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Validation of T Stage According to Depth of Invasion and N Stage Subclassification Based on Number of Metastatic Lymph Nodes for Distal Extrahepatic Bile Duct (EBD) Carcinoma

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Abstract: According to the current AJCC staging system, the T stage of distal extrahepatic bile duct carcinoma (EBD) is classified according to the extent of the tumor within or beyond the bile duct wall. However many invasive carcinoma accompany stromal desmoplasia that obscure lower boundary of bile duct wall; it is frequently difficult to clearly define the extent of tumors using the current T classification system. In this study, we validated an alternative T classification system by depth of invasion (DoI; T1: < 5 mm, T2: 5 to 12 mm, and T3: ≥ 12 mm). Specifically, we evaluated DoI in 114 cases of distal EBD carcinoma using digital scan images to achieve more objective measurements of tumor DoI. In addition, we evaluated the effect of the number of metastatic lymph nodes (LNs) as well as the number of total examined LNs on the survival rate in the same patient group, and performed a comparative analysis of these data to assess patient survival. We also analyzed 114 cases of distal EBD carcinoma using the current T and N classification of the AJCC staging system (7th edition). The T stage of the current AJCC staging system was not associated with significant differences in patient survival, especially between T2 and T3. However, T staging by DoI was associated with statistically significant differences in patient survival ($P < 0.001$ in DoI-1, $P = 0.002$ in DoI-2). With respect to N stage, we divided patients into 3 tiers comprising class 1 (no nodal metastasis), class 2 (1–3 nodal metastases), and class 3 (4 or more nodal metastases). In 3-tier classification analysis, the median survival times for classes 1, 2, and 3 were 79.2, 28.8, and 10.9 months, respectively. The difference in survival among the 3 classes was statistically significant ($P < 0.001$). We found the cut-off value of 11 LNs (1 to 10 vs ≥ 11) for N0 stage showed most significant difference ($P = 0.007$). We think at least 11 LNs should be examined for more accurate evaluation of N stage in distal EBD carcinoma. We propose an alternative T classification using DoI and 3-tier sub-classification of N stage for distal EBD carcinoma.

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Abbreviations: AJCC = American Joint Committee on Cancer, CI = confidence interval, DoI = depth of invasion, EBD = extrahepatic bile duct, HR = hazard ratio, LN = lymph node, WHO = World Health Organization.

INTRODUCTION

Bile duct carcinoma accounts for 3% of all gastrointestinal cancers worldwide,[1] and the reported incidence in the USA is 1 to 2 cases per 100,000 (3500 new cases per year).[2] Distal EBD carcinoma accounts for 20% to 30% of all bile duct carcinomas.[3] In the Republic of Korea, the age-standardized incidence rate of EBD carcinoma is 3.3 and 1.5 per 100,000 men and women, respectively,[4] which is 5 to 7 times higher than data of the USA (0.5 and 0.3 per 100,000 men and women, respectively, age-standardized incidence rate).[5] Despite the development of surgical techniques and new oncologic modalities, the survival of patients with EBD carcinoma has not been improved. The National Cancer institute's SEER data shows that the 5-year survival rates of the EBD cancer in the USA, 2000 to 2006, are 30% (localized, like AJCC stage I), 24 % (regional, including AJCC stages II and III), and 2 % (distant, the same as AJCC stage IV). The overall survival data of EBD carcinoma of the Republic of Korea has not been reported. One largest study (237 cases) which was conducted by our institute reported that the 5-year survival rate of distal EBD carcinoma patients was 48.3 % after curative resection during 1995 to 2011.[6] Curative surgical resection is the first treatment of choice for EBD carcinoma, and there is currently no beneficial biomarker for EBD carcinoma yet. Thus, tumor staging remains one of the most important parameters for predicting the survival of patients with EBD carcinoma.

In the previous World Health Organization (WHO) classification, the staging systems for bile duct carcinoma were separated into 2 groups according to anatomic location, the intrahepatic bile duct and EBD and the hilar cholangiocarcinoma was included in EBD carcinoma.[7] The EBD is surrounded by different anatomical structures according to location. Specifically, the upper EBD is partially encircled by the hilum of liver, the middle EBD is surrounded by periductal adipose tissue and exposed to the peritoneal cavity, and the distal EBD is surrounded by the pancreas. Generally, intrahepatic and perihilar cholangiocarcinomas must be treated via liver surgery, whereas the most distal EBD carcinoma requires a pancreaticoduodenectomy or Whipple resection. For this reason, the 7th

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edition of the AJCC cancer staging system divided EBD carcinomas into perihilar and distal EBD carcinomas, with the latter designated as the epicenter between the junction of the cystic duct-common hepatic duct and the ampulla of Vater.

