Surgical Innovation
2018, Vol. 25(1) 62–68

DOI: 10.1177/1553350617751451
journals.sagepub.com/home/sri
SAGE

Indocyanine Green Fluorescence Imaging-Guided Surgery in Primary and Metastatic Liver Tumors

Eva Lieto, MD, PhD[1], Gennaro Galizia, MD, PhD[1], Francesca Cardella, MD[1], Andrea Mabilia, MD[1], Nicoletta Basile, MD[1], Paolo Castellano, MD, PhD[1], Michele Orditura, MD, PhD[1], and Annamaria Auricchio, MD[1]

Abstract

Background. After surgery for liver tumors, recurrence rates remain high because of residual positive margins or undiagnosed lesions. It has been suggested that detection of hepatic tumors can be obtained with near-infrared fluorescence imaging (FI). Indocyanine green (ICG) has been used with contrasting results. The aims of this study were to explore ICG-FI-guided surgery methodology and to assess its potential applications. *Materials and Methods*. Out of 14 patients with liver tumors, 5 were not operated on, and 9 patients (3 primary and 6 metastatic tumors) underwent surgery. ICG (0.5 mg/kg) was injected intravenously 24 hours before surgery. Fluorescence was investigated prior to resection to detect liver lesions, during hepatic transection to guide surgery, on both cross-section and benchtop to assess surgical margins, and for pathological evaluation. *Results*. All operations were successful and had a short duration. ICG-FI detected all already known lesions (n = 10), and identified 2 additional small tumors (1 hepatocarcinoma and 1 metastasis, diagnostic improvement = 20%). Two hepatocarcinomas were hyperfluorescent; the remaining one, with a central hypofluorescent area and a hyperfluorescent ring, was indeed a mixed cholangiohepatocarcinoma. All metastatic nodules were hypofluorescent with a hyperfluorescent rim. In all cases, in vivo and ex vivo fluorescence revealed clear liver margins. Postoperative pathological examination greatly benefited of liver fluorescence to assess radicality. *Conclusion*. ICG-FI-guided surgery was shown to be an effective tool to improve both intraoperative staging and radicality in the surgical treatment of primary and metastatic liver tumors.

Keywords

image-guided surgery, surgical oncology, surgical education

Introduction

The liver can be affected by primary and secondary neoplasms. Among malignant primary tumors, the most frequent are hepatocellular carcinoma (HCC), which occurs in 75% of cases, and cholangiocarcinoma (6% of all cases).[1] HCC ranks among the top 5 causes of death from cancer in males of any age (7%). Hepatitis B and C are the main risk factors; other causes are alcohol abuse, some inherited metabolic diseases (hemochromatosis and α-1-antitrypsin deficiency), and metabolic syndrome (a group of risk factors capable to raise the risk for heart disease, diabetes, and stroke, namely, obesity, high fasting blood sugar, hypertriglyceridemia, low high-density lipoprotein cholesterol levels, and hypertension).[2] Secondary tumors are represented, above all, by metastases of gastrointestinal origin and also by tumors arising in the breast, uterus and ovary, lung, prostate, and skin.[3] Almost 20% to 25% of colorectal cancer patients harbor distant metastases at the time of diagnosis, and the median overall survival rate does not exceed 2 years. The liver is involved in 80% to 90% of the cases and, in almost 50% of which, is the only site of metastasis.[4]

Liver surgical resection, if feasible, results in a survival benefit for patients with primary or metastatic hepatic tumors.[5] The long-term outcome is directly linked

[1]University of Campania "Luigi Vanvitelli", Naples, Italy

Corresponding Author:
Gennaro Galizia, Division of Surgical Oncology, "Luigi Vanvitelli" University of Campania School of Medicine, Department of Surgical Sciences, c/o II Policlinico, Edificio 17, Via Pansini, 5, 80131 Naples, Italy.
Email: gennaro.galizia@unicampania.it

