comparison of group A, B, C and D

The clinicopathological characteristics of the four groups is presented in *Table 1*. There were significantly more patients with microscopic liver invasion in group D compared with those in groups A, B, and C (P=0.038, *Table 1*). Rates of combined portal vein and/or hepatic artery resection were similar among all groups (P>0.05, *Table 1*).

Among the patients, 184 had an initial negative PM, and additional resection was not performed in nine patients from this group because of false-negative diagnosis on FS; therefore, they were excluded from group A. Of the 44 patients who were initially PM(+), 37 underwent additional resection bringing about 21 PM(–) cases (group B) and 16 final PM(+) patients (group D). Additional resection was not performed in seven patients because of their unfit general condition and long operation time. Ultimately, 32 (9+16+7) (14%) of the 228 patients were PM(+), without (group C, n=16) or with additional resection (group D, n=16).

The type of hepatectomy was similar among the four groups (P=0.16). In left hepatectomy (hemihepatectomy + trisectionectomy), 24 patients were initially PM(+). Among them, 11 (45.8%) became PM(–) after additional resection. In contrast, in right hepatectomy, 13 patients were initially PM(+). Among them, 5 (38.5%) became PM(–) after

Table 1 Clinicopathological characteristics of groups A, B, C, and D

Variable, n (%)	Total (n=228)	Group A (n=175)	Group B (n=21)	Group C (n=16)	Group D (n=16)	P value
LPM ≤10 mm[a]	102 (44.7)	73 (41.7)	11 (52.4)	9 (56.3)	9 (56.3)	0.41
Age [yr][a]	61 [20–82]	61 [20–82]	62 [39–77]	63 [46–74]	55.5 [30–71]	0.27
Female/male	94/134	74/101	7/14	9/7	4/12	0.28
Preoperative CA19-9 (U/mL)[a]	195.60 (5.0–1,000)	203.4 (5.0–1,000)	142.0 (44.9–409.87)	387.60 (29.92–1,000)	193.15 (7.18–1,000)	0.41
Preoperative biliary drainage	174 (76.3)	133 (76.0)	14 (66.7)	13 (81.3)	14 (87.5)	0.49
Preoperative portal vein embolization	21 (9.2)	19 (10.9)	0 (0.0)	1 (6.3)	1 (6.3)	0.43
Bismuth type						0.41
I	19 (8.3)	15 (8.6)	2 (9.5)	1 (6.3)	1 (6.3)	
II	44 (19.3)	32 (18.3)	6 (28.6)	4 (25.0)	2 (12.5)	
III	60 (26.3)	41 (23.4)	8 (38.1)	4 (25.0)	7 (43.8)	
IV	105 (46.1)	87 (49.7)	5 (23.8)	7 (43.8)	6 (37.5)	
Type of hepatectomy						0.16
Left hepatectomy	120 (52.6)	97 (55.4)	11 (52.4)	5 (31.3)	7 (43.8)	
Left trisectionectomy	7 (3.1)	6 (3.4)	0 (0.0)	1 (6.3)	0 (0.0)	
Central bisectionectomy	39 (17.1)	23 (13.1)	5 (23.8)	5 (31.3)	6 (37.5)	
Right hepatectomy	49 (21.5)	37 (21.1)	5 (23.8)	5 (31.3)	2 (12.5)	
Right trisectionectomy	13 (5.7)	12 (6.9)	0 (0.0)	0 (0.0)	1 (6.3)	
Combined portal vein reconstruction[b]	57 (25.0)	47 (26.9)	4 (19.0)	3 (18.8)	3 (18.8)	0.79
Combined hepatic artery reconstruction[b]	32 (14.0)	27 (15.4)	3 (14.3)	1 (6.3)	1 (6.3)	0.72
pT[c]						0.80
1	11 (4.8)	10 (5.7)	1 (4.8)	0 (0.0)	0 (0.0)	
2a/2b	47 (20.6)	36 (20.5)	5 (23.8)	3 (18.8)	3 (18.8)	
3	102 (44.7)	73 (41.7)	12 (57.1)	9 (56.3)	8 (50.0)	
4	68 (29.8)	56 (32.0)	3 (14.3)	4 (25.0)	5 (31.3)	
pNc						0.11
N0	144 (63.2)	117 (66.9)	14 (66.7)	7 (43.8)	6 (37.5)	
N1	68 (29.8)	46 (26.3)	6 (28.6)	8 (50.0)	8 (50.0)	
N2	16 (7.0)	12 (6.9)	1 (4.8)	1 (6.3)	2 (12.5)	

Table 1 (*continued*)

Table 1 (*continued*)

Variable, n (%)	Total (n=228)	Group A (n=175)	Group B (n=21)	Group C (n=16)	Group D (n=16)	P value
pStage[c]						0.64
I	11 (4.8)	10 (5.7)	1 (4.8)	0 (0.0)	0 (0.0)	
II	31 (13.6)	24 (13.7)	4 (19.0)	3 (18.8)	0 (0.0)	
IIIA/IIIB	96 (42.1)	78 (44.6)	8 (38.1)	4 (25.0)	6 (37.6)	
IIIC	62 (27.2)	43 (24.6)	6 (28.6)	6 (37.5)	7 (43.8)	
IVA	13 (5.7)	10 (5.7)	1 (4.8)	1 (6.3)	1 (6.3)	
IVB	15 (6.6)	10 (5.7)	1 (4.8)	2 (12.5)	2 (12.5)	
Histological grade						0.50
G1 (well)	16 (7.0)	14 (8.0)	1 (4.8)	1 (6.3)	0 (0.0)	
G2 (moderately)	85 (37.3)	62 (35.4)	12 (57.1)	6 (37.5)	5 (31.3)	
G3 (poorly)	127 (55.7)	99 (56.6)	8 (38.1)	9 (56.3)	11 (68.8)	
Microscopic venous invasion	30 (13.2)	21 (12.0)	3 (14.3)	3 (18.8)	3 (18.8)	0.65
Microscopic perineural invasion	120 (52.6)	91 (52.0)	7 (33.3)	11 (68.8)	11 (68.8)	0.090
Microscopic liver invasion	132 (57.9)	104 (59.4)	8 (38.1)	7 (43.8)	13 (81.3)	0.038*
Positive proximal ductal margin	32 (14.0)	0 (0.0)	0 (0.0)	16 (100.0)	16 (100.0)	<0.001*
R1	45 (19.7)	11 (6.3)	2 (9.5)	16 (100.0)	16 (100.0)	<0.001*

Group A, negative proximal ductal margin (PM) without additional resection; group B, negative PM with additional resection; group C, positive PM without additional resection; group D, positive PM with additional resection. *, significantly different; LPM: length between gross tumor and proximal ductal resection margin; [a], median (range); [b], 19 patients who underwent combined portal vein and hepatic artery reconstruction overlap in each category; [c], according to the AJCC TNM classification 8th edition.

additional resection. The difference was not significantly different (P=0.74).

For final resection margin status (R), the rate of R1 resection was 6.3% (11/175) in group A, 9.5% (2/21) in group B, 100% (16/16) in group C, and 100% (16/16) in group D. Significantly more R1 resections were performed in groups C and D than in groups A and B (P<0.001). Accordingly, 183 patients (80.3%) had R0 resection, whereas 45 R1 resection patients (19.7%).

