MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 3406-3416
DOI: 10.12659/MSM.916423

Received: 2019.03.22
Accepted: 2019.04.24
Published: 2019.05.08

Improving the Surgical Effect for Primary Liver Cancer with Intraoperative Fluorescence Navigation Compared with Intraoperative Ultrasound

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Bing Liu
BC 1 Tian Liu
BCD 1 Ming Su
BCD 2 Ya-Qi Ma
BC 3 Bei-Feng Zhang
BC 4 Ye-Fei Wang
B 1 Bing-Yang Hu
ABCDEFG 1 Yong-Liang Chen

1 Department of Hepatobiliary Surgery, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China
2 Department of Pathology, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China
3 Department of Anesthesia Operation Center, Chinese People's Liberation Army (PLA) General Hospital, Beijing, P.R. China
4 Department of General Surgery, The First Hospital of Yulin, Yulin, Shaanxi, P.R. China

Corresponding Author: Yong-Liang Chen, e-mail: chenyongl301@163.com
Source of support: Departmental sources

Background: This study aimed to compare the application value of intraoperative fluorescence navigation technology (FNT) and intraoperative ultrasound (IOUS) in primary liver cancer surgery.

Material/Methods: Fifty consecutive patients with primary liver cancer scheduled to receive surgical treatment were divided into FNT group and IOUS group. FNT and IOUS were separately used to guide tumor resection and detect new cancerous lesions in the 2 groups. The complete tumor resection rate (R0) resection rate, length of the tumor distance from cutting edge, the diagnostic efficacy of cancerous nodules and the fluorescence imaging characteristics of different types tumors were recorded.

Results: The R0 resection rate was 100% (25 out of 25 patients) in the FNT group and 96% (24 out of 25 patients) in the IOUS group. In the FNT group, 1 case (4%, 1 out of 25 patients) had cancer tissue that was less than 1 cm from the cutting edge, compared to 7 cases (28%, 7 out of 25 patients) in the IOUS group (P=0.049), which was a significant difference. In the remaining livers of 50 consecutive patients, FNT found 5 new cancerous nodules with a sensitivity of 71.4%, a specificity of 11.1%, and a false-positive rate of 88.9%; for IOUS the results were 42.9%, 88.9%, 11.1%. The fluorescence imaging characteristics of all well-differentiated hepatocellular carcinomas were tumor tissue imaging, but all other types of tumors were ring imaging around the tumor.

Conclusions: FNT can improve the R0 resection rate, ensure a safe distance between tumor and cutting edge and can identify more new cancerous nodules compared to IOUS. Thus, FNT could improve the surgical treatment effect for primary liver cancer and hopefully further improve the prognosis of patients.

MeSH Keywords: Carcinoma, Hepatocellular • Hepatectomy • Indocyanine Green • Liver Neoplasms • Optical Imaging • Ultrasonography, Doppler

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/916423

4130 4 2 48

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Primary liver cancer, most of which comprises hepatocellular carcinomas (HCC), is the fifth most common cancer worldwide and the second leading cause of cancer deaths [1–5]. Hepatectomy has continued to be the best treatment method for patients with resectable HCC [6–9]. Despite the high efficacy of hepatectomy, more than 70% of patients develop recurrent tumors within 5 years after hepatectomy [10–13]. Optimizing the surgical program and improving the surgical effect has always been a goal of surgeon. Intraoperative ultrasound (IOUS) can accurately locate tumors and identify tumor anatomical relationship with surrounding blood vessels and bile ducts. Moreover, it can find new cancerous nodules that are not found by preoperative imaging and help to formulate surgical plans and guide tumor resection [14–18]. However, during the process of tumor resection, IOUS cannot continuously locate tumors in real time, and cannot assess the cutting edge, and small superficial carcinoma lesions cannot be well detected.

Recent developments in fluorescence navigation technology (FNT) based on indocyanine green (ICG) are enhancing the accuracy of liver resection and pathological diagnosis of small liver cancers. ICG was accidentally found to accumulate in HCC tissue and in normal hepatic parenchyma around metastatic adenocarcinoma nodules [19,20]. The first application of this FNT was reported in 2009 by Ishizawa et al. and Gotoh et al. In hepatobiliary surgery, it is crucial to accurately determine the location of the tumor, determine the cutting margin, and discover new cancerous nodules. Since the first report, more than 700 cases of liver resection have been reported using FNT to identify liver tumors with a high sensitivity (70% to 100%) [19,21–39].

Both FNT and IOUS can improve the surgical effect of liver tumor resection. However, more studies are needed to compare the application value of these 2 auxiliary technologies and evaluate the application value of combining them in the surgical treatment of primary liver cancer. This study will compare the advantages and disadvantages of these 2 technologies and discuss the clinical application value of combining these 2 technologies in the surgical treatment of primary liver cancer.

Material and Methods

Patients

Fifty consecutive patients with primary liver cancer who were scheduled to undergo liver tumor resection from April 2015 to June 2015 were selected from The PLA General Hospital. The patients were randomly divided into 2 groups: the fluorescence navigation technology group (FNT group) and the intraoperative ultrasound group (IOUS group). The FNT group used fluorescence navigation technology to guide the resection of liver cancer, and the IOUS group used IOUS to guide the resection of liver cancer. The basic data are shown in Table 1.

Table 1. General characteristics of the 50 patients.

	FNT group	IOUS group	*P**
Gender (Male/Female)/cases	19/6	21/4	0.725
Age/years	51.96±9.70	52.60±14.05	0.852
>50 years/cases	13	13	1.000
≤50 years/cases	12	12	
ALT/cases			
<40 U/L	14	19	0.136
>40 U/L	11	6	
AST/cases			
<40 U/L	20	22	0.702
>40 U/L	5	3	
ALB/cases			
>35 g/L	24	22	0.492
28–35 g/L	1	2	
<28 g/L	0	1	

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1 continued. General characteristics of the 50 patients.

