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RESEARCH ARTICLE

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HEPATOFLUO: A prospective monocentric study assessing the benefits of indocyanine green (ICG) fluorescence for hepatic surgery

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Background and Objectives: Fluorescence imaging using indocyanine green (ICG) is undergoing extensive development. This study aimed to assess the merits of ICG in regard to hepatic surgery.

Methods: Patients with liver lesions that required a resection were eligible. They received an injection of ICG the day before the surgery. Step 1 allowed assessment of use of the medical device under surgical conditions. Steps 2 and 3 assessed the capacity of the MD to detect known tumorous lesions and to spot a predefined area of the liver following injection of ICG into the portal vein (ICGp).

Results: The 1st step allowed for validation of the MD use with three patients. Between 04-2013 and 04-2015, 45 pts were included (40 eligible) in steps 2 and 3. All of the tumorous lesions (95/119) exhibited fluorescence. Four new metastasis were detected in 3 pts, and two missing metastases in 1 pt. False positive were 22%. The maximal depth for detection by fluorescence was 13 mm. Injection of ICGp allowed the corresponding anatomical area to be identified in 16/20 patients.

Conclusion: This study confirmed that intraoperative fluorescence is a helpful and relevant tool for the liver surgeon (NCT 01738217).

KEYWORDS
colorectal metastasis, fluorescence, hepatic surgery, indocyanine green

1 | INTRODUCTION

Fluorescence imaging using indocyanine green (ICG) is currently undergoing extensive development in medicine and surgery. While it appears to be highly suitable for clinical application in plastic surgery, in the treatment of lymphedema or for sentinel lymph node detection,[1] some others applications, as hepatic surgery, are more controversial. Nonetheless, it has several potential advantages, because ICG is taken up by hepatocytes and then fully cleared in an unaltered form by the biliary route without enterohepatic circulation and has a short half-life. It is thus a non-specific tumor marker, allowing detection of intrahepatic lesions.

In 2008, Aoki et al[2] showed the potential relevance of this fluorescence for anatomical marking of the liver by injection of ICG directly into the portal vein (ICGp). In 2009, Ishizawa et al[3] described how it could be used as a tumor marker (for primary or secondary lesions) by injection of the ICG by a peripheral venous route. Since then, there have been several retrospective and inhomogeneous

reports on the use of ICG in liver surgery, with various preoperative imaging. The aim of our study was to assess prospectively the potential benefit of ICG in liver surgery.

2 | METHODS

This prospective monocentric phase I/II study was carried out at a tertiary center. Patients ≥18 years old requiring resection of liver tumor lesions and for whom the OMS score was ≤2 were eligible. Patients who had previously undergone major liver surgery (more than three segments) were ineligible.

The protocol was approved by Ethics committee Lyon Sud-Est IV, and the study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practices. All patients provided written informed consent. The trial was registered with clinicaltrials.gov, number NCT01738217.

The Medical Device (MD) used ICG and Fluobeam® (Fluoptics, Grenoble, France) as an infrared camera. ICG is a fluorescent agent that emits in the near-infrared with an absorption peak at 822 nm. Following intravascular injection, ICG becomes bound to plasma proteins, accumulates in the intravascular space, and is taken up by hepatocytes and then fully cleared in an unaltered form by the biliary route without enterohepatic circulation.

2.1 | Procedures

The study was divided into three steps: the feasibility of MD use was first studied in step 1, and 2 parallel steps were then carried out to assess the abilities of fluorescence imaging in hepatic surgery. Thus, steps 2 and 3 were aimed at determining the ability of the Fluobeam® device to detect tumor lesions, and its ability to detect a predefined anatomical area, respectively.

All patients were evaluated at initial referral and before surgery with chest and abdominal computerized tomography (CT)-scan, gadoxetate disodium-enhanced and diffusion-weighted liver MRI and PET-CT scan. Response to chemotherapy was evaluated by CT-scan every four to six cycles.

All patients received a preoperative intravenous injection of 0.25 mg/kg ICG the day before surgery. Patients in step 3 also received an intraoperative injection of ICG in the portal vein (ICGp): 20 to 40 mL of diluted solution (1/320° = 0.0078 mg/mL) for the first seven patients, 40 to 80 mL of diluted solution (1/320°) for the next six patients, and then 40 to 80 mL of diluted solution (1/160° = 0.0156 mg/mL) for the last seven patients.

