Occult synchronous liver metastasis from perihilar cholangiocarcinoma

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ABSTRACT

Background: No authors have reported "occult" liver metastases from perihilar cholangiocarcinoma (PHCC), which are defined as intrahepatic metastases that are overlooked by preoperative workup and intraoperative inspection but are detected by final pathology. The aim of this study was to clarify the features of such unmarked metastases.

Methods: Medical records of patients with PHCC treated between 2001 and 2016 were retrospectively reviewed with attention to liver metastases.

Results: During the study interval, 945 consecutive patients with PHCC were treated, including 260 unresected and 685 resected patients (672 with hepatectomy and 13 without). Of these, 36 patients had overt liver metastases. Of the 672 hepatectomized patients, 21 (3.1%) patients had occult liver metastases with a median number of 1 (range 1 to 6). When compared between occult and overt metastases, the diameter was smaller in the former (5 mm vs 12 mm, $P<0.001$). When compared between the 21 patients with occult metastases and the 645 hepatectomized patients without liver metastases, microscopic venous invasions and lymph node metastases were frequently observed in the former. Survival for the 21 patients with occult metastases was better than that for the 36 patients with overt metastases (MST, 17.1 vs 7.4 months, $P<0.01$).

Conclusions: Occult liver metastases from PHCC are not extremely rare. Meticulous handling of the resected specimens is crucial to detect such metastases. Although patients with occult metastases had advanced stages of the disease, their survival was better than that for patients with overt metastases.

Introduction

Perihilar cholangiocarcinoma (PHCC) is a devastating disease and still considered to be the most difficult cancer to treat.[1-5] Curative resection, although technically demanding, is the only curative option with a chance of long-term survival. Therefore, in the past several decades many surgeons have aggressively challenged surgical resections and have reported outcomes with varying degrees of success.[1-5]

Several synchronous liver metastases from colorectal cancer are ideal candidates for surgery. In contrast, those from PHCC are deemed unresectable, which is a well-accepted consensus. From our experiences, however, we have noticed that small liver metastases are overlooked during laparotomy and are found by final pathology in several patients who have undergone hepatectomy. To date, no authors have reported such occult synchronous liver metastases from PHCC: thus, nothing is known about these special unmarked metastases.

The aim of the present retrospective study was to review the medical records of patients with PHCC, with a focus on liver metastases, and to clarify the incidence and features of occult synchronous liver metastases from PHCC.

Patients and Methods

Study patients

Between 2001 and 2016, consecutive patients with PHCC who were treated at the First Department of Surgery, Nagoya University Hospital, were retrospectively reviewed, with a special attention to liver metastases. This study was approved by the Human Research Review Committee of Nagoya University Hospital (Approval No. 2018-0075).

All patients were divided into the following 5 groups according to the status of liver metastasis: group A, inoperable patients due to liver metastases detected by preoperative workup; group B,

laparotomized but unresected patients due to liver metastases found by intraoperative inspection; group C, hepatectomized patients with liver metastases detected by preoperative workup or intraoperative inspection; group D, hepatectomized patients with liver metastases found by final pathology of the resected specimens; group E, hepatectomized patients without liver metastases by final pathology. Groups A, B and C were defined as patients with "overt" liver metastases, and group D was defined as patients with "occult" liver metastases.

Workup for tumor staging and preoperative management

For tumor staging, ultrasonography, computed tomography (CT), and cholangiography were routinely performed. Other imaging approaches, including magnetic resonance imaging (MRI) and positron emission tomography, were utilized in certain selected patients, when needed.

Patients who had jaundice and/or dilated bile ducts in the future remnant lobe routinely underwent biliary drainage, either by percutaneous or endoscopic approach. Portal vein embolization was performed when the liver remnant was less than 40%.[5]

Surgery

When periaortic lymph node metastasis, liver metastasis, and/or peritoneal dissemination were observed during laparotomy, resection was abandoned in principle. However, even in the presence of distant metastasis, resection was undertaken in selected patients, provided that resection was not deemed to be too risky and was considered likely to improve the patient's quality of life. Intraoperative ultrasound was not used for looking for liver metastasis.

All hepatectomies were performed after the serum total bilirubin concentrations were less than 2 mg/dl. The liver parenchyma was transected with an ultrasonic dissector (CUSA, Valleylab, Boulder, CO) via the Pringle maneuver for 15 or 20 minutes at 5-minute intervals. Combined vascular resection or combined pancreatoduodenectomy was performed when needed.[5] Bilio-enteric continuity was re-established by using a Roux-en-Y cholangiojejunostomy.