The length between the gross tumor and the proximal ductal resection margin (LPM), age, sex, pr-CA, preoperative biliary drainage, preoperative portal vein embolization, pTNM Stage, histological differentiation, and microscopic venous invasion were not statistically different between the groups (all P>0.05). In all patients, pr-CA was positively correlated with the total bilirubin level (r=0.234, P=0.000).

Relationship between local recurrence rate and ductal margin status

Local recurrence was found in 77 of the 228 patients (33.8%). When local recurrence was investigated according to the ductal margin status, local recurrence was observed in 53 patients (30.3%) in group A, in 8 patients (38.1%) in group B, in 7 patients (43.8%) in group C, and in 9 patients (56.2%) in group D. There was no significant relationship between local recurrence and ductal margin status (P=0.14, chis squared test, *Table 2*). However, an improving trend was found for the rate of local recurrence when a negative

Table 2 Local recurrence rate in 228 patients undergoing resection stratified by ductal margin status

N, (%)	Local recurrence		Total
	(–)	(+)	
PM(–) without additional resection (group A)	122 (69.7)	53 (30.3%)	175
PM(–) with additional resection (group B)	13 (61.9)	8 (38.1%)	21
PM(+) without additional resection (group C)	9 (56.2%)	7 (43.8%)	16
PM(+) with additional resection (group D)	7 (43.8%)	9 (56.2%)	16

P=0.14, by chi-squared test.

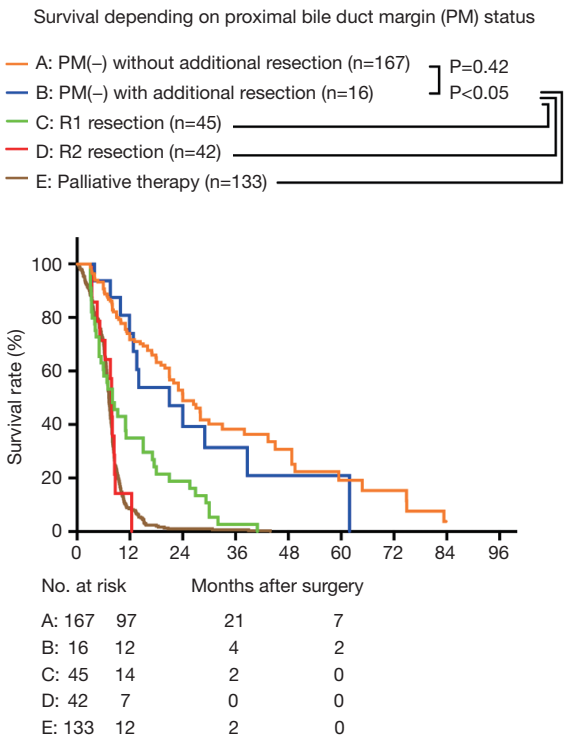

Figure 2 Survival depending on proximal bile duct margin (PM) status.

proximal bile duct resection margin could not be achieved by additional resection (*Table 2*).

Survival and prognostic factors

The median survival rates of PM(–) without additional resection, PM(–) with additional resection, R1 resection, R2 resection, and palliative therapy were 23.98 (95% CI, 19.09–28.88) months, 20.99 (95% CI, 6.67–35.31) months, 10.47 (95% CI, 8.76–12.18) months, 7.87 (95% CI, 7.49–8.25) months, and 7.23 (95% CI, 6.94–7.53) months, respectively. The difference between PM(–) without additional resection and PM(–) with additional resection under R0 resection was not significant (P=0.42). The median survival of R0 resection was significantly better than that of R1 resection, R2 resection, and palliative therapy (P<0.05). The median survival of R1 resection was also significantly better than that of R2 resection and palliative therapy (P<0.05). The survival rates of R2 resection and palliative therapy were similar (P=0.98) (*Figure 2*).

The overall survival rate of the 228 patients was 64.7% at 1 year, 28.7% at 3 years, and 13.9% at 5 years. The median survival was 20.98 (95% CI, 16.98–24.98) months, and the median follow-up time was 25.99 (3.02–88.7) months.

On univariate analysis, 10 of the 17 possible clinicopathological prognostic factors, including preoperative CA19-9, proximal additional resection, LPM, Bismuth type, tumor stage (pT), lymph nodes metastases, the TNM stage (pStage), histological grade, microscopic liver invasion, and resection margin status (R) were significant (*Table 3*). Multivariate analysis of the 10 significant factors demonstrated that LPM [hazard ratio (HR): 1.96], pT (HR: 0.18), and resection margin status (HR: 0.36) were independent prognostic factors for survival (*Table 3*).

Interactions among CA19-9, additional resection of the PM, LPM, and survival

We then analyzed the overall survival according to the final PM status, using groups A–D. The 1- and 3-year survival of groups A, B, C, and D were 70.9%, 37.0%, 66.0%, and 28.6%; and 38.1%, 0.0%, and 25.0%, and 0.0%, respectively (*Figure 3A*). The median survival of groups A–D were 23.98 (95% CI, 19.09–28.88) months, 20.99 (95% CI, 6.67–35.31) months, 11.60 (8.22–14.98) months, and 9.50 (7.07–11.92) months, respectively. The overall survival of groups A and B were similar (P=0.16), and both were significantly higher than those of groups C and D (P<0.05). The overall survival of groups C and D were similar (P=0.23) (*Figure 3A*). To better compare the overall survival of R1 resection and each PM(–) group, we separated the R1 resection individually. After separating R1 individually, the 3-year survival and median survival in groups A and B remained similar and were both significantly better than that in the R1 resection group (P<0.05, *Figure 3B*). The

Table 3 Survival analysis in all patients (n=228)

Variable	n	Survival rate (%)		Univariate, P value	Multivariate, hazard ratio (95% CI)	P value
		3-year	5-year			
Age (yr)				0.54		
<66	170	30.1	15.1			
≥66	58	24.1	8.0			
Sex				0.36		
Male	134	31.7	13.5			
Female	94	24.9	14.5			
Preoperative CA19-9 (U/mL)				0.043*		0.35
CA19-9 ≤150	102	36.3	18.2		1	
150< CA19-9	126	23.4	11.3		0.83 (0.56–1.22)	
Proximal additional resection				0.001*		0.10
Not performed	191	32.2	16.1		0.68 (0.43–1.08)	
Performed	37	15.6	5.8		1	
LPM				0.000*		0.001*
LPM ≤10 mm	102	12.8	8.5		1.96 (1.33–2.89)	
LPM >10 mm	126	40.3	18.2		1	
Preoperative biliary drainage				0.51		
Not performed	54	39.2	11.0			
Performed	174	25.1	14.3			
Bismuth type				0.002*		0.66
I/II	63	51.5	27.3		0.89 (0.54–1.47)	
III/IV	165	19.1	6.5		1	
Type of hepatectomy				0.14		0.55
Left hepatectomy	120	37.6	19.4		1	
Left trisectionectomy	7	20.0	14.3		1.52 (0.58–4.00)	
Central bisectionectomy	39	19.8	0.0		1.20 (0.50–2.90)	
Right hepatectomy	49	15.2	10.1		1.71 (0.68–4.27)	
Right trisectionectomy	13	21.9	7.7		1.23 (0.37–4.13)	
Combined PV and/or HA				0.097		0.98
Not performed	158	30.7	17.6		1	
Performed	70	22.7	0.0		1.00 (0.66–1.50)	
pT[a]				<0.001*		<0.001*
1/2a/2b	58	59.0	34.5		0.18 (0.072–0.44)	
3/4	170	13.2	0.0		1	

