Is a fluorescence navigation system with indocyanine green effective enough to detect liver malignancies?

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Abstract

[Background]   Although several reports have shown the efficacy of a fluorescence navigation system (FNS) with indocyanine green (ICG) to detect liver malignancies during hepatectomy, the real accuracy of this procedure is not yet clear. This study aimed to analyze the actual efficacy of ICG-FNS in cirrhotic and non-cirrhotic livers.

 [Methods]   Ten cirrhotic whole livers explanted from liver transplant recipients and twenty-three non-cirrhotic livers from patients who underwent hepatectomy for various kinds of liver tumors were investigated with ICG-FNS. All surgical specimens were analyzed macroscopically and pathologically.

[Results] In the patients with a cirrhotic liver, most nodules illuminated by ICG-FNS were diagnosed as regenerative nodules pathologically. The positive predictive value was 5.4%. There was a significant difference in positive predictive value to detect malignant liver tumors between cirrhotic liver and non-cirrhotic liver (5.4% vs 100%, p<0.0001). In the non-cirrhotic livers, eleven of thirty-three (32.4%) tumors were not recognized by ICG-FNS through the liver surface before resection. There was a significant difference in the depth from the liver surface to tumor between illuminated nodules and non-illuminated nodules (1.5 mm vs 11.6 mm, p<0.01).

[Conclusions] It is necessary to know the limitation of ICG-FNS when detecting liver

malignancies in both cirrhotic and non-cirrhotic livers.

Introduction

In liver surgery, in order to achieve absolute removal of tumors, it is essential to recognize even small tumors and to ensure the accurate surgical margin intraoperatively. Intraoperative ultrasonography has been the gold standard to detect liver tumors since it was introduced by Makuuchi et al.[1]. Recently, several reports have shown the efficacy of intraoperative navigation surgery using a fluorescence navigation system with indocyanine green (ICG-FNS) [2,3].

ICG-FNS has generally been used to detect the sentinel lymph nodes in the breast[4,5], gastric[6,7,8], lung[9,10], and esophageal cancers[11] and to intraoperatively assess graft patency in vascular surgery[12,13]. The adaption of ICG-FNS was expanded for hepatobiliary surgery[3,14,15]. Ishizawa et al. [2] reported that fluorescence intraoperative cholangiography with ICG is a safe and valuable procedure for a road map of biliary tract anatomy and identification of liver tumor through the visualization of the disordered biliary excretion of ICG in real time. However, the actual efficacy of ICG-FNS to detect liver tumors is not clear. For example, false positive detection might occur because regenerative nodules develop as liver damage progresses[16]. Also, small tumors located deep in the liver parenchyma might not be detected by this procedure.

Therefore, the aim of this study was to evaluate the actual efficacy of ICG-FNS in

cirrhotic or non-cirrhotic liver separately.

Materials and Methods

Patients

Ten cirrhotic whole livers were obtained from living donor liver transplant (LDLT) recipients. The original diseases included hepatitis C virus (HCV)-cirrhosis in 8, hepatitis B virus (HBV)-cirrhosis in 1, and alcoholic cirrhosis in 1. Patient characteristics are listed in Table 1. In addition, we evaluated twenty-three non-cirrhotic liver specimens obtained from patients who underwent hepatectomy for various kind of liver tumors [hepatocellular carcinoma (HCC) in 12, metastasis of colorectal cancer in 9, cholangiocarcinoma in 1, and hepatic carcinoid in 1] (Table 1).

Examination methods

As a fluorescence source, we used ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan), which had been intravenously injected before surgery at a dose of 0.5mg per of body weight as part of a routine liver function test. The intervals between the ICG injection and surgery ranged from 2 days. As a fluorescent imaging tool, we used Photo Dynamic Eye-II (PDE-II, Hamamatsu Photonics, Hamamatsu, Japan), which filtered out light with a wavelength below 820 nm, and 36 light-emitting diodes with a wavelength of 760 nm. The camera imaging head was positioned between 20 and 30 cm above the

surgical specimen. The marking suture was performed near the site of the illuminated

nodules during ten minutes while putting the emission of light situation in the video.