Chemotherapy

Gemcitabine hydrochloride and tegafur-gimeracil-oteracil potassium (S-1) were authorized for use in treating cholangiocarcinoma in Japan in around 2007. Thereafter, we have used these agents as adjuvant chemotherapy as well as chemotherapy for unresected patients. Postoperative adjuvant chemotherapy was performed in patients with nodal metastasis, a positive surgical margin, and/or occult liver metastasis, where gemcitabine hydrochloride or S-1 was given for at least 6 months after surgery. Postoperative radiotherapy combined with chemotherapy was used in selected patients with a positive surgical margin.

Pathological assessment

The extrahepatic bile duct of the resected specimen was opened longitudinally, beginning from the distal resection margin and moving up to the proximal margin, in order to accurately evaluate the ductal margin status. Then, the resected specimens were fixed in 10 % formalin for several days and serially sectioned at 5-mm intervals (**Figure 1**). Intrahepatic bilio-vascular structures were identified on the serial sections, which were documented on real-size, color-photocopies of the sections.[6] The cut surfaces of the specimen were carefully observed and, when a nodule that was suspected to have a liver metastasis was found on the cut surface, that specimen was sectioned for a microscopic examination (**Figure 2A**). The specimens were prepared in the usual manner by hematoxylin and eosin staining. Histologic findings were described using the TNM Classification of Malignant Tumors by the International Union Against Cancer (7th edition, 2009).[7]

Statistical analysis

Results are expressed as median with ranges unless otherwise specified. The statistical analysis was performed by Man-Whitney *U* test for continuous variables and by Fisher's exact probability test for categorical variables. Patient survival was calculated by the Kaplan-Meier method. Differences in survival curves were compared using the log-rank test. A multivariate analysis was performed using Cox

proportional hazards model to identify prognostic factors. A $P<0.05$ was considered statistically significant. All statistical calculations were performed using the SPSS version 22 software (IBM Japan, Tokyo).

Results

During the study period, 945 consecutive patients with PHCC were treated at the present authors' clinic: 260 patients had unresectable disease and the remaining 685 patients underwent resection. Of the 685 resected patients, 13 underwent extrahepatic bile duct resection without hepatectomy. The remaining 672 patients underwent hepatectomy, including right trisectionectomy (n=57), right hemihepatectomy (n=213), left trisectionectomy (n=177), left hemihepatectomy (n=208), central bisectionectomy (n=11), and other hepatectomies (n=6), with (n=85) or without pancreatoduodenectomy (n=587). Vascular resection was aggressively performed for locally advanced PHCC, including portal vein resection alone (n=145), hepatic artery resection alone (n=40), and simultaneous resection of the portal vein and hepatic artery (n=93).[8]

Incidence of synchronous liver metastasis

Liver metastases were found in 30 (11.5%) of the 260 unresected patients and were the fifth common cause of unresectability. Of these, 15 patients were categorized as group A, and the remaining 15 patients were as group B (**Figure 3**). Of the 672 hepatectomized patients, 6 (0.9%) patients were categorized as group C, other 21 patients were as group D, and the remaining 645 patients were as group E (**Figure 3**).

Thus, the incidence of occult synchronous liver metastases was 3.1% (21/672) in hepatectomized patients. Overall, synchronous liver metastases, including both overt and occult metastases, were observed in 57 (6.0%) of the entire group of the 945 patients treated.

Clinical features of liver metastasis

Clinical features of liver metastases in each group are summarized in **Table 1**. In group A (n=15),

all liver metastases were detected with a median number of 4 by preoperative CT, thus leading to unresectability. In group B (n=15), liver masses in 10 patients were not detected by preoperative CT: in the remaining 5 patients, liver masses were detected, but the preoperative CT diagnosis was liver abscess in 4 patients and parenchymal ischemia in one patient. During laparotomy, liver metastases were detected and confirmed by frozen section, and resections were abandoned. In group C (n=6), preoperative diagnoses of liver metastasis had been made in 5 patients. In the remaining 1 patient, an overt metastasis was found during laparotomy. Nevertheless, resections were performed under aggressive strategy at that time. In group D (n=21), no liver masses were detected by preoperative CT, and liver metastases were not found, even through the use of laparotomy. However, final pathology of the resected specimens demonstrated liver metastases with a median number of 1 (range 1 to 6) (**Figure 2B**). All occult liver metastases were located inside the liver; in other word, they were not seen on the external surface of the liver. The diameters of occult liver metastases (the largest one when multiple) was 3 mm or less in 9 patients, 4 mm to 10 mm in 11 patients and 12 mm in one patient, with a median diameter of 5 mm.

