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ORIGINAL RESEARCH

Prognostic factors for operable biliary tract cancer: serum levels of lactate dehydrogenase, a strong association with survival

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Background: Biliary tract cancers (BTCs) are uncommon but fatal, with a low 5-year survival rate after surgical resection. This study was designed to investigate the prognostic factors for operable BTC.

Methods: Baseline demographics at diagnosis were retrospectively evaluated in 341 BTC patients undergoing radical surgery at The First Affiliated Hospital of Nanjing Medical University from January 2011 to December 2015. The association between prognostic factors and overall survival (OS) was determined by multivariate analysis using the Cox proportional hazards regression model.

Results: Our study showed that 341 patients were included in the analysis, of which 166 (48.7%) were males and 175 (51.3%) were females. Older age, depth of tumor invasion, positive surgical margin, lower hemoglobin, and higher lactic dehydrogenase (LDH) were associated with significantly worse OS using multivariate analysis. In the entire cohort, the estimate of median OS in patients with LDH <271 U/L was 36.291 months (95% CI; 30.989–41.594 months), and 30.736 months (95% CI; 19.154–42.318 months) in patients with LDH ≥271 U/L (adjusted HR-1.505, 95% CI; 1.009–2.245, $P = 0.045$). Moreover, it was investigated whether serum LDH retained its significance as a prognostic marker in BTC subgroups separately. The results showed that LDH was prognostic in patients with distal bile duct (DBD) carcinoma undergoing radical surgery (HR-2.452, 95% CI; 1.167–5.152, $P = 0.018$). However, there were no statistical differences between LDH and OS in multivariate analysis in the other three individual subgroups except for DBD carcinoma. This may be due to the limited number of patients in the study, indicating that a greater number of patients may be required for statistical significance.

Conclusion: Older age, depth of tumor invasion, positive surgical margin status, lower hemoglobin levels, and elevated serum LDH level are associated with poor survival in operable BTC patients. Serum LDH level is a cost-effective prognostic biomarker in patients with operable BTC and especially DBD carcinoma.

Keywords: biliary tract cancer, lactate dehydrogenase, tumor marker, prognosis, radical surgery

Introduction

Biliary tract cancers (BTCs) are rare but fatal. In the USA in 2017, the estimated number of new cancer cases from gallbladder and other biliary tissue was 11,740 (5,320 in males; 6,420 in females) and the estimated number of deaths was 3,830 (1,630 in males; 2,200 in females).[1] In People's Republic of China in 2015, estimates of gallbladder cancer incidence and mortality were 52,800 (24,500 in males;

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28,300 in females) and 40,700 (18,800 in males; 21,800 in females), respectively.[2] Tumors of the biliary tract typically have a poor prognosis, with 5-year survival rates in the range of 5%–15%.[3,4] According to the primary site, BTCs encompass gallbladder carcinoma (GBC), distal bile duct (DBD) carcinoma, intrahepatic cholangiocarcinoma (IHC), and hilar cholangiocarcinoma.

Surgical resection is reported to be the only potential method for curative treatment of BTC.[5,6] Data supporting an adjuvant approach are sparse. The Phase III UK Advanced Biliary Cancer-02 (ABC-02) study confirmed the combination of cisplatin with gemcitabine as the standard treatment for advanced BTC.[7] Targeting angiogenesis and HER2/neu blockade have been shown to be promising treatment strategies for BTC patients.[8,9] However, prognostic factors among BTC patients remain scarce. R0 resection and adjuvant chemotherapy may be prognosticators of long-term survival for patients with cholangiocarcinoma.[10–12] Despite surgical resection, recent reports concerning postoperative prognosis are unsatisfactory, with a 5-year survival rate of 27%–37% for DBD carcinoma, 22%–44% for IHC, and 11%–41% for hilar cholangiocarcinoma.[13–17] Therefore, the prognostic factors that predict long-term survival of BTC patients undergoing radical surgery are essential.

Serum tumor markers, including carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), have been used as a complementary approach for the diagnosis of BTC in conjunction with radiology and histology/cytology.[18–20] Likewise, lactic dehydrogenase (LDH) is a glycolytic enzyme that is essential for tumor maintenance and can be used as an attractive antitumor strategy by inhibiting glucose metabolism.[21] LDH is reported to be associated with the hypoxia-inducible factor (HIF) pathway, hypoxia, acidity, and neoangiogenesis.[22] Serum LDH levels can provide prognostic information on overall survival (OS) in patients with advanced pancreatic cancer, colorectal cancer, and melanoma.[23–26] However, the prognostic roles of tumor markers including CEA, CA19-9, and LDH have not yet been clearly elucidated in operable BTC.

