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Fluorescence Imaging After Indocyanine Green Injection for Detection of Peritoneal Metastases in Patients Undergoing Cytoreductive Surgery for Peritoneal Carcinomatosis From Colorectal Cancer

A Pilot Study

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Objective: The aim of this study was to evaluate the role of fluorescence imaging (FI) using an intraoperative injection of free indocyanine green (ICG) in the detection of peritoneal metastases (PM) due to colorectal cancer (CRC).

Background: A large proportion of patients undergoing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy will have local recurrence. This is, in part, related to the presence of small undetected nodules in the peritoneal cavity. Near-infrared FI-guided surgery has provided new opportunities for detection of nonvisible lesions during cancer surgery.

Methods: Patients with PM from CRC admitted for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy were selected for participation in this study (NCT02032485). Free ICG, at 0.25 mg/kg of patient weight, was intravenous (IV)-injected intraoperatively. Tumor-to-background ratio was calculated for all suspect resected PM.

Results: Sixty-three of 78 peritoneal resected nodules in 14 patients were evaluated for fluorescence, among them, 53 were malignant (84%) and 10 benign (16%). Twenty-six were hypofluorescent, 16 moderately hyperfluorescent, and 21 hyperfluorescent. Amongst the 42 nodules of the 9 patients with nonmucinous adenocarcinoma, the mean tumor-to-background ratio was 1.92 (SD 0.67) in malignant and 1.02 (SD 0.06) in benign nodules ($P = 0.0099$). In 4 of 14 patients (29%), the surgery was modified by intraoperative ICG-FI, which detected additional PM not found using visualization and palpation.

Conclusions: This pilot study demonstrates that non-mucinous PM of CRC can be visualized intraoperatively using ICG-FI. Furthermore, ICG-FI findings resulted in modification of the planned surgery in 29% of patients.

Keywords: fluorescence imaging, indocyanine green, peritoneal carcinomatosis, colorectal cancer, peritoneal metastases, detection, intravenous, intraoperative

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Colorectal cancer (CRC) is one of the most common cancers and is a leading cause of cancer-related deaths worldwide.[1] Peritoneal metastases (PM) occur in 30% to 40% of patients with CRC, representing the only site of metastasis and the principal cause of death in approximately 25% of cases.[2,3] Currently, cytoreductive surgery (CS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC) is the only potentially curative option in patients with limited PM. This treatment results in 5-year overall survival rates ranging from 40% to 70%, comparable to results obtained in patients surgically treated for liver metastases (LM).[4–6] In patients undergoing CS for PM from a CRC origin, the radicality of surgical resection is a major prognostic factor, because incomplete resection provides no clear benefit as compared with systemic treatments.[3,7] Currently, preoperative imaging, either morphologic or metabolic, is not sensitive enough to accurately predict the extent of peritoneal disease and to guide surgery.[8] Accordingly, the completeness of CS essentially relies on the surgeon's ability to perform intraoperative staging using visual detection and palpation of the entire peritoneal surface. Therefore, in the current setting where CS is limited to clinically detectable peritoneal nodules, recurrence from unresected subclinical disease is thought to represent a significant cause of failure of the procedure.

Intraoperative fluorescence imaging (FI)-guided surgery is an emerging technology that has been shown to improve tumor detection in different oncological conditions.[9,10] Among the different probes, which have been used, indocyanine green (ICG) has been shown to be safe and useful for identifying different tumors, such as hepatocellular carcinoma and LM of CRC origin.[11,12] In these conditions, ICG-FI was able to sensitively detect small subclinical liver tumors that were not identified at surgical exploration under standard white light. Additionally, ICG-FI helped to guide the

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surgery and the establishment of resection margins by improving the visual delineation between normal and cancer tissues.[13]

On these bases, we hypothesized that FI using ICG could improve the detection of PM and the assignment of safe resection margins in patients undergoing CS for peritoneal cancer (PC) of CRC origin. We report here our preliminary results on the use of FI after intraoperative IV ICG injection during CS and HIPEC in patients with PM of CRC origin.