Table 3 (*continued*)

Table 3 (*continued*)

Variable	n	Survival rate (%)		Univariate, P value	Multivariate, hazard ratio (95% CI)	P value
		3-year	5-year			
Lymph node metastasis				0.001*		0.071
No	144	33.5	18.5		0.69 (0.46–1.03)	
Yes	84	20.0	5.0		1	
pStage[a]				<0.001*		0.62
I/II	42	60.8	39.9		1	
IIIA/IIIB/IIIC/IVA/IVB	186	18.0	3.1		1.29 (0.47–3.55)	
Histological grade				0.045*		0.25
G1 (well)	16	68.6	34.3		1	
G2 (moderately)/G3 (poorly)	212	25.3	12.4		1.66 (0.70-3.91)	
Microscopic venous invasion				0.93		
Absent	198	26.5	13.9			
Present	30	34.6	13.7			
Microscopic perineural invasion				0.13		0.80
Absent	108	34.4	16.4		0.95 (0.65–1.40)	
Present	120	18.6	9.3		1	
Microscopic liver invasion				0.041*		0.23
Absent	96	35.0	23.3		0.78 (0.52–1.17)	
Present	132	23.6	6.3		1	
Proximal ductal margin				<0.001*		
Negative	196	35.8	17.3			
Positive	32	0.0	0.0			
Distal ductal margin				0.004*		
Negative	209	29.5	15.0			
Positive	19	15.7	0.0			
R				<0.001*		<0.001*
0	183	36.3	18.5		0.36 (0.23–0.58)	
1	45	5.4	0.0		1	

*, significant difference; [a], according to the AJCC TNM classification 8th edition. LPM, length between gross tumor and proximal ductal resection margin; PV, portal vein resection; HA, hepatic artery resection.

median survival of LPM >10 mm [27.99 (95% CI, 22.54–33.45) months] was significantly better than that of LPM ≤10 mm [15.01 (95% CI, 10.20–19.83) months] (P<0.001, *Table 2*).

Next, the survival rates of the four groups were compared in subgroups stratified according to the pr-CA value [cut-off value =150 U/L (8)], The patients were divided into pr-CA >150.0 U/mL (pr-CA high (H)) and pre-CA ≤150.0 U/mL [pr-CA low (L)] subgroups. *Figure 4A,B* shows that the overall survival of group B was similar to that of group A in the two pre-CA subgroups (P>0.05), even after separating R1 resection individually (P>0.05, *Figure 4C,D*). For pr-

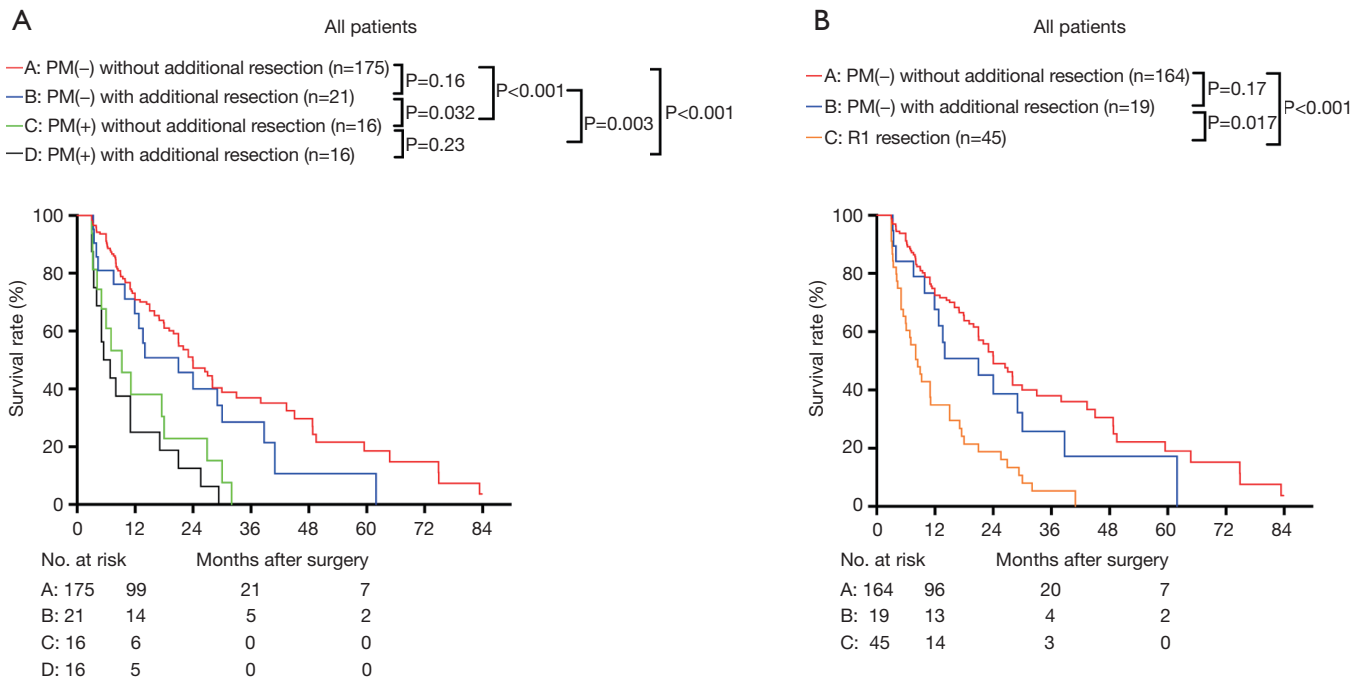

Figure 3 Overall survival for the enrolled population according to the final proximal ductal margin (PM) status.

CA19-9≤150.0 U/mL and pre-CA19-9>150.0 U/mL, the survival of groups A and B were significantly better than those of group D (both P<0.05). After separating R1 resection individually, in all pr-CA subgroups, the survival of group A and B was similar (P>0.05), and both were significantly better than that of R1 resection (P<0.05) (*Figure 4C,D*).

Figure 5 presents the survival of the enrolled patients stratified by LPM and R1 resection for different levels of pre-CA19-9: LPM ≤10 mm, and LPM >10 mm. The median survival of patients with LPM >10 mm [35.01 (95% CI, 26.54–49.49) months] was significantly better than that of patients with LPM ≤10 mm [19.06 (95% CI, 14.45–23.66) months] and R1 resection [10.47 (95% CI, 8.76–12.18) months] (*Figure 5A*). For pre-CA19-9 ≤150 U/mL, the survival of patients with LPM ≤10 mm and LPM>10 mm was similar (P=0.14) and were significantly better than that of R1 resection (P<0.05, *Figure 5B*). For pre-CA19-9 >150 U/mL, the survival of patients with LPM >10 mm was significantly better than that of patients with LPM ≤10 mm (P=0.011) and R1 resection (P<0.001). The survival of patients with LPM ≤10 mm was also significantly better than that of R1 resection (P=0.005).