The aim of this study was to investigate the role of tumor markers in predicting clinical outcome for BTC patients who have undergone radical surgery. It was hypothesized that elevated baseline serum LDH is prognostic of diminished OS in operable BTC, especially in operable DBD carcinoma.

Methods

Study population and data collection

Patients who were diagnosed with BTC at The First Affiliated Hospital of Nanjing Medical University from January 2011 to December 2015 were enrolled in this study. All patients were histologically confirmed with BTC. Due to a potentially different biology,[27] ampullary tumors were not included in this study. Exclusion criteria were as follows: 1) BTC patients with concurrent primary tumors of other types, 2) patients with missing or incomplete interest data, 3) patients with advanced BTC, and 4) patients who expired within the first month after surgery due to postoperative complications. In total, 341 patients were eligible for the analysis and were followed until December 2016 (Figure 1).

The baseline demographics that were evaluated in this study included age, sex, site of primary tumor (DBD, GBC, IHC, or hilar), depth of tumor invasion, lymph node status, metastasis, stage, histologic differentiation, lymphovascular invasion, perineural invasion, surgical margin status, hemoglobin, CEA, CA19-9, and LDH at diagnosis (prior to surgery). A CEA level of 4.7 ng/mL, CA 19-9 level of

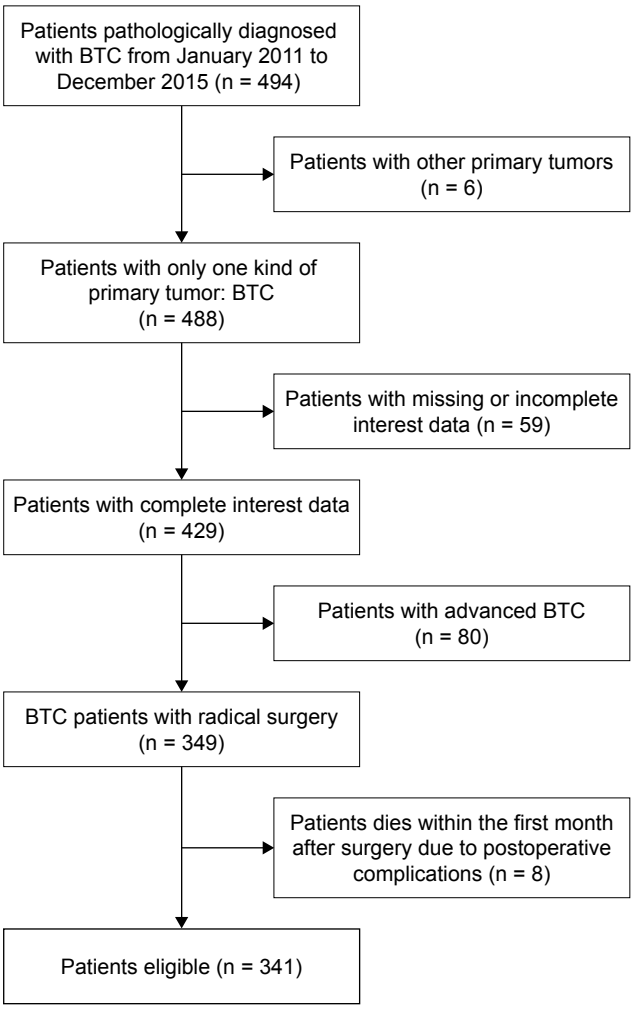

Figure 1 Flowchart on patient selection.
Abbreviation: BTC, biliary tract cancer.

39 U/mL, and LDH level of 271 U/L were defined as cutoff values for normal levels according to the historical data and manufacturer's recommendations.[28,29] Stage classification was based on pathological findings and documented according to the seventh edition of the Union for International Cancer Control tumor-node-metastasis staging system.[30] The protocol was approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University prior to study initiation and conformed to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). Written informed consent was obtained from all patients.

Statistics

Continuous variables were described as median (interquartile range [IQR]). OS was defined as the period from the day of operation to death or the last follow-up (December 2016). The OS of all patients in this study was analyzed and recorded. Survival distribution was estimated by the Kaplan–Meier method. The log-rank test was used to evaluate the equality of survivor functions across groups. The Cox proportional hazards model was used with a 95% CI for the univariate and multivariate analyses. The chi-square test was used to compare baseline characteristics of patients between the LDH groups. All statistical tests were two-sided. Statistical significance was defined as $P < 0.05$. All analyses were performed using SPSS software for Windows (version 19; IBM SPSS, Somers, NY, USA).

