

Resection of Hepatic Lesions Perfused by the Cholecystic Vein Using Indocyanine Green Navigation in Patients with cT2 Gallbladder Cancer

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Abstract

Background Various approaches to hepatectomy have been proposed for cT2 gallbladder cancers (GBC), but the optimal management strategy remains unclear. The aim of this study is to assess the effectiveness of using an indocyanine green (ICG)-based intraoperative navigation system during hepatic resection for cT2 GBC.

Methods From September 2007 to December 2017, 24 consecutive patients diagnosed with cT2 GBC underwent hepatic resection using ICG navigation. After cannulation of the cholecystic artery, ICG diluted with dissolution liquid was injected and ICG fluorescence illumination was visualized with the HyperEye Medical System. And additional histopathological examination was performed on the most recent 15 of the 24 patients for detection of microscopic liver metastasis.

Results For all patients, the disease-free survival rate was 59.1% at 5 years and overall survival rate was 86.2% at 5 years. Microscopic liver metastasis was detected in the resected liver in 3 (20%) of 15 patients, whose site of liver was S6, S5, and S5, respectively. The weight of the liver resected using ICG navigation was significantly smaller than that of S4a/S5 segmentectomy ($P < 0.0001$).

Conclusion Resected hepatic lesion using ICG imaging was possible to perform hepatectomy including liver micro-metastasis without excess or deficiency. This procedure might be novel intraoperative imaging method to provide valuable information on the optimal surgical approach to cT2 GBC.

Introduction

The prognosis of gallbladder cancer (GBC) is poor, and various treatment approaches have been adopted. Depth of invasion, presence of lymph node metastasis, and negative resection margins are significant prognostic factors that affect overall survival in patients with GBC [1–4]. However, these factors are not easily detectable preoperatively or intraoperatively. Various approaches to hepatectomy have been proposed for cT2 gallbladder cancers, from simple cholecystectomy to radical S4a/S5 hepatectomy with/without extrahepatic bile duct resection, but the optimal management strategy remains unclear [5–7].

Recently, in vivo fluorescence imaging techniques for identification of anatomical landmarks during surgery have

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been developed [8, 9]. One of the best indications for intraoperative indocyanine green (ICG)-based imaging is hepatobiliary surgery because of its ability to label bile ducts [10], hepatic tumors [11], and the boundaries of hepatic segment [12].

In the present study, we attempted to observe cholecystic venous flow using the HyperEye Medical System (HEMS; Mizuho Ikakogyo Co. Ltd, Japan) and to evaluate its efficacy for resection of hepatic lesions perfused by the cholecystic vein in patients with cT2 GBC. This same system has been recently used for the retrieval of the sentinel lymph node in breast cancer surgery [13] and for the evaluation of bowel circulation in patients with mesenteric artery aneurysms [14].

Method

Patient selection

This study was approved by the Tokyo Medical University Hachioji Medical Center Ethical Committee. From September 2007 to December 2017, 24 consecutive patients diagnosed with cT2 GBC underwent hepatic resection using ICG navigation. The patients' records were retrospectively reviewed. The data reviewed included patient demographics, clinical presentation, histopathological data, and tumor-node-metastasis (TNM) stage according to the Japanese Classification of Biliary Tract Cancers 6th Edition [15] and operative procedures. cT2 GBC was definitively diagnosed by abdominal and endoscopic ultrasonography and computed tomography.

Surgical procedure

After superselective cannulation of the cholecystic artery had been performed, ICG and indigo carmine dye, diluted with dissolution liquid to 5 ml, were injected slowly (Fig. 1a). ICG fluorescence illumination was visualized with the HEMS, which consists of a combination of custom-made optical filters and an ultra-high-sensitive closed-circuit device (CCD) image sensor with non-Bayer color filter arrays (HyperEye technology; United States Patent Application 20080251694), which can detect visible and near-infrared (NIR) rays between 380 and 1200 nm. Excitation of ICG was induced with a light-emitting diode (LED) light source (760 nm) that is part of the HEMS imaging system. The CCD camera is positioned approximately 50–80 cm above the surgical site. Both indigo carmine blue dye and ICG HEMS imaging were used to visualize the course of the cholecystic vein through the liver (Fig. 1b). ICG fluorescence range had no difference with indigo carmine dye range. The HEMS' LED produces