to complete removal of all tumor cells based on microscopic examination of the margins (defined as "R0" surgical resection). Preoperative conventional imaging using contrast-enhanced ultrasound, computed tomography scan and/or magnetic resonance imaging, and metabolic imaging using FDG-PET/CT (FDG positron emission tomography/computed tomography) are currently used for diagnosis of cancer and as an aid to guide its resection.[6] However, translation of imaging findings to the operating field is extremely difficult for oncological surgeons. Visual appearance, palpation, and intraoperative ultrasonography findings are the only available means for discriminating between tumor and normal tissue and, consequently, for determining whether or not an adequate tumor-free margin has been obtained during surgery.[7,8] Moreover, intraoperative histopathological analysis of frozen tumor margins is expensive, time consuming, and may be inadequate in the presence of large lesions.[9] In addition, identification of small and/or superficial lesions may be problematic, leading to high postoperative recurrence rates.[3,10,11]

During surgery, hepatic tumors can be identified by near-infrared (NIR) fluorescence imaging (FI) through the visualization of substances that are retained in the liver after intravenous injection.[12-14] Indocyanine green (ICG) is a water-soluble anionic probe with excitation and emission wavelengths in serum at 778 and 830 nm, respectively.[15] ICG is normally used for liver function assessment.[16] Following intravenous injection, ICG binds to plasma proteins and is then rapidly taken up by hepatocytes, thus quickly disappearing from the bloodstream (half-life ~3-4 minutes).[17] ICG is secreted unmetabolized into the bile, although in some instances, it may accumulate in particular areas making them detectable through their acquired fluorescence.[14,18] This feature, combined with its rapid clearance from the circulation, makes ICG a potentially good probe for the NIR-FI of solid tumors, including liver tumors.[19-21] In addition, it could be useful to detect superficial or subcapsular liver tumors that frequently go undiagnosed by conventional imaging.[22,23]

The aims of this study were thus to explore the methodology of ICG-FI-guided surgery and to assess its potential applications in the surgical treatment of primary and metastatic liver tumors.

Materials and Methods

Patient Cohort

All patients with primary or metastatic liver tumors suitable for hepatic resection, observed at our institution from December 1, 2016, to February 28, 2017, were initially eligible for this study. Exclusion criteria for liver surgery were the following: age older than 80 years, poor liver function (Child-Pugh score B/C and model for end-stage liver disease score >15), insufficient estimated remnant liver volume after radical resection, extrahepatic metastases, and presence of severe comorbidities with performance status >1. Further exclusion criteria were contraindications to the use of ICG: estimated glomerular filtration rate <55 mL/min, pregnancy, breastfeeding, hyperthyroidism, or allergy to iodine, shellfish, or ICG.

There were 5 patients with a primary liver tumor and 9 patients with liver metastases from colorectal cancer liver metastases. Two patients with primary liver cancer were excluded from surgical treatment because of diffuse intrahepatic tumor invasion (1 patient) or poor hepatic function (1 patient). In metastatic patients, 3 cases were not operated on because of advanced intrahepatic tumor diffusion (2 cases) or poor general condition (1 case). Ultimately, 9 patients (3 primary and 6 metastatic tumors) underwent surgery; in all cases, no contraindications to the use of ICG were recorded. Liver tumor behavior was assessed through conventional and metabolic imaging, complemented by serum levels of α-fetoprotein and carbohydrate antigen 19-9 in cases of primary liver cancer. No preoperative liver biopsy was performed in these patients. Out of 6 metastatic patients, 4 had previously undergone resection of their colorectal cancer 14 to 26 months earlier, while 2 patients had synchronous hepatic metastases. After resection of the primary bowel tumor, all patients received 5-fluorouracil + oxaliplatin-based adjuvant chemotherapy and were followed-up at 3 monthly intervals until tumor recurrence. The Ethics Committee of the "Luigi Vanvitelli" University of Campania approved the use of ICG, and all patients gave written informed consent.

ICG-FI-Guided Surgery

In all patients, ICG (PULSION Medical Systems SE, FeldKirchen, Germany) at a dose of 0.5 mg/kg was injected intravenously 24 hours before surgery. No patient underwent ICG_{15} retention test. The patients with a primary liver tumor underwent a bisubcostal laparotomy; in metastatic patients, a median laparotomy with right costal incision was preferred. The liver was exposed as usual, and the organ was carefully checked by visual inspection, manual palpation, and intraoperative ultrasound. Fluorescent images of the primary and metastatic liver tumors as well as the semiquantization of fluorescent signals were obtained using a commercially available FI system, namely, Fluobeam (Fluoptics Imaging Inc, Cambridge, MA). Fluobeam is a class IIa CE-marked medical device. It allows the visualization of fluorescent moiety with a maximum emission between 760 and 850 nm for an excitation wavelength of 750 nm. Fluobeam includes a class 1 laser source that is safe under all

Table 1. Clinicopathological Characteristics.