In the current AJCC staging system for distal EBD carcinoma, T1 and T2 are classified according to the extent of the tumor within or beyond the bile duct wall, whereas T3 is determined by adjacent organ invasion, including the pancreas.[8] However, the T classification of the current AJCC staging system is problematic for several reasons. First, distinction between ''within and beyond the bile duct wall'' can be difficult and unclear, especially when marked desmoplastic stromal reaction of EBD carcinoma obscures the lower boundary of the bile duct wall. Second, several studies have failed to show significant differences in survival rate using the T classification of the current AJCC staging system.[9–11] Hong et al suggested an alternative method of T staging using DoI and demonstrated that DoI is a more powerful prognostic factor than the current AJCC T classification system.[10,11] However, this alternative T classification by DoI has not been fully validated.

Metastasis to LNs is a well-known prognostic factor of poor outcome for carcinomas originating from various organs, including EBD carcinomas. Furthermore, the number of involved LNs is a significant prognostic factor in many carcinomas, including breast,[12] bladder,[13] esophagus,[14] stomach,[15] and rectal carcinomas.[16] Some pathologists and clinicians consider the current N staging system as a fairly straightforward factor for predicting the prognosis of distal EBD cancer patients. Although the effect of number of metastatic LNs on patient survival in EBD carcinoma has been reported, its significance remains uncertain due to the limited number of cases examined.[17,18] Furthermore, inadequate assessment of LNs, either the extent of resection or pathologic examination, can result in underestimation of the N stage. In 2007, a study using the SEER database analyzed 20,068 patients with gallbladder, ampullary, and EBD cancers and suggested that at least 10 LNs should be examined for accurate N staging of these malignancies.[19] However, the cut-off value was roughly designated, and there were few supporting data with respect to distal EBD carcinoma. Thus, a more thorough analysis for the cut-off value of total number of LNs that need to be examined may be required for more accurate N staging of distal EBD cancer.

The overall purpose of this study was to validate the prognostic value of an alternative T classification system based on DoI in distal EBD carcinoma. Additionally, we examined the prognostic power of the number of metastatic LNs, as well as the number of total examined LNs, on survival in the same patient group. Based on these results, we suggest several modifications for the current T and N stage classifications of distal EBD cancer.

MATERIALS AND METHODS

Case Selection

This study was approved by the Institutional Review Board of Samsung Medical Center. We collected all cases of pancreaticoduodenectomy (including Whipple resection) for distal EBD carcinoma from the electronic chart system of the Samsung Medical Center between 2002 and 2006. All patients underwent curative intent resection for distal EBD cancer. Only those cases in which the cancer epicenter was within the distal EBD were included in our study. Distal EBD carcinoma was defined as those in which the epicenters were between the insertion point of the gallbladder cystic duct into the common hepatic bile duct and the Ampulla of Vater. Double primary cancers involving EBD cancer were excluded from the present study. After applying the inclusion and exclusion criteria, 114 cases of surgically resected distal EBD carcinomas were enrolled for this study. Clinicopathologic parameters including age, gender, DoI, pathologic T (pT), number of metastatic LNs, number of total examined LNs, residual tumor status, and overall patient survival were assessed. There were no cases with evidence of distant metastasis at the time of surgery. The clinicopathologic findings are summarized in Table 1.

Evaluation of DoI

We reviewed histopathologic findings of all cases and selected a representative slide that showed the deepest tumor invasion. All selected slides were scanned using a slide scanner (AperioScanscope XT) and depth of tumor invasion was digitally measured with the ruler tool of the Aperio scan program for more accurate and objective measurement (Fig. 1A). DoI was measured by 2 different methods, namely, DoI-1 and DoI-2 (Fig. 1B and C). DoI-1 was defined as the distance from the basal lamina of the adjacent normal bile duct mucosa to the deepest invasive tumor cells, as described previously.[10] On the other hand, DoI-2 was measured as the distance from the top of tumor surface to the deepest invasive tumor cells, except for

TABLE 1. Characteristics of Patients With Distal EBD Carcinoma (n = 114)

Characteristics	**Mean ± Standard Deviation (Range)**
Age (years)	**61.9 ± 6.4 (40.5–85.0)**
Sex	**M:F = 75:39**
Characteristics	**Number of patients**
Histologic type	
Adenocarcinoma, NOS	99 (85.1%)
Papillary adenocarcinoma	12 (10.5%)
Adenosquamous carcinoma	1 (0.9%)
Undifferentiated carcinoma	1 (0.9%)
Signet ring cell carcinoma	1 (0.9%)
T stage	
Tis	3 (2.6%)
T1	8 (7.0%)
T2	50 (43.9%)
T3	51 (44.7%)
T4	2 (1.8%)
N stage	
N0	75 (65.8%)
N1	39 (34.2%)
Surgical procedure	
PPPD or Whipple resection	97 (85.1%)
Segmental resection	17 (14.9%)
Completeness of resection	
R0	111 (97.4%)
R1	2 (1.8%)
R2	1 (0.9%)
Alive	49 (43.0%)
Died d/t disease progression	65 (57.0%)

EBD = extrahepatic bile duct, PPPD = pylorus preserving pancreatoduodenectomy.

