Table II. Operative procedures and outcomes

	Peeling off (n = *20*)	*En bloc* (n = *29*)	P
Procedure			
Hemihepatectomy/trisectomy/central bisegmentectomy	10	13	.71
Sectoriectomy	5	11	
Subsegmentectomy	2	3	
Limited resection	3	2	
Pathology of PVTT			
Viable hepatocellular carcinoma	18	26	.93
Mostly necrotic	1	1	
Completely necrotic	1	2	
Operative outcomes			
Operation time (min)	460 (260–770)	465 (245–810)	.63
Blood loss (ml)	1,162 (350–3,659)	1,170 (180–2,420)	.19
Transfusion of red blood cells (yes/no)	3/17	4/25	.15
Alanine aminotransferase on 3POD (IU/dL)	127 (38–364)	90 (3–351)	.15
Hemoglobin on 3POD (g/dL)	9.3 (6.2–11.7)	10.0 (6–14.1)	.54
Total bilirubin on 3POD (mg/dL)	0.8 (0.3–2.2)	0.8 (0.3–1.7)	.59
Hospital stay (days)	19 (11–47)	17 (12–43)	.46
Mortality	0	0	-

Continous data are shown as median and range.
PVTT, Portal venous tumor thrombus; *POD*, postoperative day.

Moreover, postoperative anemia and liver function levels were also similar between the 2 groups, as indicated by the values of hemoglobin, total bilirubin, and alanine aminotransferase 3 days after the surgery. No abnormal deterioration of liver function, indicating postoperative rethrombosis of the portal branches in the remnant liver, was observed. The lengths of the hospital stays were similar in the 2 groups, and no mortalities occurred in either group.

The median follow-up period was similar in both groups (1.6 years [range, 0.2–10.3] in the PO group vs 1.6 years [range, 0.1–9.9] in the en bloc group; $P = .73$). The 1-, 3-, and 5-year overall survival rates were 58%, 46%, and 39% in the PO group and 65%, 41%, and 41% in the en bloc group, respectively (Fig 4, *A*). The 1-, 3-, and 5-year recurrence-free survival rates were 34%, 34%, and 23% in the PO group and 38%, 22%, and 18% in the en bloc group, respectively (Fig 4, *B*). The overall and recurrence-free survival rates were similar between the 2 groups ($P = .90$ and $P = .89$, respectively).

Recurrences developed in 14 (70%) of the 20 patients in the PO group and in 23 (79%) of the 29 patients in the en bloc group (Table III). The first recurrences developed in the remnant liver in 11 (79%) of the 14 recurrent cases in the PO group and in 18 (78%) of the 23 recurrent cases in the en bloc group. No local recurrences at the cut end of the portal vein occurred in either group. One case of peritoneal dissemination occurred in the PO group. Among the 14 recurrent cases in the PO group, the first recurrence was treated using TACE in 11 (78%) patients and by repeat resection in 3 (21%) patients; among the 19 recurrent cases in the en bloc group, TACE was performed in 12 patients (52%) and repeat resection was performed in 6 patients (26%). The median survival time after the first recurrence was 0.6 years in the PO group, which was comparable to that in the en bloc group (0.6 years; $P = .57$).

DISCUSSION

Using either the PO or the en bloc technique, we have attempted to perform radical resections for advanced HCC with PVTT, even if the PVTT has extended into the main portal trunk or the first-order branches. No mortalities occurred in either the PO or the en bloc group, indicating the safety of our operative procedures. The 5-year overall survival rates of the PO and en bloc groups were 39% and 41%, respectively; both of these rates are satisfactory and suggest that our operative strategies for treating HCC with PVTT are appropriate.

In HCC cases with PVTT extending into the main or contralateral portal veins, as shown in Fig 1 *A*, resection is the only hope for a cure and to avoid liver failure or variceal bleeding.[18,31] However, surgery using the radical en bloc technique has rarely been applied or recommended,[32]

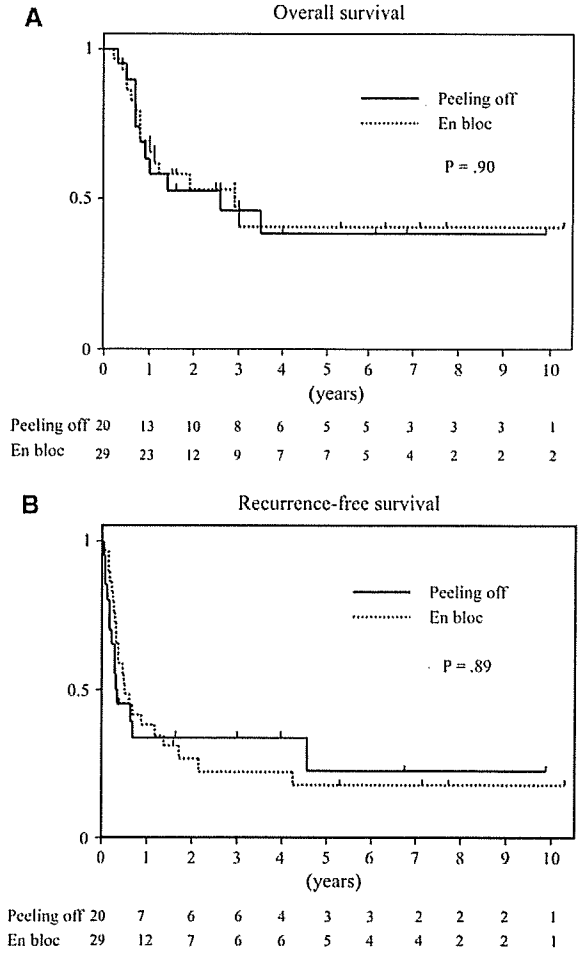

Fig 4. Survival. (*A*) Overall survival curves for the PO and en bloc groups. No significant difference was observed ($P = .90$). Numbers below the *x*-axis indicate the number of patients at risk. (*B*) Recurrence-free survival curves for the PO and en bloc groups. No significant difference was observed ($P = .89$). Numbers below the *x*-axis indicate the number of patients at risk.

possibly because this technique requires complex procedures including the reconstruction of the main portal trunk or contralateral portal branch or because the technique is associated with high postoperative morbidity and mortality rates.[19] The PO technique, on the other hand, is relatively simple because it does not require the resection and reconstruction of the portal vein.

For patients with HCC and PVTT extending into the first-order branch (Fig 2, *A*) and who have sufficient liver function, an en bloc resection of the territory including both the main HCC and the PVTT, such as a right hemihepatectomy, is usually recommended because this procedure would remove any minute intrahepatic metastases that might have scattered through the portal venous flow.[26] However, when the patient has an injured liver, such extensive hepatic resection may cause serious postoperative liver failure, which is among the most common lethal complications of this procedure.[33] The PO technique enables the resection of the tumor-bearing territory alone, preserving the PVTT-bearing territory (Fig 2, *A–D*); thus, excessive reduction of the functioning liver is avoided and portal blood flow is maintained, reducing the chances of postoperative liver failure.

Despite the use of a washout procedure during the PO technique, intraoperative blood loss and the prevalence of red blood cell transfusion in the PO group were comparable to the results in the en bloc group (Table II). No differences in postoperative anemia, liver function, or hospital stay were observed between the 2 groups. The risk of blood loss was clinically negligible, and no major complications requiring operative intervention occurred. These good short-term results without mortality support the safety of the PO technique and were mainly attributable to our strict compliance with our defined criteria for liver resection, which are based on the estimated liver functional reserve.[26,27] Ikai et al[34] reported that the removal of extended PVTT using a "simple open method," which resembles our PO technique, led to improved short-term results, with a postoperative mortality rate of 2.4% compared with a mortality rate of 33% for aggressive co-resection of the portal venous wall. Our results agree with their results with regard to the safety of these simplified procedures.