Figure 6A,B shows the survival of the four groups stratified by LPM. In the LPM ≤10 mm, the median survival of group A [20.96 (95% CI, 15.65–26.27) months] was significantly better than that of group B [9.86 (95% CI, 3.13–16.59) months, P=0.002], C [5.99 (95% CI, 3.57–8.42) months, P<0.001], and D [5.03 (95% CI, 2.12–7.94) months, P<0.001]. The survival of group B, C and D was similar (*Figure 6A*). In the LPM >10 mm subgroup, the median survival of group B [28.70 (95% CI, 17.54–39.87) months] was comparable with that of group A [29.96 (95% CI, 13.17–46.76) months] (P=0.94), and the survival of both these groups was significantly better than those of groups C [17.00 (95% CI, 0.00–38.80) months] and D [11.00 (95% CI, 3.36–18.65) months] (P<0.05). The survival of group C and D was similar (P=0.19) (*Figure 6B*). After separating R1 resection individually, in the LPM ≤10 mm subgroup, the median survival of group A [20.96 (95% CI, 15.65–26.27) months] remained significantly better than that of group B [9.86 (95% CI, 3.13–16.59) months, P=0.04] and R1 resection [5.50 (95% CI, 3.79–7.20) months, P=0.00], but there was no significant different between the survival of group B and R1 resection (P=0.19, *Figure 6C*). In the LPM >10 mm subgroup, the median survival of group B [30.03 (95% CI, 18.52–41.54) months] was comparable with that of group A [35.01 (95% CI, 20.35–55.68) months] (P=0.98), and the survival of both these groups was significantly better than that of R1 resection [15.0 (95% CI, 6.77–23.23)

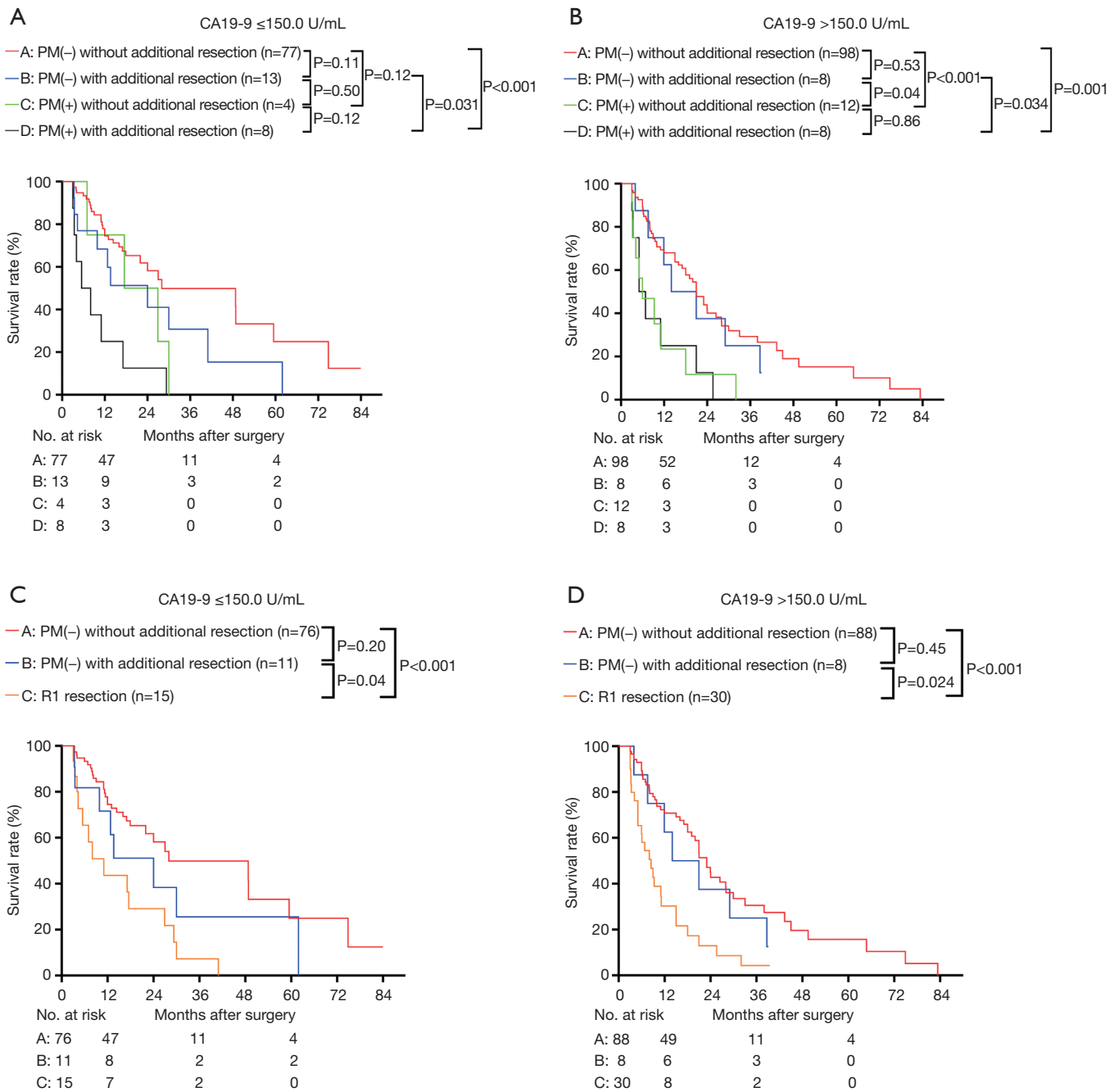

Figure 4 Overall survival according to the final proximal ductal margin (PM) status with the study population stratified according to CA19-9 ≤150.0 or CA19-9 >150.0 U/mL (A,B), and after separating R1 resection patients individually (C,D).

months] (P<0.05, *Figure 6D*). The survival of patients with LPM >10 mm was significantly better than those with LPM ≤10 mm (P<0.05, *Figure 7A,B*) except for the PM(+) patients (P>0.05, *Figure 7C,D*).

To further investigate the effect of LPM on overall survival, we further divided the patients into groups regarding the LPM: LPM ≤5 mm: 44 patients; 5 mm < LPM ≤10 mm: 58 patients; 10 mm < LPM ≤20 mm: 88 patients; 20 mm < LPM: 38 patients. We found that The OS of LPM ≤5 mm (median: 11.02 months) was