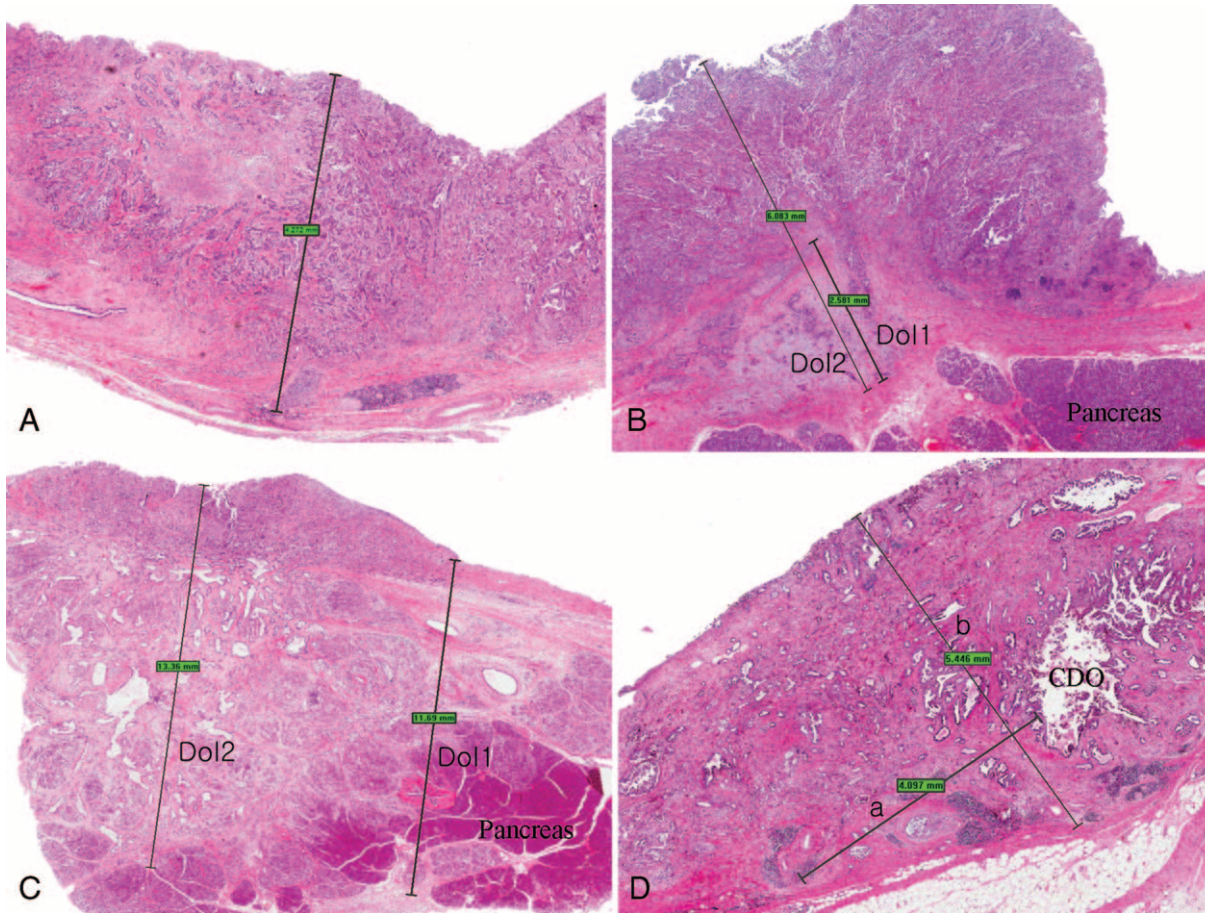

FIGURE 1. Schematic of DoI measurement. DoI-1 was defined as the distance between an imaginary line along the basal lamina of the adjacent normal bile duct mucosa and the deepest invasive tumor front. DoI-2 was defined as the distance between the top of the tumor and deepest invasive tumor front. (A) Measurement of DoI in a flat tumor. In flat tumors, DoI-1 and DoI-2 are the same. (B) Measurement of DoI in an elevated tumor. This kind of tumor produces different values according to DoI-1 and DoI-2. This case belongs to group 1 according to DoI-1 and group 2 according to DoI-2. In addition, it was difficult to establish the imaginary line along the basal lamina for this case, because the tumor pulled up the basal lamina layer. (C) Another example of an elevated tumor. This case belongs to group 2 according to DoI-1 and to group 3 according to DoI-2. (D) Tumor involving the cystic duct opening (CDO). In this situation, DoI can be overestimated as shown by line b. Thus, DoI should be measured as the distance between the basal lamina of cystic duct and the deepest level of invasion (line a). CDO = cystic duct opening, DoI = depth of invasion.