	FNT group	IOUS group	*P**
TBIL/cases			
<21.0 μmol/L	23	23	1.000
>21.0 μmol/L	2	2	
AFP/cases			
>20.0 μg/L	9	13	0.254
<20.0 μg/L	16	12	
CA199/cases			
>37 U/mL	5	6	1.000
<37 U/mL	20	19	
CEA/cases			
>5.0 μg/L	0	2	0.490
<5.0 μg/L	25	23	
None/hepatitis B/hepatitis C/case	10/13/2	10/14/1	0.831
Maximum tumor diameter/cm	6.68±4.64	6.96±4.02	0.816
≤3 cm/cases	4	4	0.648
3–5 cm/cases	9	5	
>5 cm/cases	12	16	
The operation time/minutes	202.72±68.06	195.40±62.39	0.694
Amount of blood loss during surgery/mL	548.00±426.59	366.40±247.77	0.086
Intraoperative blood transfusion/cases	10	11	0.725
Hospital days/days	18.52±5.95	19.00±6.28	0.783
Surgical complications/case	3	5	0.482
AJCC staging (8^{th})/case			
IA/IB/II/IIIA/IIIB/IVA	3/17/2/1/1/1	1/18/5/0/1/0	0.505
Pathological type (high/medium/low differentiation)			
HCC	18 (2/13/3)	20 (3/15/2)	0.580
ICC	6 (0/5/1)	5 (0/3/2)	
cHCC-CC	1 (0/0/1)	0 (0/0/0)	

FNT group – fluorescence navigation technology group; IOUS group – intraoperative ultrasound group; HCC – hepatocellular carcinoma; ICC – intrahepatic cholangiocarcinoma; cHCC-CC – combined hepatocellular carcinoma and cholangiocarcinoma. *P*>0.05, indicating no significant difference between the two groups.

The inclusion criteria were as follows: 1) initial diagnosis of primary liver cancer via preoperative imaging examination and laboratory examination were confirmed with primary liver malignancy by postoperative pathology (including HCC, cholangiocarcinoma and combined hepatocellular carcinoma and cholangiocarcinoma); 2) preoperative imaging examination showing that all tumors were diagnosed as single tumor without vascular invasion or distant metastasis; 3) surgical indications for tumor resection were present and the operation was performed successfully; and 4) Child-Pugh liver function grading A/B.

The exclusion criteria were as follows: 1) patients with abnormal bile metabolism or excretion; 2) patients with ICG allergy

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

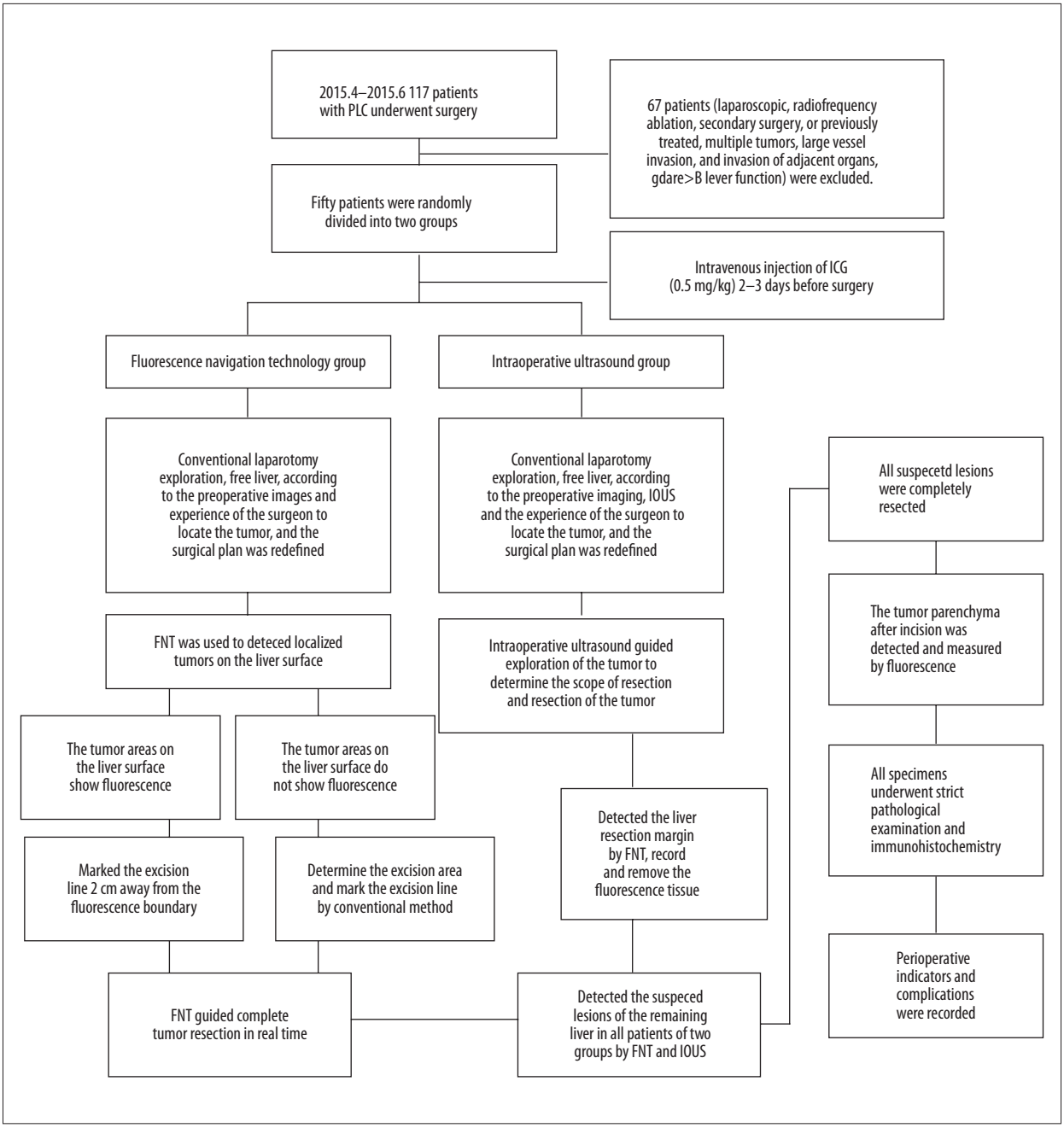

Figure 1. Flow chart of research procedure.

or iodine allergy; 3) patients with a history of laparoscopic surgery, radiofrequency ablation, secondary surgery or previous treatment; 4) patients with multiple tumors, invasion of large vessels, and invasion of adjacent organs; and 5) patients with liver function above grade B.

