Effects of microvascular invasion on clinical outcomes after resection with curative intent for cholangiocarcinoma

Bo-Hye Song, MD, Boram Cha, MD, Jin-Seok Park, MD*, Seok Jeong, MD, Don Haeng Lee, MD

Abstract
Surgery is the only curative treatment for cholangiocarcinoma, but even after surgery, survival rates are unsatisfactory. Recently, several reports have suggested microvascular invasion (MiVi) is associated with poor postoperative prognosis in hepatocellular carcinoma (HCC). We considered that MiVi might be associated with poor clinical outcomes in patients with surgically resectable cholangiocarcinoma.

The records of 91 patients who underwent resection with curative intent for cholangiocarcinoma at Inha University Hospital from 2007 to 2017 were comprehensively reviewed for clinicopathological characteristics, DFS, and overall survival (OS) relations between these factors and the presence of MiVi.

Forty-nine of the 91 study subjects had MiVi and 42 did not. Median overall survivals were 492 days in the MiVi group and 1008 days in the noMiVi group and median DFSs were 367 days and 760 days, respectively. Cumulative survival ratio and recurrence incidence rates were significantly different in the 2 groups (P= .012). Multivariable analysis showed the presence of MiVi was an independent risk factor of OS (hazard ratio [HR] 3.34; 95% confidence interval [CI], 1.40–7.97; P = .007). Cholangiocarcinoma is known to have a poor prognosis. When microvascular invasion remains after surgery it is associated with poor clinical outcomes.

Abbreviations: DFS = disease-free survival, HCC = hepatocellular carcinoma, ICC = intrahepatic cholangiocarcinoma, MaVi = macrovascular invasion, MiVi = microvascular invasion, OS = overall survival.

Keywords: cholangiocarcinoma, curative intended surgery, microvascular invasion, prognosis, prognostic factor

1. Introduction
Cholangiocarcinoma is classified by anatomical location as perihilar, distal extrahepatic, and intrahepatic tumor of bile ducts. Approximately, half of cholangiocarcinoma patients present with the perihilar type, and 40% and 10% with the distal extrahepatic and intrahepatic types, respectively. Surgical treatment is the preferred option for all types, but fewer than one-third of patients are resectable at diagnosis.[1] Reported 5-year survival rates of the perihilar, distal extrahepatic, and intrahepatic types are 11% to 41%, 27% to 37%, and 22% to 44%, respectively.[2] Surgical extent depends on the tumor site and location.[3]

Cholangiocarcinoma differs from hepatocellular carcinoma and pancreaticoduodenectomy is performed for complete resection of distal cholangiocarcinoma.[4] Intrahepatic cholangiocarcinoma (ICC) is treated by segmentectomy or hepatic lobectomy depending on tumor size and location.[5]

Known prognostic factors after surgery include local clearance (R0 no residual tumor or R1 microscopic residual tumor), lymph node metastasis, primary tumor size, and vascular invasion.[6] Reported 5-year survivals after R0 resection are perihilar (30%), distal extrahepatic (27%), and intrahepatic (63%). However, negative tumor margins are achieved in <30% of patients,[7] and the high incidence of recurrence after surgery is a major concern. Cholangiocarcinoma often involves macrovascular and microvascular invasion, whereas cholangiocarcinoma commonly spreads through the lymphatic system, which is a major prognostic factor after surgery. Some authors have recommended lymphadenectomy during ICC resection, but data supporting its prophylactic effect are insufficient.[8] Primary tumor size-associated prognostic differences are reflected by the 8th UICC/AJCC TNM staging system, which is based on a tumor size cut-off of 5 cm. Vascular invasion represents an advanced phase of cancer progression and involves macrovascular and microvascular invasion. Prognostic
differences are mentioned in the TNM staging system, but the definitions of the terms used are somewhat unclear. Microvascular invasion (MaVi) is defined as tumor invasion of a major vessel as determined by macroscopic examination or radiological imaging, but microvascular is not clearly defined, although some features such as the presence of tumor emboli in a portal radicle vein, a large capsule vessel, or a vascular space lined by endothelial cells have been mentioned. Microvascular invasion (MiVi) has been reported to predict poorer outcomes among patients with HCC after resection or liver transplantation. Based on consideration of the pathogenesis of angioinvasion, we hypothesized MiVi probably affects clinical outcomes among cholangiocarcinoma patients that undergo curative resection.