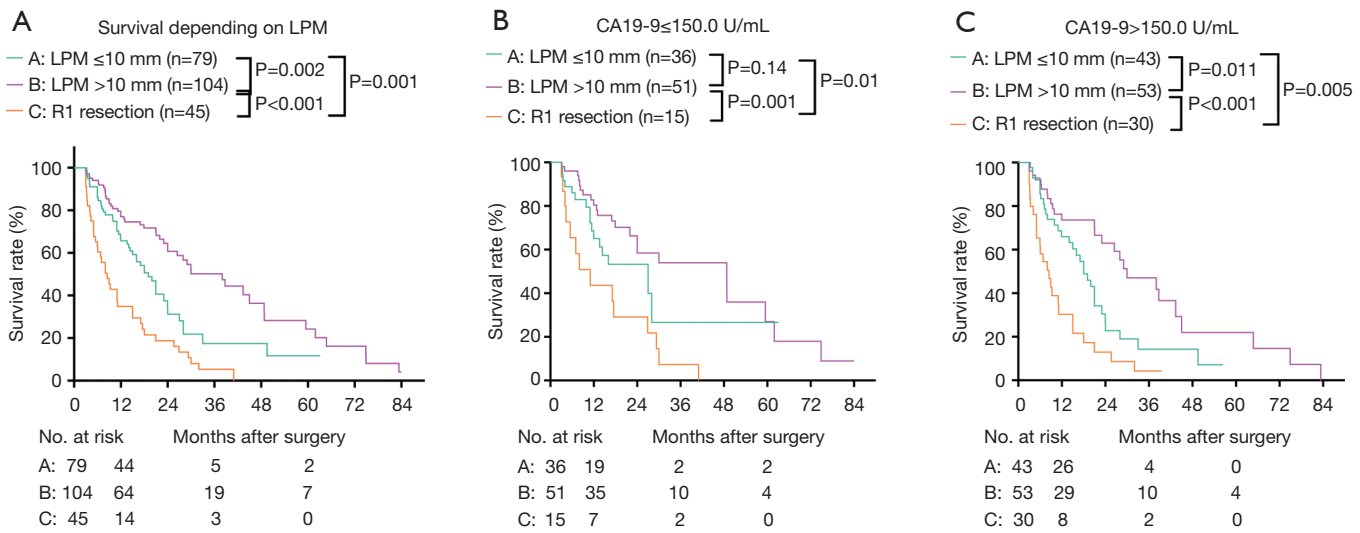

Figure 5 Overall survival according to the length between the gross tumor and the proximal ductal margin (LPM) in patients grouped according to their different levels of pre-CA19-9.

significantly worse than that of other groups (P<0.05, *Figure 8A*). The OS of 5 mm < LPM ≤10 mm was significantly worse than that of 20 mm < LPM (P=0.047, *Figure 8A*). The OS of 10 mm < LPM ≤20 mm and 20 mm < LPM was not significantly different (P=0.20, *Figure 8A,B*).

Then the survival rates of the four groups were compared in the subgroups stratified according to the value of preoperative CA19-9. When pre-CA19-9 ≤150.0 U/mL, the overall survival of LPM ≤5 mm was significantly worse than that of 10 mm < LPM ≤20 mm and 20 mm < LPM (*Figure 9A*), but not significantly differ from that of R1 resection (P>0.05, *Figure 9B*). The overall survival of 5 mm < LPM ≤10 mm, 10 mm < LPM ≤20 mm, and 20 mm < LPM were significantly better than R1 resection (*Figure 9B*); When pre-CA19-9>150.0 U/mL, the and overall survival of 20 mm < LPM was significantly better than that of LPM ≤5 mm, 5 mm < LPM ≤10 mm, 10 mm < LPM ≤20 mm, and R1 resection (*Figure 9C,D*).

Discussion

Although complete tumor resection on histological examination (R0) is the most meaningful factor influence long-term prognosis in the surgical treatment of HCCA (2), several studies have advocated that long-term survival after R0 and R1 was not significantly different (11,16,17). In the present study, the survival of R1 resection was significantly worse than R0 resection, but still better than R2 resection and palliative therapy. This is consistent with some previous reports in which the survival rates of R1 resection patients were significantly better than those with unresectable tumors (18-20).

Our findings indicated that a PM(–) achieved by further resection resulted in superior survival than a positive PM without additional resection cases. The prognostic significance of further resection of a PM(+) margin in HCCA is disputed. Although a significant survival benefit of further resection was reported by Ribero *et al.*. Endo *et al.* and Shingu *et al.* reported that additional resection did not improve survival (1,9,10). Oguro *et al.* presented that only patients with a lower level of CA19-9 and no distant metastasis could benefit from a final PM(–) achieved by further resection (6). In the present study, the survival of the 21 PM(–) patients resulting from additional resection did not differ significantly differ from 175 PM(–) patients without additional resection and was significantly better compared with all PM(+) or R1 resection patients. Oguro *et al.* thought that the effectiveness of further PM resection in bettering survival is affiliated with the degree of cancer progression, and the discrepancies in the conclusions of previous studies might be illustrated by differences in the tumor characteristics of the enrolled populations. In their report, 40% of cases were Bismuth IV disease, one of the most advanced and longitudinal wide spreading perihilar cholangiocarcinomas, which was similar to that of Shingu *et al.* (38.9%). The proportion was only 14.6% in the

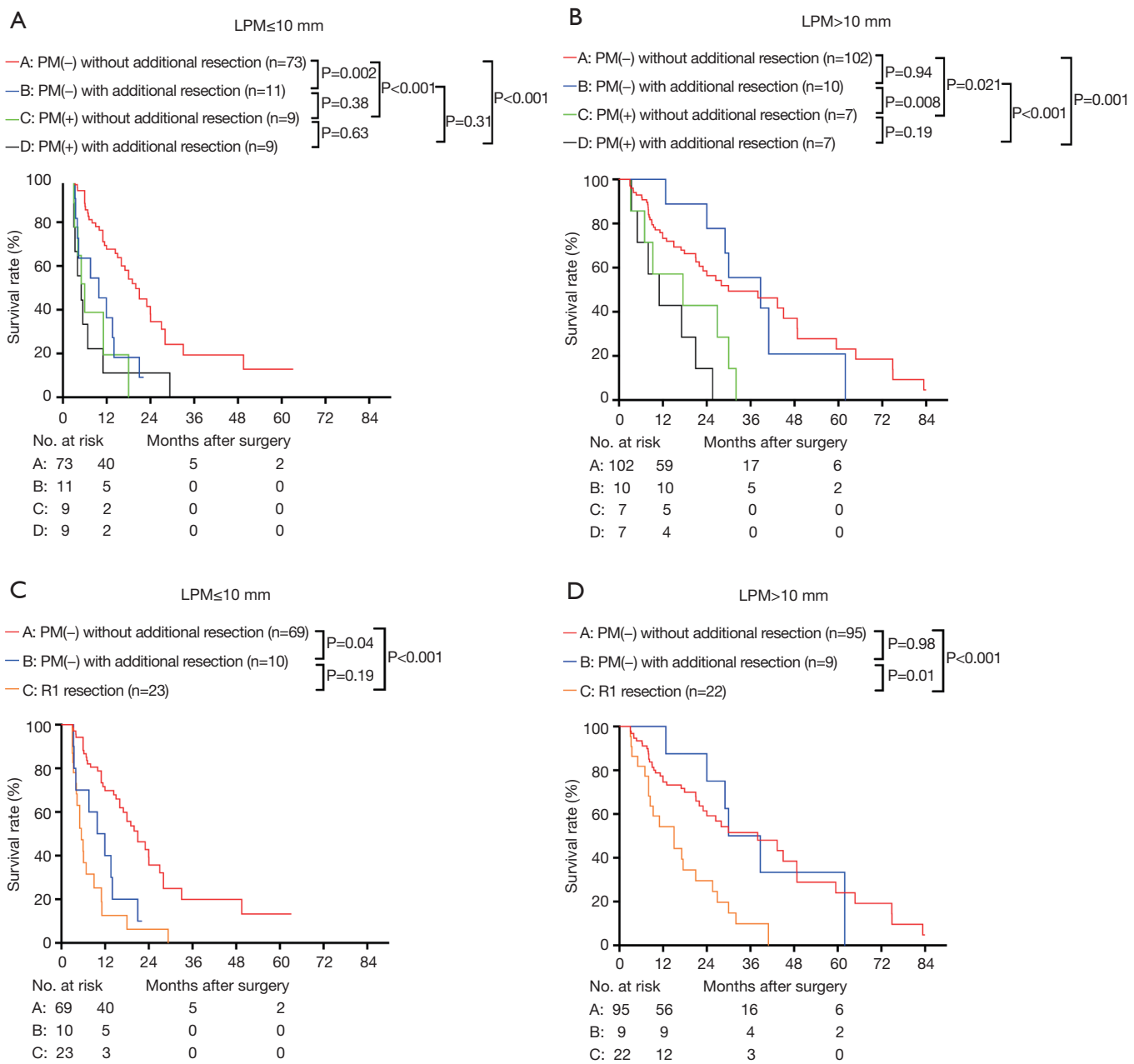

Figure 6 Overall survival according to the final proximal ductal margin (PM) status with the study population stratified by the final length of proximal ductal margin (LPM): LPM ≤10 mm or LPM >10 mm (A,B), and after separating by R1 resection (C,D).