cases of intraductal papillary neoplasm of bile duct (IPNB). In the case of IPNB, the basal lamina of the adjacent normal mucosa was used as the starting point for measurement of DoI. Thus, DoI-1 and -2 were the same for cases of IPNB. DoI was divided into 3 groups according to Hong et al's criteria: Group 1 ($DoI < 5$ mm), Group 2 ($5 \leq DoI < 12$ mm), and Group 3 ($DoI \geq 12$ mm). DoI-1 and DoI-2 data were analyzed with respect to patient survival and compared with the survival data according to the T classification of the current AJCC staging system (7th edition).

Evaluation of LN Status

We reviewed the number of metastatic LNs and total examined LNs in all 114 cases. Cases were divided into 2 classes according to the presence or absence of LN metastasis, and the cases were further subdivided with respect to nodal metastasis and according to cut-off number of metastatic LNs. To determine the cut-off value, we consecutively analyzed, divided, and compared patient survival according to the number of metastatic LNs. The association between patient survival and the number of examined LNs for cancers staged as N0 was also analyzed. In addition, N0 cases were subdivided into 6 groups according to the total number of nodes examined.

Statistical Analysis

Statistical analyses were performed using the SPSS statistical package. Survival curves were plotted using the Kaplan–Meier method, and the significances of differences were determined using the log-rank test and the Cox proportional hazards regression model. P values <0.05 were considered significant.

RESULTS

Association Between Survival and DoI

When we analyzed patient survival data with T classification of current AJCC staging system (7th edition), it was not statistically correlated with patient survival ($P = 0.07$, Fig. 2A). However, T classification according to both DoI-1 and DoI-2