The liver tumor burden was evaluated intraoperatively firstly by visual inspection, manual palpation, and ultrasonography, and secondly using the Fluobeam® imaging. For step 3, ICGp was performed either through the liver parenchyma using ultrasound imaging, or extra-hepatically by direct catheterization of the portal vein. Modifications of the operative procedure from the preoperative surgical plan were noted. All removed lesions were analyzed by anatomical pathology.

Patients were followed according to standard practices for 30 days after the surgery. The postoperative complications were graded according to the Dindo classification.[4]

2.2 | Outcome

The primary endpoint for step 1 was a composite of the feasibility criteria based on the ability to detect fluorescence and to explore the mobilized liver.

For step 2, the primary endpoint was determination of the proportion of patients for whom all of the lesions (benign or malignant, superficial/deep) were detected using the MD during surgery or pathological examination, while for step 3 it was the proportion of patients for whom it was possible to detect a specific liver area.

Secondary endpoints included the ability to detect tumor lesions which were not previously known, the extent of modification of the surgical plan, the depth limit for detection of the fluorescence, the number of asepsis failures due to MD use, the ability to administer two doses of ICG to the same patient as one injection before and one during the surgery (for tumor detection and segment targeting, respectively).

2.3 | Statistical analysis

Step 1 was performed according to a "3 + 3" scheme, with inclusion of three patients at first. Three additional patients were included if a failure occurred with any of the first three patients. A failure was defined as the absence of fluorescence detection, or of aseptic conditions, or if it did not meet the needs of surgeon or the nurse.

For steps 2 and 3, the number of patients was calculated using a two-stage Simon's phase II procedure.[5] Success was defined for each patient as detection of all known hepatic lesions (step 2) and by detection of a specific liver area (step 3). Assuming $p_0 = 60\%$ (the highest rate which, if true, would imply that the MD does not warrant further use in the indication) and $p_1 = 85\%$ (the lowest rate that would imply that the MD warrants further use in the indication), a type I error alpha of 0.05 and 80% power, 20 patients had to be enrolled of each step. An interim analysis was performed. The step was considered to be successful if at least 16 successes were observed in both stages.

Statistical analyses were performed with SAS software (version 9.1). The continuous data are presented as median value with range.

2.4 | Role of the funding source

As the sponsor, the institution was responsible for conception and management of the trial, data analysis, and publishing of the results. Funding was provided by Lyon Auvergne Rhône-Alpes cancer research cluster (CLARA).

3 | RESULTS

From April 2013 to April 2015, 48 patients were included in the study. Five patients were excluded from the analysis: one due to vanishing of hepatic lesions following chemotherapy in step 2; three in step 3, for whom a hepatectomy had not been performed, and 1 in step 3 for whom perioperative injection of ICG was not possible due to technical reasons (Figure 1).

3.1 | Patient characteristics

No patient had a prior history of underlying hepatopathy. The main histological subtype was liver metastases from colorectal cancer ($n = 36$) (Table 1).

Among the 40 patients included in steps 2 and 3, the surgical procedure was modified from the preoperative surgical plan for 13 patients (32.5%). For seven patients the modification was related to use of the MD, and lesions were malignant for 3/7 patients. Thus three patients (7.5%) benefit from the MD.

All of the metastatic lesions exhibited a fluorescent halo (Figure 2). The fluorescence was visible intra-operatively for superficial lesions or during anatomical pathology examination for the deepest lesions. The two hepatocellular carcinomas (HCC) were poorly differentiated and exhibited a fluorescent halo.

3.2 | Primary and secondary endpoints

For step 2, the primary objective was achieved for all the 20 patients (100%, CI95% [86.1;-]): all known lesions ($n = 51$) were detected during or following the surgery (i.e. at pathology examination) using the Fluobeam®. Of note, 10 of these lesions were not malignant. Seven malignant lesions could only be detected during the anatomical pathology examination: two were superficial but posterior lesions, and five lesions were at depths greater than 10 mm.

For step 3, the primary objective was achieved for 16/20 patients (80%, CI95% [59.9;]): one segment (2, 3, 6, 7, 8) was detected for eight patients, the right posterior section for three the anterior section for 1, and the right hemiliver for 4. No interference between the pre- and intra-operative injections of ICG was noted.

A total of 119 lesions were fluorescent per and/or post-operatively in the 40 patients included in steps 2 and 3 (Table 2). Among them, 95 lesions were proven malignant. The pathological analysis showed any lesions ($n = 10$) or benign lesions (biliary cyst, biliary hamartoma, angioma, nodular, and focal hyperplasia, sinusoidal dilatation, and peliosis) for the 24 remaining lesions.