Patient Number	Age (Years)	Sex	Preoperative Diagnosis	Number of Lesions: Conventional Procedures, n (Ø mm)	Number of Lesions: ICG-FI, n (Ø mm)	Operation Time (Minutes)	Time ICG-FI (Minutes)	ICG-FI Findings
1	64	Male	Metastasis	1 (45)	1 (45)	190	20	Hypofluorescent
2	72	Male	HCC	1 (40)	1 (40)	200	15	Hyperfluorescent
3	65	Male	Metastasis	1 (30)	1 (30)	170	20	Hypofluorescent
4	65	Female	HCC[a]	1 (60)	1 (60)	220	20	Mixed cholangio-hepatocarcinoma
5	65	Female	Metastasis	1 (25)	1 (25)	150	15	Hypofluorescent
6	70	Female	Metastasis	1 (40)	2 (10-40)	210	30	Hypofluorescent
7	64	Female	Metastasis	2 (20-30)	2 (20-30)	240	25	Hypofluorescent
8	79	Female	HCC	1 (35)	2 (7-35)	230	30	Hyperfluorescent
9	70	Male	Metastasis	1 (30)	1 (30)	180	10	Hypofluorescent
			Total nodules	10 nodules	12 nodules			

Abbreviations: ICG-FI, indocyanine green-fluorescence imaging; HCC, hepatocellular carcinoma.
[a]At postoperative pathology the nodule was a mixed cholangio-hepatocarcinoma.

conditions of normal use. The laser light is conveyed from the control box to the optical head via an optical fiber. Two laser beams are emitted from the optical head shedding light on an elliptic area of 15 × 10 cm at distance of 20 cm from the optical head. The Fluobeam allows visualizing, on a computer screen, the accumulation of NIR fluorescent agents in lesions located up to 8 mm from liver surface.[24] Hepatic transection was guided by ICG-FI to obtain free tumor margins; after resection, the hepatic surgical margins were checked again to evaluate residual fluorescence. In the operating room immediately after liver resection, all specimens were observed ex vivo on the benchtop with Fluobeam to confirm their previous appearance and to investigate the margins of the resected tissue. Finally, postoperative pathological examination was performed using optical and NIR confocal microscopy, along with conventional methods, to assess fluorescence behavior and surgical radicality.

Results

The clinicopathological characteristics are shown in Table 1. Five patients were females. The median age was 65 years (range = 64-79 years; interquartile range [IQR] = 64-70 years). All operations were successful and no complications were recorded; the postoperative course was uneventful and all patients were discharged between the 7th and the 14th postoperative days. The operation time ranged from 150 to 240 minutes (median = 200 minutes; IQR = 180-220 minutes). ICG-FI took a median time of 20 minutes (range = 10-30 minutes; IQR = 15-25 minutes); all images collected by Fluobeam were converted into pictures and videos. Since 1 patient presented with 2 hepatic metastases, the total number of lesions diagnosed with current pre- and intraoperative diagnostic procedures was 10. However, ICG-FI identified 2 additional subcapsular hepatic nodules with a diameter of 7 and 10 mm, respectively. Eventually, a total of 12 lesions were detected (diameter ranging from 7 to 60 mm; median = 35 mm; IQR = 25-45 mm). On Fluobeam examination, all livers, including cirrhotic livers, showed a diffuse and homogenous slight fluorescence. In the 3 patients with primary liver tumors, Fluobeam examination revealed intense and homogenous fluorescent (hyperfluorescent) nodules in 2 patients, one of whom had also a small (7 mm) subcapsular undiagnosed nodule in a different segment (Figure 1A). In the remaining patients, ICG-FI identified a large nodule (60 mm) with central hypofluorescent areas and a thin hyperfluorescent ring surrounding the lesion (Figure 2A). This nodule was radically resected; on postoperative pathological examination, it was shown to be a mixed neoplasm with central areas of cholangiocarcinoma surrounded by a peripheral ring of hepatocellular tumor cells. On the contrary, all metastatic lesions appeared as hypofluorescent nodules with an intense fluorescent rim; in 1 patient, ICG-FI identified a further small and subcapsular nodule, which was demonstrated to be a metastatic lesion on postoperative pathological examination (Figure 3).