2. Patients and methods

2.1. Patients

Patients with symptomatic or accidentally discovered laboratory imaging abnormalities underwent further evaluation. Cholangiocarcinoma are basically included chest and abdominal CT, laboratory tests which including liver function test and tumor marker (CA 19-9, CEA). Some people need to further work up such as esophagogastroduodenoscopy, EUS, endoscopic retrograde cholangiopancreatography (ERCP), and magnetic resonance imaging (MRI). Depending on the location of the lesion and the extent of involvement, surgical resection could consider primary treatment. If resection is impossible or metastatic, chemotherapy and radiation therapy are considered.

A retrospective review of all medical records including imaging, pathologic reports, and laboratory results was performed between 2007 and 2017. A total of 128 patients were diagnosed and underwent surgery with curative intent for cholangiocarcinoma at the Inha University School of Medicine. All patients were pathologically confirmed, but only patients that achieved curative resection (R0 or R1 resection) were included in the study. The patient exclusion criteria applied were as follows: receipt of palliative surgery or open/closed surgery due to an advanced stage, death due to a postoperative complication (eg, hepatic failure or infection), HCC as determined by postoperative biopsy, or a double primary cancer. Patient underwent surgical treatment based on staging according to standard medical guidelines. Of the 128 patients, 91 met these criteria in this study. This study is a retrospective analytical study using medical records. The consent was exempted from the consent of the subjects because the risk to the subjects was very low, and no personally identifiable information was collected. The study protocol was approved under the approval of the institutional review board of Inha university hospital. (Approval No 2019-11-031)

2.2. Data collection

Preoperative evaluation included imaging (ultrasonography, computed tomography [CT], ERCP, magnetic resonance cholangiopancreatosgraphy, and positron emission tomography-CT) to evaluate primary tumor extension. All resected tumors were evaluated for size, number, histologic type, differentiation, adjacent organ invasion, and margin vascular, perineural invasion, and lymph node statuses. MaVi was defined as the presence of vessel invasion by gross examination and MiVi as tumor invasion of hepatic veins, the portal system or lymphatic ducts visible only by microscopy. Surgical resection margins were classified by a pathologist as R0 resection (defined as the complete absence of cancer cells as determined microscopically) or R1 resection (defined as a microscopically positive margin).

2.3. Statistical analysis

The student t test was to determine the significances of differences between the MiVi and non-MiVi groups. Survival curves were constructed using the Kaplan-Meyer method, and survival curve differences were analyzed using the univariate log-rank test. Multivariate analysis was conducted using a Cox proportional hazards model to identify factors associated with OS or DFS. To confirm the proportional risk assumption, all significant factors determined by univariate analysis putting one risk factor into the Cox proportional risk model, and then entered into a multivariate analysis using the Cox proportional hazard regression model. Factors found to be related to OS and DFS with p values between 0.05 and 0.2 were entered into the multivariate analysis. Hazard ratios and 95% confidence intervals were estimated, and statistical significance was accepted for P values<.05. The analysis was performed using SPSS version 19.0 (IBM SPSS Inc, Chicago, IL)

3. Results

3.1. Baseline characteristics

One hundred and twenty-eight patients underwent curative surgery for cholangiocarcinoma from 2007 to 2017 at the authors’ institute. Patients were confirmed not have: retropancreatic or paraceliac nodal metastases or distant liver metastases, invasion of the main hepatic artery, extrahepatic adjacent organ invasion, or disseminated disease before surgery by preoperative imaging. Thirty-seven patients were excluded for the following reasons: 12 were switched to a palliative operation, 8 underwent open/closure, 9 succumbed to a postoperative complication, and 8 were excluded based on pathologic findings (4 were diagnosed with HCC, 1 patient had colon cancer and liver metastasis, 1 patient had double primary cancer, and 2 patients had no pathologic report) (Fig. 1).