The modification of the surgical plan ($n = 3$), consecutive to the MD use, was due to detection of four unknown malignant lesions. Furthermore two hepatic metastases that disappeared in one patient following chemotherapy were spotted by fluorescence.

Among the 119 lesions, 93 (78%) were fluorescent intra-operatively (of which 13 were not previously known). Among these 93 lesions, 73 were malignant (including the four newly detected), corresponding to a false positive rate of 22%. The 93 lesions fluorescent intra-operatively were at an average depth of 1.2 mm, the deepest was at 13 mm. The 26 lesions non-fluorescent intra-operatively were at an average depth of 30.6 mm (Table 3).

There was no mortality. Twenty-five patients (58.1%) had at least one postoperative complication, mostly related to surgery (30% were postoperative anemia, 12% postoperative ileus). Six patients (seven complications) had grade ≥3 postoperative complications. None of the stated complications were related to use of the MD or to the injection of the ICG.

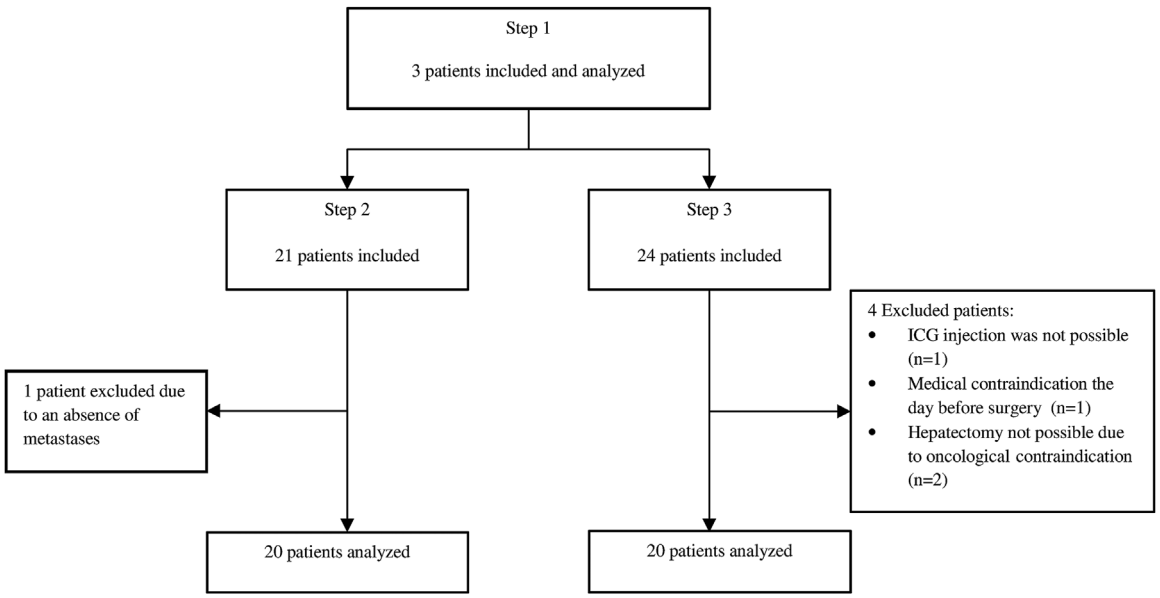

FIGURE 1 Flowchart

TABLE 1 Baseline patient demographics, clinical characteristics

	Step 1 (*N* = 3)	Step 2 (*N* = 20)	Step 3 (*N* = 20)	Total (*N* = 43)
Baseline characteristics				
Age (years)	71.4 [46.0–74.8]	67.0 [38.4–84.1]	64.8 [46.3–81.2]	66.2 [38.4–84.1]
Gender (*n* %)				
Female	1 (33.3)	9 (45.0)	9 (45.0)	19 (44.2)
Male	2 (66.7)	11 (55.0)	11 (55.0)	24 (55.8)
PS (*n* %)				
0	3 (100.0)	16 (80.0)	18 (90.0)	37 (86.0)
1	0 (0.0)	4 (20.0)	2 (10.0)	6 (14.0)
Medical history				
Type of liver lesion (n %)				
Primary	1 (33.3)	2 (10.0)	1 (5.0)	4 (9.3)
Secondary	2 (66.7)	18 (90.0)	19 (95.0)	39 (90.7)
Hepatocellular carcinoma	0 (0.0)	2 (10.0)	0 (0.0)	2 (4.7)
Cholangiocarcinoma	1 (33.3)	0 (0.0)	1 (5.0)	2 (4.7)
Colorectal adenocarcinoma	2 (66.7)	18 (90.0)	16 (80.0)	36 (83.6)
Other (renal cell carcinoma, GIST)	0 (0.0)	0 (0.0)	3 (15.0)	3 (7.0)
Prior liver surgery	2 (66.7)	3 (15.0)	2 (10.0)	7 (16.3)
Prior chemotherapy	2 (66.7)	19 (95.0)	18 (90.0)	39 (90.7)
Median number of courses	29.5 [20.0–39.0]	8.0 [1.0–51.0]	10.0 [4.0–17.0]	9.0 [1.0–51.0]