study by Ribero *et al.* Therefore, Oguro *et al.* concluded that patients with less advanced tumors might show better survival after re-resection of the positive PM. However, the proportion of Bismuth IV disease was 0% in the study by Endo *et al.* The proportion of Bismuth IV cases was a relatively high 46.9% in our study. Endo *et al.* and Shingu *et al.* reported that the survival of patients with a short proximal negative ductal margin achieved by additional resection was worse than that for patients who underwent an R0 resection with a longer ductal margin and was similar to that for patients who underwent an R1 resection. However, Ribero *et al.* and Oguro *et al.* did not investigate the width of their proximal bile ductal margin. It is possible that the width of the PM could explain the discrepancy in

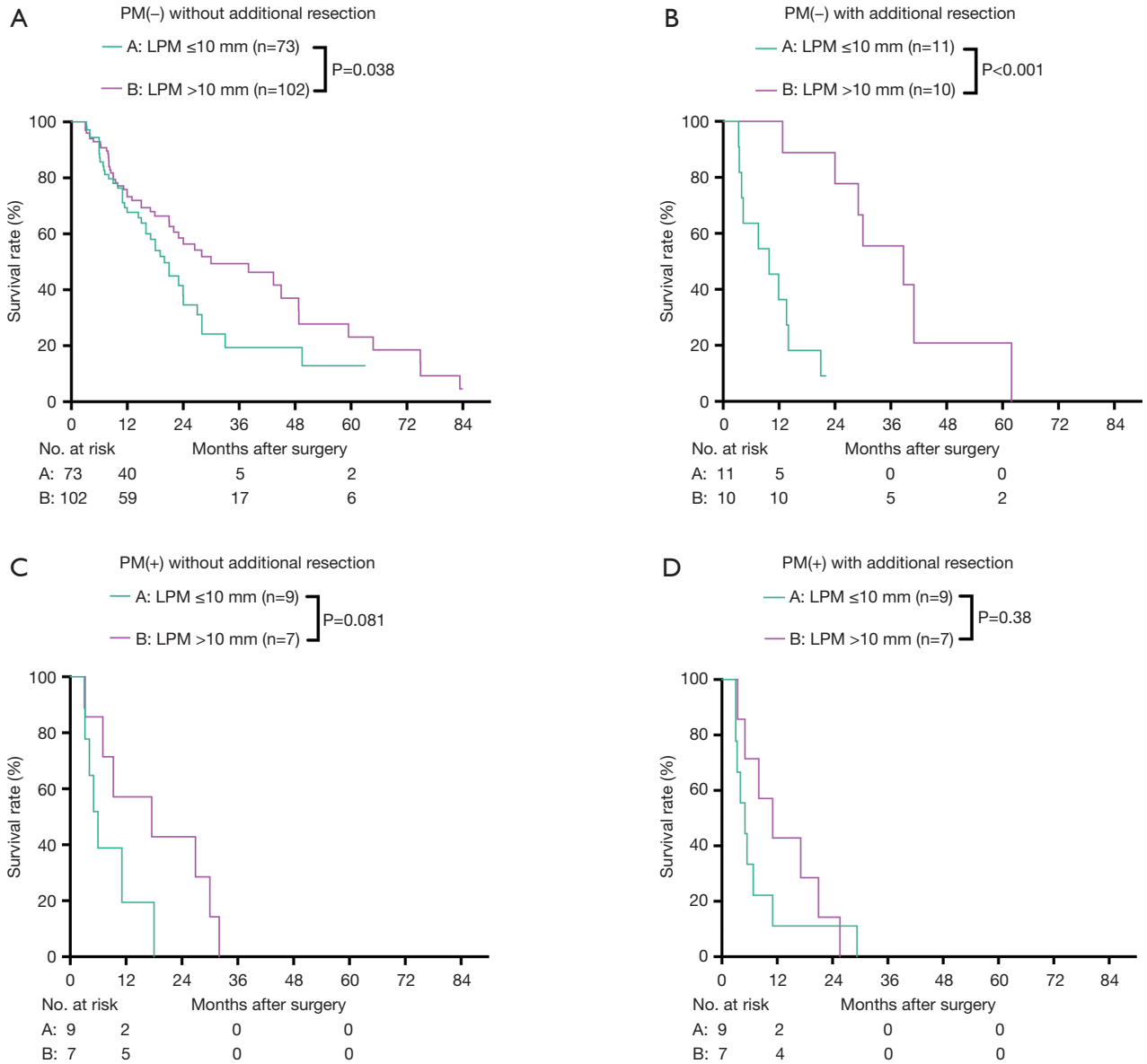

Figure 7 The survival of patients with an LPM >10 mm was significant better than that of the patients with LPM ≤10 mm (P<0.05), except for those patients with PM(+) (P>0.05).

the results of among the studies. In our study, we found that The OS of LPM ≤5 mm was significantly worse than that of other groups (*Figure 8A*). The OS of 5 mm < LPM ≤10 mm was significantly worse than that of 20 mm < LPM (P=0.047, *Figure 8A*). The OS of 10 mm < LPM ≤20 mm and 20 mm < LPM was not significantly different (P=0.20, *Figure 8A*). Therefore, a wider proximal bile ductal margin could help to increase the overall survival.

In our study, 16 PM(+) patients who underwent additional resection did not achieve a negative proximal DM, which may be attributed to a false-negative diagnosis on intra-operative FS analysis. Generally, the intraoperative status of the ductal margin is assessed using histopathological FS analysis (9). However, the inaccuracy of FS analysis to determine the presence of invasive carcinoma or epithelial atypia at the bile duct margin should be considered when compared with permanent histopathological analysis, especially after biliary drainage