The 3- and 5-year overall survival rates of the PO group reached 46% and 39%, respectively, and were comparable with those of the en bloc group (41% and 41%, respectively). We believe that these results are satisfactory; PVTT arising from HCC generally carries a miserable prognosis, with a reported median survival time of 2.7 months when the disease is left to follow its natural course.[1] Other therapies for HCC with PVTT, such as TACE,[4,5] irradiation,[6] and chemotherapy,[10-12] are not regarded as effective, whereas liver transplantation[23-25] and local ablation[13] are not indicated for PVTT in the major branches. Among previous reports regarding the surgical resection of HCC with PVTT,[14,16,35-39] some liver surgeons have reported acceptable results for the resection of HCC with PVTT, with 3-year overall survival rates of 33.2%[14] and 48.9%.[37] We previously reported a 5-year overall survival rate of 42% using a combination of TACE with subsequent resection.[16] The current study suggests that the PO technique may be

Table III. Recurrence

	Peeling off (n = 20)	*En bloc* (n = 29)	P
Recurrence (yes/no)			.51
Site of the first recurrence	14/6	23/6	
Intrahepatic (solitary)	2	7	
Intrahepatic (multiple)	9	12	
Intrahepatic and peritoneal dissemination	1	0	
Intrahepatic and other abdominal organs	1	0	
Intrahepatic and lung	0	3	
Lung	1	1	
Total	14	23	
Treatment for the first recurrecnce			
Repeat resection	3	6	
TACE	10	12	
TACE and irradiation	1	0	
Systemic chemothepary	0	2	
Conservative therapy	0	3	
Total	14	23	

TACE, Transcatheter arterial chemoemborization.

comparable with the en bloc technique as a treatment for HCC with PVTT with regard to the long-term results, even though the liver functional reserve was poorer in the PO group (ICG R 15, 17% vs 12%, respectively; $P = .049$). Liver function is known to be a significant prognostic factor for all treatment modalities, with the exception of liver transplantation.

The first possible drawback of the PO technique is the risk of recurrence in the remnant liver, because this technique preserves the PVTT-bearing sector, which may contain micrometastases that could then be scattered through the portal flow.[20,26,40] However, the incidences of intrahepatic metastasis after resection were similar between the 2 groups (Table III). Possible reasons for this encouraging result may include technical matters, such as the performance of the thrombectomy before the other procedures and the adequacy of the procedures for clamping the portal branches. Another possible reason is that our hypothesis regarding the similarity of the risks of spreading into the remnant liver for massive PVTT reaching around the root of the tumor-bearing branch, regardless of whether the PVTT crosses the root line, may be correct. For patients with intrahepatic metastasis, we positively performed TACE or repeat resection. As a result, the median survival time after the first recurrence in the PO group was comparable with that in the en bloc group.

The second drawback of the PO procedure may be the risk of cancer cell residue on the portal venous wall. The macroscopically incomplete extraction of PVTT after a "direct removal method" resulted in rapid regrowth and a miserable prognosis.[36] In the current series, however, the regrowth of PVTT from the stump of the portal vein did not occur in the PO group, and the lateral invasion of PVTT at the portal stump was not described in either group, although most of the PVTTs contained viable HCC cells. These results support our second hypothesis. The most important factor to be noted is not the lateral invasion at the tip of the PVTT, but the minute intrusion of PVTT into tiny portal branches. Thanks to the specific biological behavior of HCC, which grows and extends expansively, and well-refined techniques including the thorough removal of PVTT from tiny portal branches like the caudate branches, the risk of local recurrence can be minimized.

The third drawback is the risk of peritoneal dissemination, because this technique includes the exposure of the PVTT and the washout of cancer cells from the portal vein to the surgical field. If such contamination of the surgical field were to occur, peritoneal dissemination would likely be observed as the first recurrence. In the present series, however, only 1 case (4.9%) of peritoneal dissemination was observed in the PO group, and this recurrence was accompanied by numerous intrahepatic recurrences. Thus, the potential risk of peritoneal dissemination may be a clinically minor problem if the procedure is performed carefully.

The option of en bloc resection after preoperative portal venous embolization (PVE) should be considered; PVE enlarges the future remnant liver and can expand the resection margin.[41] In this

series, however, PVE could be applied in only 3 patients in the en bloc group. This result can be explained as follows. Patients with PVTT often require urgent treatment. The waiting period after PVE, which may be longer in patients with impaired liver function than in those with normal liver function, may be associated with more demerits than benefits. Moreover, the portal branches to the territory to be resected have already been occupied by the PVTT itself, which may partially function as a type of "spontaneous PVE." Adding preoperative TACE selective to that territory, which we routinely perform for PVTT patients to suppress tumor growth,[16] may boost this "spontaneous PVE" effect. The other problem associated with PVE in PVTT cases is the risk of PVTT fragmentation and spreading during catheter manipulation. At any rate, the efficacy of preoperative PVE for PVTT patients remains unclear and requires further investigation. If hypertrophy of the remnant liver is imperative, ethanol injection into the patent portal branches into which the PVTT has budded may be preferable.

In conclusion, the PO technique is a safe and useful treatment for patients with advanced HCC and PVTT, providing an acceptable long-term survival rate comparable to that of the en bloc technique.

The authors thank Drs Hiroshi Ohtsu and Masataka Taguri, Department of Clinical Trial Data Management, The University of Tokyo, Tokyo, Japan, for valuable advice regarding the statistical analysis.

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Original Article

Real-Time Identification of Liver Cancers by Using Indocyanine Green Fluorescent Imaging

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BACKGROUND: We have often encountered difficulties in identifying small liver cancers during surgery. Fluorescent imaging using indocyanine green (ICG) has the potential to detect liver cancers through the visualization of the disordered biliary excretion of ICG in cancer tissues and noncancerous liver tissues compressed by the tumor. **METHODS:** ICG had been intravenously injected for a routine liver function test in 37 patients with hepatocellular carcinoma (HCC) and 12 patients with metastasis of colorectal carcinoma (CRC) before liver resection. Surgical specimens were investigated using a near-infrared light camera system. Among the 49 subjects, the 26 patients examined during the latter period of the study (20 with HCC and 6 with metastasis) underwent ICG-fluorescent imaging of the liver surfaces before resection. **RESULTS:** ICG-fluorescent imaging identified all of the microscopically confirmed HCCs (n = 63) and CRC metastases (n = 28) in surgical specimens. Among the 63 HCCs, 8 tumors (13%, including 5 early HCCs) were not evident grossly unless observed by ICG-fluorescent imaging. Five false-positive nodules (4 large regenerative nodules and 1 bile duct proliferation) were identified among the fluorescent lesions. Well-differentiated HCCs appeared as uniformly fluorescing lesions with higher lesion-to-liver contrast than that of moderately or poorly differentiated HCCs (162.6 [71.1-218.2] per pixel vs 67.7 [-6.3-211.2] per pixel, $P < .001$), while CRC metastases were delineated as rim-fluorescing lesions. Fluorescent microscopy confirmed that fluorescence originated in the cytoplasm and pseudoglands of HCC cells and in the noncancerous liver parenchyma surrounding metastases. ICG-fluorescent imaging before resection identified 21 of the 41 HCCs (51%) and all of the 16 metastases that were examined. **CONCLUSIONS:** ICG-fluorescent imaging enables the highly sensitive identification of small and grossly unidentifiable liver cancers in real time, enhancing the accuracy of liver resection and operative staging. **Cancer 2009;115:2491–504. © 2009 American Cancer Society.**

KEY WORDS: hepatocellular carcinoma, early hepatocellular carcinoma, liver metastasis, liver resection, diagnosis.

Although liver resection has played a leading role in the treatment of hepatocellular carcinoma (HCC) and metastases of colorectal carcinoma (CRC), the intraoperative diagnosis of small tumors

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This study was approved by the Ethics Committee of Tokyo University Hospital and registered in UMIN-CTR (no. 000001075, https://center.umin.ac.jp/ctr/index.htm); written informed consent was obtained from all patients.

The authors thank T. Ogura, H. Tsumura, and Y. Kinoshita for setting up the fluorescent microscopy system.