Results

Patients

A total of 341 BTC patients were available for our analysis. Patient and tumor characteristics of the entire cohort are presented in Table 1. Among the 341 patients, 146 (42.8%) patients were above 65 years of age and 195 (57.2%) patients were below 65 years of age. The study cohort consisted of 166 (48.7%) males and 175 (51.3%) females. The median serum levels of CEA, CA19-9, and LDH of the entire cohort were 3.40 ng/mL (IQR 2.11–5.27 ng/mL), 83.2 U/mL (IQR 24.8–417.2 U/mL), and 215 U/L (IQR 184–273 U/L), respectively.

Patient characteristics by primary site are reported in Table 2. Malignancies included 98 (28.7%) DBD carcinoma, 127 (37.2%) GBC, 38 (11.1%) IHC, and 75 (22.0%) hilar cholangiocarcinoma.

Outcomes in the entire cohort

To investigate whether the patient baseline characteristics and laboratory factors were associated with survival of

Table 1 Demographics for entire cohort of patients with biliary tract cancer (N = 341)

Variable	Entire cohort (N = 341) N (%)
Age	
<65 years	195 (57.2)
≥65 years	146 (42.8)
Sex	
Male	166 (48.7)
Female	175 (51.3)
Primary tumor site	
Distal bile duct	98 (28.7)
Gallbladder	127 (37.2)
Intrahepatic	38 (11.1)
Hilar	75 (22.0)
NA	3 (0.9)
Depth of tumor invasion	
T1	23 (6.7)
T2	132 (38.7)
T3	158 (46.3)
T4	15 (4.4)
NA	13 (3.8)
Lymph node status	
Negative	223 (65.4)
Positive	109 (32.0)
NA	9 (2.6)
Metastasis	
Negative	299 (87.7)
Positive	33 (9.7)
NA	9 (2.6)
Seventh UICC TNM Stage	
I	41 (12.0)
II	138 (40.5)
III	100 (29.3)
IV	46 (13.5)
NA	16 (4.7)
Histologic differentiation	
Well and moderately differentiated	151 (44.2)
Poorly differentiated	169 (49.6)
NA	21 (6.2)
Lymphovascular invasion	
Negative	296 (86.8)
Positive	39 (11.4)
NA	6 (1.8)
Perineural invasion	
Negative	172 (50.4)
Positive	163 (47.8)
NA	6 (1.8)
Resection margin	
Negative	258 (75.7)
Positive	77 (22.6)
NA	6 (1.8)
Hemoglobin g/L, median (IQR)	125 (114–135)
CEA ng/mL, median (IQR)	3.40 (2.11–5.27)
CA19-9 U/mL, median (IQR)	83.2 (24.8–417.2)
LDH U/L, median (IQR)	215 (184–273)

Abbreviations: NA, not available; UICC, Union for International Cancer Control; TNM, tumor node metastasis; IQR, interquartile range; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.

Table 2 Demographics by primary site for entire cohort of patients with biliary tract cancer (N = 338)