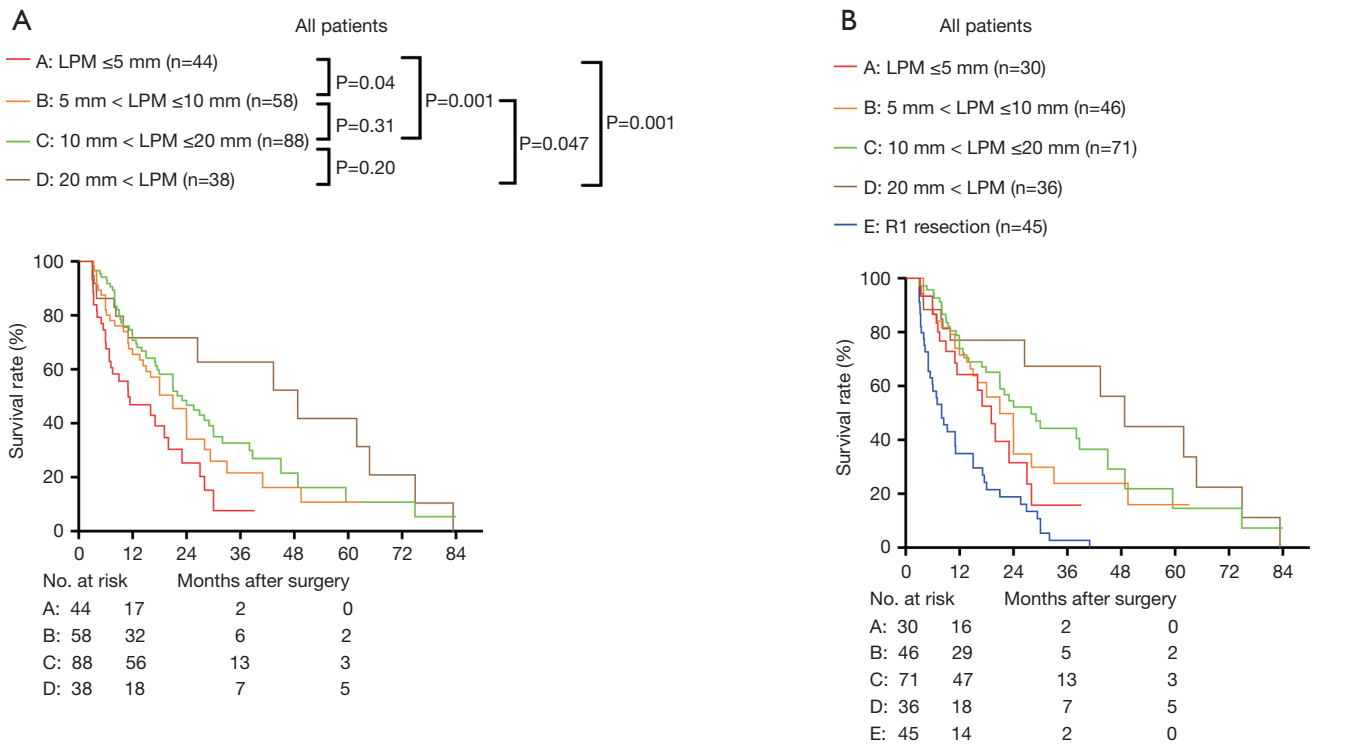

Figure 8 Overall survival for the entire study population according to the length between gross tumor and proximal bile duct resection margin (LPM).

procedures (9). The sensitivity of intraoperative FS was reported as 68–75% (13,21). The inconsistency between FS and permanent histopathological analyses of bile ductal margin may explained by several factors. Inflammatory stromal infiltration of the tumor into the surrounding duct is an inherent characteristic of cholangiocarcinoma (22). the mistaken margin assessment of intraoperative FS analysis may attributed to the presence of atypical cells within the boundary zone between the tumor and the normal duct epithelium, and the propensity for submucosal tumor extension (9).

The pre-CA19-9 level has been used as a useful prognostic marker in patients with gastrointestinal cancers (23-25). Unfortunately, only a few studies have reported the prognostic value of the pre-CA19-9 level in patients with biliary carcinomas. As coexisted obstructive jaundice is generally a feature of HCCA, which has an impact on the serum CA19-9 level. It is complicated to clarify the effect of an elevated CA19-9 level is (26-29). Thus, assessing the pre-CA19-9 level after suitable biliary drainage is recommended to predict long-term survival. The CA19-9 level might be a significant prognostic factor only in limited patients with resected HCCA (6) Previously, we reported that preoperative CA 19 - 9 (>150 U/mL) levels is associated with poor resectability, poor survival, and higher tendency for early recurrence (7,8). We employed the pr-CA19-9 value in present study population to investigate its prognostic value on the proximal tumor-free margin. Regardless of the pr-CA19-9 level, the survival of group A and B patients were similar. The survival of patients in group B was significantly higher than that of group D ($P<0.05$), but not significantly different with that of patients in group C. The survival effectiveness of further resection of a positive PM might be associated with the value of pre-CA19-9. Individuals with a Lewis^{a-b} phenotype (lacking the Lewis antigen glycosyl-transferase) are unable to synthesize CA19-9 (30). Approximately 10% of the Japanese population are Lewis^{a-b} and these individuals do not express CA19-9 at all (31). Consequently, we excluded patients with pr-CA <5.0 U/mL to avoid false negatives.

To better compare the effects of different LPMs on survival, we divided the enrolled patients into LPM ≤10 mm and LPM >10 mm subgroups. The results supported the view that a wider proximal bile duct margin could achieve