Received: July 28, 2008; **Revised:** November 21, 2008; **Accepted:** November 26, 2008

Published online: March 26, 2009 © 2009 American Cancer Society

DOI: 10.1002/cncr.24291, www.interscience.wiley.com

Table 1. Patients' Background Characteristics

Variable	HCC, n=37	Metastasis of CRC, n=12
Age, y*	67 (22-79)	64 (52-73)
ICG retention rate at 15 minutes (%)*	13.9 (3.7-46.5)	7.4 (2.2-13.0)
Child-Pugh class, A/B (%)	34 (92)/3 (8)	12 (100)/0 (0)
Hepatitis B virus surface antigen, positive/negative (%)	12 (32)/25 (68)	0 (0)/12 (100)
Hepatitis C virus antibody, positive/negative (%)	14 (38)/23 (62)	0 (0)/12 (100)
Background liver, cirrhosis/noncirrhosis (%)	12 (32)/25 (68)	0 (0)/12 (100)

HCC indicates hepatocellular carcinoma; CRC, colorectal carcinoma; ICG, indocyanine green.
*Median with range.

remains insufficient. Despite recent advances in imaging modalities, 3%-17% of HCCs[1,2] and metastases of CRC[1,3-5] can be detected only by microscopic examinations. In particular, we have often encountered difficulties in identifying small (< 2.0 cm) HCCs with indistinct margins (early HCC[6-9] or very early HCC[10]). Such grossly unidentifiable liver cancers may be overlooked by operative or even pathological diagnosis because thorough microscopic examinations of entire specimens are practically unfeasible. Novel intraoperative imaging techniques are needed to enhance the accuracy of liver resection and the pathological diagnosis of small liver cancers.

Recently, fluorescent imaging techniques using indocyanine green (ICG) have been used to assess coronary artery bypass graft patency during surgery.[11,12] These techniques are based on the finding that ICG binds to plasma proteins and protein-bound ICG emits light with a peak wavelength of around 830 nm when illuminated with near-infrared light.[13] Initially, we tried to apply fluorescent imaging techniques to intraoperative cholangiography.[14] While performing intraoperative cholangiography procedures in HCC patients, we happened to discover that HCCs fluoresced strongly even before the intrabiliary injection of ICG for cholangiography. Because these patients had received an intravenous injection of ICG for a routine liver function test before surgery and intravenously injected ICG is excreted exclusively by the liver,[15] we hypothesized that the HCCs fluoresced because they had retained the preoperatively injected ICG as a result of biliary excretion disorders in the cancerous tissues. Such fortuitous experiences have led us to develop the novel fluorescent imaging techniques described in this article; these techniques enable the highly sensitive identification of liver cancers through the visualization of the disordered biliary excretion of ICG that exists in HCC and noncancerous liver tissues surrounding CRC metastases.

MATERIALS AND METHODS

Patients

The subjects comprised 49 patients who had undergone liver resection for HCC (n = 37) or CRC metastasis (n = 12) at the University of Tokyo Hospital during the months between July and September 2007 and in March 2008, when a fluorescent imaging system was available at the hospital. Among the patients with HCC, the ICG retention rate at 15 minutes ranged from 3.7% to 46.5% (median, 13.9%) and 3 Child-Pugh class B patents were included, whereas the patients with metastases had normal liver function (Table 1).

Before surgery, all patients underwent abdominal ultrasonography and contrast-enhanced helical computed tomography (CT) examinations. In patients with HCC, resection was indicated for tumors in which the CT examination revealed both early hyperenhancement and delayed hypoenhancement.[7,16] During surgery, the liver was evaluated by visual inspection, manual palpation, and intraoperative ultrasonography.[17] Newly detected HCCs or metastases were resected whenever possible.[17,18]

ICG-Fluorescent Imaging of Surgical Specimens and Subsequent Pathological Examinations

As a fluorescent source, we used ICG (Diagnogreen, Daiichi Sankyo, Tokyo, Japan), which had been

intravenously injected before surgery at a dose of 0.5 mg per kg of body weight as part of a routine liver function test to determine the operative indications and procedures.[16,19] The intervals between the ICG injection and surgery ranged from 1 to 7 days (median, 3 days) in patients with HCC and from 1 to 14 days (median, 3 days) in patients with metastasis.

Surgical specimens were cut to include each tumor's maximum diameter based on gross inspection immediately after liver resection. The remaining parts of the specimens were also sliced into 10-mm sections using a long blade. All of the cut surfaces were grossly examined and subsequently investigated using the fluorescent imaging system in the operation room, without referring to the pre- or intraoperative findings. Any fluorescing lesions were marked with needles for subsequent microscopic examination if they were larger than 5 mm in size, because routine pathological examinations to discriminate small liver cancer from large regenerative nodules or dysplastic nodules are often performed for lesions with a diameter of greater than 5 mm.[20]

All the surgical specimens were formalin fixed and examined by experienced pathologists (N.F. and J.S.). All grossly identified nodules were microscopically evaluated irrespective of the presence or absence of ICG-fluorescence. A diagnosis of HCC was made according to the Japanese classification system[8] and the criteria proposed by the International Working Party.[20] The definition of early HCC was as follows: grossly, the tumor exhibits indistinct margins and does not destroy the preexisting hepatic framework; microscopically, it comprises uniformly distributed well-differentiated cancerous tissues with little cellular and architectural atypia and grows at tumor-nontumor boundaries as if it is replacing the neighboring liver cell cords.[6-9]

Fluorescent Imaging System

The fluorescent imaging system (PDE, Hamamatsu Photonics, Hamamatsu, Japan) comprised a small control unit ($322 \times 283 \times 55$ mm) and a camera unit ($80 \times 181 \times 80$ mm). The camera unit comprised a charge-coupled device camera, 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 20 cm above the surgical specimen, which was set in a lightproof box, with the output of light emitting diodes set at 1 mW per cm^2. Data were transmitted to a personal computer via an image capture card, and fluorescent images were created and stored at 72 pixels per inch.

Measurement of Signal Intensity on Fluorescent Images

The signal intensities of the lesions and noncancerous liver tissues were determined by calculating the mean brightness of an operator-defined region of interest on monochromatic fluorescent images of surgical specimens with the help of Photoshop version 7.0 software (Adobe Systems, San Jose, Calif). The brightness of each pixel was measured within a range of 0-255. The lesion-to-liver contrast was calculated by subtracting the signal intensity of the noncancerous liver parenchyma from that of the lesion.

Fluorescent Microscopy

Surgical specimens were fixed with formalin, sectioned at 10 μm, and stained with hematoxylin only (eosin was avoided because of the fluorescence of the dye itself). Fluorescent microscopic examinations were performed using an upright epifluorescence microscope (Eclipse 55i with a J-FL attachment, Nikon Instruments, Tokyo, Japan) with a cooled charge-coupled device camera (Retiga EXi, QImaging, Surrey, BC, Canada), a xenon light source (MAX-302, Asahi Spectra, Tokyo, Japan), and filters (excitation 775 ± 50 nm and emission 810 nm longpass and 845 ± 55 nm bandpass, Chroma Technology, Rockingham, Vt).

ICG-Fluorescent Imaging Before Resection

Among the 49 subjects, the latter 26 patients (20 with HCC and 6 with CRC metastases) underwent ICG-fluorescent imaging before resection: After the mobilization of the liver, the camera imaging head was positioned 20 cm above the liver surface (the output of the light emitting diodes was 4 mW per cm^2), the surgical lights were turned off (the ceiling lights were kept on), and the fluorescent images on the liver surfaces were displayed on a television monitor in the operation room.

Table 2. Characteristics of the Lesions Detected Only by Indocyanine Green-Fluorescent Imaging

Case	ICG Retention Rate at 15 Minutes, %	Child-Pugh Class	Preoperative Diagnosis*	Intraoperative Ultrasonography	Tumor Size, mm	Microscopic Diagnosis
1	8.0	A	HCC	ND	2	Moderately differentiated HCC
2	4.8	A	HCC	Hyperechoic	4	Well-differentiated HCC
3	26.4	B	ND	ND	12	Well-differentiated HCC
4	17.1	A	ND	Hypoechoic	19	Early HCC
5	3.8	A	ND	Hypoechoic	12	Early HCC
5	3.8	A	ND	ND	10	Early HCC
6	15.1	A	ND	ND	8	Early HCC
7	9.8	A	ND	ND	6	Early HCC
8	20.7	A	ND	Hyperechoic	7	Large regenerative nodule
9	23.4	A	ND	ND	7	Large regenerative nodule
3	26.4	B	ND	ND	6	Large regenerative nodule
10	21.3	A	ND	Hyperechoic	5	Large regenerative nodule
11	15.1	A	ND	ND	5	Bile duct proliferation

ICG indicates indocyanine green; HCC, hepatocellular carcinoma; ND, not detected.
* According to ultrasonography and contrast-enhanced helical computed tomography.

Statistical Analysis

Continuous data are expressed as the median (range). Categorical data and continuous data were compared using a Fisher exact test and the Wilcoxon rank sum test, respectively. We assigned statistical significance at $P < .05$. The calculations were performed with the help of JMP version 5.1.1 software (SAS Institute, Cary, NC).