All 50 patients in the FNT group and IOUS group were scheduled to undergo radical liver tumor resection under general anesthesia. All patients were intravenously injected with ICG (0.5 mg/kg) 2 to 3 days before surgery, and routine preoperative examination, evaluation and surgical planning were conducted. All patients included in the study received informed consent and signed informed consent documents. The study was approved by The PLA General Hospital ethics review committee, No. S2014-052-01.

ICG-based fluorescence imaging system

The fluorescence imaging system adopts the handheld optical molecular imaging surgery navigation system independently

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

developed by the Institute of Automation, Chinese Academy of Sciences, Key Laboratory of Molecular Imaging, Chinese Academy of Sciences and Beijing Digital Precision Medicine Technology Co., Ltd. The system is equipped with a portable hand-held handle, and the front-end probe of the handle integrates an excitation light source transmitter and a camera device. The excitation light source can emit light at approximately 785-nm excitation. The camera can receive the 840-nm near-infrared light emitted by the excited ICG molecules, transmit it to the host computer through the data line, and then use special software to analyze the data and obtain the fluorescence imaging image. During the operation, disposable sterile bags were placed on the handle probe to ensure a sterile environment. The operating lamp was turned off (the light in the operating room was retained), and the distance between the probe and surface of the liver was approximately 30 cm. Fluorescence signals could be collected.

Intraoperative ultrasound was performed using the Hitachi Aloka Color Ultrasound HI VISION instrument (model Noblus; Shanghai Chuangxun Medical Equipment Co., Ltd., China) with a probe frequency of 7.5 mHz.

Treatment by surgery

After admission, the patients were randomly divided into the FNT group and the IOUS group according to a random number table. All the patients underwent liver tumor resection under general anesthesia, and intravenous injection of ICG (0.5 mg/kg) was administered 2 to 3 days before surgery. ICG for injection (Dandong Yichuang Pharmaceutial Co., Ltd., China) was selected as the fluorescence reagent. Routine preoperative examination, surgical planning, preoperative preparation, general anesthesia and surgical area sterilization were performed (Figure 1).

In the FNT group, FNT was used for real-time visualization of the known tumor, recording whether the tumor could be visualized in the corresponding tumor projection area on the liver surface. The imaging range was marked with an electric knife, the tumor resection range was demarcated 1 to 2 cm away from the imaging range, and the tumor resection was conducted under the real-time guidance of fluorescence imaging. If no fluorescence imaging of the tumor projection area was observed on the liver surface, the tumor surface resection area can be determined according to the preoperative imaging results and surgeon's experience. During the whole process of resection, the fluorescence imaging system was still used to locate the tumor boundary for the surgeon in real time, and all the fluorescence imaging tissues were removed.

Patients in the IOUS group were guided by IOUS to locate the tumor, and tumor resection was conducted at a distance of 1 to 2 cm from the calibrated tumor range. After complete resection of the tumor, fluorescence navigation technology was used to detect the liver resection margin *in vivo*, and the number of suspected lesions showing fluorescence was recorded. All suspected lesions were detected by rapid intraoperative frozen pathological examination, and any residual cancer cells was positive at the resection margin *in vivo*, while other results were recorded as negative (Figure 1).

Detection of new suspected lesions

The FNT and IOUS were used to detect suspected lesions in the remaining livers of the 50 patients. All suspected lesions detected by the 2 methods were examined by rapid intraoperative frozen pathological examination, and the number, size, nature, degree of differentiation and depth of distance from the surface of the liver were recorded (Figure 1).

Fluorescence imaging characteristics of the tumors *in vitro*

After e complete resection of the tumor detected in the preoperative images of all 50 patients, the pathologists carried out rapid intraoperative frozen pathological examination on the cutting edges of the isolated specimens and recorded the pathological report results. The tumor was dissected *in vitro*, fluorescence imaging was performed, and the maximum diameter, shortest distance from the tumor to the cutting edge and fluorescence imaging characteristics of the tumor were recorded. Imaging of the tumor parenchyma and surrounding tissues was considered fluorescence imaging, while imaging of other areas was considered non-fluorescence imaging. Routine pathological examination was performed on the specimens to record the tumor type and degree of differentiation.

Statistical analysis

The data were analyzed using IBM SPSS Statistics 23.0 software. A parametric test (*t*-test) compared the 2 groups of conforming parameter data, and non-parametric tests (chi-squared test and Fisher's exact test) compared the 2 groups of non-parametric data. $P<0.05$ was considered statistically significant.

Results

Result 1. FNT improved the surgical resection effect for the major tumor

FNT was used to conduct real-time guided resection of preoperative known tumors in 25 patients in the FNT group. Fluorescence was detected in the corresponding projection area of the tumor on the liver surface (Figure 2A/a, 2B/b, 2C/c) in 21 patients (21 out of 25 patients, 84.0%), with an average