When compared between the overt liver metastases (groups A, B, and C) and the occult liver metastases (group D) (**Table 1**), no between-group differences were observed in age, gender, Bismuth type, tumor marker, and location of the liver metastases. Conversely, the diameters of liver metastases were significantly smaller in the occult metastasis groups than in the overt metastasis groups (5 mm vs 12 mm, $P<0.001$).

Next, the 21 resected patients with occult liver metastases were compared with the 645 resected patients without synchronous liver metastases (**Table 2**). Bismuth type IV was significantly predominant in the former than in the latter. Microscopic venous invasions, microscopic liver invasions, and lymph node metastases were also significantly frequently observed in the former than in the latter. As anticipated, the patients with occult liver metastases had more advanced stages of the

disease.

Survival

In groups C and D, 90-day mortality after hepatectomy was 0%, while it was 2.0% (13/645) in group E. Survivals were compared among the 5 patients groups (groups A-E, **Figure 3**). Survivals for the group A, B, and C patients were almost identical and dismal: most of the patients died of the disease within 2 years. On the other hand, survival for group D patients was worse than that for group E patients, but better than those for group A, B, and C patients (**Figure 4**). The median survival time was 7.4 months in groups A-C, 17.1 months in group D, and 45.2 months in group E.

Of the 21 group D patients, 15 patients had single occult metastases and the remaining 6 had multiple occult metastases: their survivals were almost identical (median survival time 19.6 vs. 16.7 months, P=0.789). Eleven patients, most of whom underwent surgery after 2007, received adjuvant chemotherapy, and their survival was significantly better than that of the remaining 10 patients without adjuvant chemotherapy (median survival time 25.5 vs. 10.0 months, P=0.008). At the time of this writing, 19 patients died of recurrence including liver (n=14), peritoneum (n=8), lung (n=4), locoregional (n=3), brain (n=1), and bone (n=1) recurrences, with overlap. The remaining 2 patients are still alive without recurrence, for 47 and 33 months, respectively.

Prognostic factors in patients with occult liver metastasis

Prognostic factors in the 21 patients with occult liver metastasis were analyzed. Of these, 11 patients underwent adjuvant chemotherapy, and the remaining 10 patients did not. The regimens used were gemcitabine hydrochloride (n=5), S-1 (n=5), and gemcitabine with cisplatin (n=1). The reasons of no use of adjuvant chemotherapy in the remaining 10 patients were cases before 2007 (n=6), refusal by patients themselves (n=3), and unknown cause (n=1).

On univariate analysis, 2 of 9 possible clinicopathologic prognostic factors were significant (**Table 3**). Multivariate analysis using these significant factors revealed that adjuvant chemotherapy

and lymph node metastasis were independent prognostic factors in patients with occult liver metastasis.

Discussion

The current study is the first report on occult synchronous liver metastases from PHCC, which are defined as intrahepatic metastases that are overlooked by preoperative diagnostic imaging and intraoperative inspection but that are detected by final pathology. Under the definition, such liver metastases were observed in 3.1% (21/672) of hepatectomized patients. This incidence was low, but not extremely rare, and higher than we anticipated. Strictly speaking, as only resected segments of the liver were histologically examined, it is unclear whether occult liver metastases existed in the remnant liver or not. In addition, if the specimens were cut into more fine sections (3 mm intervals), the incidence may have increased. Thus, the incidence of "at least 3.1%" is correct. Occult liver metastases could be identified because hepatectomy had been performed as resectional procedure. These metastases, if any, have never been detected in other gastrointestinal malignancies in which hepatectomy is not necessary for curative resection. Consequently, the term "occult" synchronous liver metastasis can be specifically applied only for hepatobiliary malignancies that require a hepatectomy or a liver transplant.