RESULTS

Cancer Detection by Using ICG-Fluorescent Imaging of Surgical Specimens

Microscopic examinations confirmed a total of 91 cancers (63 HCCs and 28 metastases of CRC). All of the HCCs and metastases were identified using our ICG-fluorescent imaging technique on the cut surfaces of the surgical specimens.

Table 2 summarizes the 13 lesions that were detected using ICG-fluorescent imaging technique but that were not evident during gross examination of the cut surfaces of the specimens. These 13 lesions comprised 8 HCCs (13% of the 63 HCCs, Fig. 1), including 5 early HCCs (45% of the 11 early HCCs in our series, Fig. 2), and 5 false-positive nodules (4 large regenerative nodules and 1 bile duct proliferation). Our series also included 10 nonfluorescent nodules (found in 6 patients with HCC), which microscopically proved to be regenerative nodules (n = 8) and dysplastic nodules (n = 2). All 28 CRC metastases were identified by both gross examination and fluorescent imaging.

The sensitivities and positive predictive values of the ICG-fluorescent imaging on surgical specimens for HCC were thus 100% and 93% (63 cancers of the 68 fluorescing lesions), respectively, while those for the CRC metastases were 100% and 100%, respectively.

Fluorescent Patterns of Liver Cancers

The fluorescent patterns of the 63 HCCs on the cut surfaces of the surgical specimens were classifiable into the following 3 types: a total fluorescent type (all of the cancer tissues showed a uniform fluorescence, n = 33, Fig. 3A), a partial fluorescent type (part of the cancer tissues showed fluorescence, n = 26, Fig. 3B), and a rim fluorescent type (the cancer tissues were negative for fluorescence but the surrounding liver parenchyma showed fluorescence, n = 4, Fig. 3C). All of the 28 CRC metastases displayed rim fluorescence (Fig. 3D).

Between the total fluorescent-type HCCs and the partial-fluorescent-type HCCs, the former group comprised smaller tumors ($12^{2\text{-}40}$ mm vs $30^{7\text{-}150}$ mm; $P = .001$) and, microscopically, well-differentiated carcinomas and pseudogland formation were more common among the total fluorescent-type HCCs (58% vs 0%; $P < .001$, and 85% vs 50%; $P = .005$, respectively; Table 3). The rim fluorescent-type HCCs comprised only poorly differentiated carcinomas.

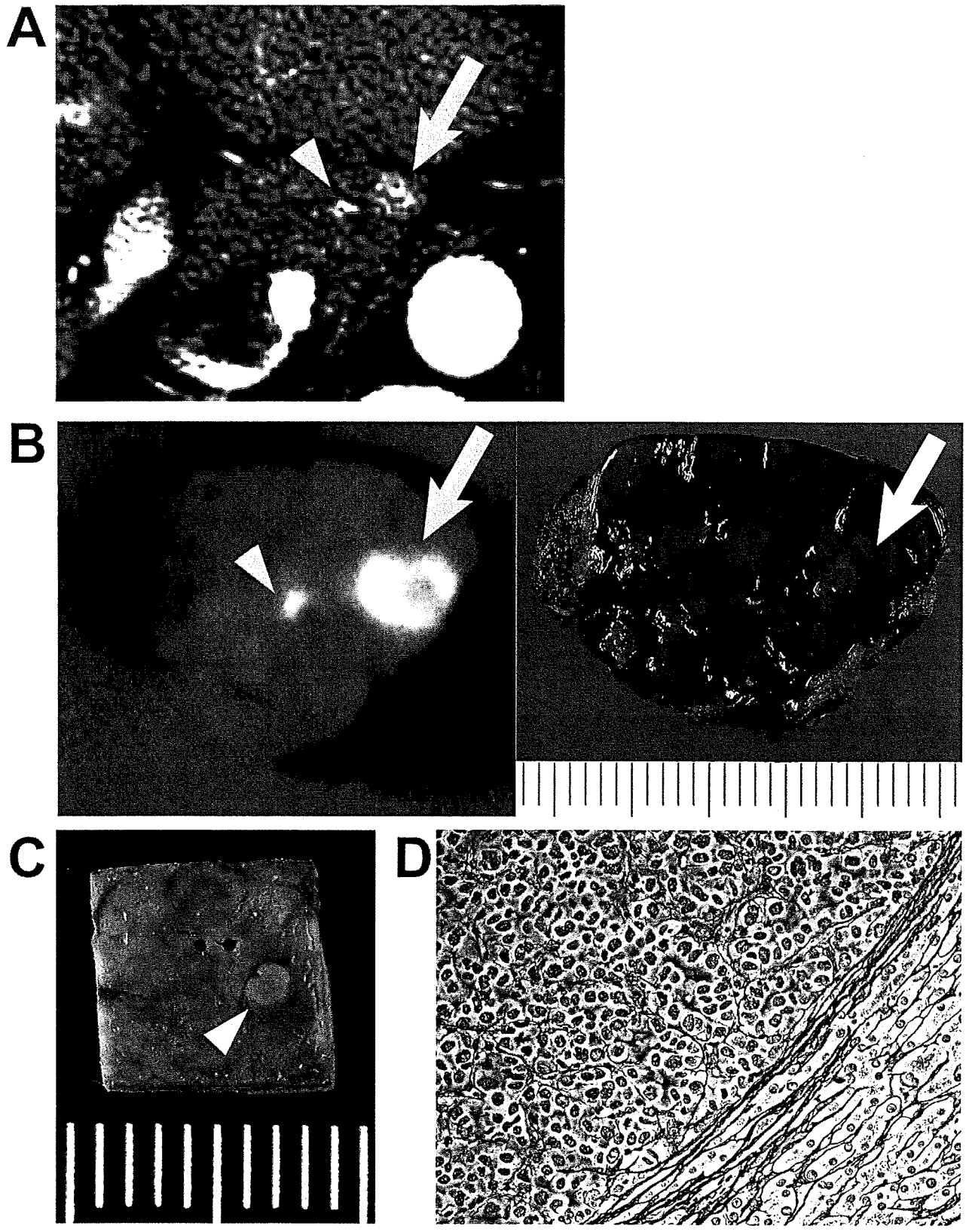

FIGURE 1. Hepatocellular carcinomas that were not evident grossly unless observed using the fluorescent imaging technique (Case 1 in Table 2). (A) Preoperative contrast enhanced computed tomography (CT) revealed 2 hypervascular tumors in Couinaud's segment I (arrow and arrowhead). (B) On the resected specimens, fluorescent imaging identified 2 fluorescing lesions corresponding to the lesions identified on the preoperative CT (left), although only the larger 1 (arrow) was identifiable by gross examination (right). (C) When the smaller fluorescing lesion (arrowhead) was cut deeper after formalin fixation, cancerous tissues with a diameter of 2 mm appeared (arrow). (D) Microscopically, the smaller tumor proved to be a moderately differentiated hepatocellular carcinoma (silver reticulin stain, original magnification, ×40).