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

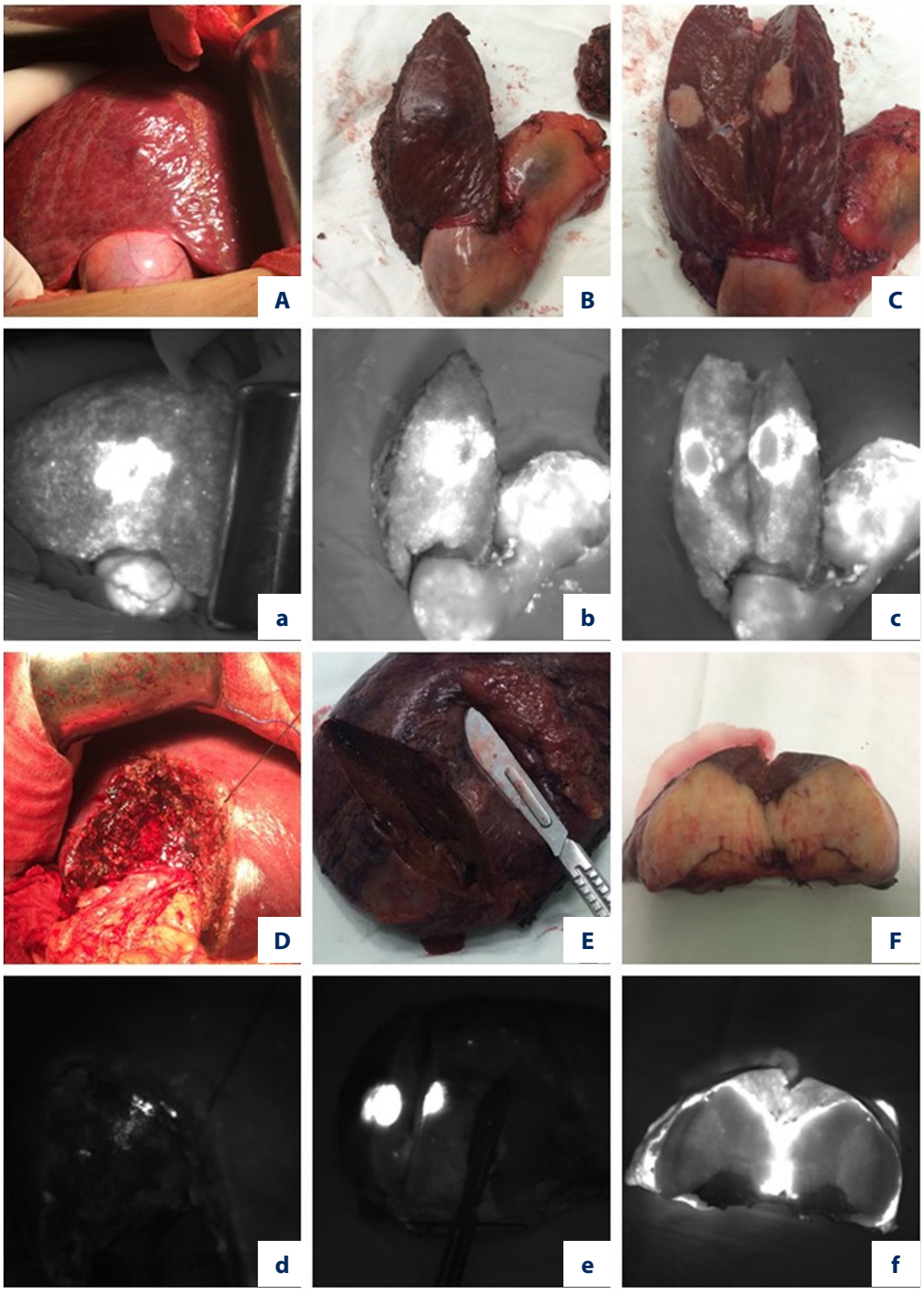

Figure 2. (**A/a, B/b, C/c**) In a male 52-year-old patient, the tumor was located at the junction of S4, S5, and S8 segments of the liver. (**A/a**) Direct vision and fluorescence imaging image of the liver surface. (**B/b, C/c**) Direct vision and fluorescence imaging images of the isolated specimens, in which ICG residues were detected in the gallbladder. (**D/d**) In a female 61-year-old patient, the tumor was located in the right liver. After resection of the tumor by conventional surgery, fluorescence imaging was used to detect the suspected lesion at the resection margin of the liver. (**E/e**) In a male 51-year-old patient, the tumor was located under the liver capsule, the size was approximately 2.0×1.5×0.9 cm, the pathological result was highly differentiated hepatocellular carcinoma, and fluorescence imaging was characterized by tumor tissue imaging. (**F/f**) In a male 72-year-old patient, tumor in S8 segment, size 6.0×5.0×3.5 cm, medium-low differentiated cholangiocarcinoma, fluorescence imaging characteristics of peripheral imaging.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Lengths of cancer tissues from the liver resection margin in the 2 groups of isolated specimens.

	>1.0 cm/cases	<1.0 cm/cases	*P*
FNT group	24 (96.0%)	1 (4.0%)	0.049*
IOUS group	18 (72.0%)	7 (28.0%)	

FNT group – fluorescence navigation technology group; IOUS group – intraoperative ultrasound group. * Fisher's exact test exact significant. (2-sided). *P*<0.05, indicating a significant difference between the 2 groups.

tumor depth of 0.51 cm (range, 0 to 0.7 cm) and an average tumor maximum diameter of 6.7 cm. No fluorescence was detected on the liver surface in 4 patients (4 out of 25 patients, 16.0%), with an average tumor depth of 1.90 cm (range, 1.4 to 3.0 cm) and an average tumor maximum diameter of 3.5 cm. The whole process of tumor resection of the 21 patients with fluorescence on the liver surface were guided by FNT. While the 4 patients with no fluorescence had tumors located by preoperative examination and operator's experience, and the FNT was used to detect the cutting edge in real-time during the whole tumor resection process, and all the fluorescence imaging tissues were removed. Pathological examination results showed that, in all 25 patients in the FNT group, no residual cancer cells were detected at the cutting edge of the isolated specimens after tumor resection, therefore the complete tumor resection rate (R0) was 100% (25 out of 25 patients). In specimens, the shortest distance between the tumor and resection margin was greater than 0.8 cm in all cases, less than 1 cm in 1 case (4%,1 out of 25 patients), and greater than 1 cm in 24 cases (96%, 24 out of 25 patients), which was significantly superior to IOUS (*P*=0.049 <0.05) (Table 2).