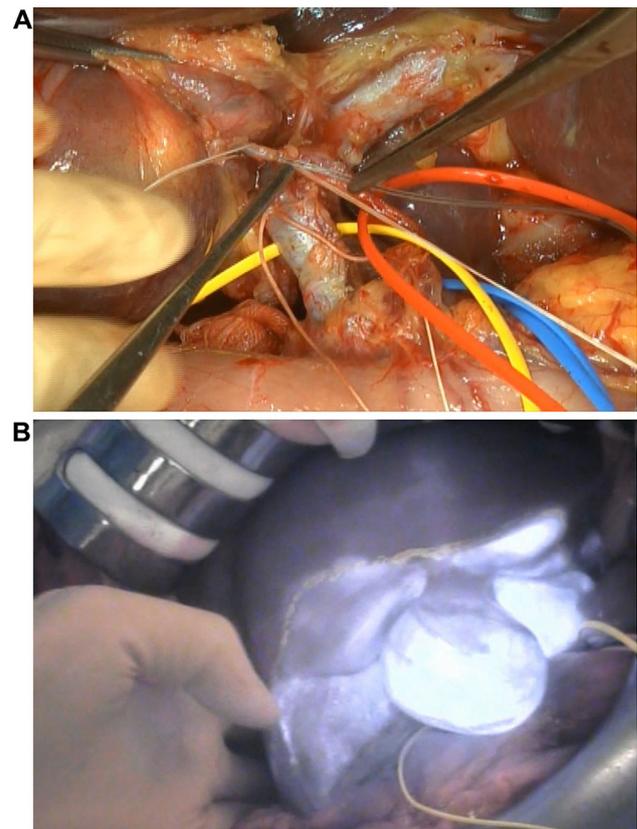


Fig. 1 Surgical procedure of ICG navigation surgery. After cannulation of the cholecystic artery, ICG was injected slowly (a). ICG fluorescence illumination was visualized with the HEMS. Both indigo carmine blue dye and ICG HEMS imaging were used to visualize the course of the cholecystic vein through the liver (b)

a limited amount of heat during use, resulting in an easier and safer surgical procedure. Extrahepatic bile duct resection was performed if the cystic duct margin was positive on frozen section. Nodal dissection was performed, including at least the portal nodes and often the celiac nodes. Pringle maneuver was performed during injection of ICG and liver parenchymal dissection. In this study, all cases were intended to perform this procedure, because all cT2 cases were diagnosed as T1 or T2 stage.

Histopathological examination

Additional histopathological examination was performed on the most recent 15 of the 24 patients for detection of microscopic liver metastasis. Multiple-step sections (5 mm) of the resected specimens were embedded in paraffin. The primary lesion and the resected liver were subjected to hematoxylin–eosin staining. All sites of possible microscopic metastasis to the liver were examined. Microscopic liver metastasis was defined as a distant metastatic nodule including cancer cell nests in the lumen

of the portal vein and discrete nodular lesions in the liver less than 5 mm in diameter. Cancer cells were verified by multiple-step sections that confirmed that cells were discontinuous from the primary lesion. The number, size, and distance from the gallbladder bed were recorded for each metastasis.

Statistical analysis

Our univariate analysis used *t* test analysis. A *P* value of 0.05 or less was considered statistically significant. The biostatistics package SPSS software (SPSS, Chicago, IL) was used for all analyses.

Results

Clinicopathological characteristics

The clinicopathological characteristics of all 24 patients are summarized in Table 1. There were 13 men and 11 women, aged 56–82 years (median age, 73 years). Of them, seven patients (29%) received additional extrahepatic bile duct resection for positive cystic duct margins. A part or all of S4a and S5 was stained by ICG in all patients, and a part of S6 stained in three patients. All patients received lymph node dissection. Postoperative complications (over Clavien–Dindo Grade III) occurred in one patient (bile leakage), and there was no postoperative mortality. Pathologically, five patients (21%) were classified as having T1b tumors and five (21%) had regional lymph node metastasis. All T1b cases had no lymph node metastasis, and the final stage was 1, while the five patients with lymph node metastasis were at a final stage of 3b. Recurrence occurred in seven patients (29%) in the lymph nodes ($n = 3$), liver ($n = 1$), lung ($n = 1$), bone ($n = 1$), and tumor dissemination ($n = 1$).