Any illuminated nodules clearly visualized from the liver surface regardless of signal

intensity, size and illuminated pattern were marked. The recognition of the illuminated

nodules was clarified by two independent surgeons (T.T. and I. M.). Surgical specimens

were observed from every angle, and cut to include each tumor's maximum diameter

based on gross inspection. After the operation, the number of the illuminated lesions in

the video and the number of the marking sites were counted and macroscopically

analyzed. In addition, the number of illuminated lesions which were detected through

the liver surface and the number of identifiable tumors by pathological examination

were analyzed.

Statistical analysis

Results for continuous variables are expressed as the median (range). The

positive predictive value was calculated as the number of pathological tumors in

illuminated nodules. Data for continuous variables were compared using the

Mann-Whitney U test. We set statistical significance at $p < .05$.

Results

The efficacy of ICG-FNS to detect HCCs in a cirrhotic liver

The median number of preoperatively detected tumors with various

modalities in the 10 patients with cirrhotic liver was 2 (0-4). However, as shown in

Figure 1, the median number of illuminated nodules was 20 (7-37), and the median

number of nodules to be macroscopically considered as tumors was 16 (3-25). The

number of pathologically diagnosed as tumors was 2 (0-8), so the positive predictive

value was only 5.4% (0-24.2%). The number of false negatives was 14(4-17). There

were significant differences between the numbers of preoperatively detected tumors and

the number of both illuminated nodules and those macroscopically recognized as tumors,

as shown in Figure 1 (p<0.001). Moreover, there were also significant differences

between the numbers of nodules pathologically diagnosed as tumors and the number of

both illuminated nodules and nodules macroscopically recognized as tumors (p<0.001).

HCC on the liver surface with ICG-FNS revealed fluorescence in Fig 2A. The area that

was actually illuminated was also macroscopically observed as HCC in Fig 2B. The

same part was pathologically diagnosed as HCC in Fig 2C. However, the other areas

that were illuminated and observed as nodules were diagnosed as regenerative nodules

(Fig 2D-F).

The efficacy of ICG-FNS to detect malignant tumors in a non-cirrhotic liver

The median number of preoperatively detected tumors with various modalities

in twenty-three patients with non-cirrhotic liver was 1 (1-4). The median number of

illuminated nodules and nodules macroscopically recognized as tumors was 1 (0-3) and

1 (1-4), respectively. Finally, the median number of nodules pathologically diagnosed as

liver tumors was 1 (1-4), so that the positive predictive value to detect malignant tumors

was 100%. There were no significant differences between the groups (Supplemental

Figure 1). There was a significant difference in positive predictive value to detect HCC

or malignant tumors between the cirrhotic livers and the non-cirrhotic livers (5.4% vs

100%; p<0.0001). Although some tumors could be detected from the liver surface (Fig

3A, B) and positive predictive value to detect malignant tumors in the non-cirrhotic

livers was 100%, other tumors could not be detected from the liver surface (11/34,

32.4%). These nodules were finally recognized after the liver was cut, including tumors

(Fig3C, D) and defined as non-illuminated nodules. There was a significant difference

in the median depth from the liver surface between illuminated nodules (n=23) and

non-illuminated nodules (n=11) (1.5mm vs 11.6mm, p<0.01) (Fig 4A). Although there

was no significant difference, non-illuminated nodules tended to be smaller than

illuminated nodules (illuminated nodules, 32.4mm vs non-illuminated nodules,

20.7mm; p=0.058) (Fig 4B). Furthermore, the tumor size of almost all non-illuminated

nodules located within 10mm depth tended to be smaller than 10mm (Figure 4C).