Accordingly, 91 patients who underwent curative-intent resection for cholangiocarcinoma constituted the study cohort. Forty-nine (53.8%) had MiVi (53.8%). Average study subject age was 62 years and males accounted for 63%~64%. No significant intergroup difference was observed between blood liver functions or tumor marker (CA 19-9, alpha fetoprotein) levels. However, pathological characteristics after surgery differed significantly. The percentage of moderate to poorly differentiated cancers was higher in the MiVi group (NoMiVi: WD 35.7%, MD 42.8%, PD 11.9%/ MiVi: WD 10.2%, MD 40.8%, PD 42.8% (P=.002), the R0 resection rate was lower in the MiVi group (NoMiVi 42.9%, MiVi 10.2%, P < .001), and the lymphatic invasion rate was higher (NoMiVi 4.7%, MiVi 63.2%, P < .001). Tumors were also more
invasive in the MiVi group ($P < .001$), and for this reason, a greater percentage of patients received adjuvant chemotherapy (NoMiVi 59.5%, MiVi 83.7%, $P = .006$) and radiotherapy (NoMiVi 26.2%, MiVi 46.9%, $P = .015$) after surgery. Furthermore, mean DFS and OS were significantly shorter in the MiVi group (DFS: NoMiVi 760 days, MiVi 367 days/OS: NoMiVi 1008 days, MiVi 492 days) (Table 1).

### 3.2. DFS and OS of patients

A comparison of cumulative incidences of death and recurrence survival curves showed MiVi was associated with significantly poorer prognoses (Fig. 2A and B, $P = .012$).

Univariate analysis showed lymphatic invasion and MiVi significantly influenced OS and DFS, but multivariable analysis

---

**Table 1**

|                     | No vascular invasion (n = 42) | Microvascular invasion (n = 49) | $P$   |
|---------------------|-------------------------------|---------------------------------|-------|
| Age, y              | 62.3 (55–66)                  | 62.1 (56–65)                    | .92   |
| Male sex            | 64.2% (n = 27)                | 63.2% (n = 31)                  | .89   |
| CA 19–9, U/mL       | 166.5 (45.5–337.2)            | 228.1 (80.7–534.9)              | .47   |
| AFP, ng/mL          | 3.5                           | 1444.6                          | .34   |
| AST, U/L            | 134.2 (74–155)                | 135.1 (72–149)                  | .97   |
| ALT, U/L            | 20.6 (65–167)                 | 20.4 (90–186)                   | .84   |
| ALP, U/L            | 489.9 (283–677)               | 746.6 (140–1114)                | .13   |
| Histologic type     |                               |                                 | .01   |
| Adenocarcinoma      |                               |                                 |       |
| Well-differentiated | 35.7% (n = 15)                | 10.2% (n = 5)                   |       |
| Moderately differentiated | 42.8% (n = 18) | 40.8% (n = 20) |       |
| Poor differentiated  | 11.9% (n = 5)                 | 42.3% (n = 21)                  |       |
| Others              | 9.5% (n = 4)                  | 6.1% (n = 3)                    |       |
| R0 resection        | 42.9% (n = 18)                | 10.2% (n = 9)                   | <.01  |
| Lymphatic invasion  | 4.7% (n = 2)                  | 63.2% (n = 31)                  | <.01  |
| Perineural invasion | 47.6% (n = 20)                | 48.9% (n = 24)                  | .92   |
| AJCC T stage        |                               |                                 | <.01  |
| T1                  | 50% (n = 21)                  | 4.08% (n = 2)                   |       |
| T2                  | 33.3% (n = 14)                | 59.2% (n = 29)                  |       |
| T3                  | 9.5% (n = 4)                  | 26.6% (n = 14)                  |       |
| T4                  | 4.7% (n = 2)                  | 8.1% (n = 4)                    |       |
| Missing             | 2.3% (n = 1)                  |                                 |       |
| Total bilirubin     | 3.4 (1.3–8.4)                 | 4.6 (1.8–5.5)                   | .24   |
| Adjuvant chemotherapy| 59.5% (n = 25)                | 83.7% (n = 41)                  | .01   |
| Adjuvant radiotherapy| 26.2% (n = 11)               | 46.9% (n = 23)                  | .01   |
| DFS, days           | 760.4 (460–1060)              | 367.9 (212–523)                 | .02   |
| OS, days            | 1008.0 (607–1408)             | 492.4 (371–613)                 | .03   |

**Note:**

| $P$ = probability, R = residual tumor, T = tumor.
showed only MiVi significantly and independently predicted OS \((P = .007)\) (Tables 2 and 3).