PATIENTS AND METHODS

Study and Patients

The present interventional and diagnostic study was approved by the Investigational Review Board of the Institut Jules Bordet (CE2008), and registered at the Clinical Trials.gov Protocol Registration System (NCT02032485; https://register.clinicaltrials.gov). All patients gave written informed consent before inclusion.

The primary objective of the study was to evaluate whether the ICG-FI technique is able to detect PM in patients undergoing CS for PC. The secondary objective was to evaluate the sensitivity of ICG-FI to detect additional subclinical PM not identified during intraoperative exploration under white light. Patients with PC of colorectal origin who were candidates for CS and HIPEC were approved for the study. All patients underwent routine work-up to exclude extra-abdominal metastases. Exclusion criteria included: age <18 years, inability to give informed consent, hyperthyroidism, documented coronary disease, significant renal failure, history of allergy, pregnancy, breastfeeding, and extensive PC.

Surgery

All patients underwent an explorative laparotomy by xypho-pubic incision to evaluate the extent and resectability of PC. We systematically measured the peritoneal cancer index (PCI) score after exposure of the entire abdominal cavity. Surgery was performed using standard techniques for peritoneal resection surgery.[14,15]

Indocyanine Green Injection Modalities and Intraoperative Indocyanine Green Fluorescence Imaging (Clinical Scale)

In the first patient, 0.25 mg/kg of free ICG (Indocyanine Green; Pulsion, Paris, France) were injected IV 24 hours before surgery. In this case, no fluorescence of the peritoneal structures was detected during exploratory surgery. Thereafter, we adapted our protocol and ICG was injected intraoperatively by central venous line after complete exposure of the abdominal cavity and confirmation of the indication for CS. This allowed intraoperative detection of fluorescent PM and was therefore established as standard procedure for this study. As a consequence, the first patient was excluded for further analyses. ICG-FI was performed using a dedicated near-infrared (NIR) camera system (Photodynamic Eye, PDE; Hamamatsu Photonics, Hamamatsu, Japan). In the operating room and for intraoperative imaging, the camera unit of the device was covered with a sterilized transmissive cover and the camera was held directly by the surgeon. ICG redosing was not performed in this study.

In the first step, the abdominal cavity was systematically explored for detection of macroscopic peritoneal nodules using visual inspection and palpation. After ICG injection, these clinically identified nodules were specifically explored for fluorescence then excised and selectively collected for pathological analysis. In the next step, the entire peritoneal surface was explored with the PDE camera for detection of additional subclinical peritoneal nodules undetected by palpation or direct vision. When detected, these nodules were separately excised and sent for pathological analysis, together with surrounding tissue whenever possible, to allow further calculation of fluorescence index compared with the background. Nodules were classified according to their fluorescence intensity: hyperfluorescent nodules were defined as showing an obvious hyperfluorescence compared with surrounding tissue. Hypofluorescent nodules were defined as showing hypofluorescence compared with surrounding tissue. Moderately hyperfluorescent nodules were defined when their fluorescence could be only poorly distinguished from surrounding tissue.

Quantitative Image Analysis of Fluorescence Video Images (Quantitative Scale)

The specific fluorescence of each peritoneal nodule was evaluated according to 2 methods, by reviewing intraoperatively recorded videos (in vivo calculation) and by reviewing videos recorded on the back table on the freshly resected operative specimens (ex vivo calculation). For each specimen, the fluorescence ratio between identified nodules and background tissue was evaluated. For this calculation, regions of interest (ROIs) were delineated over the fluorescent nodules and on the adjacent background peritoneum. For each ROI, fluorescence intensity expressed in arbitrary units was measured with the IC-Calc 2.0 program (Pulsion Medical System), allowing calculation of TBR.

All specimens were imaged under standard conditions with the PDE camera by the same person. These images were correlated with intraoperative findings and with anatomo–pathological reports. For patients with extensive peritoneal disease (PCI score >17), we performed excision of the most representative tumoral nodules as it was impossible to explore, excise, and analyze all of the peritoneal nodules. These patients were excluded for sensitivity and specificity calculation.