The IOUS group was used as the control group, and for these 25 patients, IOUS was mainly to guide tumor resection. Pathological results showed that 1 patient had residual cancer cells on the cutting edge of the *in vitro* specimen, and the R0 was 96% (24 out of 25 patients) and the rate of microscopic residual cancer (R1) was 4% (1 out of 25 patients). The shortest distance from the tumor to the cutting edge was 0.1 cm (range, 0.1 to 3.2 cm). The shortest distance between the tumor and the cutting edge was less than 1 cm in 7 cases (28%, 1 case <0.1 cm) and >1 cm in 18 cases (72%), a finding that was significantly different from that in the fluorescence navigation group. This indicated that fluorescence navigation technology could better ensure the safe distance between tumor and resection margin and could improve the R0 resection rate of the tumor.

Result 2: FNT assisted IOUS to detect the resected margin of the remaining liver

In the IOUS group, 25 patients received IOUS-assisted tumor resection, and then FNT was used to detect the resected margin of the remaining liver. Five suspected nodules were detected by fluorescence detection technology (Figure 2D/d), and all suspected lesions were resected and underwent intraoperative frozen pathological examination. The results showed that there were residual cancer cells on the cutting edge of 1 lesion with a diameter of 0.2 cm, 2 lesion were cirrhotic nodules with an average diameter of 0.27 cm, 1 lesion was an inflammatory nodule with a diameter of 0.3 cm, and 1 lesion was normal liver tissue with a diameter of 0.2 cm.

Result 3: Detection rate of cancer nodules by FNT

After major tumor resection, the remaining livers of all 50 patients were examined separately by fluorescence navigation and intraoperative ultrasound to identify suspected nodules. The results (Table 3) show that the fluorescence imaging system found 21 new suspected nodules. Between those nodules and their liver surface, the shortest length average was 0.35 cm (range, 0 cm to 0.7 cm), and the average diameter was approximately 0.5 cm (range, 0.2 cm to 1.4 cm). The rapid pathological examination results showed that 4 of 21 nodules proved to be cancerous, 1 showed atypia hyperplasia, 6 were cirrhotic nodules, 2 were liver tissue inflammatory nodules, 2 displayed steatosis, and 6 were normal liver tissue. The sensitivity, specificity, and false-positive rate of FNT in detecting new cancerous nodules were 71.4%, 11.1%, and 88.9%, respectively.

IOUS identified 5 new suspected nodules. Between those nodules and their liver surface, the shortest length average was 1.36 cm (range, 0.5 to 2.5 cm), the average diameter was approximately 1.5 cm (range, 0.8 to 2.3 cm). Rapid pathological examination results showed that 3 of those 5 nodules proved to be cancerous and 2 showed cirrhotic nodules. The sensitivity, specificity and false-positive rate of IOUS for detecting new cancerous nodules were 42.9%, 88.9% and 11.1%, respectively. Detecting the residual liver by both FNT and IOUS, 7 new cancerous nodules were identified (Table 3).

Result 4. Fluorescence imaging features of the *in vitro* tumor specimens

The fluorescence imaging results of 50 isolated samples showed that all well-differentiated HCC (100%, 5 out of 5 HCC samples) and some moderately differentiated HCC (12%, 3 out of 25 samples) represented fluorescence imaging of tumor

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. Diagnostic efficacy of the 2 technologies for new cancer nodules.

	Cancerous tissue (+/–)	Non-cancerous tissue (+/–)	SE	SP	FPR	FNR	PV+	PV–
FNT	5/2	16/2	71.4%	11.1%	88.9%	28.6%	23.8%	50%
IOUS	3/4	2/16	42.9%	88.9%	11.1%	57.1%	60.0%	90%

FNT – fluorescence navigation technology; IOUS – intraoperative ultrasound; SE – sensitivity; SP – specificity; FPR – false-positive rate; FNR – false-negative rate; PV+ – positive predictive value; PV– – negative predictive value.

Table 4. Fluorescence imaging features of 50 isolated tumors detected preoperatively.

Degree of differentiation	Fluorescence imaging features		
	Tumor tissue imaging/cases	Ring imaging around tumor/cases	Total number/cases
HCC	8	30	38
Well	5	0	5
Moderate	3	25	28
Poor	0	5	5
ICC	0	11	11
Moderate	0	8	8
Poor	0	3	3
cHCC-CC	0	1	1
Poor	0	1	1

HCC – hepatocellular carcinomas; ICC – intrahepatic cholangiocarcinoma; cHCC-CC – combined hepatocellular carcinoma and cholangiocarcinoma.

tissue imaging (Figure 2E/e). Most moderately differentiated HCC (88%, 22 out of 25 samples), all cholangiocarcinomas (Figure 2F/f) (100%, 11 out of 11 samples), and mixed types (100%, 1 out of 1 sample) represented the circumhepatic tissue annular imaging. Specimens and cutting edges were sent for pathological routine examination, followed by hemostasis and abdominal closure and then conventional surgical procedures (Table 4).

Discussion

HCC is a major health problem that is increasing in both developing and developed countries [1–5]. Long-term survival after surgery depends on several factors: the completeness of resection, the number of tumors, the presence of vascular invasion, and lymph node metastases [14]. Compared with other treatment methods, surgery has shown a better prognosis for primary liver cancer. The ideal goal of hepatectomy is the unification of therapeutic effectiveness, surgical safety and minimally invasive intervention. Intraoperative ultrasound (IOUS) can help surgeons optimize surgical procedures, increase the safety and thoroughness of surgery, and reduce the risk of surgery. Makuchi et al. first used ultrasound in liver surgery in 1979 [15]; since then, IOUS has developed rapidly in hepatobiliary surgery. Shukla et al. conducted IOUS in the surgical treatment of 48 patients with liver tumors, and the results showed that for 14 patients (29.2%) new cancerous nodules were found, 5 patients could not be resected due to multiple liver lobules involved, and in 21 patients (43.7%) surgical plans were changed [17]. Intraoperative ultrasound-guided liver cancer resection can improve the complete resection rate of tumors, identify new cancerous nodules, and reduce the possibility of postoperative liver failure [18]. However, during the process of tumor resection, IOUS cannot continuously locate the tumor in real-time, it cannot assess the cutting edge, and small superficial carcinoma lesions cannot be well detected. Thus, the surgeon can only perform resection by experience. Presently, there is no effective method to determine whether there is residual cancer tissue in liver sections under direct vision. Although rapid intraoperative pathological examination can detect local suspected tissues on the liver resection margin, it cannot carry out comprehensive and detailed examination of the resection margin. In recent years, FNT based on ICG has shown unique advantages in the process of liver tumor resection. However, the advantages of FNT and how to implement it are still being explored. FNT can compensate for the limitations of IOUS due to its advantages including continuous

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

real-time localization of tumors, detection of small superficial tumor lesions, and intraoperative assessment of the cutting edge. Therefore, combined FNT and IOUS can better guide surgical operations and improve the complete resection rate of tumors, thus improving the surgical effect and prognosis of patients with primary liver cancer.