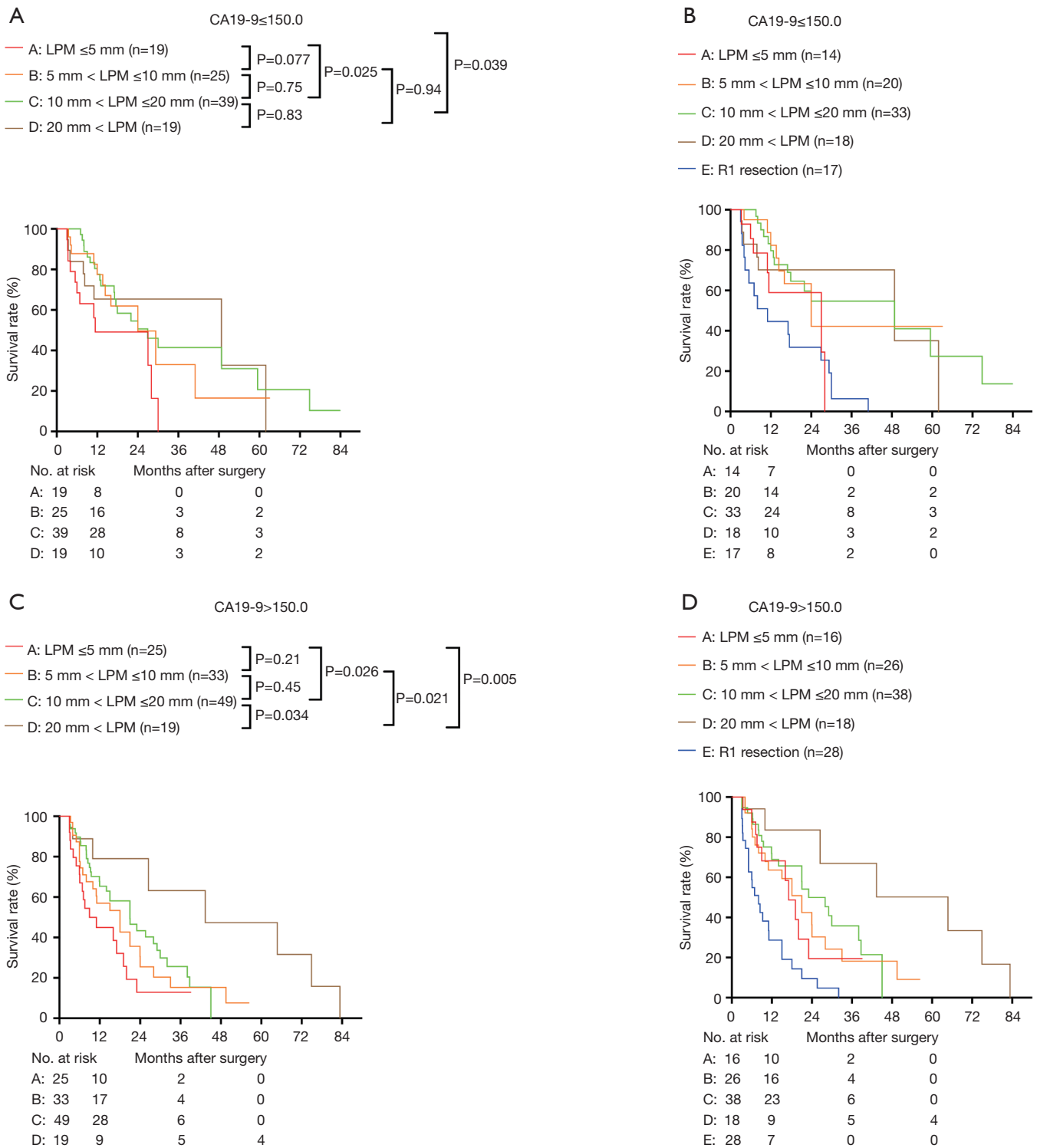

Figure 9 Overall survival according to the final length of proximal ductal margin (LPM) with the study population stratified according to preCA19-9 ≤150.0 U/mL or pre-CA19-9>150.0 U/mL.

better overall survival. The patients with LPM>10 mm survived longer than patients with LPM ≤10 mm especially those with pr-CA19-9 >150 U/mL. Further resection of the PM(+) margin was operated to secure a clear margin whenever technically possible. Ebata *et al.* suggested that a 10-mm, even a 20-mm margin is required to eradicate invasive bile duct carcinoma (32). Seyama *et al.* showed that the survival of patients with surgical tumor-free margin >5 mm was significantly better than that of patients with a margin <5 mm. However, the survival of patients after R0 resection did not significantly differ from those of patients with a narrow margin (<5 mm) or received R1 resection (16). Sakamoto *et al.* suggested a 5-mm tumor-free margin due to anastomotic recurrences never happened if a proximal tumor-free resection margin >5 mm was achieved (33). Additionally, the proximal longitudinal invasion of HCCA tumors range from 0.6 to 18.8 mm in the submucosal layer (2). These reports suggested that the resection margin status should be redefined when a proximal tumor-free resection margin <5 mm, and an LPM >10 mm is recommended to achieve significantly better survival. The different survival results between studies by Ribero *et al.*, Endo *et al.*, Shingu *et al.* and Oguro *et al.* might be attributed to the different LPMs. However, the LPM was not presented or investigated in previous studies on additional resection (1,6,9-11,13). In our study, the survival of patients with LPM ≤10 mm was significant worse than that of patients with LP >10 mm ($P<0.05$), which is an independent prognostic factor for survival. With an LPM ≤10 mm, there was no significant difference among the survival rates of groups B, C, and D, or between group B and R1 resection after separating R1 resection individually. The survival of LPM>10 mm patients was significantly better than LPM ≤10 mm patients ($P<0.05$) in group A and B. However, the survival of LPM>10 mm and LPM ≤10 mm patients were similar in group C and D. To further investigate the effect of different LPM on overall survival, we compared the survival of different LPM according to the value of preoperative CA19-9. When pre-CA19-9 ≤150.0 U/mL, the overall survival of LPM ≤5 mm was significantly worse than that of 10 mm < LPM ≤20 mm and 20 mm < LPM, but not significantly differ from that of R1 resection. The overall survival of 5 mm < LPM ≤10 mm, 10 mm < LPM ≤20 mm, and 20 mm < LPM were significantly better than R1 resection; When pre-CA19-9 >150.0 U/mL, the overall survival of 20 mm < LPM was significantly better than that of LPM ≤5 mm, 5 mm < LPM ≤10 mm, 10 mm < LPM ≤20 mm, and R1 resection. The above findings show that, for R0 resection, the pr-CA19-9 level is more associated with the LPM, rather than with whether additional resection is performed when a positive proximal margin is found intraoperatively. When pre-CA19-9 ≤150.0 U/mL, the proximal margin wider than 10 mm potentially achieve a better survival benefits; When pre-CA19-9 >150.0 U/L, the LPM wider than 20 mm may achieve a significant better survival benefit.

It is technically difficult to perform further resection at the proximal margin in PM(+) patients as only a few millimeters of bile duct can be respected. Addition resection of more than 1 cm is difficult in most patients (10). In our study, 47.6% (10/21) patients received additional resection >1 cm. Additional resection of more than 0.5–1 cm of the proximal bile duct means additional resection of a liver segment, or that additional choledochojejunostomy is needed, which significantly increases the damage to the patients. Therefore, it is necessary to consider the patient's general condition comprehensively when performing an additional resection.

Most cases of HCCA are encountered after invasion into the periductal connective tissue or surrounding liver (34). It is commonly to encounter that the periductal hilar tissues and adjacent liver tissue were directly invaded, even in well-differentiated adenocarcinoma. Somer *et al.* identified that the tumor had a very prominent, direct extension into the liver (35). Once a tumor infiltrates beyond the serum, perineural invasion could be seen in 81.4% cases (36), and vascular structures are also often invaded by tumors. Hepatic invasion could be observed in up to 76% of HCC patients under surgery (37). The mean distance of microscopic invasion beyond the gross margin toward the liver is 16.8 mm, making it difficult to evaluate and obtain an R0 resection (38). This was supported by the present study, in which the rate of microscopic liver invasion was significantly higher in group D.

There are several limitations in the current study. First, the sample size was small, especially the additional resection and PM(+) groups, because it often depends on the accuracy, sensitivity, and specificity of intraoperative FS to decide whether additional resection should be performed. The invasion of critical vessels and adjacent liver parenchyma of HCCA also increases the difficulty of additional resection to achieve R0 status. Second, this study was drawn from a single geographical area. Third, HCCA is generally diagnosed at an advanced stage and many patients have hyperbilirubinemia. The pr-CA is always associated with increased total bilirubin level, which affects the results

analysis. Thus, further research and multicenter studies should be carried out to support the clinical utility of our findings.

In conclusion, the survival benefit of further resection of the positive proximal margin in HCCA was not significantly different to negative proximal margin without additional resection. It has a limited association with the pr-CA19-9 level. An LPM ≥10 mm is potentially more associated with the survival benefit compared with additional resection of the positive proximal margin, when performed under different pr-CA19-9 levels.

Acknowledgements

Wen-Jie Ma wants to thank, in particular, the invaluable support received from C.B. Hu over the years.
Funding: We acknowledge the Science & Technology Support Project of Sichuan Province (Nos. 2018JY0019, 2015FZ0076, and 2014SZ0191).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study protocol was approved by the ethics committee review board of Sichuan University. Informed consent was obtained from all patients for surgical treatment. Data collection and analysis were performed according to the ethical standards of the Helsinki Declaration.

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Cite this article as: Ma WJ, Wu ZR, Shrestha A, Yang Q, Hu HJ, Wang JK, Liu F, Zhou RX, Li QS, Li FY. Effectiveness of additional resection of the invasive cancer-positive proximal bile duct margin in cases of hilar cholangiocarcinoma. HepatoBiliary Surg Nutr 2018;7(4):251-269. doi: 10.21037/hbsn.2018.03.14