Recently, Aufhauser et al. investigated the incidence of radiologically unrecognized (occult) intrahepatic metastases in explant hepatectomy specimens from orthotopic liver transplants for hepatocellular carcinoma.[9] Even in patients having single tumor with Model of End-stage Liver Disease (MELD) score $\leq$10, occult multifocality was found in as many as 35 % (452/1287) of the patients, with a median number of 1 (range, 1 to 5). Compared to this result, the incidence in the present study was much lower, although biological behaviors as well as surgical procedures are largely different between PHCC and hepatocellular carcinoma.

The current study has shown that thorough investigations of the resected specimens are important in detecting occult liver metastases. As mentioned in the Methods section, all of the resected specimens were "routinely" serially sectioned at 5-mm intervals, following 10 % formalin fixation.[6] We carefully observed the cut surfaces of the resected specimens, with attention paid to "small nodules" that were suspected to have liver metastases. When such lesions were found on the cut surfaces, they were histologically investigated. The 21 patients with occult liver metastases were eventually categorized as having stage IVB cancer diagnoses because a liver metastasis is defined as a pM1 disease.[7] If occult liver metastases were not found, then they must have been categorized into other stages. Meticulous handling of the resected specimens, with careful observation, is crucial, in order to detect small intrahepatic metastases, which will then lead to more accurate tumor staging.

Preoperative diagnoses of liver metastases in the present series were unexpectedly poor. Of the 57 patients with liver metastases including occult metastases, only 20 (35.1%) patients were definitively diagnosed with liver metastases by using preoperative CT. In the remaining 37 patients, metastases were never detected or, if detected, they presented as small, space occupying lesions, thus resulting in non-definitive diagnoses. One possible reason is that few patients with PHCC underwent MRI as part of the preoperative workup, due to the institutional strategy. Specifically, no patients with liver metastases underwent MRI. Although it must have been difficult to detect occult liver metastases even with MRI, improvement of the diagnostic accuracy for liver metastases is an important task.

We purposely performed hepatectomy in 6 patients with overt metastases, expecting that hepatectomy might improve their qualities of life. However, all of the patients died of the disease within 2 years, and their survival curve was almost identical to that for the unresected patients. These findings clearly show that patients with overt liver metastases are beyond surgical indication. These "excessive" hepatectomies were performed in the early 2000s and, thereafter, have never again been performed.

In the resected patients with occult liver metastases, the survival was significantly better than that in the patients with overt liver metastasis. Actually, 8 patients with occult liver metastasis survived for more than 2 years, while almost all patients with overt metastasis died within 2 years. This noticeable finding may be attributed primarily to "lead time" bias, meaning that the patients with occult liver metastasis are earlier in their disease progression timeline. On the other hand, multivariate analysis revealed that the adjuvant chemotherapy is an independent prognostic factor. The survival of the 10 patients without adjuvant chemotherapy was dismal, being similar to that of groups A-C patients (**Table 3**). Thus, a possibility may also exist that adjuvant chemotherapy using gemcitabine or S-1[10-12] is somewhat effective for the treatment of occult liver metastasis. Anyway, as the number of analyzed patients is limited, further studies are needed. At present, we routinely use S-1 as adjuvant chemotherapy for resected patients with lymph node metastasis, R1 resection, and occult liver metastasis. As S-1 is oral drag, it is easy to use as adjuvant chemotherapy. Adjuvant radiotherapy is also used in patients with R1 resection according to surgeon's preference.

The present study has some limitations, including its retrospective nature and the use of a single center; therefore, unexpected biases cannot be completely ruled out. The small number of patients with occult liver metastasis is also a limitation. However, due to the rarity and lack of awareness about this metastasis, conducting a study with a large sample size will be very difficult. Nevertheless, the present study comprises one of the largest series of patients with PHCC and is the first report on this issue. Another limitation is low degree of use of preoperative MRI. In Japan, most surgeons have traditionally preferred CT, rather than MRI.[5] In contrast, MRI has been widely used in Western countries[1-4]: therefore, studies from such countries are expected.

In conclusion, occult liver metastases from PHCC are not extremely rare. Meticulous handling of the resected specimen presented here is important to detect such metastases and leads to more accurate tumor staging. Patients with occult liver metastasis have advanced stages of the disease,

however, their survival may be improved by the use of adjuvant chemotherapy.

Disclosure

The present authors declare no conflict of interest.

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Figure Legends

Figure 1. Handling of the resected specimen. The resected specimen was serially sectioned at 5-mm intervals, and the cut surface was carefully observed.

Figure 2. Representative case of occult synchronous liver metastasis.