The prognosis of liver cancer patients after tumor resection is closely related to whether the tumor is completely resected. In 1978, American Joint Committee on Cancer (AJCC) first introduced the concept of residual tumor classification (R classification), in 1994, Hermanek et al. [40] elaborated the definition of R classification and its relationship with prognosis. The degree of radical resection of a malignant tumor, namely, the grade of residual tumor, is an indicator to evaluate the status of residual tumor after malignant tumor resection, which is represented by complete tumor resection (R0), microscopic residual (R1) and gross residual tumor (R2). Therefore, the R0 rate of surgery has become one of the criteria to judge the effect of surgery. According to the Result 1 section, the fluorescence imaging system can visualize the tumor and present a clear fluorescence image in the tumor projection area on the liver surface. The surgeon can determine the scope of tumor resection according to the imaging area and can accurately locate and remove the tumor in real time during the process of tumor resection. In hepatectomy, a safe resection line 1 to 2 cm away from the tumor boundary on the surface of the liver is easy to achieve. However, when deep liver tissue is removed, the safe distance between the tumor and cutting edge cannot be well determined under direct vision, which sometimes leads to an insufficient safe distance between the deep tumor and cutting edge of the liver. The fluorescence transmission of ICG to the liver tissue is less than 1.0 cm. Thus, in the process of tumor resection, when the fluorescence can be clearly detected at the cutting edge, the tumor is not sufficiently long for liver section (<1.0 cm), and we need to adjust the direction of liver section resection to leave an adequate safe distance. Therefore, FNT in liver resection is significantly superior to IOUS in ensuring a safe distance between the tumor and cutting edge(P=0.049 <0.05), which is expected to reduce the rate of postoperative tumor recurrence *in situ*. FNT provides a rapid and comprehensive judgement of the liver resection margin *in vivo*, compensating for the disadvantages of being time consuming and incomplete sampling of the intraoperative pathological examination.

Many tumors are not visually observable on the surface of the liver. Although IOUS can help surgeons identify tumors, it is difficult to find small superficial lesions. FNT can clearly fluoresce the tumor and transmit to the corresponding area on the surface of the liver, which is conducive to the clear location of the tumor (Figure 2A). However, because ICG fluorescence can only penetrate the liver tissue less than 1 cm, the technique can only detect superficial tumors on the liver surface, thus missing the deeper tumors (Result 1 section). Since IOUS showed its unique advantages in deep tumor localization, the combination of the 2 techniques can better locate the tumor before the liver tumor resection and make the surgery more accurate. Although deeper tumors cannot be detected on the surface of the liver, FNT can still be used for continuous navigation during tumor resection. If the excised section showed fluorescence, it indicated that the scope of resection was not enough, and the direction of resection needed to be adjusted to ensure a safe distance between the tumor and the section, and finally reached 100% R0 resection rate (96% with IOUS) (Result 1 section). Because the IOUS could not detect the small residual cancer tissue at the cutting edge, after the tumor resection guided by IOUS, FNT was used to supplement the detection of the cutting edge, which could better detect the small residual cancer tissue, thus ensuring that the cutting edge had no residual cancer tissue (Result 2 section). FNT and IOUS can compensate for each other's disadvantages; thus, the combined use of these 2 technologies can better guide tumor resection and improve the complete tumor resection rate. Therefore, in the process of hepatectomy for primary liver cancer, both using FNT alone and using them in combination can achieve the goal of tumor R0 resection rate.

As shown in the Result 3 section, FNT identified more undetected cancerous nodules than IOUS in the detection of residual suspected liver lesions, with a higher sensitivity (FNT: 71.4% versus IOUS: 42.9%). More patients (FNT: 10% versus IOUS: 6%) changed surgical procedures. However, a higher false-positive rate (FNT: 88.9% versus. IOUS: 11.1%) means that more benign tissue is sacrificed to detect and remove all cancerous lesions. FNT can be used to guide the detection and resection of suspected lesions in patients with sufficient residual liver to achieve the goal of resection of all cancerous lesions. However, in patients with poor liver function (especially in patients with cirrhosis), if suspected fluorescence nodules are found in the remaining liver, the resectability of suspected lesions needs to be evaluated in detail. Fluorescence can be detected by ICG on liver nodules (including HCC, ICC, metastatic carcinoma, focal nodular hyperplasia (FNH), hemangioma, cirrhotic nodules, inflammatory nodules, steatosis and some normal liver tissues), which will interfere with the intraoperative judgement of the nature of suspected lesions and lead to an increased false-positive rate. The reason for the fluorescence of benign nodules derived from the liver parenchyma may be the dysfunction in the excretion of bile by the hepatocytes contained therein and destruction of the microbile duct structure in the liver tissue, which leads to the obstruction of bile excretion and retention of ICG that is excreted through the bile duct [20,21]. The fluorescence of nodules not derived from liver parenchyma might be due to occlusion of microbile ducts in peripheral liver tissues compressed by the

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

tumor, leading to ICG deposition [41]. Further research and technical improvements are needed to reduce the incidence of these false positives. As the Results 3 section shows, the new cancerous nodules detected by FNT were all superficial tumors located within 0.7 cm from the liver capsule, while those detected by IOUS were all deep tumors located above 0.7 cm. This is related to the limitation that ICG fluorescence can only penetrate thin human tissues [20–23]. The 2 techniques complement each other and improve the detection rate of cancerous nodules. Therefore, we recommend that the combined use of FNT and IOUS detection of residual liver *in vivo* can achieve a more comprehensive and complete detection rate of new cancerous nodules.