4 | DISCUSSION

To the best of our knowledge, we report herein the results of the first prospective registered study assessing the use of ICG fluorescence in liver surgery.

Our study showed that all of the cancerous lesions were fluorescent (sensitivity of 100%). This means that if a lesion does not fluoresce, it is obviously not cancerous. It is also important to notice that chemotherapy, administered in 39 patients, did not alter the reliability of the fluorescence imaging.

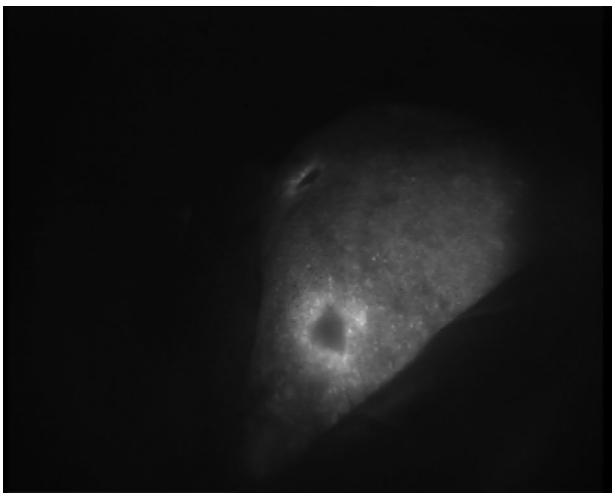

FIGURE 2 Colorectal cancer fluorescence, typical fluorescent halo

The appearance of the fluorescence differs according to the type of lesion.[6] HCCs exhibit entirely homogenous fluorescence with well differentiated lesions and a halo occurs with poorly differentiated lesions. For metastases, the fluorescence appears as a halo. Peritumoral fluorescence may be explained by an accumulation and retention of ICG around the tumorous lesions. Proliferation of the biliary microtubules can be seen around the metastases, and this could explain the accumulation of ICG.[7] At the microscopic level, van der Vorst et al[8] have claimed that the fluorescent signal can be present both in the intracellular section as well as in the extracellular section at the level of the transition area between the tumor and the normal hepatic parenchyma.

On the other hand, the study revealed a false positive rate of 20%. This high rate could be partly explained by the learning curve. Ten out of 24 fluorescent but benign lesions corresponded to point like fluorescent images systematically resected in the first patients. The pathological examination did not find any lesions. The 14 other false-positive lesions were benign tumors and parenchymal alterations of the liver. Ishizawa et al[3] reported five false positives with 26 patients (19%), but this was largely in regard to HCC. Lim et al[6] reported 40% to 50% of false positives with HCC surgery do to dysplastic and/or regeneration nodules. By contrast there was no false positive in the study by van der Vorst et al[8] who analyzed 100 lesions among 40 patients who underwent surgery for colorectal liver metastases, with ICG injection 24-48 h prior to surgery. This author found 25 benign

TABLE 2 Number of known lesions and lesions detected using the MD Fluobeam®

Number of lesions	Known before surgery	Detected during surgery[a]	Only detected by fluorescence during surgery	Only detected by pathological examination[b]	Total
Benign	12	3	9	0	24
Malignant	76	7	4	8	95[c]
Total	88	10	13	8	119
	98 "known lesions"		21 previously unknown lesions		

[a]New lesions detected by visual inspection, manual palpation, and intraoperative ultrasound.
[b]Metastases detected upon macroscopic pathological examination.
[c]All malignant lesions were detected intraoperatively by fluorescence (for superficial lesions) or upon pathological examination (for deep lesions).