Statistical Analyses

Fluorescence intensity was assessed with a clinical qualitative scale and a quantitative scale (TBR). TBR of the peritoneal nodules were analyzed as a continuous and categorical variable using cut-off 1.3 ($<1.3/\geq 1.3$). The cut-off of 1.3 was determined to have the best agreement between the clinical and the quantitative scale. The size of the nodules was analyzed as a continuous and categorical variable using 10 mm as cut-off (≤ 10 mm/>10 mm). As the unit of analysis is the nodule and not the patient, and the data are not independent, we used a mixed linear model to test our hypothesis (for continuous variables) and a generalized estimating equations (GEE) model (for categorical variables) to take into account the correlation structure within the patient. In the mixed linear model, compound symmetry was used as a type of covariance matrix. To have a symmetrical distribution of dependent variables, we used a log transformation of the ratio of the fluorescence. In the GEE analyses, empirical instead of model-based standard errors were used because they are more robust against misspecification of the correlation structure. An exchangeable covariance matrix was used. Odds ratios are presented with their 95% confidence intervals. Fluorescence was analyzed in univariate and multivariate GEE (adjustment for the nodule size). Cohen Kappa test was used to assess the agreement between the clinical scale and the TBR scale.

RESULTS

Patients

From September 2013 to November 2014, we obtained informed consent from 17 consecutive patients to participate in the study (see Supplemental Digital Content 1_ Figure, http://links.lww.com/SLA/A948). Three patients were excluded for different reasons.

TABLE 1. Characteristics of Evaluable Patients (n = 14)

Patient age, mean (range)	59.4 (39–70) y
Male	6
Female	8
Location of primary tumor	
Appendix	2
Right colon	6
Transverse colon	1
Left colon	2
Sigmoid colon	2
Rectum	1
Peritoneal carcinomatosis	
Synchronous	6
Metachronous	8
PCI score	
Mean	9.6
Median	8
Range	2–24
AJCC T and N	
T0N0*	1
T4a/b N0	2
T2N1	1
T4aN1	3
T3N2	4
T4aN2	3
Neoadjuvant chemotherapy	
yes	2
no	12
Pathological diagnosis availability before FI	
yes	12
no	2

AJCC T and N indicates American Joint Committee on Cancer Tumor and Node.
*Low grade appendiceal mucinous neoplasm.

Clinical and pathological characteristics of the 14 evaluated patients are detailed in Table 1. There were 8 females and 6 males, with a median age of 59.5 years (range: 39–70). Nine patients presented well- or moderately differentiated CRC and 5 had mucinous tumors, including 3 colorectal and 2 of appendix origin. Two patients received neoadjuvant chemotherapy (2 and 8 courses of Folinic Acid, 5-Fluorouracyl and Irinotecan (FOLFIRI)). The majority of patients had limited peritoneal disease with a mean PCI score of 9.6 (range: 2–24), 3 of them had a PCI score >17. In these 3 patients, only a limited number of surgical specimens were removed for pathological analysis and they were further excluded for the analysis of the sensitivity and specificity of the TBR.

Fluorescence Imaging in Patients With Peritoneal Metastases From Nonmucinous and Mucinous Colorectal Cancer

In the 14 patients with non-mucinous and mucinous CRC, a total of 78 peritoneal nodules were resected and analyzed by histopathology. Sixty-three of them were evaluated for their fluorescence (81%). Fifteen nodules could not be evaluated for their fluorescence intensity due to insufficient normal tissue around the nodule impairing TBR calculation (n = 3), impossibility to measure fluorescence due to the proximity of high fluorescent background from the liver (saturation) (n = 4), absence of residual fluorescence due to the delay (>6 hours) between ICG injection and nodule resection (n = 4), and excision of the nodules for extemporaneous pathological confirmation before ICG injection (n = 4). Among the 63 evaluable peritoneal nodules, 53 were malignant (84%) and 10 benign (16%). The duration of intraoperative imaging in which we detect hyperfluorescence of PM varied from 5 to 40 minutes after ICG administration. However, it does not mean that nodules were no longer hyperfluorescent.