Discussion

ICG is usually used to directly measure the actual functional state of the liver [17]. ICG is a water-soluble, inert compound that is injected intravenously. It mainly binds to plasma proteins, is taken up by hepatocytes, and is excreted unchanged into the bile. Moreover, ICG is known to absorb infrared rays[18]. Lights, and specially near-infrared light (NIR) in the biological window (700-900nm), can be exploited for intraoperative imaging guidance. Tanaka et al. demonstrated that (1) the appearance of the surgical field is not altered, (2) it is safe, (3) wavelengths in the 800nm range penetrate relatively deeply into living tissue, and (4) there has been a tremendous recent effort in developing general-purpose NIR fluorophores that can be conjugated to targeting or other molecules, thus creating "contrast agents" matched to any desired surgical application[19]. Therefore, ICG injection combined with FNS is widely used to detect sentinel lymph nodes [4)-11], because this technique is convenient and safe for assessing lymph node status in the oncological field. Recently, adaptation of ICG-FNS was expanded further to hepatobiliary surgery and there have been some reports of its capability in detecting tumors [2)3]. Furthermore, several reports also showed that ICG-FNS was effective to check the bile leakage and the surgical margin [2)3)14)15].

Our team sectioned whole livers with HCC removed from recipients who

underwent living donor liver transplantation and fully investigated them to detect small

HCCs[20]. Generally, hepatocytes have the ability to reproduce by themselves even if the

hepatocytes are exposed to disorders. However, when hepatocytes are exposed to

inflammation for a long time and repeatedly, hyperplasia of the collagen fiber deposits

occurs strongly and many benign nodules, such as regenerative nodules, in a cirrhotic

liver appear surrounded by collagen fiber in the extra-cellular matrix. In addition, the

structure of the hepatic lobule is disturbed and the function of hepatocytes is affected [21].

On the other hand, as described above, ICG binds to plasma proteins, one of which is

ligandin. Ligandin is the binding protein of ICG and is uniformly distributed over

hepatocytes in a normal liver. The expression of ligandin deviates with liver damage,

and it is not expressed in areas of necrosis, fibrosis, or severe inflammation, so that the

expression of ligandin becomes relatively rich in the regenerative area[22]. Finally, the

coloration of ICG is thought to accumulate in the regenerative nodules [16].In fact, in our

series of cirrhotic livers, many false positive nodules were illuminated, possibly because

of the severe liver function disorder and the biliary excretion disorders[2)16)21)22)].

However, Ishizawa et al. demonstrated that the signal intensity of the noncancerous

liver parenchyma was higher in patients with an unfavorable ICG retention rate and in

patients who had received the ICG injection within 24 hours before surgery [2]. Although

the interval longer than 2 days might be better to obtain a good lesion-to-liver contrast,

especially in patients with advanced cirrhosis, we routinely perform the ICG test when

the patients hospitalized generally 2 days before the operation. Also, the optimal

interval between ICG injection and surgery to detect tumors remains controversial.

Secondly, we attempted to evaluate liver resection cases with non-cirrhotic livers.

The aim of this study was to determine whether fluorescence navigation system was

effective or not to detect tumors. Therefore, we did not evaluate the illuminated pattern

of tumors. However, we got the same results as Ishizawa's report concerning with

illuminated pattern of liver tumors (data not shown)[2].It was clear that although false

positive nodules were not observed in the non-cirrhotic livers, smaller tumors and

tumors that were located deeper than 10mm from liver surface to tumor were difficult to

recognize as illuminated nodules. Kim et al have already described that near-infrared

light penetrates human tissues to a depth of about 5-10 mm and Ishizawa et al. have also

described that cancer detectability using fluorescent imaging technique seems mainly to

depend on the depth of the tumors from the liver surface because of the limited tissue

penetration of near-infrared light [2,23]. Therefore, in our study, ICG-FNS wasn't able to

detect tumors located deeper than 10mm. Possibly, 10mm is the maximum limit of

depth for ICG-FNS to detect tumors. Based on these results, we suggested that the

capability of detecting tumors with ICG-FNS alone was not sufficient in both cirrhotic

and non-cirrhotic livers.