Variable	Distal bile duct (N = 98) N (%)	Gallbladder (N = 127) N (%)	Intrahepatic (N = 38) N (%)	Hilar (N = 75) N (%)
Age				
<65 years	57 (58.2)	68 (53.5)	25 (65.8)	45 (60.0)
≥65 years	41 (41.8)	59 (46.5)	13 (34.2)	30 (40.0)
Sex				
Male	52 (53.1)	47 (37.0)	19 (50)	47 (62.7)
Female	46 (46.9)	80 (63.0)	19 (50)	28 (37.3)
Depth of tumor invasion				
T1	2 (2.0)	7 (5.5)	12 (31.6)	2 (2.7)
T2	29 (29.6)	48 (37.8)	13 (34.2)	42 (56.0)
T3	62 (63.3)	64 (50.4)	9 (23.7)	23 (30.7)
T4	2 (2.0)	6 (4.7)	1 (2.6)	6 (8.0)
NA	3 (3.1)	2 (1.6)	3 (7.9)	2 (2.7)
Lymph node status				
Negative	66 (67.3)	78 (61.4)	28 (73.7)	51 (68.0)
Positive	31 (31.6)	48 (37.8)	7 (18.4)	22 (29.3)
NA	1 (1.0)	1 (0.8)	3 (7.9)	2 (2.7)
Metastasis				
Negative	93 (94.9)	110 (86.6)	28 (73.7)	67 (89.3)
Positive	4 (4.1)	16 (12.6)	7 (18.4)	6 (8.0)
NA	1 (1.0)	1 (0.8)	3 (7.9)	2 (2.7)
Seventh UICC TNM stage				
I	20 (20.4)	11 (8.7)	8 (21.1)	2 (2.7)
II	58 (59.2)	37 (29.1)	10 (26.3)	33 (44.0)
III	13 (13.3)	56 (44.1)	4 (10.5)	27 (36.0)
IV	4 (4.1)	20 (15.7)	11 (28.9)	11 (14.7)
NA	3 (3.1)	3 (2.4)	5 (13.2)	2 (2.7)
Histologic differentiation				
Well and moderately differentiated	49 (50.0)	56 (44.1)	16 (42.1)	30 (40.0)
Poorly differentiated	43 (43.9)	65 (51.2)	20 (52.6)	41 (54.7)
NA	6 (6.1)	6 (4.7)	2 (5.3)	4 (5.3)
Lymphovascular invasion				
Negative	88 (89.8)	109 (85.8)	35 (92.1)	64 (85.3)
Positive	10 (10.2)	17 (13.4)	2 (5.3)	10 (13.3)
NA	0	1 (0.8)	1 (2.6)	1 (1.3)
Perineural invasion				
Negative	45 (45.9)	84 (66.1)	22 (57.9)	21 (28.0)
Positive	53 (54.1)	42 (33.1)	15 (39.5)	53 (70.7)
NA	0	1 (0.8)	1 (2.6)	1 (1.3)
Resection margin				
Negative	76 (77.6)	100 (78.7)	31 (81.6)	51 (68.0)
Positive	22 (22.4)	26 (20.5)	6 (15.8)	23 (30.7)
NA	0	1 (0.8)	1 (2.6)	1 (1.3)
Hemoglobin g/L, median (IQR)	124.50 (111.00–134.25)	125.00 (113.00–134.00)	127 (117.75–139.25)	124.00 (113.00–135.00)
CEA ng/mL, median (IQR)	3.65 (2.32–4.93)	3.42 (1.90–6.93)	3.30 (2.00–6.44)	3.10 (2.13–5.15)
CA19-9 U/mL, median (IQR)	130.90 (49.20–418.90)	40.01 (11.93–279.75)	69.60 (24.90–1,000)	204.00 (48.60–606.40)
LDH U/L, median (IQR)	219.50 (183.00–300.25)	204.00 (177.00–250.00)	222.50 (182.00–273.00)	222.00 (194.00–267.0)

Abbreviations: NA, not available; UICC, Union for International Cancer Control; TNM, tumor node metastasis; IQR, interquartile range; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.

BTC patients undergoing radical surgery, univariate and multivariate analyses were performed. Univariate analysis revealed that better survival was associated with age <65 years, with a tumor primary site from DBD, depth of tumor invasion, negative lymph node status, early stage, well and moderately differentiated histological differentiation, absence of lymphovascular invasion, absence of perineural invasion, negative surgical margin, hemoglobin ≥110 g/L, and LDH <271 U/L

Table 3 Univariate and multivariate analyses of patients with biliary tract cancer (N = 341)

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age ≥65 years vs <65 years	1.457	1.087–1.953	**0.012**	1.491	1.060–2.099	**0.022**
Female vs male	1.247	0.930–1.672	0.140	1.094	0.764–1.565	0.624
GBC vs DBD	1.547	1.066–2.246	**0.022**	1.440	0.928–2.236	0.104
IHC vs DBD	1.321	0.778–2.242	0.303	1.826	0.905–3.686	0.093
Hilar vs DBD	1.470	0.957–2.257	0.078	1.326	0.791–2.223	0.284
Depth of tumor invasion						
T2 vs T1	2.565	1.032–6.377	**0.043**	2.730	0.931–8.001	0.067
T3 vs T1	3.376	1.372–8.304	**0.008**	3.343	1.138–9.818	**0.028**
T4 vs T1	5.827	2.020–16.806	**0.001**	5.381	1.565–18.505	**0.008**
Lymph node status (positive vs negative)	1.727	1.275–2.338	<**0.001**	1.357	0.948–1.944	0.096
Metastasis (positive vs negative)	1.383	0.867–2.205	0.173	1.018	0.587–1.767	0.949
Stage II vs I	2.599	1.339–5.044	**0.005**	/	/	/
Stage III vs I	3.627	1.862–7.067	<**0.001**	/	/	/
Stage IV vs I	4.218	2.060–8.637	<**0.001**	/	/	/
Histologic differentiation (poorly vs well and moderately differentiated)	1.745	1.287–2.366	<**0.001**	1.409	0.991–2.005	0.056
Lymphovascular invasion (positive vs negative)	1.741	1.155–2.626	**0.008**	1.211	0.739–1.984	0.447
Perineural invasion (positive vs negative)	1.387	1.031–1.867	**0.031**	0.973	0.673–1.407	0.885
Resection margin (positive vs negative)	1.557	1.108–2.187	**0.011**	1.500	1.011–2.227	**0.044**
Hemoglobin (<110 g/L vs ≥110 g/L)	1.805	1.280–2.546	**0.001**	1.551	1.010–2.382	**0.045**
CEA (≥4.7 ng/mL vs <4.7 ng/mL)	1.216	0.875–1.691	0.244	1.442	0.999–2.082	0.051
CA19-9 (≥39 U/mL vs <39 U/mL)	1.340	0.945–1.899	0.101	0.972	0.646–1.463	0.893
LDH (≥271 U/L vs <271 U/L)	1.435	1.038–1.982	**0.029**	1.505	1.009–2.245	**0.045**