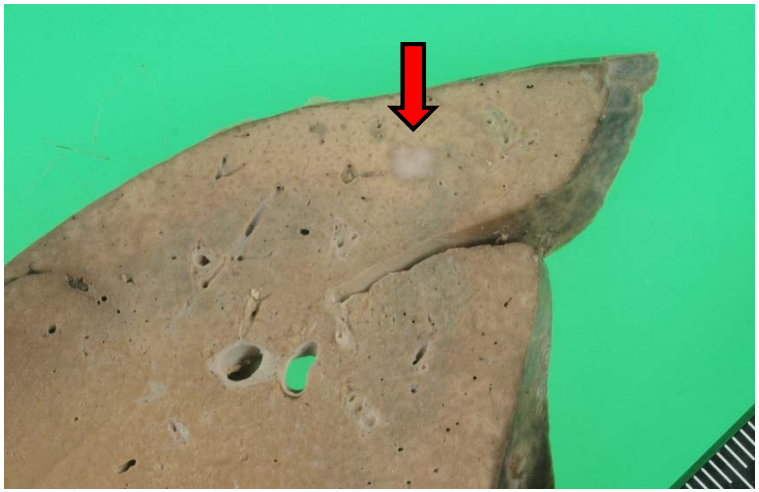
A: Cut surface of the resected specimen. A small intrahepatic nodule (red arrow) was observed.

B: Microscopic finding of the liver nodule (Hematoxylin-eosin stain).

Figure 3. Overview of the patients treated during the study period, according to resectability and liver metastasis.

Figure 4. Survival curves for patients with or without liver metastases. Group A, inoperable patients due to liver metastases detected by preoperative workup; group B, laparotomized but unresected patients due to liver metastases detected by intraoperative inspection; group C, hepatectomized patients with overt liver metastases; group D, hepatectomized patients with occult liver metastases; group E, hepatectomized patients without liver metastases (Please see **Figure 3**)