All the *in vitro* specimens were sectioned, and fluorescence imaging was performed. The results showed that all well-differentiated HCC and some moderately differentiated HCC represented tumor tissue imaging with fluorescence imaging characteristics, while all other nodules represented peripheral liver parenchyma imaging (Table 4). Some scholars have reported the molecular mechanism of HCC fluorescence imaging [42]: well-differentiated HCC cells still have the uptake function of ICG, but the functional and/or morphological changes caused by bile excretion disorders lead to the deposition of ICG in tumor tissues. However, the ICG deposition mechanism of liver parenchyma around the cancer remains unclear likely because the ductules in the liver tissue surrounding the tumor blockage result in ICG deposits, but Van Der Vorst et al. [43] recently reported increased immature liver cells and organic anion channel protein expression can also lead to damaged ICG in liver tissue surrounding the tumor deposits. This conclusion was supported by the results of microfluorescence imaging of the liver tissue around the tumor by Miyata et al. [44]. ICG fluorescence imaging was first detected in the cytoplasm of smaller hepatocytes rather than in the intercellular space around the tumor. Since the tumor was not dissected during surgery, the difference in fluorescence imaging characteristics of the tumor had little influence on the localization of the tumor. However, the imaging of liver tissue around the tumor can ensure a safe distance for tumor resection.

In summary, FNT can be used to visualize primary liver cancer tumors, guide tumor resection, ensure a safe distance between the tumor and cutting edge and detect the residual liver resection edges after conventional methods such as IOUS-assisted tumor resection, which can achieve a high R0 tumor resection rate. The combination of FNT and IOUS to detect the remaining suspected liver lesions can achieve the goal of finding more cancerous lesions. The standardized application of FNT can improve the surgical effect for patients with primary liver cancer and is expected to improve the prognosis. In addition to x-ray, computed tomography, magnetic resonance imaging, and positron emission tomography, it will also provide important aid to FNT in advancing the detection of deep tumors [45–47] and the possibility of using an FNT-guided multimodality method to precisely excise tumors has already been demonstrated [48].

Conclusions

FNT can improve the R0 resection rate of tumors and identify more new cancerous nodules, thus, improving the surgical treatment effect for primary liver cancer and hopefully further improve the prognosis of patients. With the development of computer technology and imaging technology, FNT will become an important auxiliary technology in cancer surgery.

References:

1. Morris-Stiff G, Gomez D, de Liguori Carino N, Prasad KR: Surgical management of hepatocellular carcinoma: is the jury still out? Surg Oncol, 2009; 18(4): 298–321
2. Yuichi N, Takeaki Ishizawa, Akio S: Fluorescence-guided surgery for liver tumors. J Surg Oncol, 2018; 118: 324–31
3. Altekruse SF, McGlynn KA, Reichman ME: Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol, 2009; 27: 1485–91
4. Bosch FX, Ribes J, Diaz M, Cleries R: Primary liver cancer: Worldwide incidence and trends. Gastroenterology, 2004; 127(Suppl. 1): S5–S16
5. Giannini EG, Farinati F, Ciccarese F et al: Prognosis of untreated hepatocellular carcinoma. Hepatology, 2015; 61: 184–90
6. Llovet JM, Burroughs A, Bruix J: Hepatocellular carcinoma. Lancet, 2003; 362: 1907–17
7. Balsells J, Charco R, Lazaro JL et al: Resection of hepatocellular carcinoma in patients with cirrhosis. Br J Surg, 1996; 83: 758–61
8. Shuto T, Kinoshita H, Hirohashi K et al: Indications for, and effectiveness of, a second hepatic resection for recurrent hepatocellular carcinoma. Hepatogastroenterology, 1996; 43: 932–37
9. Takenaka K, Kawahara N, Yamamoto K et al: Results of 280 liver resections for hepatocellular carcinoma. Arch Surg, 1996; 131: 71–76
10. Sugimachi K, Maehara S, Tanaka S et al: Repeat hepatectomy is the most useful treatment for recurrent hepatocellular carcinoma. J Hepatobiliary Pancreat Surg, 2001; 8: 410–16
11. Matsuda M, Fujii H, Kono H, Matsumoto Y: Surgical treatment of recurrent hepatocellular carcinoma based on the mode of recurrence: Repeat hepatic resection or ablation are good choices for patients with recurrent multicentric cancer. J Hepatobiliary Pancreat Surg, 2001; 8: 353–59
12. Suenaga M, Sugiura H, Kokuba Y et al: Repeated hepatic resection for recurrent hepatocellular carcinoma in 18 cases. Surgery, 1994; 115: 452–57
13. Neeleman N, Andersson R: Repeat liver resection for recurrent liver cancer. Br J Surg, 1996; 83: 893–901
14. Weber SM, Ribero D, O'Reilly EM: Intrahepatic cholangiocarcinoma: Expert consensus statement. HPB, 2015; 1: 669–80
15. Makuuchi M, Hasegawa HC, Yamazaki S: Newly devised intraoperative probe. Image Technol Info Display Med, 1979; 11(3): 1167–69
16. Sietses C, Meijerink MR, Meijer S et al: The impact of intra-operative ultrasonography on the surgical treatment of patients with colorectal liver metastases. Surg Endosc, 2010; 24(8): 1917–22