### 3.3. Follow up results

During the 11-year study period, 41 patients died, 6 were referred for hospice care, and 25 patients were lost to follow-up. Nineteen patients remained alive at the time of writing. Twelve patients survived for \(>5\) years after diagnosis. Cholangiocarcinoma locations were: 6 intrahepatic, 5 extrahepatic, and 1 perihilar area; and degrees of tissue differentiation were: 6 well-differentiated, 4 moderately differentiated, and 2 poorly differentiated. Postoperative resection margins were R0 in 8 and R1 in 4. Only 1 patient with MiVi survived for \(>5\) years (Table 4). Three of the 12 patients that survived for \(>5\) years experienced recurrence. A 69-year-old man\(^{10}\) currently under hospice care had peritoneal seeding at time of relapse. A 68-year-old woman\(^{9}\) underwent re-operation (pylorus-preserving pancreaticoduodenectomy) due to recurrence and did not develop further recurrence over 2 years and 4 months of subsequent follow-up. The other was 69-year old female patient\(^{10}\) that underwent Rt. Hepatic lobectomy and adjuvant chemotherapy. Recurrence occurred 907 days after surgery and was treated by additional surgery and biliary stent insertion. Of the 12 patients, a 64-year-old woman\(^{11}\) with the poorest prognosis had stage IIIA disease with portal vein invasion at diagnosis. She underwent hepaticojejunostomy with cholecystectomy and portal vein resection followed by adjuvant chemoradiotherapy, and no recurrence was subsequently observed (Table 4).

### 4. Discussion

Surgical treatment is the preferred curative treatment option for cholangiocarcinoma, but recurrence and mortality rates are high after surgery. The T classification of the 8th AJCC (American Joint Committee on Cancer) guideline, divides cholangiocarcinoma by tumor size and number and by the presence or absence of vascular invasion, but unfortunately vascular invasion is not well defined. Usually, vascular invasion includes MaVi and MiVi, and MaVi can be detected using various imaging procedures before treatment, for example, as a tumor thrombus in a major portal or hepatic vein, whereas MiVi must be detected by

---

**Table 2**

| Variable                  | Univariate analysis |          | Multivariate analysis |          |
|---------------------------|---------------------|----------|-----------------------|----------|
|                           | \(P\)               | HR (95% CI) | \(P\)               | HR (95% CI) |
| R0 resection              | .12                 | 2.16 (0.8–5.8) | .86                 | 0.91 (0.42–2.06) |
| Metastatic LN             | .11                 | 1.72 (0.8–3.3) | .002                | 3.19 (1.55–6.55) |
| Lymphatic invasion        | .03                 | 2.03 (1.05–3.95) | .332                | 0.74 (0.40–1.36) |
| Microvascular invasion    | .002                | 3.19 (1.55–6.55) | .007                | 3.34 (1.40–7.97) |
| Perineural invasion       | .332                | 0.74 (0.40–1.36) | .100                | 1.28 (0.95–1.72) |
| Stage                     | .100                | 1.28 (0.95–1.72) | .062                | 1.00 (1.00–1.01) |
| CA 19–9                   | .618                | 1.169 (0.63–2.16) | .82                 | 0.89 (0.52–2.51) |