Figure 1



A

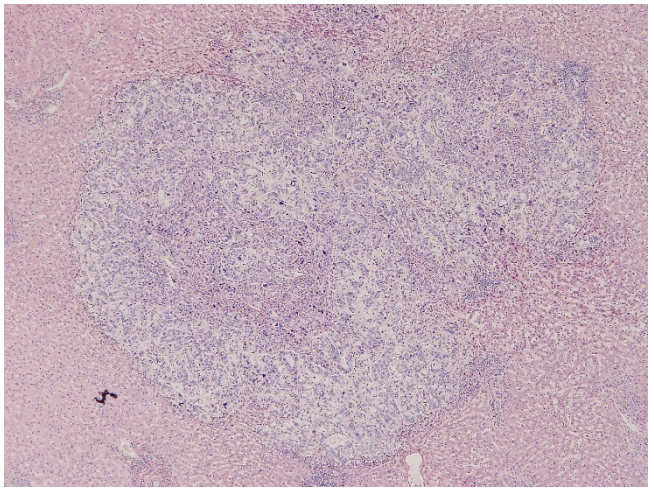


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Figure 2

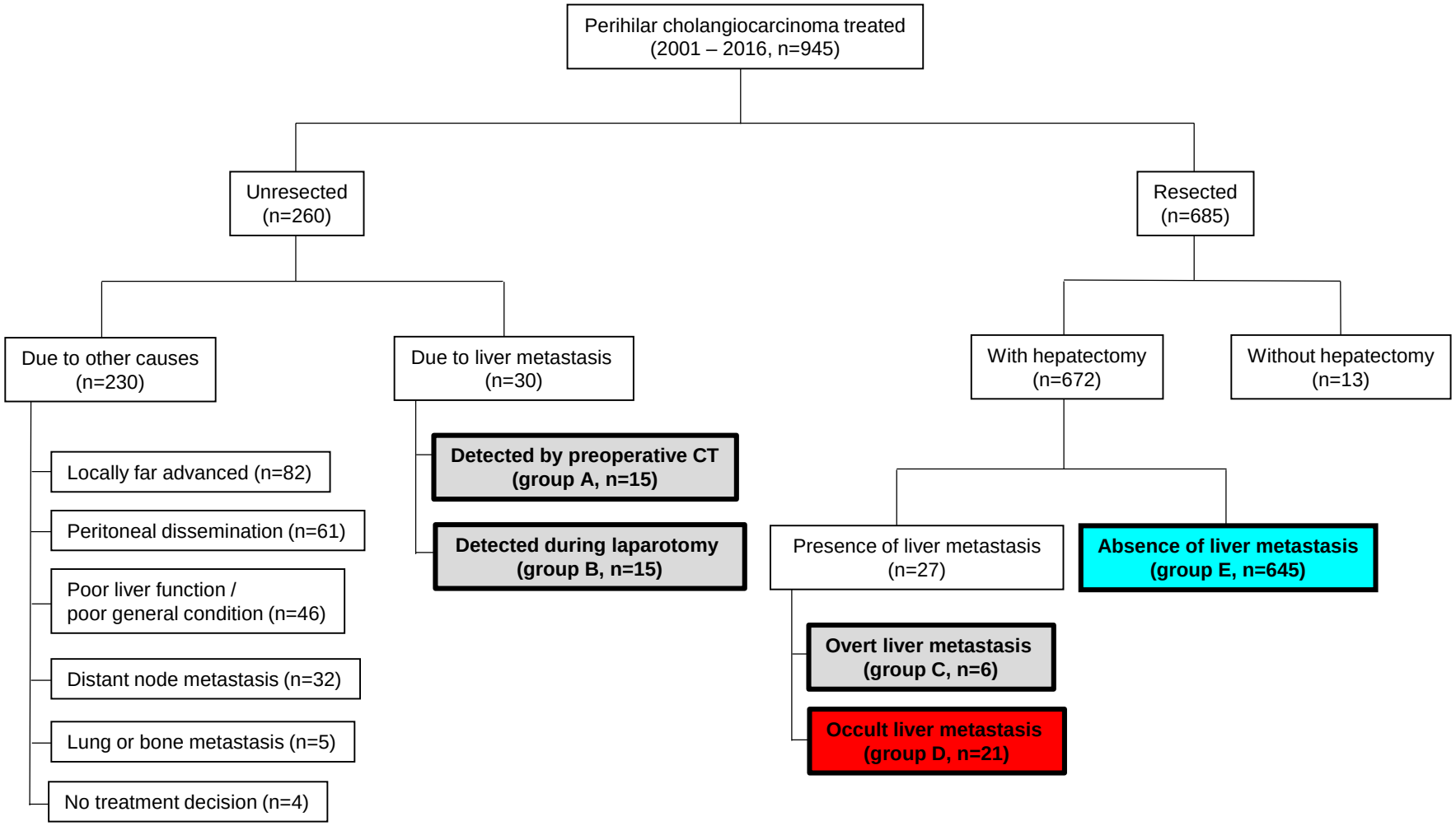
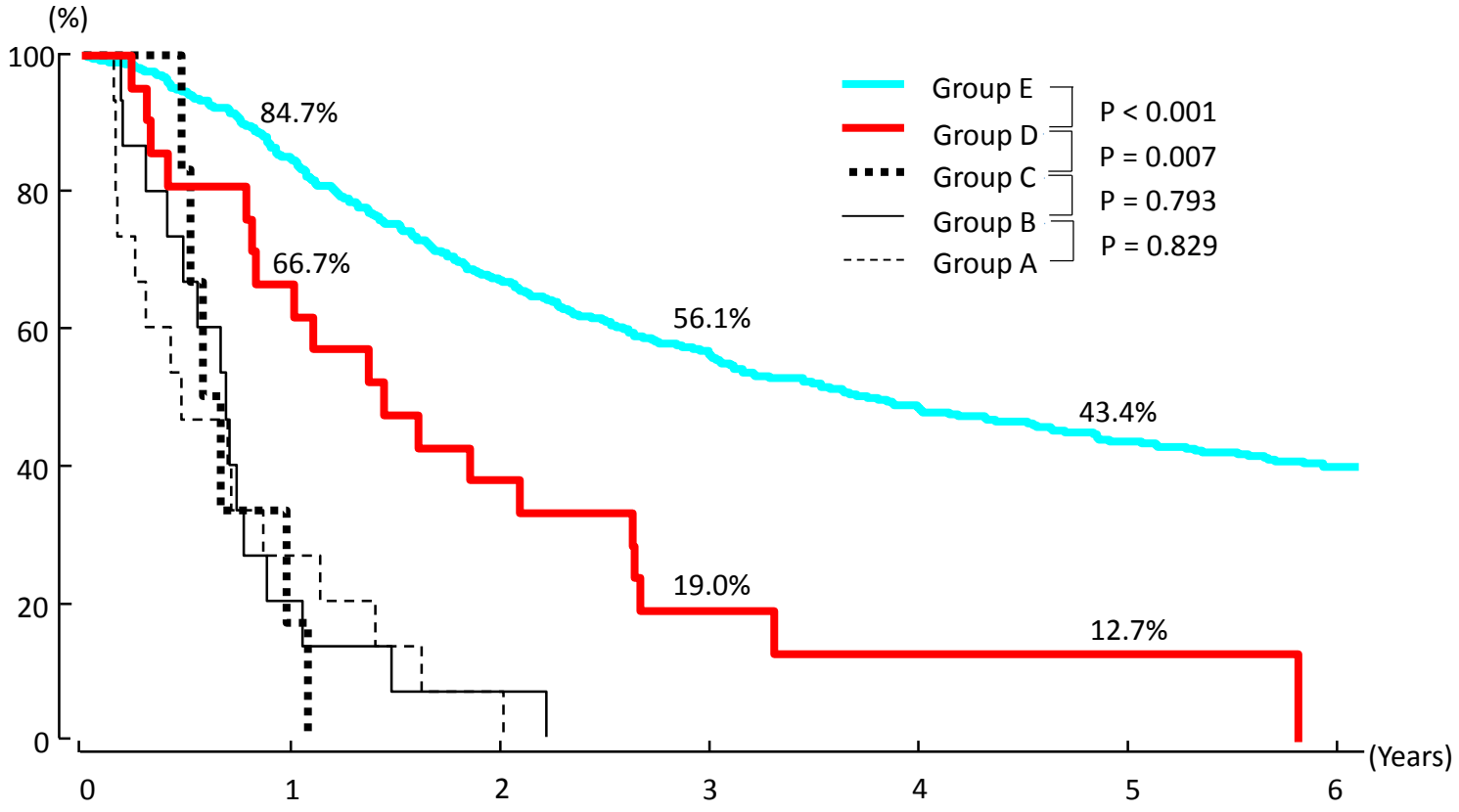