lesions among 13 patients without any peritumorous fluorescence. The delay between the preoperative injection and the surgery appears to be key to explaining the number of false positives, due to inadequate wash-out from the noncancerous liver tissue.[9] In our study the injection was done the day before the intervention. It would appear that a delay of at least 24 h is necessary, with the maximal delay that can be considered being 14 days, and the ideal delay being from 48 to 72 h.[9,10] Preoperative assessment by conventional imaging is recognized as being insufficient to detect all metastases, as intraoperative ultrasound allows detection of new metastases in up to 19% of patients, resulting in alteration of the surgical procedure from the initially planned procedure in 12%.[11] This lack of accuracy in preoperative, and probably intra-operative, assessment is likely to explain that more than 50% of patients with colorectal liver metastases will have a recurrence. It is of importance, since 40% of the patients have liver-limited recurrence, accessible to curative-intent treatment. The fluorescence imaging could be complementary to intraoperative ultrasound imaging to improve the detection of new lesions.[12] Peloso et al[13] assessed fluorescence imaging with intraoperative ultrasound, and suggested that combining these methods were of great value for detection of small lesions (less than 3 mm) at a depth of less than 10 mm.

Radiological disappearance of colorectal liver metastases is a major and frequent issue after chemotherapy,[14] and close to half of these patients will suffer from intrahepatic relapses. Fiducial placement for tracking of metastases is feasible for deep lesions rather than for superficial lesion, in which fluorescence might be helpful to detect disappeared metastases. Indeed, the remaining fluorescence could be explained by the properties of ICG, while nearly 80% of the disappeared metastases still contain viable tumor cells.

One aim of our study was to assess the feasibility of anatomical staining of the liver by fluorescence imaging. In 2008, Aoki et al[2] showed that such staining was feasible following ultrasound-guided injection by the intraportal route of 1 ml of ICG (5 mg/ml). Kobayashi et al. demonstrated in 2017 the feasibility of portal vein territory identification using ICG with five types of fluorescence staining techniques.[15] In a preclinical study in pigs (data not shown), we determined the volume and the dilution of the ICGp, the lack of interference between preoperative venous injection of ICG and ICGp and the correlation between anatomical fluorescent staining and ischemic staining. Following this initial experimental assessment, we used a volume of ICG between 20 to 40 mL that was diluted 1/320° (0.0078 mg/mL). In light of non-homogenous fluorescence we changed the volume that was injected (40 to 80 mL) and the dilution (1/160°, or 0.0156 mg/mL). The injection was performed in the portal vein (direct insertion or after using ultrasound) with clamping of the hepatic pedicle. We did not notice interference between the preoperative ICG venous injection and ICGp, thus allowing detection of tumor lesions while also proceeding to anatomical staining of the liver. In 2015, Inoue et al[16]compared this fluorescent staining by ICG to conventional staining (ischemic and indigo carmine), and they were able to show that fluorescent staining was superior, with a more clear demarcation and longer persistence over time, particularly with livers that have an irregular surface. This point is all the more true for livers altered by chemotherapy (blue liver syndrome) or by surgical procedures (repeat hepatectomies) for which the use of blue or ischemic staining might be less reliable and less effective. In 2014, Sakoda et al[17] again showed the feasibility and the relevance of this staining method for laparoscopic hepatectomy, with a ultrasound-guided injection of 1 mL of ICG (5 mg/mL) in a portal branch.

The strength of this study lies in its prospective, registered evaluation with rigorous pre and intra-operative staging. This study has some limits as it is a monocentric study and both primary and

TABLE 3 Characteristics of the lesions according to their intra-operative fluorescence

Intraoperative fluorescence	Yes	No	Total
N	93	26	119
Average depth (mm)	1.2 (max = 13)	30.6	7.7
Malign/Benign (*n*, %)	73 (78%)/20 (22%)	22 (85%)/4 (15%)	95 (80%)/24 (20%)
Average size (mm)	17	30	16
Median size (mm)	10 (0–200)	30 (1–70)	9.5 (0–200)

secondary tumors were included. However, we mainly included patients with colorectal liver metastases during steps 2 and 3 (90%).

5 | CONCLUSION

This study confirmed that intraoperative fluorescence is a helpful and relevant tool for the liver surgeon. It can help by identifying anatomical area, especially on altered liver. It might decrease the risk of liver relapse by detecting new metastases especially small and superficial lesions where ultrasound imaging is deficient. Although the benefit in survival will be difficult to be further demonstrated, we recommend the use of fluorescence imaging during liver surgery.

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SYNOPSIS

We assessed the utility of fluorescence during liver surgery in a prospective study. We showed that fluorescence is helpful to guide anatomical resection and to detect additional metastases despite modern preoperative multimodal imaging.