According to the fluorescence detected by the NIR camera, the surgeon was able to make real-time decisions as to how to modify the cut surface away from the tumor in order to perform a complete surgical resection. In all cases, postresection Fluobeam examination of the hepatic cross-section displayed clear margins (Figure 1B). On the benchtop, a complete correspondence between in vivo and ex vivo observations was found; furthermore, for each specimen, a boundary of normal tissue around each

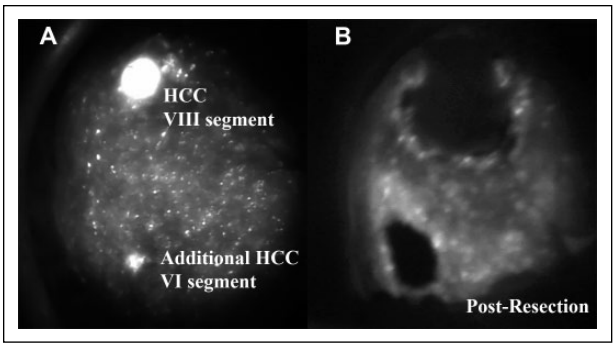

Figure 1. Intraoperative indocyanine green-fluorescence imaging (ICG-FI) in a hepatocellular carcinoma (HCC) patient (case number 8). (A) Before hepatic resection. Twenty-four hours after the intravenous injection of ICG 0.5 mg/kg, the liver showed a diffuse and homogenous slight fluorescence. The already diagnosed HCC in the VIII segment is hyperfluorescent. A small, subcapsular nodule undetectable even with manual palpation was detected in the VI segment. (B) After ICG-FI-guided hepatic resection. Complete absence of fluorescence around the hepatic margins.

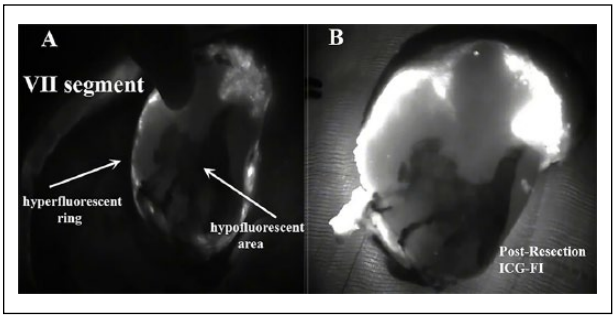

Figure 2. Mixed cholangio-hepatocarcinoma preoperatively diagnosed as hepatocellular carcinoma (HCC; case number 4). (A) Intraoperative indocyanine green-fluorescence imaging (ICG-FI). A large nodule in the VII segment with a central hypofluorescent area and a hyperfluorescent ring is visible. (B) ICG-FI on the benchtop. Same aspect as the intraoperative one. Beyond the fluorescent ring, the hypofluorescent liver (clear hepatic margins) is clearly detectable.

lesion was identified (Figures 2B and 4). On postoperative pathological examination, all surgical resections could be shown to be potentially radical with free hepatic margins. All analyses confirmed that ICG fluorescence was retained in the cancer cells of HCCs and in the noncancerous liver cells surrounding the metastatic nodules. Although tumor fluorescence was low, its presence was a remarkable tool to evaluate surgical radicality. In primary liver tumors, ICG-FI allowed to establish a clear demarcation from peripheral noncancerous tissue; instead, in metastatic nodules, a fluorescent ring confirmed complete removal of the lesion.