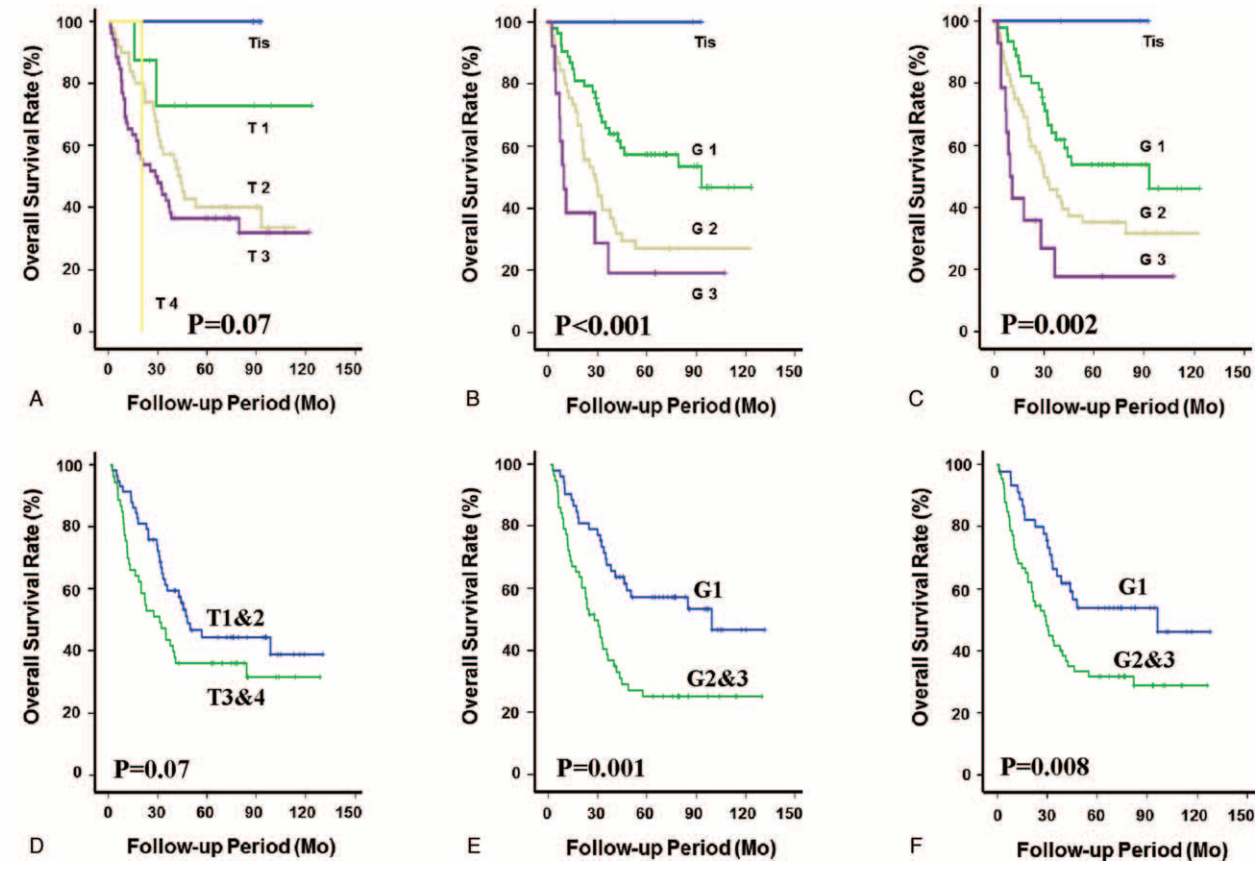

FIGURE 2. (A) Kaplan–Meier survival analysis based on current T stage (AJCC 7th Ed.) ($P = 0.07$). (B) Overall survival analysis according to classification by DoI-1 ($P < 0.001$). (C) Overall survival analysis according to classification by DoI-2 ($P = 0.002$). (D) Survival analysis comparing T1-2 and T3-4c ($P = 0.07$). (E) Survival analysis comparing G1 and G2-3 according to classification by DoI-1 ($P = 0.001$). (F) Survival analysis comparing G1 and G2-3 according to classification by DoI-2 ($P = 0.008$). AJCC = American Joint Committee on Cancer, DoI = depth of invasion.

revealed a statistically significant difference in the survival rate among groups ($P < 0.001$ in DoI-1; Fig. 2B) ($P = 0.002$ in DoI-2: Fig. 2C). Specifically, the pairwise comparison of group 1 versus group 2 showed a significant difference in survival for both DoI-1 and DoI-2 ($P = 0.004$ in DoI-1, $P = 0.04$ in DoI-2) (Table 2), although comparison between groups 2 and 3 did not reveal a significant difference in survival ($P = 0.16$ in DoI-1, $P = 0.06$ in DoI-2). In contrast, none of the pairs of groups defined according to the current T classification staging system were significantly different from each other (Table 2). We also analyzed each system after stage grouping (T1–2 vs T3–4 and group 1 vs group 2–3) and found that the current T stage system

TABLE 2. Overall Survival Analysis Between 2 Adjacent Groups According to AJCC 7th, DoI-1, and DoI-2

AJCC 7th	No.	DoI-1	No.	DoI-2	No.
Tis	3	Tis	3	Tis	3
Tis vs T1 (P=0.35)		Tis vs G1 (P=0.19)		Tis vs G1 (P=0.19)	
T1	8	Group 1	53	Group 1	45
T1 vs T2 (P=0.16)		G1 vs G2 (P=0.004)		G1 vs G2 (P=0.04)	
T2	50	Group 2	45	Group 2	52
T2 vs T3&4 (P=0.18)		G2 vs G3 (P=0.16)		G2 vs G3 (P=0.05)	
T3&4	53	Group 3	13	Group 3	14
T1&2 vs T3&4 (P=0.07)		G1 vs G2&3 (P=0.001)		G1 vs G2&3 (P=0.008)	

AJCC = American Joint Committee on Cancer, DoI = depth of invasion.
Group 1 = DoI < 5 mm, Group 2 = 5 ≤ DoI < 12 mm, Group 3 = DoI ≥ 12 mm.

failed to reveal a significant survival difference (Fig. 2D), whereas classification by DoI (both DoI-1 and DoI-2) exhibited a significant difference in survival analysis (Fig. 2E–F).

Association Between Survival and Number of Metastatic LNs

We first divided patients into groups based on a cut-off value of 3 nodal metastases (1–3 vs ≥4). There was a significant survival difference when compared by 1 to 3 versus 4 or more nodal metastases (log-rank test, $P = 0.02$) (Table 3). Specifically, the median survival time of cases with 1 to 3 versus 4 or more nodal metastases was 28.8 months and 10.9 months, respectively (Table 3). Other cut-off values showed no statistical differences according to survival analysis (Table 3).

We also divided patients into 3 tiers as follows: class 1 (cases with no LN metastasis), class 2 (cases with 1–3 metastatic LNs), and class 3 (cases with 4 or more metastatic LNs). The median survival times for class 1, 2, and 3 were 79.2 months, 28.8 months, and 10.9 months, respectively (Fig. 3A). The difference in survival among these 3 classes was statistically significant (log-rank test, $P < 0.001$) (Fig. 3A). In addition, survival was significantly different among these groups according to pair-wise comparison ($P = 0.04$ for class 1 vs 2 and $P = 0.02$ for class 2 vs 3) (Fig. 3A).