CA = cancer, CI = confidence interval, CTx = chemotherapy, HR = hazard ratio, LN = lymph node, \(P\) = probability, R = residual tumor, RTx = radiotherapy.
microscopy. Furthermore, the NCCN (National Comprehensive Cancer Network) guidelines do not provide recommendations for the classification of microvascular invasion or provide guidance for additional adjuvant chemotherapy. In HCC, MiVi has been well associated with poor outcomes after surgical resection and liver transplantation, and it has been proposed that tumor cells spread intrahepatic dissemination through the portal circulation.[12,14] Shao et al and Jonas et al compared the pathologic findings of patients with ICC and HCC that underwent surgical exploration. In ICC patients the appearance of vessels at a tumor distance of >1 cm was found to significantly and adversely affect prognosis. However, in ICC only a MiVi to tumor distance of >1 cm was shown to be prognostic. The authors suggested these differences are due to the different invasion and metastasis pathways of HCC and ICC.[12,14]

The present study shows that MiVi was associated with poor histological differentiation, low R0 resection rates, more lymph node invasion, and advanced stage disease (Table 1). These findings suggest the presence of MiVi reflects the progression of advanced cancer and are consistent with the findings of a previous study, in which tumor size, MiVi, poor tumor grade, and poor tumor differentiation were independently associated with what in cholangiocarcinoma.[15] These associations mean that greater understanding of cholangiocarcinoma is needed at the pathophysiological level.

In the present study, perineural invasion was not a significant prognostic factor not only in OS but also DFS. Perineural invasion was defined as the presence of cancer cells extending along perineural spaces. Some have suggested perineural invasion is a prognostic factor in ICC and extrahepatic cholangiocarcinoma.[16,17] Anatomically, the biliary system is closely located to the peripheral nerve plexus and the celiac plexus, and this proximity may facilitate peripheral nerve invasion by biliary tumors. Murakawa et al suggested that rich autonomic nerve supply to the biliary system might also facilitate perineural invasion.[18]

However, Kim et al showed that in cases where adequate dissection was performed, perineural invasion appeared to have no influence on survival[19] which was in accordance with our result. The reason for these conflicting results is that cholangiocarcinoma associated morbidities are relatively low compared to the individual diversity, which makes the topic difficult to analyze given the potential influences of a multitude of factors. Because little has been achieved in terms of improving the prognosis of cholangiocarcinoma, many studies have been initiated to identify risk factors, and some authors have suggested nomograms be used to predict survival.[20]

We suggest meta-analysis and large-scale studies be conducted to identify the risk factors involved.

In the present study, cholangiocarcinoma at all locations was included, and this may have introduced bias into our prognostic assessment of the impact of microvascular invasion. Cholangiocarcinomas differ in terms of epidemiology, origin, etiology,
pathogenesis, and ICC is histologically consists of biliary epithelial and hepatic progenitor cells that are distinct from other types of cholangiocarcinoma.

In some studies conducted on animal models, it has been proposed ICC results from the transdifferentiation and neoplastic conversion of normal hepatocytes into malignant cholangiocytes. In contrast, distal extrahepatic and perihilar cholangiocarcinoma arise from biliary epithelium and peribiliary glands. Due to these pathophysiological differences, the metastasis mechanisms of bile duct cancer and HCC and of bile duct cancer in different locations probably differ. Zhang et al suggested ICC is a more aggressive type of cholangiocarcinoma that is associated with poorer outcomes after curative resection than peripheral ICC or Klatskin tumor. However, Ercolani et al reported that in patients with comparable pathologic characteristics and stages, the outcomes of all 3 tumors at similar locations were indistinguishable. The authors concluded cholangiocarcinomas with different sites of origin have different tendencies to invade bordering structures. Many opinions angiocarcinomas with different sites of origin have different locations were indistinguishable. The authors concluded chol-