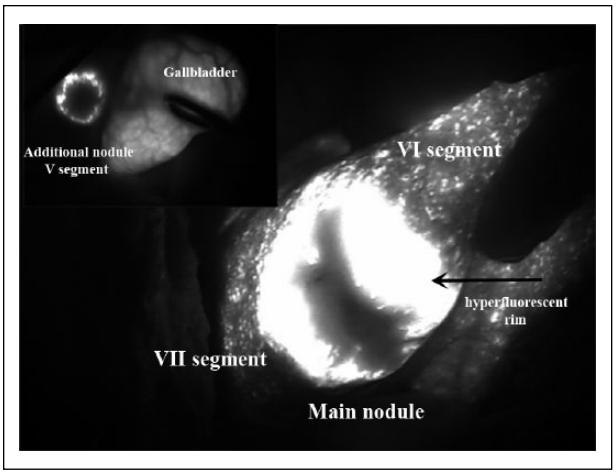

Figure 3. Intraoperative indocyanine green-fluorescence imaging (ICG-FI) in patients with a metastatic hepatic lesion from colorectal cancer (case number 6). ICG-FI detected the already known lesion in the VII segment as a hypofluorescent nodule with a hyperfluorescent rim. In cartouche: An additional undetected subcapsular nodule was identified by ICG-FI on the right side of the gallbladder (V segment).

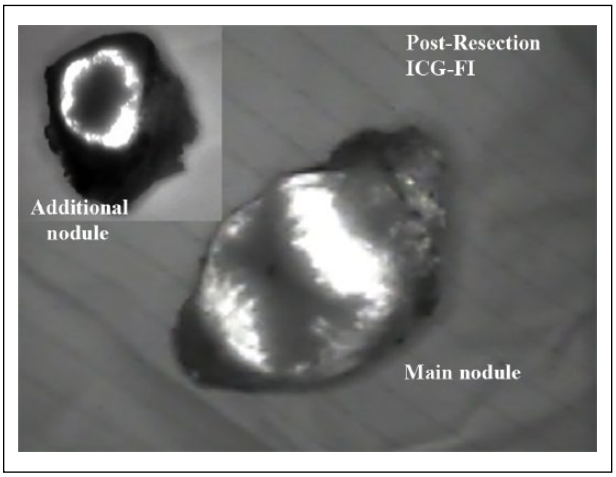

Figure 4. Same case as Figure 3 showing indocyanine green-fluorescence imaging (ICG-FI) on the benchtop after hepatic resection. Both metastatic lesions (main nodule of the VII segment and an additional nodule of the V segment–*cartouche*) had the same aspect as the one observed intraoperatively, with a hypofluorescent ring around the hyperfluorescent rim (clear hepatic margins).

Discussion

This study shows that ICG-FI correctly identified primary and metastatic liver tumors previously diagnosed with current conventional methods. In addition, ICG-FI was demonstrated to be a promising and useful intraoperative tool to detect additional small, superficial, and

subcapsular tumor nodules that have gone unrecognized by other diagnostic tools. Moreover, this procedure seemed to be particularly useful during liver transection in order to obtain free surgical margins and may constitute a remarkable help to assess radicality both immediately after resection and during pathological analysis.

Surgical resection of primary and metastatic liver tumors has been demonstrated to be the cornerstone of treatment along with other combined therapies, such as ablation and systemic chemotherapy. However, intra- and extrahepatic recurrence rates remain high, even after potentially curative surgery, with disappointing long-term outcome.[10,25-28] It has been suggested that the presence of undetected lesions during liver surgery could lead to disease underestimation, particularly for small and subcapsular lesions that are very difficult to detect.[29] Therefore, improvements in pre- and intraoperative diagnostic tools are believed to be a crucial issue.

The fluorophores are substances that, under light excitation, emit fluorescence in the NIR light spectrum ranging from about 700 to 850 nm. This range has the advantage of avoiding the absorption of light by hemoglobin in the visible light spectrum (<600 nm) and other molecules in the infrared spectrum (> 900 nm), thus offering a better contrast. The detection of tumoral tissue depends on the tumor-to-background ratio, which is the ratio between the fluorescence intensity of the tumoral tissue and the surrounding normal tissue.[30] Several fluorescent agents have been investigated in animal models, but only a few have reached the clinic, and none has obtained Food and Drug Administration approval. On the contrary, ICG is a registered and Food and Drug Administration–approved fluorescent agent for optical imaging, and is a safe and inexpensive NIR fluorescent probe. Because of its rapid blood clearance, ICG is normally used for liver function assessment and for visualization of the biliary tree.[16] However, thanks to the serendipitous experiences of Ishizawa et al, ICG has been noted to be retained in primary and metastatic liver tumors after preoperative intravenous injection.[12] Therefore, ICG has emerged as a potentially useful tool for intraoperative detection and evaluation of hepatic neoplasms.[13,14,18] However, experiences with NIR ICG-FI are still limited and mechanisms underlying the accumulation of this fluorescent agent have not been clearly elucidated.[14,30]