Association Between Survival and Number of Total Examined LNs

A total of 75 of 114 cases were classified as N0. The N0 group was further divided into 6 classes according to examined LNs (class 1: 0 to 3, class 2: 4 to 6, class 3: 7 to 9, class 4: 10 to 12, class 5: 13 to 15, class 6: ≥ 16) and compared using survival data (Fig. 3B). The former 3 classes (1 to 3) had a similar degree of slope, whereas the latter classes (4 to 6) also exhibited similar characteristics. To establish an optimum cut-off value for total number of examined LNs, we varied the cut-off value according to number of LNs examined (Table 4). There was no difference in survival when patients were analyzed by 1 to 8 versus 9 or more examined LNs. However, there was a significant difference in survival with cut-off value of 10 (1 to 9 vs. 10 or more, log-rank test, $P = 0.01$) (Fig. 3C). In addition, the lowest P value ($P = 0.007$) was observed with a cut-off value of 11 (1 to 10 vs 11 or more LNs examined) (Fig. 3D).

TABLE 3. Comparison for Cut-off Value Based on Number of Metastatic LNs (n = 39)

No. of Metastatic LNs	No. of Cases	Median Survival (CI)	*P*
1	12	36.5 (11.3–61.7)	0.06
2 or more	27	14.6 (10.2–19.0)	
1 to 2	22	28.8 (11.1–46.5)	0.17
3 or more	17	14.9 (4.3–25.5)	
1 to 3	28	28.8 (9.9–47.7)	0.02*
4 or more	11	10.9 (2.8–19.0)	
1 to 4	32	27.5 (14.7–40.3)	0.15
5 or more	7	10.9 (2.7–19.1)	

CI = 95% confidence interval, LN = lymph node.
* Significant difference ($P < 0.05$).

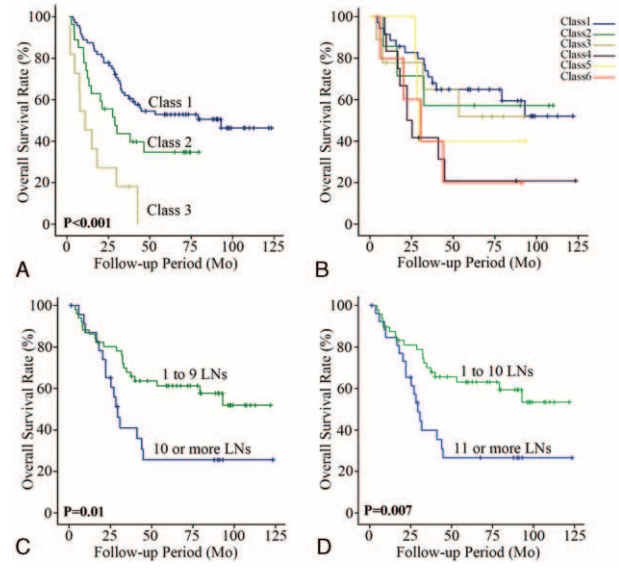

FIGURE 3. (A) Kaplan–Meier survival analysis based on 3-tier metastatic lymph node (LN) classification: class 1 (no LN metastasis), class 2 (1–3 metastatic LNs), and class 3 (4 or more metastatic LNs). The median survival time for classes 1, 2, and 3 was 79.2 months, 28.8 months, and 10.9 months, respectively. The difference in survival among the 3 classes was statistically significant ($P < 0.001$). (B) The N0 group was divided into 6 classes according to the number of examined LNs (class 1: 0–3, class 2: 4–6, class 3: 7–9, class 4: 10–12, class 5: 13–15, class 6: ≥ 16) and survival rates were analyzed. The former 3 classes (class 1–3) had a similar degree of slope, and the latter (class 4–6) exhibited similar findings. (C) A significant difference in survival was noted when the cut-off value was 1 to 9 versus 10 or more LNs ($P = 0.01$). (D) The lowest P value ($P = 0.007$) was observed for the comparison of 1 to 10 versus 11 or more LNs. LN = lymph node.

Multivariate Analysis of Clinicopathologic Features

The independent prognostic significance of DoI-1 and other clinicopathologic parameters was determined using the Cox proportional hazards models. According to multivariate analysis, DoI-1 ($P = 0.009$, HR 2.03), the total number of examined LNs for N0 (10 or less, $P < 0.004$, HR 2.63), number of metastatic LNs (≥4, $P < 0.001$, HR 5.24), and completeness of resection (R2, $P = 0.003$, HR 66.41) remained significant (Table 5), although when the number of metastatic LNs was between 1 and 3, the P-value was only marginally significant ($P < 0.08$, HR 1.85).