Notes: Data in bold indicates $P < 0.05$. / indicates not included in the multivariate analysis.
Abbreviations: GBC, gallbladder carcinoma; DBD, distal bile duct; IHC, intrahepatic carcinoma; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.

(Table 3 and Figure 2). However, using univariate analysis, sex, metastasis, CEA, and CA19-9 were not statistically significant in OS in patients with radical surgery of BTC. Compared to LDH <271 U/L, LDH ≥271 U/L was associated with a worse OS using univariate analysis (HR-1.435, 95% CI; 1.038–1.982, $P = 0.029$). Moreover, LDH retained its significance as a prognostic marker in multivariate analysis (HR-1.505, 95% CI; 1.009–2.245, $P = 0.045$) in the entire cohort along with age, depth of tumor invasion, resection margin, and hemoglobin (Table 3).

Comparison between high and low LDH groups

For all patients included in the analysis, the estimate of median OS in patients with LDH <271 U/L was 36.291 months (95% CI; 30.989–41.594 months) and 30.736 months (95% CI; 19.154–42.318 months) in patients with LDH ≥271 U/L (log-rank $P = 0.028$) (Figure 2E).

Patients showing a pretreatment serum LDH <271 U/L were classified as LDH-low patients (251 patients, 73.6%, group A), whereas patients with pretreatment serum LDH level ≥271 U/L were classified as LDH-high patients (90 patients, 26.4%, group B). The patients with positive lymphovascular invasion and positive perineural invasion were more frequently found in the high LDH group ($P = 0.010$, $P = 0.031$, respectively) (Table 4).

Outcomes by BTC site

Considering the tumor primary site, it was further determined whether serum LDH retained its significance as a prognostic marker in BTC subgroups separately. The results showed that LDH was prognostic in multivariate analysis for the DBD subgroup (HR-2.452, 95% CI; 1.167–5.152, $P = 0.018$) (Table 5). The data showed a nonsignificant improvement in survival with LDH <271 U/L compared with LDH ≥271 U/L in multivariate model in individual subgroups: GBC (Table S1) or hilar cancer (Table S2). Moreover, in multivariate analysis, age ($P = 0.003$) and depth of tumor invasion ($P = 0.020$) showed a significant association with OS in GBC (Table S1). Since there was a limited number of patients in the IHC subgroup, the corresponding multivariate analysis was not available. Details of multivariate analysis outcomes for OS by BTC site are provided in Tables 5, S1, and S2.