In HCC, neoplastic hepatocytes can take up ICG but are unable to eliminate the fluorescent probe because of biliary excretion disorders at their biliary pole, as demonstrated by evidence of fluorescence in the canalicular side of cancer cells by fluorescent microscopy. Therefore, unlike noncancerous tissue, tumoral pseudoglands tend to accumulate ICG and, as a result, HCC is characterized by a strong staining.[12,14,18] Another possible explanation for hyperfluorescence is represented by the enhanced permeability effect. Abnormally permeable tumoral vessels are thought to underlie the enhanced permeability effect, with consequent ICG passage and accumulation in the extravascular space, leading to tumoral tissue hyperfluorescence as compared with surrounding normal tissues.[31] There is now sufficient evidence for HCC to behave as a hyperfluorescent nodule on ICG-FI, although poorly differentiated HCCs may not accumulate ICG, thus appearing as hypofluorescent areas.[13,14] In our series, both HCCs displayed an intense fluorescence; since none of them was poorly differentiated, we cannot add our contribution to this issue. To date, the role of ICG-FI in cholangiocarcinoma has been described in very few cases.[7,18,20] In contrast with some authors,[20] and in agreement with others,[7,18] our case showed a hypofluorescent pattern. Indeed, hepatocytes accumulating ICG are usually absent in this tumor; thus, cholangiocarcinoma appears as a hypofluorescent area surrounded by a hyperfluorescent ring. This latter has been attributed to compressed thin biliary ducts containing ICG, with concomitant intrahepatic cholestasis produced by the tumor itself. Interestingly, our case showed a central hypofluorescent area (constituted by neoplastic biliary cells) surrounded by a thin hyperfluorescent ring represented by HCC without areas of cholestasis. To the best of our knowledge, this is the first time that the ICG-FI pattern of a mixed cholangio-hepatocarcinoma has been reported. Like cholangiocarcinoma, metastatic liver tumors also appear as hypofluorescent nodules because they do not accumulate ICG.[14,20,21,24,30] The characteristic hyperfluorescent rim surrounding the tumor is believed to be constituted by compressed hepatocytes, with increased ductular transformation and severe disorders of bile excretion.[32]

Timing of injection and optimal dosage of ICG are important issues for standardization of the technique. Some authors have suggested to inject ICG before surgery, and time interval ranged from 1 to 14 days, while others proposed an intraoperative injection.[7,8,18] In agreement with the majority of authors, we preferred to inject ICG 24 hours before surgery, in order to consistently reduce physiological hepatic uptake and to allow the drug to concentrate in the tumor.[21,30,33] Accordingly, 0.5 mg/kg represents the most commonly used dose.

In our experience, ICG-FI identified all previously known hepatic lesions and detected 2 further small subcapsular tumors undiagnosed by conventional procedures, with a resulting 20% diagnostic improvement. This finding confirms the utility of ICG-FI for more accurate disease staging. However, in our present and previous experiences, with the technology currently available, the total sensitivity of ICG-FI still remains fairly limited because lesions at

greater than 8 mm of liver depth may escape detection.[14,30] On the other hand, ICG-FI confers the surgeon the remarkable advantages to guide hepatic resection in a real-time fashion and to immediately evaluate surgical margins as a benchtop system.[8,9,34] In addition, previous experiences have already demonstrated that ICG-FI-guided surgery during laparoscopic approach to several hepatic tumors may improve staging and oncologic radicality, and avoid unnecessary laparotomies.[8,22,35,36] Finally, ICG-FI may also contribute to improvements in pathological examination because identification of fluorescent regions before microscopic evaluation would allow focused attention to highly suspicious areas.[8,37]

This study presents some weakness. The limited sample size of patients and conflicting results with previous studies require caution. However, ICG-FI-guided surgery seems a promising and effective tool to improve intraoperative staging and radicality in the surgical treatment of primary and metastatic liver tumors. Further studies are needed to standardize the technique and to determine its role in this patient population.