DISCUSSION

TNM staging is one of the most powerful prognostic indicators for the majority of cancer survival analyses. In addition, pathologic staging can provide more precise prognostic information than clinical staging. In general, the pathologic T stage is defined based on anatomic and histopathologic findings. Gastrointestinal tract (GI) organs have a relatively well-defined anatomic layering that makes it easy to differentiate T stages of each organ. Specifically, tumors confined to the mucosa or submucosa are defined as T1, tumor invasion into the muscle layer is defined as T2, extension of the tumor beyond the muscle layer or subserosa is defined as T3, and tumor involvement of the serosa and/or invasion to adjacent

TABLE 4. Comparison for Cut-Off Point Based on Number of Examined LNs in N0 Patients (n = 75)

No. of Sampling LNs	No. of Cases	Median Survival	*P*
0 to 7	16	29.4	0.10
8 or more	59	93.0	
0 to 8	21	30.7	0.11
9 or more	54	93.0	
0 to 9	24	29.4	0.01*
10 or more	51	NA	
0 to 10	27	29.4	0.007*
11 or more	48	NA	
0 to 11	32	31.8	0.03*
12 or more	43	NA	
0 to 12	33	31.8	0.03*
13 or more	42	NA	

LN = lymph node, NA = not applicable.
* Significant difference ($P < 0.05$).

organs is defined as T4. Likewise, for the hollow viscera of the esophagus, stomach, and intestine, it is not difficult to differentiate the T stage by anatomic layering. However, the biliary tree is different from the GI tract with respect to anatomic layering. Indeed, for the EBD, the submucosal layer is absent and the bile duct wall, which corresponds to the muscle layer of GI tract organs, is thin and muscle components are sparse, and can be difficult to identify. Under normal conditions, the lower boundary of the bile duct wall is easily distinguished from the surrounding periductal adipose tissue and pancreas. However the lower boundary of distal bile duct is frequently obscured by desmoplastic tissue reaction of invasive carcinoma, which makes microscopic distinction between T2 and T3 more difficult in distal EBDcarcinoma.[20] Notwithstanding the importance of pathologic staging, little attention has been paid to improvement and validation of pathologic T stage of EBD carcinoma, especially for distal EBD carcinomas. This is partially due to the relatively low incidence of cholangiocarcinoma in Western countries and also its anatomic complexity, which makes appropriate T and N classification a relatively difficult task. Hong et al previously reported that the different T classifications of the current AJCC staging system (7th edition) are not associated with significant differences in patient survival.[9–11] Consistently, we also found no significant difference in survival between T2 and T3 patients classified by the current AJCC staging system, even though distal EBD carcinoma accounted for the majority of these cases (88.6 %). In addition, comparison between early (T1–T2) and advanced (T3–T4), patients according to the current AJCC staging system failed to reveal a statistically significant association with patient outcome ($P = 0.07$, Fig. 2D). In contrast, when we analyzed by DoI, the survival curves of Group 1 (<5 mm of DoI) and Group 2 (5–12 mm of DoI) were clearly separated from each other with statistically significant difference ($P = 0.004$). On the other hand, there was no significant difference between Group 2 and Group 3 (> 12 mm of DoI), which was probably due to the small number of Group 3 cases ($P = 0.16$ in DoI-1; $P = 0.06$ in DoI-2). When DoI-based staging was analyzed for Group 1 versus Groups 2–3, both of the DoI methods utilized in this study were strongly associated with patient prognosis. With respect to the different DoI methods, when comparing DoI-1 to DoI-2, DoI-1 exhibited a slightly lower *P* value ($P = 0.001$ vs $P = 0.008$, Fig. 2E–F). Importantly, our results suggest that classification based on the tumor invasion depth was more useful than T classification of current AJCC staging system with respect to patient prognosis.

TABLE 5. Multivariate Survival Analysis for the Overall Survival

Parameter	*P* Value	HR	95% CI
Age	0.84	1.06	0.62–1.82
Gender	0.62	0.87	0.51–1.51
DoI (group1 vs 2&3)	0.009*	2.03	1.19–3.45
No. of metastatic LN			
0 (Total LN 11 or more)	–	1	–
0 (Total LN 10 or less)	0.004*	2.62	1.36–5.05
1–3	0.08	1.85	0.93–3.67
≥4	<0.001*	5.24	2.36–11.66
Completeness of resection			
R0	–	1	–
R1	0.35	2.01	0.46–8.77
R2	0.003*	66.41	4.00–1102.54

CI = confidence interval, DoI = depth of invasion, HR = hazard ratio, LN = lymph node.
* Statistically significant ($P < 0.05$).

In practical view, accurate measurement of tumor invasion depth can be tricky in some cases. The basal lamina of the adjacent normal mucosa should be the starting point for the measurement of tumor invasion depth, and Hong et al used an imaginary line from the adjacent normal epithelium as a starting point for measurement of tumor DoI. However, this method has some limitations. Tumors can occasionally distort the normal bile duct structure making it difficult to identify basal lamina of adjacent normal mucosa. In addition, representative slides may not contain normal bile duct epithelium in some cases. As shown in Figure 1C, the tumor often pulls up the basal lamina layer. In the present study, we overcame these problems by evaluating serial sections and alternative representative slides in which the basal lamina of the adjacent normal mucosa of the bile duct was present. Likewise, we used 2 different methods to measure tumor invasion depth: DoI-1, which comprised the distance from the basal lamina of the adjacent normal bile duct mucosa to the most deeply invasive tumor cells, and DoI-2, which comprised the distance from the top of the tumor surface to the most deeply invasive tumor cells. We initially thought that DoI-2 may be more reliable than DoI-1, because we suspected that measurements from the top of the tumor surface may be more consistent and reliable than measurements from the basal lamina of adjacent normal mucosa due to the subjective nature of the imaginary line representing the basal lamina. Even though tumor staging according to both DoI-1 and DoI-2 was able to demonstrate survival differences, staging according to DoI-1 was more statistically significant. Importantly, the results of the present study support the previous findings by Hong et al.[10,11]