[2] Khan SA, Davidson BR, Goldin RD, et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. Gut 2012;61:1657–69.
[3] Jarnagin WR, Shoup M. Surgical management of cholangiocarcinoma. Semin Liv Dis 2004;24:189–99.
[4] Weber SM, Jarnagin WR, Klimstra D, et al. Intrahepatic cholangio-
carcinoma: resectability, recurrence pattern, and outcomes. J Am Coll Surg 2001;193:384–91.
[5] Yamamoto M, Ariizumi S-I. Surgical outcomes of intrahepatic cholangiocarcinoma. Surg Today 2011;41:896–902.
[6] Yang et al reported that in patients with comparable pathologic limitations that included patients with R1 resection.
[7] DeOliveira ML, Cunningham SC, Cameron JL, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg 2007;245:755–62.
[8] Amini N, Ejaz A, Spolverato G, et al. Management of lymph nodes during resection of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: a systematic review. J Gastrointest Surg 2014;18:2136–48.
[9] Edge SB. AJCC Cancer Staging Manual. Springer 2010;7:97–100.
[10] Rodríguez-Perálvarez M, Luong TV, Andreana L, et al. A systematic review of microvascular invasion in hepatocellular carcinoma: diagnostic and prognostic variability. Ann Surg Oncol 2013;20:325–39.
[11] Roayae S, Blume IN, Thung SN, et al. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. Gastroenterology 2009;137:830–5.
[12] Jonas S, Bechstein WO, Steinmüller T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology 2001;33:1080–6.
[13] Rajagopalan V, Grossbard ML, Kozuch P. Gallbladder and biliary tract carcinoma: a comprehensive update, part 2. Liver 2004;18:1180–9.
[14] Shao C, Chen J, Chen J, et al. Histological classification of microvascular invasion to predict prognosis in intrahepatic cholangiocarcinoma. Int J Clin Exp Pathol 2017;10:7674–81.
[15] Spolverato G, Ejaz A, Kim Y, et al. Tumor size predicts vascular invasion and histologic grade among patients undergoing resection of intrahepatic cholangiocarcinoma. J Gastrointest Surg 2014;18:1284–91.
[16] Shirai K, Ebata T, Oda K, et al. Perineural invasion is a prognostic factor in intrahepatic cholangiocarcinoma. World J Surg 2008;32:402.
[17] Murakami Y, Uemura K, Sudo T, et al. Perineural invasion in extrapancreatic cholangiocarcinoma: prognostic impact and treatment strategies. J Gastrointest Surg 2013;17:1429–39.
[18] Murakawa K, Tada M, Takada M, et al. Prediction of lymph node metastasis and perineural invasion of biliary tract cancer by selected features from cDNA array data. J Surg Res 2004;122:184–94.
[19] Kim HJ, Kim CY, Hur HY, et al. The prognostic factors for survival after curative resection of distal cholangiocarcinoma: perineural invasion and lymphovascular invasion. Surg Today 2014;44:1879–86.
[20] Hyder O, Marques H, Pulitano C, et al. A nomogram to predict long-
term survival after resection for intrahepatic cholangiocarcinoma: an Eastern and Western experience. JAMA Surg 2014;149:432–8.
[21] Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology 2013;145:2125–29.
[22] Pan B, Malato Y, Calvisi DF, et al. Cholangiocarcinomas can originate from hepatocytes in mice. J Clin Invest 2012;122:2911–5.
[23] Komuta M, Goyaere O, Vandecaveye V, et al. Histological diversity in cholangiocarcinoma: resectability, recurrence pattern, and outcomes. J Am Coll Surg 2001;193:384–91.
[24] Yamamoto M, Ariizumi S-I. Surgical outcomes of intrahepatic cholangiocarcinoma. Surg Today 2011;41:896–902.
[25] Ercolani G, Dazzi A, Giovinazzo F, et al. Intrahepatic, peri-hilar and extrahepatic cholangiocarcinoma: three different locations of the same tumor or three different tumors? Eur J Surg Oncol 2015;41:1162–9.
[26] Hu LS, Weiss M, Popescu I, et al. Impact of microvascular invasion on clinical outcomes after curative-intent resection for intrahepatic cholangiocarcinoma. J Surg Oncol 2019;119:21–9.

Author contributions
Conceptualization: Jin-Seok Park.
Supervision: Seok Jung, Don Haeng Lee.
Writing – original draft: Bo-Hye Song, Boram Cha.
Writing – review & editing: Boram Cha, Jin-Seok Park.

References
[1] Blechacz B, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. Hepatology 2008;48:308–21.