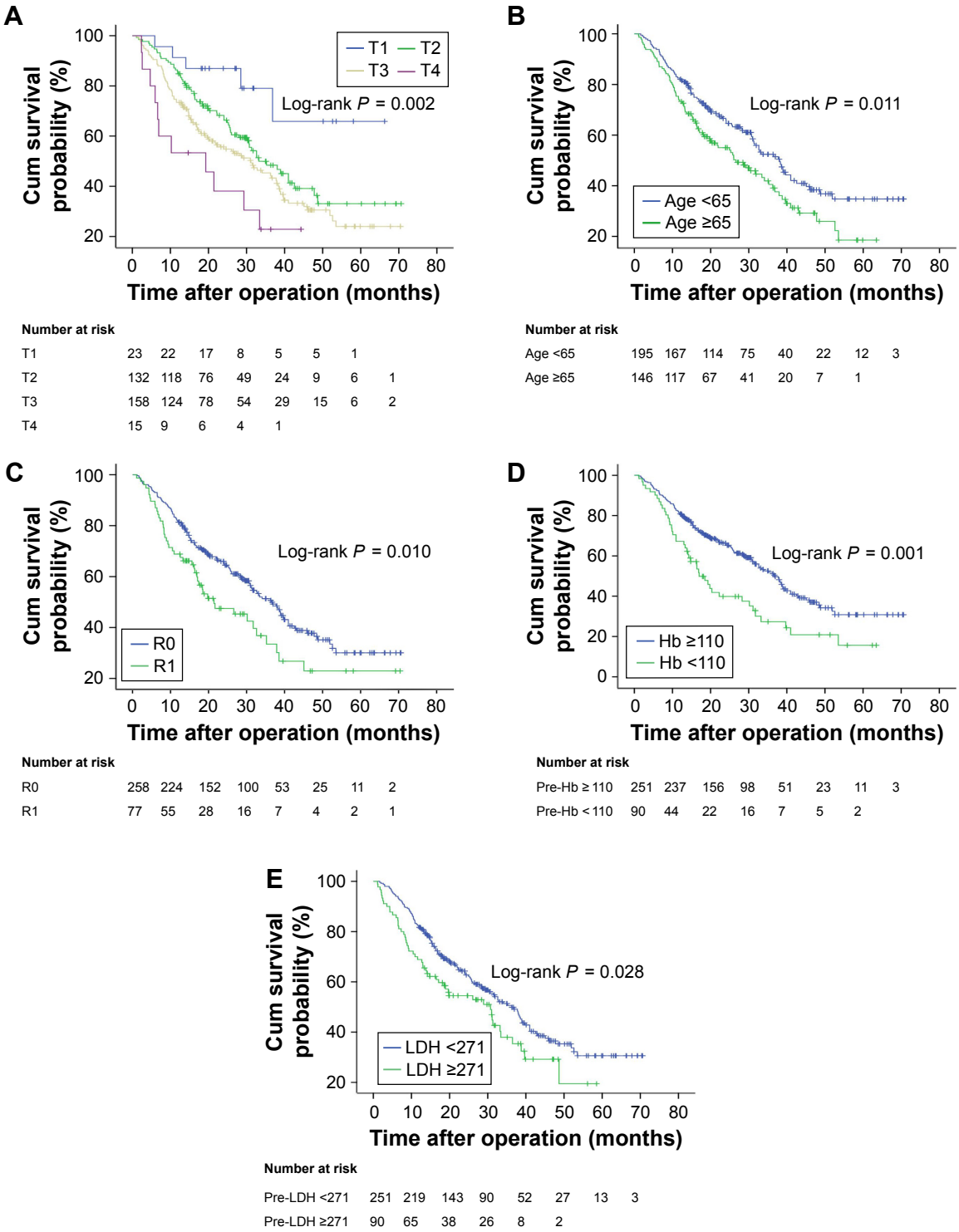

Figure 2 Kaplan–Meier analysis of overall survival.
Notes: Unadjusted overall survival curves for independent factors by univariate analyses: depth of tumor invasion (**A**), age (**B**), resection margin (**C**), pre-Hb (**D**), and pre-LDH (**E**).
Abbreviations: pre-, preoperative; Hb, hemoglobin; LDH, lactic dehydrogenase; T, depth of tumor invasion; R0, positive resection margin; R1, negative resection margin.

Discussion

Surgical resection offers the only potentially curative option for BTC,[5] while postoperative prognosis is unsatisfactory after extensive surgical resection.[13–16] Moreover, the prognostic role of tumor markers has not yet been clearly elucidated in BTC patients undergoing radical surgery. Therefore, it is significant to identify prognostic factors that predict long-term survival of BTC patients who have undergone radical surgery.

Table 4 Comparison between the high LDH and low LDH groups (N = 341)

Variable	LDH <271 (N = 251) N (%)	LDH ≥271 (N = 90) N (%)	*P*-value
Age			
<65 years	144 (57.4)	51 (56.7)	0.908
≥65 years	107 (42.6)	39 (43.3)	
Sex			
Male	123 (49.0)	43 (47.8)	0.842
Female	128 (51.0)	47 (52.2)	
Primary tumor site			
Gallbladder	99 (39.8)	28 (31.5)	0.154
Distal bile duct	64 (25.7)	34 (38.2)	
Hilar	58 (23.3)	17 (19.1)	
Intrahepatic	28 (11.2)	10 (11.2)	
Depth of tumor invasion			
T1	20 (8.4)	3 (3.4)	0.064
T2	102 (42.7)	30 (33.7)	
T3	105 (43.9)	53 (59.6)	
T4	12 (5.0)	3 (3.4)	
Lymph node status			
Negative	170 (70.2)	53 (58.9)	0.050
Positive	72 (29.8)	37 (41.1)	
Metastasis			
Negative	219 (90.5)	80 (88.9)	0.664
Positive	23 (9.5)	10 (11.1)	
Seventh UICC TNM stage			
I	30 (12.7)	11 (12.4)	0.752
II	104 (44.1)	34 (38.2)	
III	69 (29.2)	31 (34.8)	
IV	33 (14.0)	13 (14.6)	
Histologic differentiation			
Well and moderately differentiated	113 (48.5)	38 (43.7)	0.442
Poorly differentiated	120 (51.5)	49 (56.3)	
Lymphovascular invasion			
Negative	224 (91.1)	72 (80.9)	**0.010**
Positive	22 (8.9)	17 (19.1)	
Perineural invasion			
Negative	135 (54.9)	37 (41.6)	**0.031**
Positive	111 (45.1)	52 (58.4)	
Resection margin			
Negative	194 (78.9)	64 (71.9)	0.182
Microscopic positive	52 (21.1)	25 (28.1)	
Hemoglobin g/L, median (IQR)	125.00 (114.00–134.75)	125 (111.5–137.0)	0.426
CEA ng/mL, median (IQR)	3.10 (2.06–5.22)	3.80 (2.30–5.50)	0.503
CA19-9 U/mL, median (IQR)	68.15 (19.35–336.90)	132.50 (55.60–707.40)	0.192