The clinicopathological characteristics of the 15 patients that had additional pathological examination results are shown in Table 2. Of the 15, three (20%) were found to have liver micro-metastasis, and as a result were classified as stage 4b. There were two cases of recurrence in the patients with liver micro-metastasis, one in the lymph nodes and one tumor dissemination. Among the patients with liver micro-metastasis, three are still alive.

Disease-free and overall survival

The disease-free survival rate was 59.1% at 5 years with a median disease-free survival of 105 months. The overall survival rate for the 24 patients who underwent R0 resection for cT2 GBC was 86.2% at 5 years with a median survival of 110 months.

Table 1 Clinicopathological features of cT2 gallbladder cancer patients who underwent ICG navigation surgery

Valuables	cT2 patients ($n = 24$)
Age	Median 73 Range 56–82
Gender	
Female	11 (46%)
Male	13 (54%)
Resection of EHBD	
(+)	7 (29%)
(–)	17 (71%)
Pathological findings	
<i>T</i> factor	
T1b	5 (21%)
T2	19 (79%)
<i>N</i> factor	
N0	19 (79%)
N1	5 (21%)
<i>f</i> Stage	
1	5 (21%)
2	14 (58%)
3b	5 (21%)
Stained area of segment	
S4	24
S5	24
S6	3
Recurrence site	
Lymph node	3 (13%)
Liver	1 (4%)
Lung	1 (4%)
Bone	1 (4%)
Dissemination	1 (4%)
Survival	
Alive	19 (79%)
Dead	5 (21%)

EHBD extrahepatic bile duct

Assessment of liver micro-metastasis by additional histopathological examination

Microscopic liver metastasis was detected in the resected liver in 3 (20%) of 15 patients (Fig. 2a). The number of sliced specimens was 41, 23, and 44, respectively, and the metastases were detected within 3 cm of the gallbladder bed. Micro-lymph vessel invasion was found in one patient and the sites of liver metastasis were S6, S5, and S5, respectively. Lymph node recurrence and tumor dissemination occurred in two of the three patients, and all remained alive after the initial operation. No patient suffered a remnant liver recurrence. A representative case

Table 2 Clinicopathological features of cT2 gallbladder cancer patients who underwent additional pathological examination

Valuables	cT2 patients (n = 15)
Number of sliced specimen	
Average	20
Range	7–47
Pathological findings	
<i>T</i> factor	
T1b	2 (13%)
T2	13 (87%)
<i>N</i> factor	
N0	10 (67%)
N1	5 (33%)
<i>M</i> factor (microscopic liver metastasis)	
M0	12 (80%)
M1	3 (20%)
fStage	
1	2 (13%)
2	6 (40%)
3b	4 (27%)
4b (microscopic liver metastasis)	3 (20%)

with liver micro-metastasis is summarized in Fig. 2b. A 77-year-old woman was diagnosed with cT2 GBC and underwent liver resection without extrahepatic bile duct resection using ICG navigation. In this case, the ICG-visualized area constituted part of S4 and S5 (Fig. 2b-1); however, these sites were approximately 4 cm from the gallbladder bed (Fig. 2b-2). The histopathological specimens are shown in Fig. 2b-3 with the micro-metastasis indicated by the asterisk (Fig. 2b-4). Microscopic liver metastasis of this patient is found in Fig. 2b-5, 6.