We conclude that although ICG-FNS have the capability of detecting tumors

and checking the bile leakage and surgical margin, it is necessary to know the limitation

of ICG-FNS when searching liver malignancies in cirrhotic livers or small and deep

liver malignancies in non-cirrhotic livers. Additional modalities including ultrasound

should be adapted for use with this procedure to detect small liver tumors.

Author Contribution

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Figure Legends

Figure 1: The number of detected nodules in a cirrhotic liver

The median number of preoperative, illuminated, macroscopic, pathological tumors

with cirrhotic liver was, in order, 2 (0-4), 20 (7-37), 16 (3-25), and 2 (0-8). There were

significant differences between preoperative tumors and both illuminated nodules and

macroscopic tumor (p<0.001). Moreover, there were also significant differences

between pathological tumor and both illuminated nodules and macroscopic tumor

(p<0.001).

Figure 2: ICG-FNS for patients with cirrhotic liver and pathological results

A, HCCs on the liver surface with ICG-FNS revealed the illuminated nodules. B, The

area that actually emitted light was also macroscopically observed as liver tumor. C,

The same area was pathologically diagnosed as HCC. D, The other areas on the liver

surface in the same case also revealed the illuminated nodules. E, The areas that emitted

light were macroscopically observed as nodules. F, Finally, these areas were diagnosed

as regenerative nodules.

Figure 3: ICG-FNS for patients with liver resection with non-cirrhotic liver.

Liver tumors in illuminated nodules could be observed as illuminated nodules on the

liver surface (Figure 3A-B); liver tumors in non-illuminated nodules could not be

observed as illuminated lesions on the surface, and finally recognized after the liver was cut including the tumor under US guidance (Fig3C-D).

Figure 4: Comparison between illuminated nodules and non-illuminated nodules on the liver surface in a non-cirrhotic liver

A: There was a significant difference in median depth from the liver surface between illuminated nodules and non-illuminated nodules (1.5mm vs 11.6mm, p<0.01). B: Non-illuminated nodules tended to be smaller than illuminated nodules (illuminated nodules, 32.4mm vs non-illuminated nodules, 20.7mm; p=0.058). C: Correlation diagram of the depth and tumor size presented. The tumor size of almost all non-illuminated nodules located within 10mm depth tended to be smaller than 10mm.

Supplemental Figure 1: Number of detected nodules in a non-cirrhotic liver

The median number of preoperative, illuminated, macroscopic, and pathological tumors with non-cirrhotic liver was, in order, 1 (1-4), 1 (0-3), 1 (1-4), and 1 (1-4). There were no significant differences in each group.

Table 1: The charactaristics of patients with a cirrhotic liver and a non-cirrhotic liver

	cirrhotic liver for LDLT (n=10)	non-cirrhotic liver for liver resection (n=23)
Gender (Male: Female)	6:4	16:7
Age*	61(38-72)	67.5(60-90)
liver disease	HCV:8,　HBV:1,alchohol:1	HCV:5, HBV:5, NBNC:4, NL:9
liver tumor	HCC:9	HCC:12,　metastases：9, CCC：1, Carcinoid：1
Total-bilirubin(mg/dl)*	3.2(0.5-8.8)	0.8(0.3-1.4)
AST(IU/l)*	52(30-89)	28(13-285)
ALT(IU/l)*	29(15-75)	27(10-100)
Albumin(g/dl)*	2.6(2.1-3.8)	4.2(2.9-5.2)
Platetlet(x10^4/mm^3)*	6.4(2.3-13.4)	16.4(10.8-27.6)
PT(INR)*	1.47(1.07-1.95)	1.00(0.87-1.13)
ICGR15(%)*	44(24-70)	11(2-15)
Child-Pugh score*	9(5-12)	5(5-7)
MELD score*	14(7-22)	-

HCV: Hepatitis C virus, HBV: Hepatitis B virus, NBNC:non-HBV non HCV,

NL: normal liver, HCC: Hepatocellular carcinoma, CCC: cholangiocarcinoma,

AST: aspartate aminotransferase, ALT: alanine aminotransferase,

PT: prothorombin time, INR: international normalized ratio,

ICGR15: indocyanine green retention rate at 15 minutes,

MELD score: model for endstage liver disease score

*median　(range)