Note: Data in bold indicates $P < 0.05$.

Abbreviations: LDH, lactic dehydrogenase; UICC, Union for International Cancer Control; TNM, tumor node metastasis; IQR, interquartile range; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

LDH has been reported to be a prognosticator in various types of tumors.[22,24,26] Some studies suggest a prognostic role of LDH in patients with advanced BTC receiving certain chemotherapy.[31,32] However, the prognosis of LDH in BTC patients undergoing radical surgery and in different tumor primary sites remains unclear. The present study revealed that high serum LDH level is significantly associated with worse survival in patients with operable BTC.

In our study, we discovered the prognostic roles of age, depth of tumor invasion, surgical margin status, hemoglobin, and serum LDH level for OS in multivariate analysis in the entire cohort of 341 patients surgically treated for BTC. Pradeep et al[33] found that age was a significant predictor of survival by multivariate analysis in analyzing prognostic factors in 87 patients with GBC. Cubertafond et al[34] reported in a survey of 724 patients surgically treated

Table 5 Multivariate analysis of the patients with distal bile duct carcinoma (N = 98)

Variable	Multivariate analysis		
	HR	95% CI	*P*-value
Age ≥65 years vs <65 years	0.857	0.386–1.902	0.704
Female vs male	1.897	0.896–4.018	0.094
Depth of tumor invasion			
T3 vs T2	1.378	0.634–2.994	0.418
T4 vs T2	1.142	0.111–11.715	0.911
Lymph node status (positive vs negative)	2.282	1.133–4.594	**0.021**
Metastasis (positive vs negative)	0.625	0.141–2.774	0.537
Histologic differentiation			
Poorly vs well and moderately differentiated	1.135	0.529–2.436	0.746
Lymphovascular invasion (positive vs negative)	0.426	0.124–1.472	0.177
Perineural invasion (positive vs negative)	1.620	0.730–3.598	0.236
Resection margin (positive vs negative)	1.330	0.583–3.038	0.498
Hemoglobin (<110 g/L vs ≥110 g/L)	1.671	0.723–3.866	0.230
CEA (≥4.7 ng/mL vs <4.7 ng/mL)	1.069	0.478–2.392	0.871
CA19-9 (≥39 U/mL vs <39 U/mL)	0.919	0.335–2.521	0.870
LDH (≥271 U/L vs <271 U/L)	2.452	1.167–5.152	**0.018**

Note: Data in bold indicates $P < 0.05$.
Abbreviations: CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.

for GBC that the long-term survival correlated with cancer stage: >60 months, >22 months, and 8 months for Tis, T1 to T2, and T3 to T4, respectively. R0 resection is reported to be prognosticator of long-term survival for patients with cholangiocarcinoma.[10] These studies were consistent with our findings.

CA19-9 and CEA have been used as a complementary approach for the diagnosis of BTC in conjunction with radiology and histology/cytology,[18–20] while the prognostic roles for better survival remain controversial. Lee et al[35] suggested that CA19-9 but not CEA served as a predictor of better survival in patients with advanced cholangiocarcinoma on gemcitabine-based chemotherapy. Peixoto et al[36] reported that CA19-9 and CEA were not significantly associated with OS in 106 patients with advanced BTC using univariate analysis. Our study showed a nonsignificant trend for better survival of patients with a low level of CA19-9 or CEA. The prognostic roles of CA19-9 and CEA for survival in BTC patients have to be further validated with a larger number of patients and prospective studies.