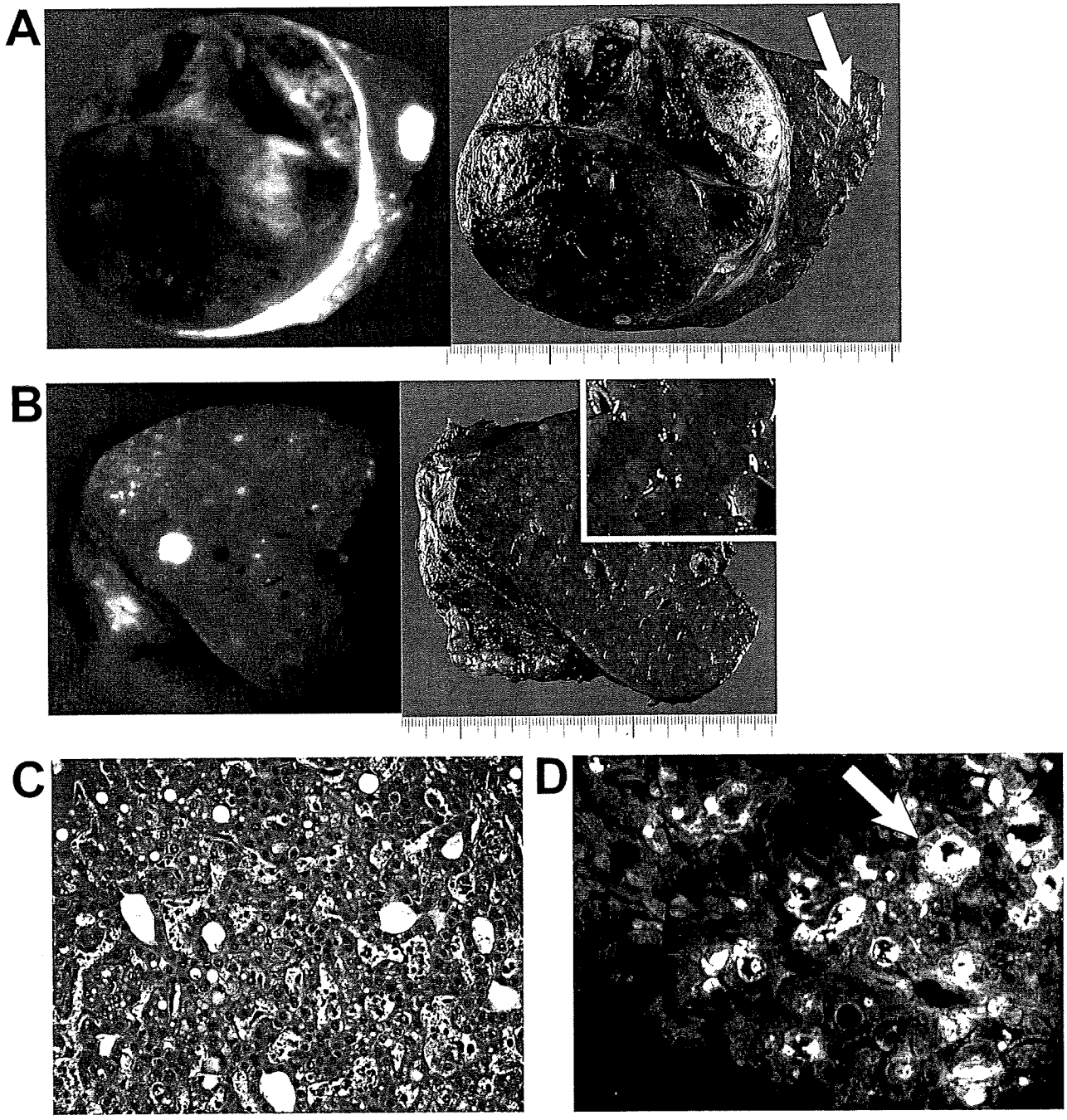

FIGURE 2. Early hepatocellular carcinomas that were detected using the fluorescent imaging technique (Case 5 in Table 2). (A) Fluorescent imaging (left) delineated a fluorescing lesion at a site where no cancerous lesion had been grossly identified (arrow on right side). Fluorescence was also demonstrated in the viable cancer tissues of the main tumor and in liver parenchyma compressed by the tumor. (B) Fluorescent imaging detected another grossly unidentifiable lesion apart from the main tumor (inset shows a magnified view of the lesion on gross examination). (C) Microscopic examinations revealed early hepatocellular carcinomas comprising well-differentiated cancer cells with little cellular and structural atypia and pseudogland formation in both of the 2 fluorescing lesions (H&E, originally × 40). (D) Fluorescent microscopy confirmed the origin of the fluorescence in the cancer cell cytoplasm and pseudoglands (arrow).

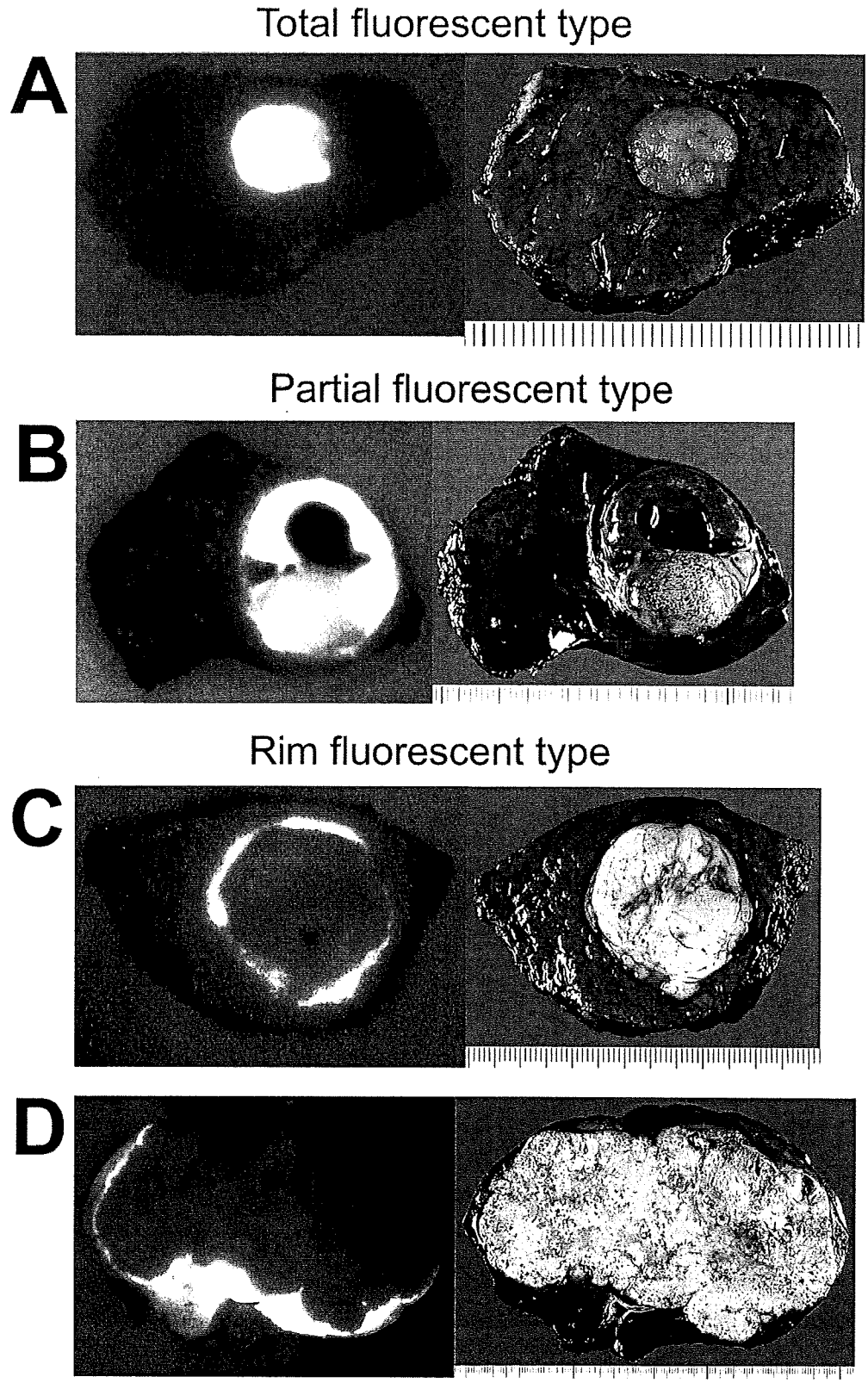

FIGURE 3. Fluorescent patterns of liver cancers on surgical specimens (left) and their gross appearances (right). (A) Total fluorescent type; well-differentiated hepatocellular carcinoma (HCC), 7 mm in diameter. (B) Partial fluorescent type; moderately differentiated HCC with well-differentiated components and hemorrhagic necrosis at the upper half of the tumor, 36 mm in diameter. (C) Rim fluorescent type; poorly differentiated HCC, 30 mm in diameter. (D) Rim fluorescent type; metastasis of colon carcinoma, 130 mm in diameter.

Table 3. Fluorescent Patterns of HCCs*

Variable	Total Fluorescent Type, n=33	Partial Fluorescent Type, n=26	*P*
ICG retention rate at 15 min (%)†	16.2 (4.3-46.5)	10.2 (3.7-23.4)	.056
Interval from ICG injection to surgery			
Within 24 h	5 (15%)	4 (15%)	>.999
More than 24 h	28 (85%)	22 (85%)	
Tumor size (mm)†	12 (2-40)	30 (7-150)	<.001
Cancer cell differentiation			
Well	19 (58%)	0 (0%)	<.001
Moderate or poor	14 (42%)	26 (100%)	
Early HCC	11 (33%)	0 (0%)	.001
Pseudoglands			
Present	28 (85%)	13 (50%)	.005
Absent	5 (15%)	13 (50%)	

HCC indicates hepatocellular carcinoma; ICG, indocyanine green.
* Excluding 4 HCCs classified as rim-fluorescent type.
† Median with range.