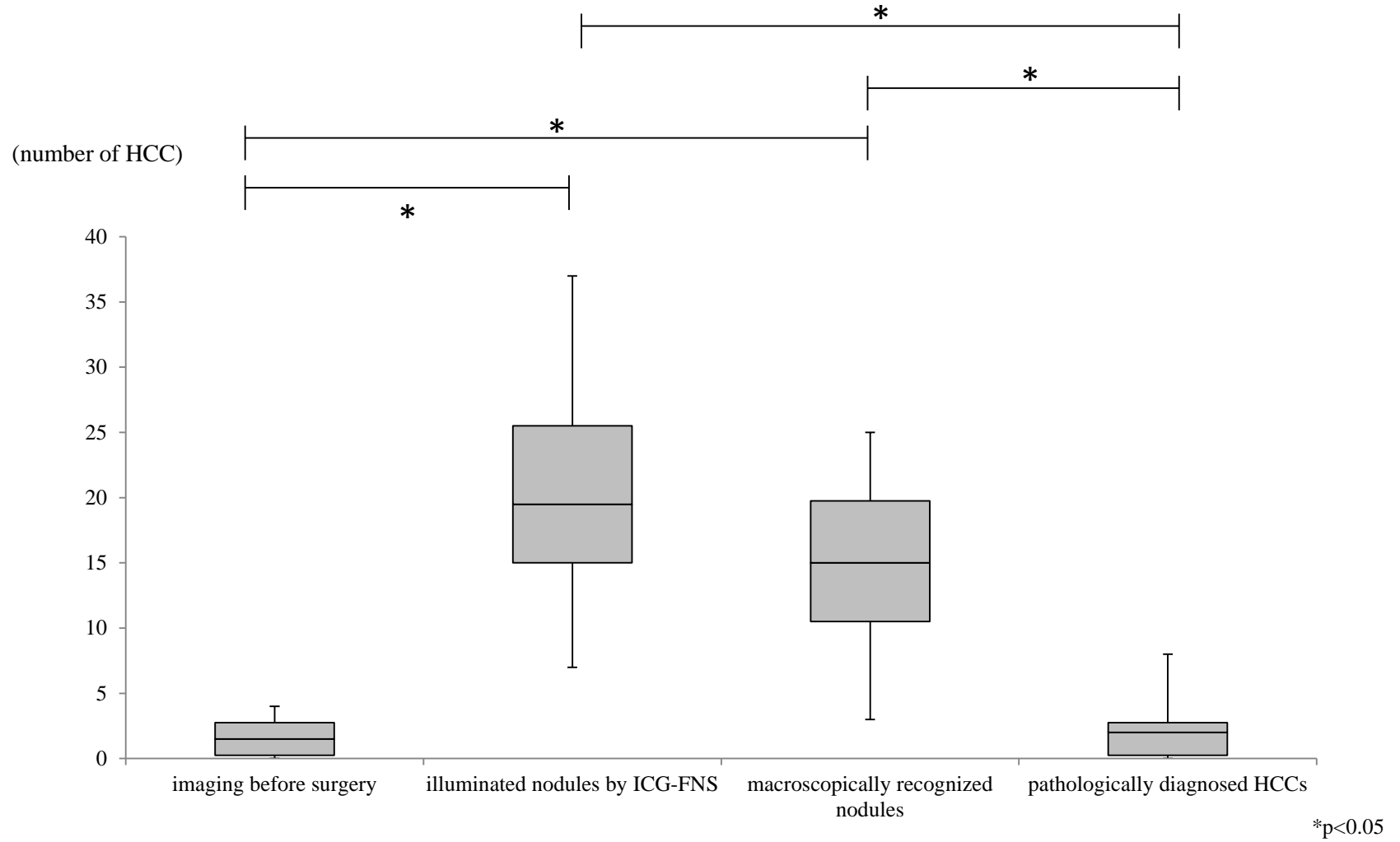Figure 1: The number of detected nodules in a cirrhotic liver.