Perihilar cholangiocarcinoma treated
(2001 – 2016, n=945)
Unresected
(n=260)
Resected
(n=685)
Due to other causes
(n=230)
Due to liver metastasis
(n=30)
With hepatectomy
(n=672)
Without hepatectomy
(n=13)
Locally far advanced (n=82)
Peritoneal dissemination (n=61)
Poor liver function /
poor general condition (n=46)
Distant node metastasis (n=32)
Lung or bone metastasis (n=5)
No treatment decision (n=4)
Detected by preoperative CT
(group A, n=15)
Detected during laparotomy
(group B, n=15)
Presence of liver metastasis
(n=27)
Absence of liver metastasis
(group E, n=645)
Overt liver metastasis
(group C, n=6)
Occult liver metastasis
(group D, n=21)

Figure 3



No. at risk

Group E	645	539	401	304	231	184
Group D	21	14	8	3	2	1
Group C	6	2	0			
Group B	15	2	1			
Group A	15	4	1			

Figure 4

Table 1. Comparison between patients with overt liver metastasis and those with occult liver metastasis

Variables	Overt metastasis			Occult metastasis	P†
	Group A	Group B	Group C	Group D	
Number of patients, n	15	15	6	21	-
Age, years (range)	63 (41 - 78)	66 (57 - 75)	67 (55 - 72)	70 (30 -77)	0.908
Gender (male / female), n	11 / 4	11 / 4	5 / 1	15 / 6	0.766
Bismuth type (I - III / IV), n	6 / 9	7 / 8	3 / 3	6 / 15	0.272
CA19-9, U/ml (range)	516 (1 - 14240)	396 (16 - 219784)	2583 (24 - 15683)	232 (1 - 18150)	0.243
Carcinoembryonic antigen, ng/ml (range)	4.2 (1 - 28.5)	3.1 (1.8 - 19.1)	4.2 (1.7 - 18.4)	4.1 (0.5 - 131.6)	0.734
Number of liver metastasis detected by preoperative CT, n (range)	4 (1 - 10)	0	1.5 (0 -10)	0	-
Number of liver metastasis detected at laparotomy, n (range)	-	1 (1 - 10)	1.5 (1 - 10)	0	-
Number of liver metastasis detected by final pathology, n (range)	-	-	1.5 (1 – 15)	1 (1 - 6)	-
Location of liver metastasis (unilobar / bilobar), n	8 / 7	14 / 1	5 / 1	19 / 2	0.185
Diameter of liver metastasis*, mm (range)	14 (7 – 26)	9 (2 - 40)	13 (6 - 25)	5 (1 - 12)	**<0.001**