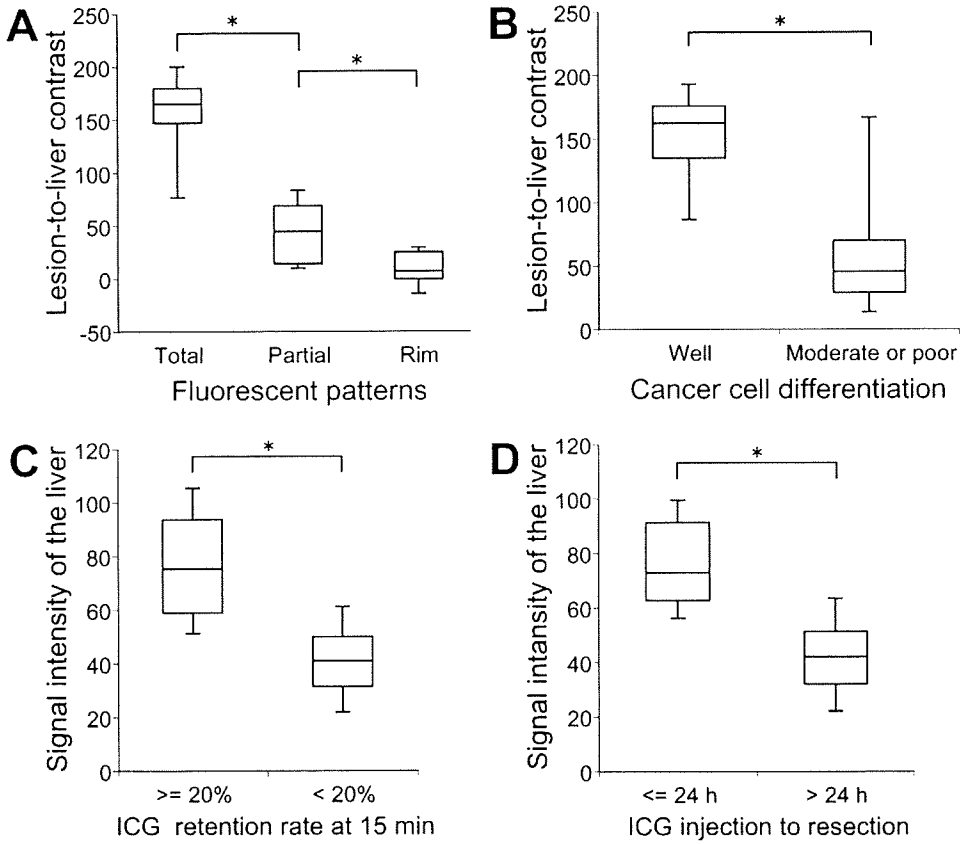

FIGURE 4. Signal intensity assessment. (A) Lesion-to-liver contrast among total fluorescent type (n=33), partial fluorescent type (n=26), and rim fluorescent type (n=32) tumors. (B) Lesion-to-liver contrast between well-differentiated (n=19) and moderately or poorly differentiated (n=44) hepatocellular carcinoma. (C) Signal intensity of the noncancerous liver parenchyma around the tumors, which were classified according to the patients' indocyanine green (ICG) retention rate at 15 minutes (equal to or greater than 20%, n=14; less than 20%, n=77). (D) Signal intensity of the noncancerous liver parenchyma around the tumors, which were classified according to the intervals between the ICG injection and resection (equal to or shorter than 24 hours, n=10; longer than 24 hours, n=81). Boxes reflect the median (2fifth-7fifth percentile). The horizontal bars show the limits of the 10^{th} and 90^{th} percentiles and the asterisk indicates $P < .001$.

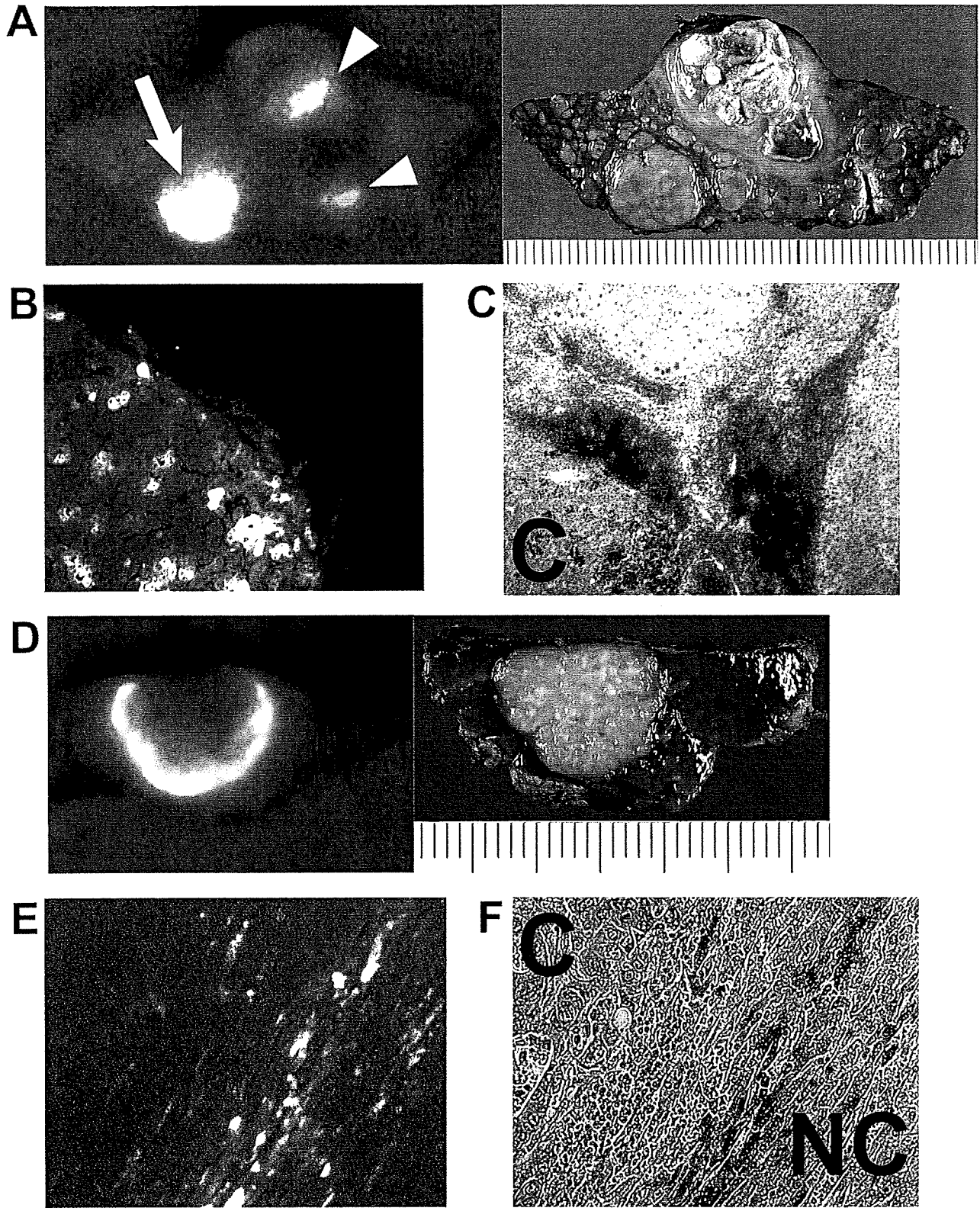

FIGURE 5. Fluorescent microscopic imaging of hepatocellular carcinoma (HCC) and metastasis of colorectal carcinoma (CRC). HCC (Case 3 in Table 2): (A) Fluorescent imaging revealed fluorescence in the newly detected well-differentiated HCC (arrow in left) as well as in a viable component of the moderately differentiated HCC (yellow arrowhead) and in a large regenerative nodule (white arrowhead). (B) Fluorescent microscopy showed fluorescence only in the cancer cell cytoplasm and pseudoglands (originally × 40). (C) A merged image of the fluorescence (demonstrated in red) on a hematoxylin-stained cross section confirmed fluorescence in the cancer tissues (C) but not in the surrounding noncancerous liver tissues (originally × 10). CRC metastasis: (D) Fluorescent imaging revealed a fluorescing rim around a tumor. (E) Fluorescent microscopic image (originally × 10). (F) A merged image of the fluorescence (demonstrated in red) on a hematoxylin-stained cross section confirmed that fluorescence was emitted not from the cancer tissues (C) but from the surrounding noncancerous liver tissues (NC) compressed by the tumor.