In the present study, we noted that it was possible to overestimate DoI in cases of EBD carcinoma involving cystic duct opening to the common hepatic bile duct. Specifically, if diffuse mucosal high-grade dysplasia/carcinoma in situ is present in both the distal cystic duct lumen and cystic duct opening to EBD lumen, and invasive carcinoma occurs predominantly around the cystic duct wall, DoI can be easily overestimated as

being from the surface lumen of the EBD to the front of the invasive carcinoma around the cystic duct wall. In such cases, it is more appropriate to measure DoI from the basal lamina of the cystic duct lumen to the deepest portion of invasion within cystic duct wall (Fig. 1D).

LN metastasis is associated with a poor prognosis in the majority of cancers. Furthermore, subdivided N stages can be used to establish different prognoses in many kinds of cancer. Thus, a subdivided N stage system has been applied to many types of carcinomas such as breast, stomach, and colon. Recently, Balci et al reported that subclassified nodal status based on the number of metastatic LNs (N0, N1; 1–2 metastatic LNs or N2; ≥3 metastatic LNs) has a significant prognostic value in ampullary carcinomas.[21] However, only 2 studies to date have reported the effect of the number of metastatic LNs on patient survival in EBD carcinoma. Yoshida et al showed that patients with 3 or more LN metastases have a worse survival rate than those with 2 or fewer in distal EBD carcinoma,[17] although they only evaluated 26 cases. Likewise, Hong et al found that patients with 5 or more metastatic LNs have a much worse rate of survival compared to patients with 4 or fewer LNs.[18] In addition, they showed that grouping patients with 1 to 3 versus 4 or more LNs was borderline significant ($P = 0.05$).[18] Despite the large number of EBD carcinoma cases (n = 209) evaluated by Hong et al, the results of their study were limited in that distal EBD cases were not separately analyzed.

In the present study, there was a significant survival difference with respect to patients with 1 to 3 versus 4 or more nodal metastases (log-rank test, $P = 0.02$). Specifically, the median survival time of cases with 1 to 3 versus 4 or more nodal metastases was 28.8 months and 10.9 months, respectively. On the other hand, when comparing patients according to the presence of 1 to 4 versus 5 or more nodal metastases, the difference in survival was not significant ($P = 0.15$). We also divided the entire group into class 1 (no LN metastasis), class 2 (1–3 metastatic LNs), and class 3 (4 or more metastatic LNs). The difference in survival among this 3-tier classification system was significant (log-rank test, $P < 0.001$). In addition, there was a significant survival difference in pair-wise comparison of these classes ($P = 0.04$ for class 1 vs 2 and $P = 0.02$ for class 2 vs 3). Based on these results, we propose that the current N1 stage should be subdivided into N1 (metastasis in 1 to 3 regional LNs) and N2 (metastasis in 4 or more regional LNs) classifications.

We also evaluated the effect of the number of metastatic LNs, as well as the number of total examined LNs, on survival in the same patient group. Previous studies have reported conflicting results using a similar approach. For example, Hong et al reported no significant survival difference according to the number of retrieved LNs.[18] On the other hand, Schwarz et al concluded that survival prediction of EBD cancer is strongly influenced by the total LN count and the number of negative LNs obtained based on the SEER 1973–2004 database.[19] In the present study, we found that the lowest *P*-value ($P = 0.007$) was observed for the comparison of 1 to 10 versus 11 or more LNs. Based on these results, we concluded that minimum 11 LNs should be subjected to pathologic examination for EBD carcinoma.

In summary, we confirmed that DoI is a powerful prognostic factor for T classification of EBD carcinoma. Thus, the current T classification system should be updated to utilize DoI and measuring from the basal lamina of the adjacent normal mucosa to the invasive tumor front is a more reliable method. N staging also requires subclassification into a 3-tier system. Based on our data, subclassification as N0 (cases with no LN metastasis), N1 (metastasis in 1 to 3 regional LNs), and N2 (metastasis in 4 or more regional LNs) was found to be useful. Lastly, dissection and examination of at least 11 LNs should be considered for accurate evaluation of N stage of distal EBD carcinoma.

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