Group A, unresected patients with liver metastasis detected by preoperative CT;
Group B, unresected patients with liver metastasis detected during laparotomy;
Group C, hepatectomized patients with overt liver metastasis;
Group D, hepatectomized patients with occult liver metastasis (See Figure 3).
Continuous data were expressed as median (range).
*, diameter of the largest metastasis in case of multiple metastases
†, indicating the difference between overt (groups A - C) and occult (group D) metastases

Table 2. Comparison between patients with occult liver metastasis and those without liver metastasis

Variables	Occult liver metastasis (Group D*)	No liver metastasis (Group E*)	P
Number of patients , n	21	645	-
Age, years (range)	70 (30 - 77)	68 (31 - 89)	0.370
Gender, n (%)			0.645
Male	15 (71.4)	416 (64.5)	
Female	6 (28.6)	229 (35.5)	
Body mass index, kg/m^2 (range)	21.4 (16.0 - 30.8)	21.3 (13.1 - 35.2)	0.452
CA19-9, U/ml (range)	232 (1 - 18150)	81 (1 - 52831)	0.163
Carcinoembryonic antigen, ng/ml (range)	4.1 (0.5 - 131.6)	2.3 (0.3 -174.0)	0.054
Bismuth classification, n (%)			**0.024**
1, 2, 3	6 (28.6)	360 (55.8)	
4	15 (71.4)	285 (44.2)	
Extent of liver resection, n (%)			
<50%	7 (33.3)	214 (33.2)	0.999
≥50%	14 (66.7)	431 (66.8)	
Combined vascular resection, n (%)	11 (52.4)	268 (41.6)	0.373
Operative time, min (range)	595 (459 - 845)	600 (344 - 1150)	0.643
Blood loss, mL (range)	1161 (370 - 3423)	1333 (46 - 11115)	0.631
Histopathological classification, n (%)			**0.037**
Well	1 (4.8)	167 (25.9)	
Moderately / Poorly / Others	20 (95.2)	478 (74.1)	
Microscopic lymphatic invasion, n (%)	18 (85.7)	461 (71.5)	0.217
Microscopic venous invasion, n (%)	17 (81.0)	300 (46.5)	**0.003**
Microscopic perineural invasion, n (%)	19 (90.5)	550 (85.3)	0.754
Microscopic liver invasion, n (%)	19 (90.5)	392 (60.8)	**0.005**
Pathological tumor category, n (%)			**0.010**
is / 1 / 2	2 (9.5)	239 (37.0)	
3 / 4	19 (90.5)	406 (63.0)	
Lymph node metastasis, n (%)	16 (76.2)	303 (47.0)	**0.013**
R1 resection, n (%)	6 (28.6)	134 (20.8)	0.415
90-day mortality, n (%)	0	13 (2.0)	0.999

Continuous data were expressed as median (range).

*, See Figure 3.

Table 3. Univariate and multivariate analyses for prognostic factors in patients with occult liver metastasis

Variables	n	Median survival time (months)	Univariate	Multivariate	
			P	HR(95% CI)	*P*
Age			0.257		
< 70 years	10	17.6			
≥ 70 years	11	10.0			
Sex			0.443		
Male	15	16.7			
Female	6	17.6			
CA19-9			0.781		
< 100 IU/L	8	17.6			
≥ 100 IU/L	13	15.4			
Extent of liver resection			0.296		
< 50%	7	6.0			
≥ 50%	14	17.6			
Blood loss			0.422		
< 1200 mL	11	19.6			
≥ 1200 mL	10	12.4			
Number of occult liver metastasis			0.789		
Single	15	19.6			
Multiple	6	16.7			
Lymph node metastasis			**0.019**		**0.045**
Absent	5	70.0		1	
Present	16	12.4		3.41 (1.19 – 9.83)	
R status (curability)			0.470		
R0	15	17.6			
R1	6	10.0			
Adjuvant therapy			**0.008**		**0.023**
Absent	10	10.0		1	
Present	11	25.5		0.21 (0.04 – 0.97)	