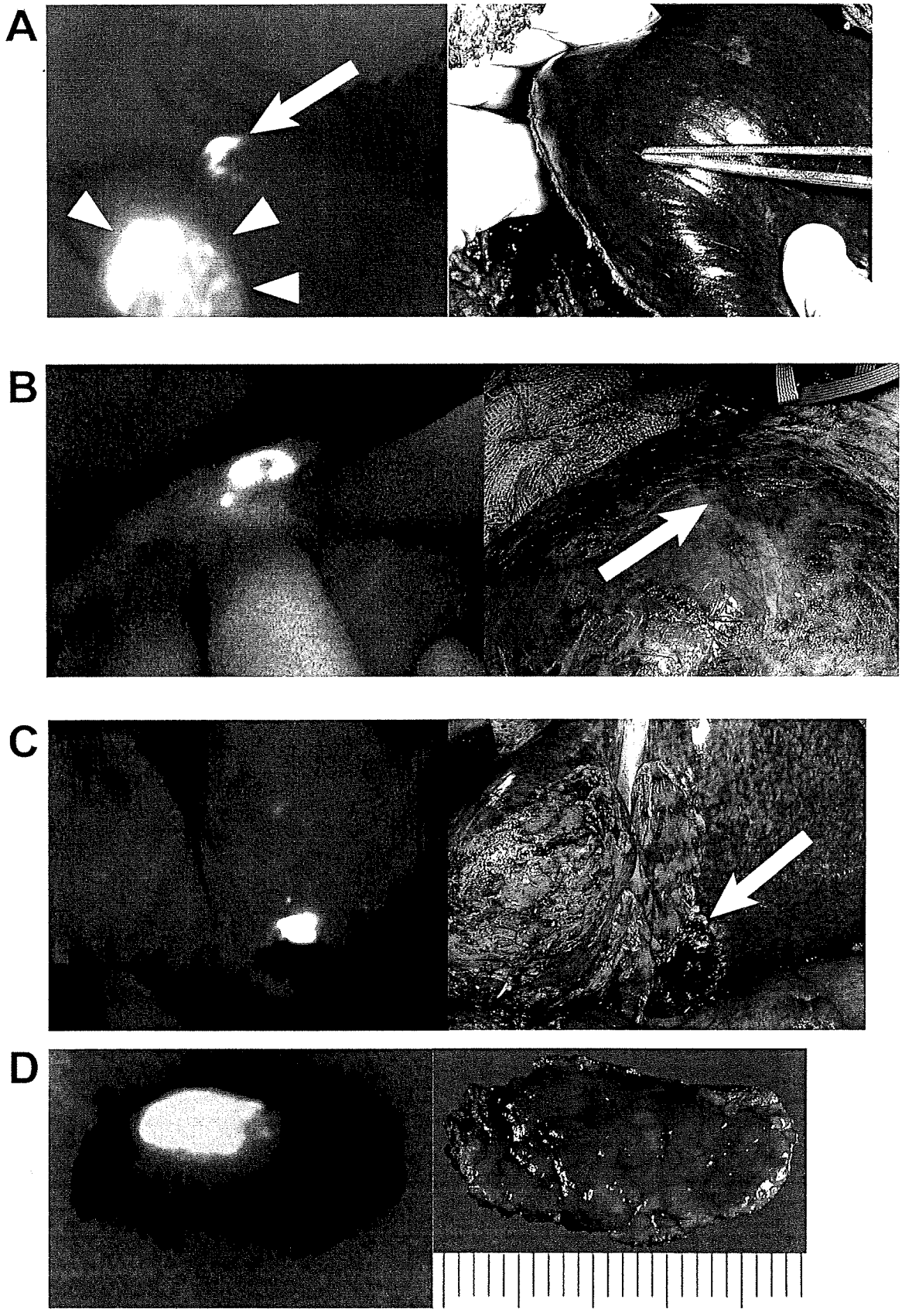

FIGURE 6.

Signal Intensity Assessment on Fluorescent Images

The lesion-to-liver contrast was significantly higher among the total fluorescent-type tumors (165^{54-218} per pixel) than among the partial fluorescent-type tumors (45 [-6-87] per pixel, $P < .001$) and was also higher among the partial fluorescent-type tumors than among the rim fluorescent-type tumors (7 [-25-45] per pixel, $P < .001$; Fig. 4A). Among the 63 HCCs, well-differentiated cancers were associated with a significantly higher lesion-to-liver contrast than moderately or poorly differentiated cancers (163^{71-218} per pixel vs 68 [-6-211] per pixel, $P < .001$; Fig. 4B). The signal intensity of the noncancerous liver parenchyma was significantly higher among patients with an ICG retention rate at 15 minutes of equal to or greater than 20% compared with those with an ICG retention rate at 15 minutes of less than 20% (75^{46-107} per pixel vs 41^{12-91} per pixel, $P < .001$; Fig. 4C), and it was also higher among patients in whom ICG had been injected within 24 hours before surgery than among those in whom the ICG had been injected more than 24 hours before surgery (73^{56-105} per pixel vs 42^{12-107} per pixel, $P < .001$; Fig. 4D).

Fluorescent Microscopy

Fluorescent microscopy confirmed the presence of fluorescence in the cytoplasm and pseudoglands of the HCC cells in areas where the ICG-fluorescent imaging of the surgical specimens had demonstrated fluorescence (Fig. 2D and Fig. 5A-C). In the rim fluorescence type-HCCs and metastases, the fluorescence was confirmed to exist not in the cancerous tissues, but in the surrounding noncancerous liver tissues where the cellular density had been increased as a result of compression by the tumor (Fig. 5D-F).

Cancer Detection by Using ICG-Fluorescent Imaging Before Resection

ICG-fluorescent imaging technique also identified 21 of the 41 HCCs that were examined (51%, Fig. 6A) and all of the 16 CRC metastases (Fig. 6B) on the liver surfaces before resection. Among the 20 tumors that were not identified by intraoperative ICG-fluorescent imaging, the tumor size was smaller than that of the 37 identifiable tumors (11^{2-40} mm vs 18^{3-130} mm, $P = .019$) and the tumor location was deeper (10^{3-35} mm vs 2^{0-8} mm, $P < .001$). When the 41 HCCs and the 16 metastases were compared, the locations of the HCCs were significantly deeper than those of the metastases (5^{0-35} mm vs 1^{0-6} mm, $P < .001$), while the tumor size of the HCCs (12^{2-102} mm) was not different from that of the metastases (18^{7-130} mm, $P = .471$). None of the tumors at a depth of more than 8 mm from the liver surfaces were identifiable.

Intraoperative ultrasonography revealed 33 of the 41 HCCs (80%) and all of the 16 metastases. Among the remaining 8 HCCs that were unidentifiable by intraoperative ultrasonography, ICG-fluorescent imaging on the liver surfaces detected 3 HCCs located at a depth of 1-2 mm from the liver surfaces.

In 1 patient with HCC (Case 4 in Table 2), intraoperative fluorescent imaging after resection demonstrated residual cancer tissues, which were unidentifiable by gross examination or intraoperative ultrasonography, on the raw surface of the remnant liver, requiring an additional resection to be performed (Fig. 6C, D).