Moreover, we found that LDH is predictive of poor prognosis and clinical outcome in BTC patients undergoing radical surgery. In addition, Faloppi et al[37] showed a possible prognostic role of pretreatment serum LDH levels in advanced BTC patients treated with first-line chemotherapy, confirming our hypothesis. In a Phase II study, Furuse et al[31] revealed that elevated serum LDH level was associated with a significantly shorter survival in 85 patients with unresectable BTC receiving combination chemotherapy of uracil-tegafur and doxorubicin ($P = 0.043$). Moreover, these findings are in accordance with previously published analyses suggesting a relationship between LDH levels and a worse outcome in other tumor types.[22,23,38] Furthermore, our study revealed that serum LDH can be a prognostic marker for DBD carcinoma. However, in terms of other primary tumors originating from the biliary tract, there were no statistically significant differences between LDH and OS in multivariate analysis in individual subgroups. The results indicated the possibility of different origins and biological behavior among these primary sites. Alternatively, this may be due to the limited number of patients in this study. Future studies may require greater numbers of patients to be considered statistically significant. The ABC-02 trial demonstrated that the site of the primary tumor within the biliary tract did not affect survival.[7]

Hypoxia-induced angiogenesis is probably the mechanism involved in high serum LDH levels and poor prognosis.[22] Published studies demonstrated the association between LDH5 up-regulation and HIF (HIF1α/HIF2α) accumulation, which is linked with activating transcription of multiple genes including those encoding glycolytic enzymes and vascular endothelial growth factor.[21,39] In addition, activated HIF pathway and angiogenic factor production are correlated with enhanced tumor aggressiveness.[40,41]

Conclusion

These data shed light on older age, depth of tumor invasion, positive surgical margin status, lower hemoglobin, and elevated serum LDH level as prognostic factors for poor survival in operable BTC patients. Moreover, serum LDH could be used as a cost-effective prognostic biomarker for DBD cancer. A limitation of this study is its retrospective nature, which means it lacks availability of some data, but it is reflective of the clinical spectrum of Chinese patients and is a relatively large study on this rare tumor type. In addition, this analysis is a single institutional series. These findings might provide new opportunities for prospective multi-institutional trials toward clinical applications of serum LDH in BTC patients undergoing radical surgery.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Multivariate analysis of the patients with gallbladder carcinoma (N = 127)

Variable	Multivariate analysis		
	HR	95% CI	*P*-value
Age ≥65 years vs <65 years	2.389	1.356–4.209	**0.003**
Female vs male	0.589	0.338–1.025	0.061
Depth of tumor invasion			
T2 vs T1	4.782	0.592–38.614	0.142
T3 vs T1	5.566	0.683–45.376	0.109
T4 vs T1	15.464	1.548–154.453	**0.020**
Lymph node status (positive vs negative)	1.281	0.688–2.383	0.434
Metastasis (positive vs negative)	1.601	0.752–3.406	0.222
Histologic differentiation			
Poorly vs well and moderately differentiated	1.005	0.533–1.896	0.987
Lymphovascular invasion (positive vs negative)	1.642	0.746–3.612	0.218
Perineural invasion (positive vs negative)	1.336	0.749–2.384	0.326
Resection margin (positive vs negative)	1.715	0.887–3.314	0.109
Hemoglobin (<110 g/L vs ≥110 g/L)	1.651	0.772–3.533	0.196
CEA (≥4.7 ng/mL vs <4.7 ng/mL)	1.462	0.767–2.786	0.249
CA19-9 (≥39 U/mL vs <39 U/mL)	0.864	0.454–1.646	0.657
LDH (≥271 U/L vs <271 U/L)	1.735	0.864–3.483	0.121

Note: Data in bold indicates $P < 0.05$.
Abbreviations: CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.

Table S2 Multivariate analysis of the patients with hilar carcinoma (N = 75)

Variable	Multivariate analysis		
	HR	95% CI	*P*-value
Age ≥65 years vs <65 years	0.697	0.268–1.815	0.460
Female vs male	1.528	0.591–3.948	0.382
Depth of tumor invasion			
T2 vs T1	4.281	0.331–55.335	0.265
T3 vs T1	2.712	0.147–50.016	0.502
T4 vs T1	11.119	0.396–311.937	0.157
Lymph node status (positive vs negative)	0.618	0.140–2.731	0.525
Metastasis (positive vs negative)	0.140	0.015–1.289	0.083
Histologic differentiation			
Poorly vs well and moderately differentiated	1.132	0.397–3.231	0.817
Lymphovascular invasion (positive vs negative)	1.493	0.348–6.409	0.590
Perineural invasion (positive vs negative)	0.300	0.115–0.782	**0.014**
Resection margin (positive vs negative)	1.676	0.662–4.239	0.276
Hemoglobin (<110 g/L vs ≥110 g/L)	1.373	0.262–7.196	0.708
CEA (≥4.7 ng/mL vs <4.7 ng/mL)	1.889	0.697–5.119	0.211
CA19-9 (≥39 U/mL vs <39 U/mL)	2.370	0.570–9.853	0.235
LDH (≥271 U/L vs <271 U/L)	2.200	0.561–8.627	0.258

Note: Data in bold indicates $P < 0.05$.
Abbreviations: CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; LDH, lactic dehydrogenase.