(number of HCC)

40
35
30
25
20
15
10
5
0

imaging before surgery | illuminated nodules by ICG-FNS | macroscopically recognized nodules | pathologically diagnosed HCCs

*p<0.05

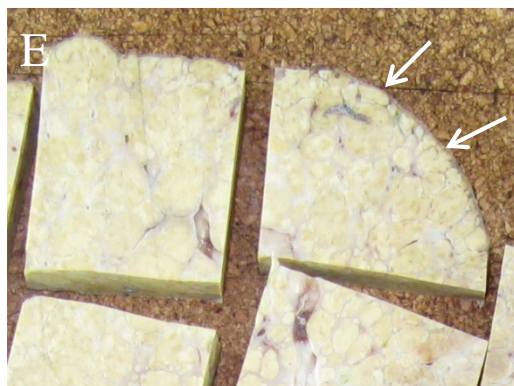Figure 2: ICG-FNS for patients with cirrhotic liver and pathological results.
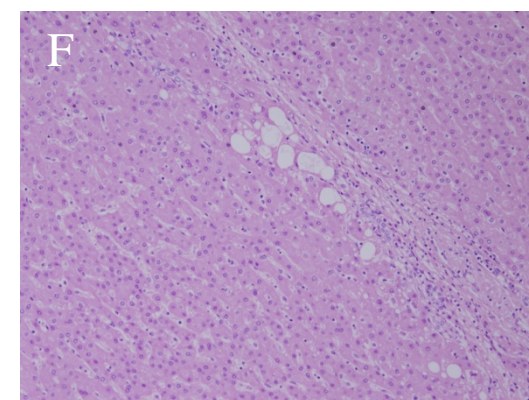
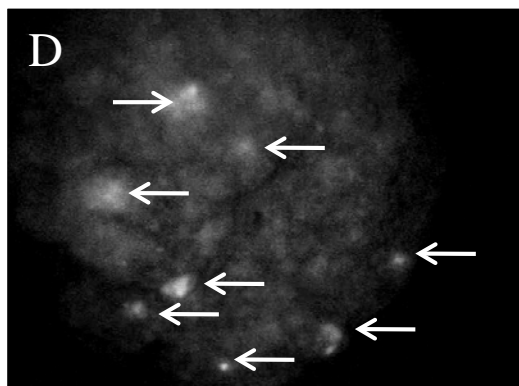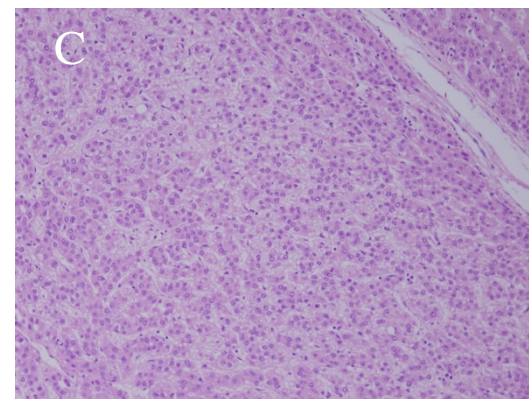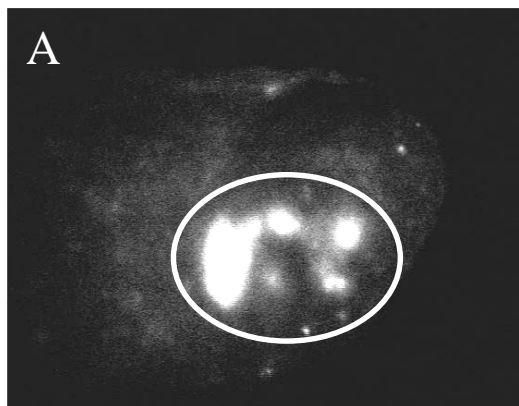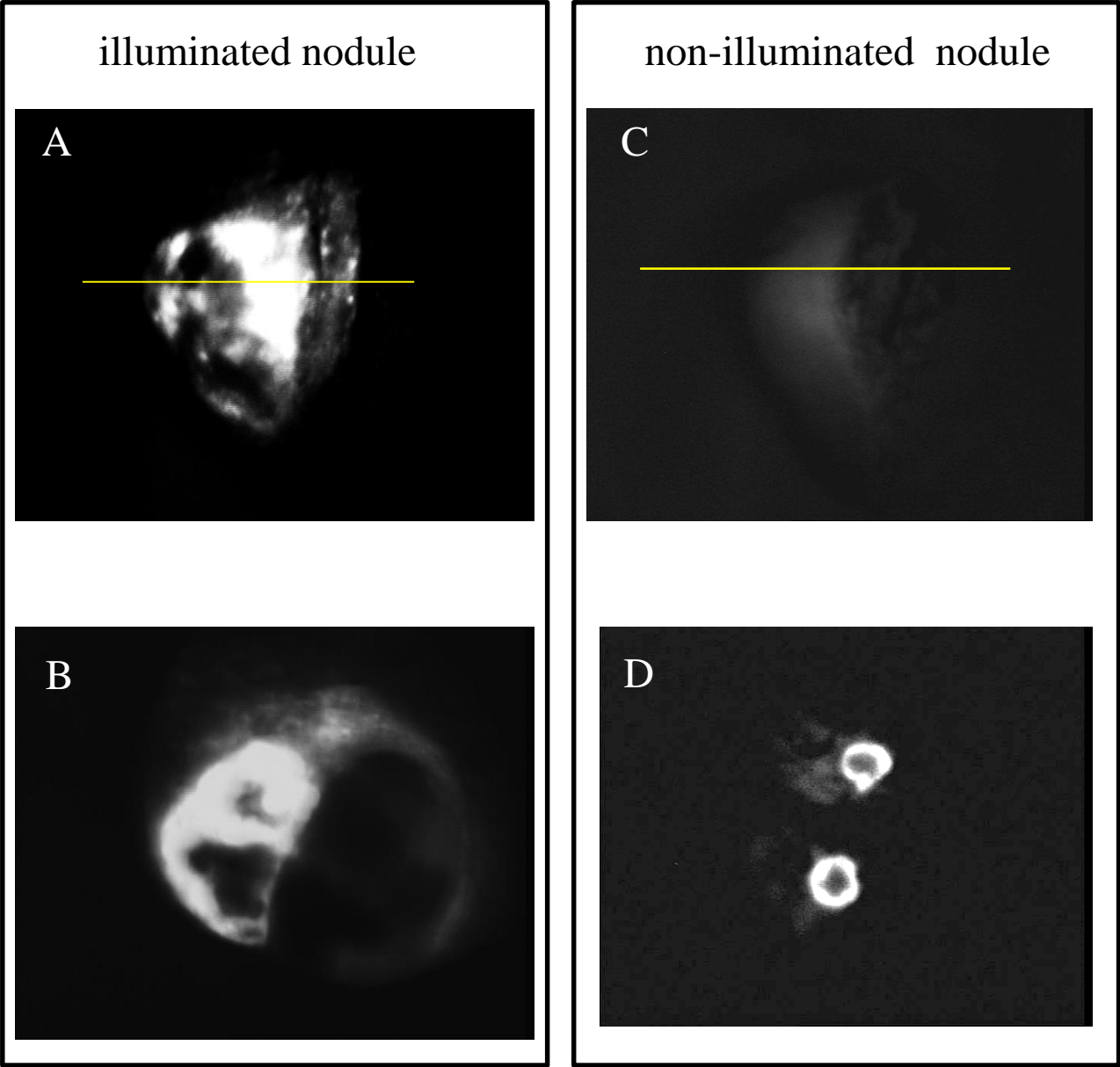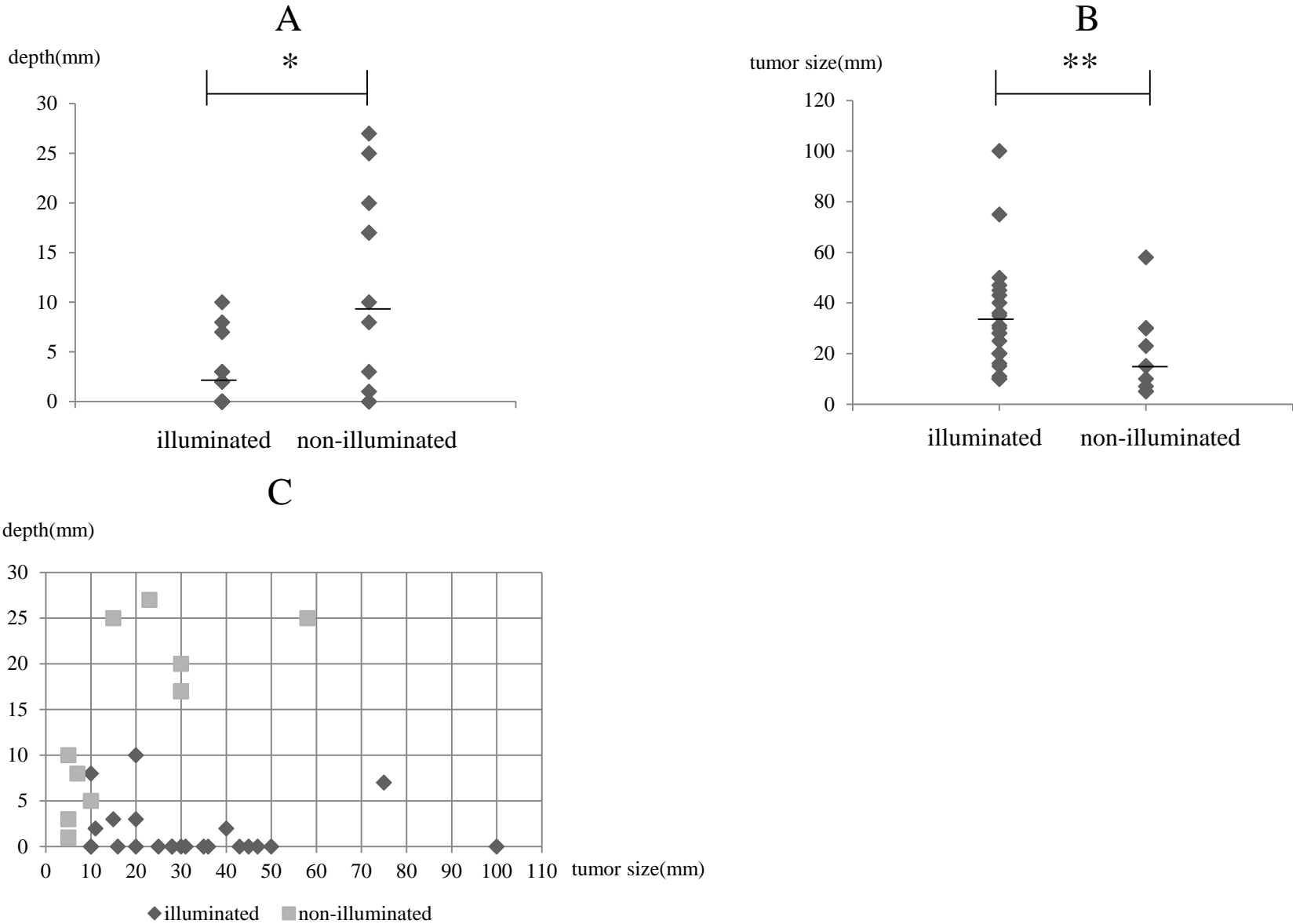Figure 3: ICG-FNS for patients with liver resection with non-cirrhotic liver.

Figure 4: The comparison between illuminated nodules and non-illuminated nodules on the liver surface in a non-cirrhotic liver.

*p<0.01   **p=0.058

Supplemental figure: The number of detected nodules in a non-cirrhotic liver.