Author Contributions

Study concept and design: Eva Lieto, Michele Orditura, Gennaro Galizia
Acquisition of data: Annamaria Auricchio, Francesca Cardella, Andrea Mabilia, Nicoletta Basile, Paolo Castellano
Analysis and interpretation: Eva Lieto, Paolo Castellano, Michele Orditura, Gennaro Galizia
Study supervision: Gennaro Galizia

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Supplementary Materials

Supplementary materials are in the database of our department and can be requested by writing to the following e-mail address: dip.scienzecardiotoraciche@unicampania.it.

ORCID iDs

Gennaro Galizia http://orcid.org/0000-0002-6897-0391
Annamaria Auricchio http://orcid.org/0000-0002-7548-2126

References

1. Waghray A, Murali AR, Menon KNV. Hepatocellular carcinoma: from diagnosis to treatment. *World J Hepatol*. 2015;7:1020-1029.
2. Cholankeril G, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma in non-alcoholic steatohepatitis: current knowledge and implications for management. *World J Hepatol*. 2017;9:533-543.
3. Ryerson AB, Eheman CR, Altekruse SF, et al. Annual report to the nation on the status of cancer, 1975-2012, featuring the increasing incidence of liver cancer. *Cancer*. 2016;122:1312-1337.
4. Galizia G, De Vita F, Lieto E, et al. Conversion chemotherapy followed by hepatic resection in colorectal cancer with initially unresectable liver-limited metastases. *Oncol Rep*. 2013;30:2992-2998.
5. Choti MA, Sitzmann JV, Tiburi MF, et al. Trends in long-term survival following liver resection for hepatic colorectal metastases. *Ann Surg*. 2002;235:759-766.
6. Nowicki TK, Markiet K, Szurowska E. Diagnostic imaging of hepatocellular carcinoma—a pictorial essay. *Curr Med Imaging Rev*. 2017;13:140-153.
7. Harada N, Ishizawa T, Muraoka A, et al. Fluorescence navigation hepatectomy by visualization of localized cholestasis from bile duct tumor infiltration. *J Am Coll Surg*. 2010;210:e2-e6. doi:10.1016/j.jamcollsurg.2010.02.052.
8. Takahashi H, Zaidi N, Berber F. An initial report on the intraoperative use of indocyanine green fluorescence imaging in the surgical management of liver tumors. *J Surg Oncol*. 2016;114:625-629.
9. Tipirneni KE, Warram JM, Moore LS, et al. Oncologic procedures amenable to fluorescence-guided surgery. *Ann Surg*. 2017;266:36-47.
10. Park YK, Song SK, Kim BW, et al. Conditional survival analysis demonstrates that recurrence risk of surgically treated hepatocellular carcinoma evolves with time. *J Gastrointest Surg*. 2017;21:1237-1244. doi:10.1007/s11605-017-3437-7.
11. Kusano M, Honda M, Okabayashi K, et al. Randomized controlled Phase III study comparing hepatic arterial infusion with systemic chemotherapy after curative resection for liver metastasis of colorectal carcinoma: JFMC 29-0003. *J Cancer Res Ther*. 2017;13:84-90.
12. Ishizawa T, Fukushima N, Shibahara J, et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. *Cancer*. 2009;115:2491-2504.
13. Lim C, Vibert E, Azoulay D, et al. Indocyanine green fluorescence imaging in the surgical management of liver cancers: current facts and future implications. *J Visc Surg*. 2014;151:117-124.
14. 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;21:440-448.
15. Gurfinkel M, Thompson AB, Ralston W, et al. Pharmacokinetics of ICG and HPPH-car for the detection of normal and tumor tissue using fluorescence, near-infrared reflectance imaging: a case study. *Photochem Photobiol*. 2000;72:94-102.
16. Sakka SG. Assessing liver function. *Curr Opin Crit Care*. 2007;13:207-214.

17. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol*. 1976;40:575-583.
18. 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;41:257-264.
19. Polom K, Murawa D, Rho YS, Nowaczyk P, Hünerbein M, Murawa P. Current trends and emerging future of indocyanine green usage in surgery and oncology: a literature review. *Cancer*. 2011;117:4812-4822.
20. Bekheit M, Vibert E. Fluorescent-guided liver surgery: Paul Brousse experiences and perspective. In: Dip FD, Ishizawa T, Kokudo N, Rosenthal R, eds. *Fluorescence Imaging for Surgeons: Concepts and Applications*. Cham, Switzerland: Springer International; 2015:117-126.
21. Barabino G, Porcheron J, Cottier M, et al. Improving surgical resection of metastatic liver tumors with near-infrared optical-guide fluorescence imaging. *Surg Innov*. 2016;23:354-359.
22. Kudo H, Ishizawa T, Tani K, et al. Visualization of subcapsular hepatic malignancy by indocyanine green fluorescence imaging during laparoscopic hepatectomy. *Surg Endosc*. 2014;28:2504-2508.
23. Yamamichi T, Oue T, Yonekura T, et al. Clinical application of indocyanine green (ICG) fluorescent imaging of hepatoblastoma. *J Pediatr Surg*. 2015;50:833-836.
24. Handgraaf HJM, Boogerd LSF, Höppener 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;43:1463-1471. doi:10.1016/j.ejso.2017.04.016.
25. Abdalla EK, Vauthey JN, Ellis LM, et al. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg*. 2004;239:818-825.
26. Wei AC, Greig PD, Grant D, Taylor B, Langer B, Gallinger S. Survival after hepatic resection for colorectal metastases: a 10-year experience. *Ann Surg Oncol*. 2006;13:668-676.
27. Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Ann Surg*. 2008;247:125-135.
28. Joliat GR, Allemann P, Labgaa I, Demartines N, Halkic N. Treatment and outcomes of recurrent hepatocellular carcinomas. *Langenbecks Arch Surg*. 2017;402:737-744. doi:10.1007/s00423-017-1582-9.
29. Karanjia ND, Lordan JT, Fawcett WJ, Quiney N, Worthington TR. Survival and recurrence after neoadjuvant chemotherapy and liver resection for colorectal metastases: a ten year study. *Eur J Surg Oncol*. 2009;35:838-843.
30. Liberale G, Bourgeois P, Larsimont D, Moreau M, Donckier V, Ishizawa T. Indocyanine green fluorescence-guided surgery after IV injection in metastatic colorectal cancer: a systematic review. *Eur J Surg Oncol*. 2017;43:1656-1667. doi:10.1016/j.ejso.2017.04.015.
31. Maeda H. The link between infection and cancer: tumor vasculature, free radicals, and drug delivery to tumors via the EPR effect. *Cancer Sci*. 2013;104:779-789.
32. van der Vorst JR, Schaafsma BE, Hutteman M, et al. Near-infrared fluorescence-guided resection of colorectal liver metastases. *Cancer*. 2013;119:3411-3418.
33. Miyashiro I, Miyoshi N, Hiratsuka M, et al. Detection of sentinel node in gastric cancer surgery by indocyanine green fluorescence imaging: comparison with infrared imaging. *Ann Surg Oncol*. 2008;15:1640-1643.
34. Kawaguchi Y, Nomura Y, Nagai M, et al. Liver transection using indocyanine green fluorescence imaging and hepatic vein clamping. *Br J Surg*. 2017;104:898-906.
35. Ishizawa T, Bandai Y, Harada N, et al. Indocyanine green fluorescent imaging of hepatocellular carcinoma during laparoscopic hepatectomy: an initial experience. *Asian J Endoscop Surg*. 2010;3:42-45.
36. Boogerd LSF, Handgraaf HJM, Lam HD, et al. Laparoscopic detection and resection of occult liver tumors of multiple cancer types using real-time near-infrared fluorescence guidance. *Surg Endosc*. 2017;31:952-961.
37. Parrish-Novak J, Holland EC, Olson JM. Image-guided tumor resection. *Cancer J*. 2015;21:206-212.