Comparison between liver resection using ICG navigation and S4a/S5 segmentectomy

Figure 3a shows the compared analysis between ICG group and S4a/S5 group. As shown in Fig. 3a, there are few cases of T2 in S4a/S5 group, and in our institute, S4a/S5 resection was indication for advanced cases such as T3 or T4 cases. All patients who performed S4a/S5 segmentectomy for T2 were incidental gallbladder cancer after laparoscopic cholecystectomy. The background and staging of the patients were significantly different from that of ICG navigation group. And microscopic liver metastasis was not surveyed in S4a/S5 group, because stage in S4a/S5 group is advanced. Moreover, the median weight of the liver resected using ICG navigation was 45 g. In contrast, the median S4a/S5 segmentectomy weighed 80 g. Thus, the liver specimen resected using ICG navigation was significantly smaller than that of S4a/S5 segmentectomy ($P < 0.0001$) (Fig. 3b).

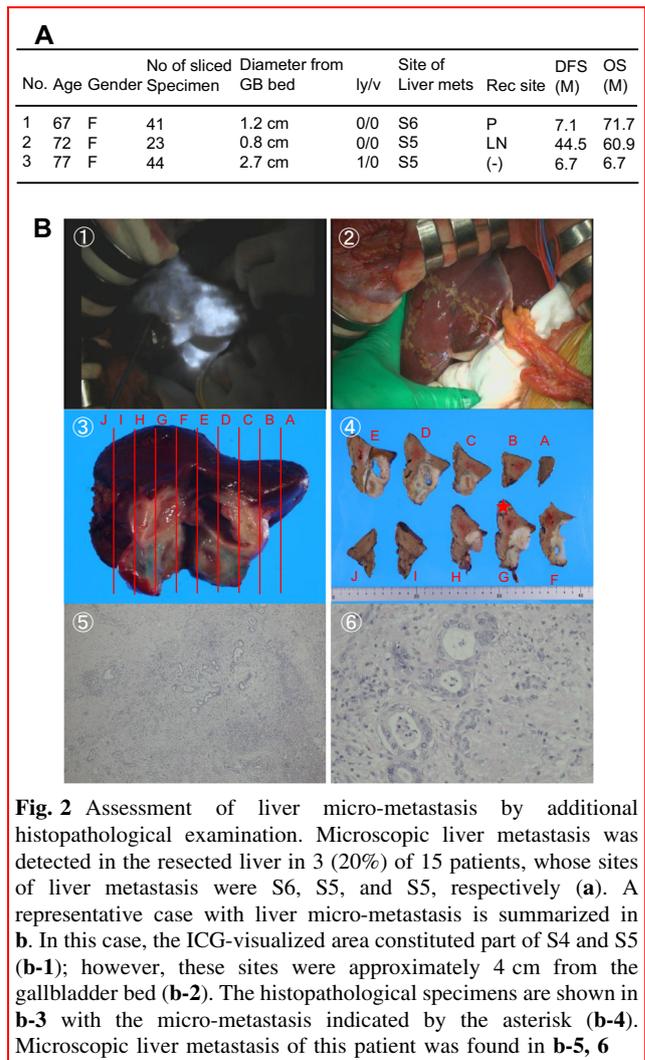


Fig. 2 Assessment of liver micro-metastasis by additional histopathological examination. Microscopic liver metastasis was detected in the resected liver in 3 (20%) of 15 patients, whose sites of liver metastasis were S6, S5, and S5, respectively (a). A representative case with liver micro-metastasis is summarized in b. In this case, the ICG-visualized area constituted part of S4 and S5 (b-1); however, these sites were approximately 4 cm from the gallbladder bed (b-2). The histopathological specimens are shown in b-3 with the micro-metastasis indicated by the asterisk (b-4). Microscopic liver metastasis of this patient was found in b-5, 6

Discussion

Liver metastasis is believed to occur via two pathways: a lymphatic route and a venous route. It is thought that there is no lymph flow from the gallbladder to the liver through the gallbladder bed, although some have reported that lymphatic backflow can occur in cases in which the cystic lymph nodes are involved [16]. Several investigators have documented the significance of vascular metastasis via the cystic vein [17]. The cystic veins vary greatly in number, size, and course; some of the veins accompany the branches of the cystic artery toward the neck of the gallbladder, which runs into the venous plexus surrounding the extrahepatic bile duct. These vessels terminate in the portal vein, which flows into both lobes. Other cystic veins carry blood directly into the liver through the gallbladder fossa. It has been found that cystic veins on the hepatic side flow almost into the portal branches of S4 or S5 when examining venous drainage of the gallbladder using ICG [18]. In