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

17. Shukla PJ, Pandey D, Rao PP et al: Impact of intraoperative ultrasonography in liver surgery. Indian J Gastroenterol, 2005; 24(2): 62–65
18. Ruzzenente A, Valdegamberi A, Campagnaro T et al: Hepatocellular carcinoma in cirrhotic patients with portal hypertension: Is liver resection always contraindicated? World J Gastroenterol, 2011; 17(46): 5083–88
19. Ishizawa T, Fukushima N, Shibahara J et al: Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer, 2009; 115: 2491–504
20. Ishizawa T, Fuknshima N, Shibahara J et al: Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer, 2009; 115(11): 2491–504
21. Gotoh K, Yamada T, Ishikawa O et al: A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. J Surg Oncol, 2009; 100: 75–79
22. Ishizawa T, Masuda K, Urano Y et al: Mechanistic background and clinical applications of indocyanine green fluorescence imaging of hepatocellular carcinoma. Ann Surg Oncol, 2014; 21: 440–48
23. Kudo H, Ishizawa T, Tani K et al: Visualization of subcapsular hepatic malignancy by indocyanine-green fluorescence imaging during laparoscopic hepatectomy. Surg Endosc, 2014; 28: 2504–8
24. Terasawa M, Ishizawa T, Mise Y et al: Applications of fusion fluorescence imaging using indocyanine green in laparoscopic hepatectomy. Surg Endosc, 2017; 31: 5111–18
25. Uchiyama K, Ueno M, Ozawa S et al: Combined use of contrast-enhanced intraoperative ultrasonography and a fluorescence navigation system for identifying hepatic metastases. World J Surg, 2010; 34: 2953–59
26. Yokoyama N, Otani T, Hashidate H et al: Real-time detection of hepatic micrometastases from pancreatic cancer by intraoperative fluorescence imaging: Preliminary results of a prospective study. Cancer, 2012; 118: 2813–19
27. Ishizuka M, Kubota K, Kita J et al: Intraoperative observation using a fluorescence imaging instrument during hepatic resection for liver metastasis from colorectal cancer. Hepatogastroenterology, 2012; 59: 90–92
28. Morita Y, Sakaguchi T, Unno N et al: Detection of hepatocellular carcinomas with near-infrared fluorescence imaging using indocyanine green: Its usefulness and limitation. Int J Clin Oncol, 2013; 18: 232–41
29. Peloso A, Franchi E, Canepa MC et al: Combined use of intraoperative ultrasound and indocyanine green fluorescence imaging to detect liver metastases from colorectal cancer. HPB (Oxford), 2013; 15: 928–34
30. Tummers QRJG, Verbeek FPR, Prevoo HAJM et al: First experience on laparoscopic near-infrared fluorescence imaging of hepatic uveal melanoma metastases using indocyanine green. Surg Innov, 2015; 22: 20–25
31. Shimada S, Ohtsubo S, Ogasawara K, Kusano M: Macro- and microscopic findings of ICG fluorescence in liver tumors. World J Surg Oncol, 2015; 13: 198
32. Abo T, Nanashima A, Tobinaga S et al: Usefulness of intraoperative diagnosis of hepatic tumors located at the liver surface and hepatic segmental visualization using indocyanine green-photodynamic eye imaging. Eur J Surg Oncol, 2015; 41: 257–64
33. Kawaguchi Y, Nagai M, Nomura Y et al: Usefulness of indocyanine green-fluorescence imaging during laparoscopic hepatectomy to visualize subcapsular hard-to-identify hepatic malignancy. J Surg Oncol, 2015; 112: 514–16
34. Kaibori M, Matsui K, Ishizaki M et al: Intraoperative detection of superficial liver tumors by fluorescence imaging using indocyanine green and 5-aminolevulinic acid. Anticancer Res, 2016; 36: 1841–49
35. Takahashi H, Zaidi N, Berber E: An initial report on the intraoperative use of indocyanine green fluorescence imaging in the surgical management of liver tumors. J Surg Oncol, 2016; 114: 625–29
36. Masuda K, Kaneko J, Kawaguchi Y et al: Diagnostic accuracy of indocyanine green fluorescence imaging and multidetector row computed tomography for identifying hepatocellular carcinoma with liver explant correlation. Hepatol Res, 2017; 47: 1299–307
37. Handgraaf HJM, Boogerd LSF, Happened DJ et al: Long-term follow-up after near-infrared fluorescence-guided resection of colorectal liver metastases: A retrospective multicenter analysis. Eur J Surg Oncol, 2017; 43: 1463–71
38. Boogied LUFF, Handcraft HAM, Lam HD et al: Laparoscopic detection and resection of occult liver tumors of multiple cancer types using real-time near-infrared fluorescence guidance. Surg Ends', 2017; 31: 952–61
39. Zhang YOM, Shi R, Hou JC et al: Liver tumor boundaries identified intraoperatively using real-time indocyanine green fluorescence imaging. J Cancer Res Clin Oncol, 2017; 143: 51–58
40. Song TJ, Ip EW, Fong Y: Hepatocellular carcinoma: Current surgical management. Gastroenterology, 2004; 127: S248–60
41. de Graaf W, Hausler S, Heger M et al: Transporters involved in the hepatic uptake of (99m)Tc-mebrofenin and indocyanine green. J Hepatology, 2011; 54(4): 738–45
42. Ishizawa T, Masuda K, Urano Y et al: Mechanistic background and clinical applications of indocyanine green fluorescence imaging of hepatocellular carcinoma. Ann Surg Oncol, 2014; 21: 440–48
43. Van der Vorst JR, Schaafsma BE, Hutteman M et al: Near-infrared fluorescence-guided resection of colorectal liver metastases. Cancer, 2013; 119(18): 3411–18
44. Akinori M, Takeaki I, Mako K et al: Photoacoustic tomography of human hepatic malignancies using intraoperative indocyanine green fluorescence imaging. PLoS One, 2014; 9(11): e112667
45. Peloso A, Franchi E, Canepa MC et al: Combined use of intraoperative ultrasound and indocyanine green fluorescence imaging to detect liver metastases from colorectal cancer. HPB (Oxford), 2013; 15: 928–34
46. Luo T, Huang P, Gao G et al: Mesoporous silica-coated gold nanorods with embedded indocyanine green for dual mode X-ray CT and NIR fluorescence imaging. Opt Express, 2011; 19: 17030–39
47. Yuasa Y, Seike J, Yoshida T et al: Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative CT lymphography for superficial esophageal cancer. Ann Surg Oncol, 2012; 19: 486–93
48. Chi CW, Du Y, Ye JZ: Intraoperative imaging-guided cancer surgery: From current fluorescence molecular imaging methods to future multi-modality imaging technology. Theranostics, 2014; 4(11): 1072–84

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]