DISCUSSION

ICG-fluorescent imaging identified all of the microscopically confirmed HCCs and CRC liver metastases in the surgical specimens. Well-differentiated HCCs were

FIGURE 6. Fluorescent images of cancers on liver surfaces before resection. (A) Intraoperative fluorescent imaging clearly identified a hepatocellular carcinoma (HCC) on the liver surface (arrow) that was not visible or palpable (tumor location was determined by intraoperative ultrasonography and is indicated by the tweezers). A cross section of the tumor is demonstrated in Figure 3A. The transverse colon was also fluorescing (arrowheads) as a result of indocyanine green in the stool. (B) A rim-fluorescing metastasis was delineated on the liver surface (its cross section was shown in Fig. 5D). Palpation of the tumor was difficult because of the presence of thick connective tissues on the liver surface from a previous surgery. (C) Intraoperative fluorescent imaging revealed that a fluorescing lesion remained on the raw surface of the remnant liver after resection (left), although the lesion was not identified by gross examination or manual palpation (right). The residual fluorescing lesion was in addition resected. (D) A cross section of the in addition resected liver specimen. Fluorescent imaging confirmed the presence of the lesion (left), which was still unidentifiable by gross examination of the cut surface. Subsequent microscopic examinations revealed an early HCC in the fluorescing area.

detected as uniformly and highly fluorescing lesions, while poorly differentiated HCCs and metastases of CRC were detected as rim-fluorescing lesions with a low lesion-to-liver contrast. Microscopically, fluorescence was confirmed to originate in the cytoplasm and pseudoglands of HCC cells and in the noncancerous liver parenchyma surrounding poorly differentiated HCCs and metastases. These results suggest that well- or moderately differentiated HCC produces tumorous fluorescence because the cancer tissues retain the portal uptake of ICG despite the functional and/or architectural destruction of biliary excretion due to cancer progression. In contrast, poorly differentiated HCCs and metastases produce rim fluorescence probably because of biliary excretion disorders in the surrounding noncancerous liver tissues that have been compressed by the tumor. Such mechanisms for the ICG-fluorescent imaging of liver cancers are consistent with those for delayed (10-24 hours) magnetic resonance imaging using biliary-excreted contrast material, which can be used to demonstrate tumorous hyperenhancement in well-differentiated HCCs and rim enhancement in metastases.[21-24]

The major expected role of ICG-fluorescent imaging in clinical settings is the identification of small liver cancers, especially early HCC, which have been shown to be the earliest clinical entity of HCC and to have a high cure rate.[7,9,10] Theoretically, our fluorescent imaging technique could be used to delineate any lesions retaining ICG in surgical specimens cut into 10-mm sections because near-infrared light penetrates human tissues to a depth of about 5-10 mm.[25] Indeed, 8 of the 63 HCCs (13%) in our series were not evident grossly unless observed by ICG-fluorescent imaging because of their deep tumor locations and/or indistinct tumor margins. Furthermore, 5 of the 11 early HCCs (45%) were macroscopically detectable only by fluorescent imaging. These results indicate that ICG-fluorescent imaging enables the highly sensitive identification of small liver cancers on surgical specimens, enhancing the accuracy of operative cancer staging. This technique may also rewrite the incidence of early HCC and provide new aspects for research on hepatocarcinogenesis.[26-29]

ICG-fluorescent imaging was also useful for identifying liver cancers before resection: 51% of the HCCs and all of the CRC metastases were delineated on the liver surfaces. Although the reason for the lower detectability of the HCCs compared with the metastases is not totally clear, cancer detectability using this 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,[25] as described earlier, rather than the characteristics of the cancerous tissues. Indeed, none of the tumors at a depth of more than 8 mm from the liver surfaces were identifiable in our series. Despite this limitation in detecting deeply located cancers, however, intraoperative ICG-fluorescent imaging is still useful because it not only aids visual inspections and palpation, but it also compensates for drawbacks in intraoperative ultrasonography in observing small lesions located just beneath the liver surface. Furthermore, this technique can be used to detect residual cancerous tissues on the raw surface of the remnant liver after resection (Fig. 6C, D). With the future application of fluorescent imaging systems to laparoscopes, ICG-fluorescent imaging may become an indispensable means of identifying liver cancers during laparoscopic hepatectomies,[30,31] in which surgeons cannot palpate the liver surface.

Other advantages of our ICG-fluorescent technique are its safety and feasibility. ICG is already used worldwide to evaluate liver function before resection. The reported incidence of adverse reactions after the intravenous injection of ICG is quite small (approximately 0.003%).[15] Furthermore, our fluorescent imaging technique can use the ICG that is injected for routine liver function tests. Once ICG is administered within 14 days before surgery, we can obtain fluorescent images of liver cancers in real time by simply placing the camera imaging head on the liver surface or on surgical specimens.

Our study has several limitations. First, our results indicated 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. These findings suggest that the interval between the ICG injection and surgery should be longer than at least 2 days to obtain a good lesion-to-liver contrast, especially in patients with advanced cirrhosis. Further studies are needed to determine the optimal dosage of ICG for fluorescent imaging based on the patient's liver function. Second, ICG-fluorescent imaging revealed 5 false-positive lesions in our HCC series. The incidence and characteristics of false-positive lesions should be clarified in larger

study populations, although the presented techniques are already useful in clinical settings for the intraoperative identification of lesions suspected of being cancerous.

In conclusion, ICG-fluorescent imaging can be used to observe HCC lesions based on their tumorous fluorescence because the portal uptake of ICG is preserved in well- or moderately differentiated cancer tissues, despite the lack of biliary extraction, and to delineate poorly differentiated HCC and CRC metastases as rim-fluorescing lesions because of biliary excretion disorders in the surrounding noncancerous liver tissues that are compressed by the tumor. Our fluorescent imaging technique enables the highly sensitive identification of small and grossly unidentifiable liver cancers in real time, enhancing the accuracy of liver resection and operative cancer staging.

Conflict of Interest Disclosures

This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant nos. 18790955 and 17591377), the ministry of Health, Labor, and Welfare of Japan (grant no. 18230201) and Japanese Society for Advancement of Surgical Techniques.

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Predeposit Autologous Plasma Donation in Liver Resection for Hepatocellular Carcinoma: Toward Allogenic Blood-Free Operations

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BACKGROUND: The aim of this study was to evaluate the safety of predeposit autologous plasma donation (PAPD) and its efficacy in avoiding allogenic blood transfusions and albumin infusion in liver resection for hepatocellular carcinoma.

STUDY DESIGN: PAPD was adopted in 20 patients in whom liver function remained within Child-Pugh's class A and an indocyanine green retention rate at 15 minutes was ≤ 15% (PAPD group). Up to 1,200 mL of autologous fresh frozen plasma was collected through three blood donation sessions. Allogenic blood transfusion rates, albumin infusion rates, and postoperative courses were compared between the PAPD group and a historic control (no PAPD) group (n = 36).

RESULTS: Serum albumin levels after the last blood donation session were not significantly different from those before PAPD. The prothrombin activity even increased through the blood donation sessions (from median 80.9% [range 70.0% to 100%] to median 89.2% [range 71.2% to 100%]; $p < 0.001$). Allogenic blood transfusion rate and albumin infusion rate were lower in the PAPD group than in the no PAPD group (11% versus 75%; $p < 0.001$ and 16% versus 47%; $p = 0.038$, respectively). Wastage rate of the autologous fresh frozen plasma products was 9%.

CONCLUSIONS: PAPD was safe in patients with underlying liver disease and can be beneficial in simulating the liver synthetic function in advance of operation. Autologous fresh frozen plasma transfusion was effective for avoiding allogenic blood products in liver resection for hepatocellular carcinoma. (J Am Coll Surg 2009;209:206–214. © 2009 by the American College of Surgeons)

Liver resection has been established as a safe and effective treatment for hepatocellular carcinoma (HCC) in patients with preserved liver function: it offers a 5-year overall survival rate of >50%, with an operative mortality of <5%.[1-6] Nonetheless, liver resection for HCC still involves a high rate of red blood cell transfusion (23% to 49%).[1,2,7-11] This remains the major disadvantage of liver resection because allogenic blood transfusion reduces cost-effectiveness, increases risks of transfusion reactions and transfusion-transmitted infections, and induces immunosuppression, which can increase risk of postoperative infections[12-14] and tumor recurrence.[15-17]

In our institution, the red blood cell transfusion rate in liver resection for HCC has been brought down to <5% because of our strict policy of "restricted red blood cell transfusion"[18,19] to avoid hepatic bilirubin overload and the subsequent hyperbilirubinemia that can occur in cirrhotic patients.[20,21] In contrast, the perioperative fresh frozen

Disclosure Information: Nothing to disclose.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 18790955), a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (grant no. 17591377), a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare of Japan (grant no. 18230201), and JSPS Fujita Memorial Fund for Medical Research.

Received January 20, 2009; Revised February 23, 2009; Accepted March 3, 2009.

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ISSN 1072-7515/09/$36.00
doi:10.1016/j.jamcollsurg.2009.03.004