Valuables	ICG surgery (n=24)	S4a/S5 resection (n=12)	P value
Age			0.092
Median	73	68	
Range	56 - 82	59 - 82	
Gender			0.995
Male	11 (46%)	7 (58%)	
Female	13 (54%)	5 (42%)	
Resection of the EHBD	7 (29%)	8 (67%)	0.037
Pathological findings			
T factor			0.002
T1	5 (21%)	0	
T2	19 (79%)	3(25%)	
T3	0	6 (50%)	
T4	0	3 (25%)	
N factor			0.777
N0	19 (79%)	9 (75%)	
N1	5 (21%)	3 (25%)	
Stage			0.278
1	5 (21%)	0	
2	14 (58%)	1 (8%)	
3	5 (21%)	8 (67%)	
4	0	3 (25%)	
Operative time (min)			0.048
Median	280	366	
Range	255 - 374)	279 – 460	
Postoperative complications	1 (4%)	1 (8%)	0.853

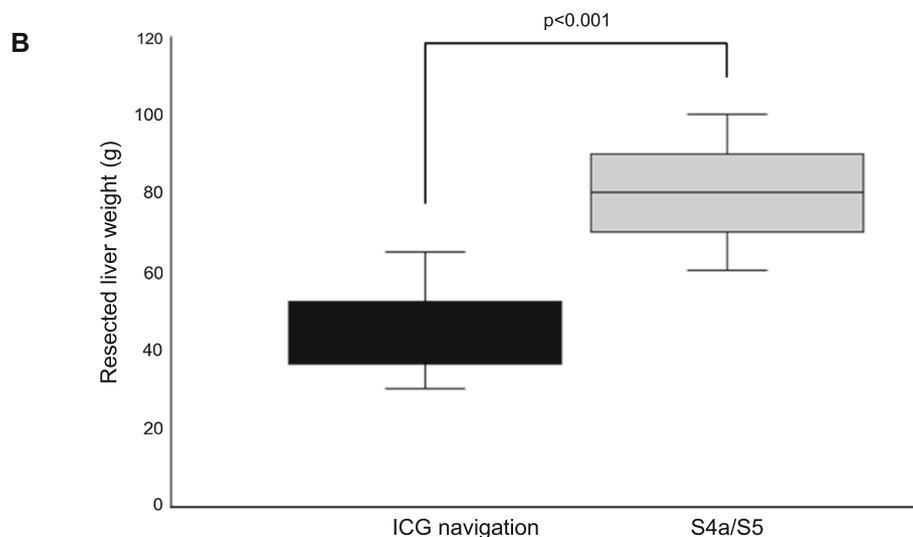


Fig. 3 Comparison between liver resection using ICG navigation and S4a/S5 segmentectomy. As shown in the figure **a**, there are few T2 cases in S4a/S5 group, and in our institute, S4a/S5 resection was indication of advanced cases such as T3 or T4. All patients who performed S4a/S5 segmentectomy for T2 were incidental gallbladder cancer after laparoscopic cholecystectomy. The background and staging of the patients were significantly different from that of ICG navigation group. Figure **b** shows the comparison of resected liver weight between ICG navigation surgery and S4a/S5 resection case. The liver specimen resected using ICG navigation was significantly smaller than that of S4a/S5 segmentectomy ($P < 0.0001$)

another report using analysis of angio CT, the cystic veins took either of two routes: one into the liver through the hepatic hilum (29%), taken mainly by the portal branch for sub-segment 4a (P4a), the anterior portal branch, and the umbilical portion of the portal branch; the other through the hepatic bed (71%), taken mainly by S4a sinusoid, S5

sinusoid, P4a, and P5 [19]. Our study found cases in which staining by ICG did not appear in the liver, but in such cases, area drained through liver bed was stained by blocking the hepatic inflow with Pringle maneuver. It might be appropriate to visualize the area perfused by the

gallbladder veins using ICG navigation and search for possible liver micro-metastasis.

Various types of hepatectomy have been performed for cT2 GBC because this lesion often spreads to the liver. The extent of microscopic portal tract invasion has been reported to correlate well with the depth of direct invasion of the liver [20]. It was found that extended right hepatectomy should be performed in patients with direct invasion to a depth of more than 2 cm into the parenchyma [21]. Anatomic resection of S4a and S5 was recommended for cT2 GBC as well as part of S6 if the gallbladder is attached to it [22]. However, using our technique, it was possible to individualize the extent of hepatectomy for each patient without excess or deficiency. In this study, one liver micro-metastasis was more than 2 cm from the gallbladder bed and liver resection within this distance may have been insufficient. On the other hand, the volume of the resected liver was significantly smaller than a standard S4a/S5 resection. Therefore, an insufficient remnant liver volume can be avoided, which may reduce postoperative complications. In our institute, indication for S4a/S5 resection was the advanced cases such as T3 or T4 cases. Therefore, it is difficult to compare prognosis and evaluation of microscopic liver metastasis between ICG group and S4a/S5 group.

ICG fluorescence is highly sensitive and has the advantage of detecting blood flow in real time. Adequate fluorescence images are obtained with small amounts of ICG diluted with dissolution liquid. However, this procedure does have some limitations. The blood flow was observed from the surface of the liver, because fluorescence located deep within the liver could not be detected by the NIR camera system. Although it will be different for individual patients, ICG staining areas can be identified even in the deep part of the liver if the time from ICG injection is short. However, it is still worthwhile to attempt to use ICG for assessment of the deep parenchyma for the resection plane when the time required for visualization with HEMS is short, because Pringle maneuver performed during ICG injection and liver parenchymal dissection. Although almost all of the cholecystic veins flowed through the gallbladder bed into S4a and S5, other venous courses are also possible [18]. Although, theoretically, ICG may go through deep side of S4 and flow into MHV, a part or all of S4a and S5 was stained by ICG in all patients, and a part of S6 was stained in a few cases. Most of area drained through liver bed was S4 and S5, but another segment detected by angio CT was only S6 [19]. By using our method, it is thought to be useful in terms of detecting such case without leaking out. Considering the mechanism of liver metastasis to the region draining from the gall bladder vein to the liver, ICG navigation can be very useful.

Our results suggest that wedge resection 1 or 2 cm from the gallbladder bed may miss liver micro-metastasis. On the other hand, there is no need to perform S4a/S5 resection in all cases; however, even such a resection may be insufficient when liver micro-metastasis exists in S6. In our study, the patient who had been preoperatively diagnosed with cT2 GBC and found to have a deeper tumor at laparotomy than initially thought did not undergo ICG navigation. ICG navigation appears to be the optimal method for determining the extent of liver resection, including resection of liver micro-metastasis, for cT2 GBC. Our results should be interpreted with caution as our sample size was small. Larger trials are needed to determine the survival outcomes of these patients. Moreover, overall survival and disease-free survival were favorable as compared to other reports [4, 5]. It was suggested that our procedure by ICG navigation contributed to this reason.

Conclusion

In this study, the effectiveness of using an indocyanine green (ICG)-based intraoperative navigation system was assessed during hepatic resection for cT2 gallbladder cancer. The resected lesion of liver using ICG imaging was possible to perform appropriate hepatectomy including liver micro-metastasis. Our surgical procedure might be a novel intraoperative imaging method to provide valuable information on the optimal surgical approach to these complex tumors. Further, our ICG imaging system enabled us to perform oncologically effective resections while preserving the normal hepatic parenchyma for cT2 GBC.

Authors contribution N Chiba, M Shimazu, and S Kawachi were involved in study design. N Chiba was involved in acquisition of data. Operation procedures were performed by all authors. N Chiba helped in analysis and interpretation. N Chiba contributed to drafting manuscript. N Chiba and S Kawachi helped in revision.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This research has been approved by Tokyo Medical University Hachioji Medical Center Ethics Committee.

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