Visual Evaluation of Intraoperative Fluorescence Imaging (Clinical Scale) and Correlation With Pathology

Intraoperative data for nodule fluorescence during intraoperative exploration are reported in Table 2. Among the 63 peritoneal nodules evaluated, 26 were hypofluorescent, 16 moderately hyperfluorescent, and 21 hyperfluorescent. Among the 26 hypofluorescent nodules, 9 were benign and 17 were malignant, all these were later determined to be of mucinous tumor origin. Fifteen out of the 16 moderately hyperfluorescent nodules were malignant, 4 of them from mucinous tumors and 11 from nonmucinous adenocarcinoma. All of the 21 hyperfluorescent nodules were malignant, all from nonmucinous adenocarcinoma.

Pattern of Fluorescence Imaging in Patients With Peritoneal Metastases From Nonmucinous Colorectal Cancer

Among the 42 nodules of the 9 patients with nonmucinous adenocarcinoma, the mean TBR was 1.92 (SD 0.67) in malignant nodules and 1.02 (SD 0.06) in benign nodules ($P = 0.0099$).

Ex vivo images obtained of PM from nonmucinous CRC showed a homogenous hyperfluorescent pattern (Fig. 1A–B). After adjustment of the ROI for in vivo versus ex vivo samples, the *P* value was still significant ($P = 0.002$). Of note, the sizes of malignant and benign nodules were not significantly different, 14.1 mm (SD 10.7) and 8.3 mm (SD 4.7), respectively ($P = 0.16$).

Pattern of Fluorescence Imaging in Patients With Peritoneal Metastases From Mucinous Colorectal Cancer

Among the 22 nodules of the 5 patients with mucinous CRC, the mean TBR was 0.98 (SD 0.23) in malignant nodules (n = 21) and the TBR for the only benign nodule was 0.73. Mucinous malignant nodules seemed clearly hypofluorescent at FI (Fig. 2A–B). When FI was performed ex vivo on the back table after resection of the nodule, a specific distribution of the fluorescence was observed, as indicated by a hyperfluorescent peripheral rim and a hypofluorescent center (Fig. 2C–D). These FI findings correlate with pathological images showing the presence of cancer cells at the periphery while mucin accumulates in the center of the tumor (Fig. 2E–G).

Sensitivity and Specificity of Fluorescence Imaging

To compute the intrinsic characteristics of the TBR test, we excluded the 3 patients with a PCI ≥ 17 for which only a minority of

TABLE 2. Intraoperative Near-infrared imaging Fluorescence Evaluated by Visualization

		Hypofluorescent	Moderately Hyperfluorescent	Clearly Hyperfluorescent
Benign		9	1	0
Malignant				
	Nonmucinous	0	11	21
	Mucinous	17	4	0

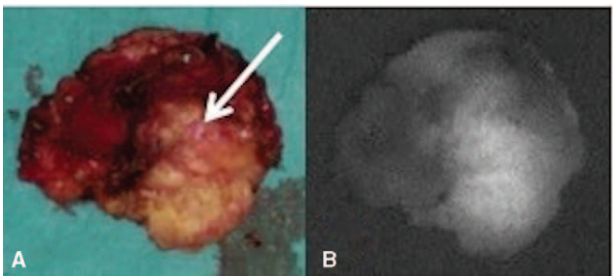

FIGURE 1. Ex vivo images obtained of peritoneal metastase from nonmucinous colorectal cancer in standard white light A and by fluorescence imaging B.

nodules were removed despite the fact that all those nodules were fluorescent. Table 3 shows the crosstabs between fluorescence and nodule status among the 33 nodules left. With a cut-off TBR value of 1.3, sensitivity and specificity in patients with nonmucinous adenocarcinoma were 87.5% (21/24) and 100% (9/9). Three nodules from nonmucinous adenocarcinoma were not hyperfluorescent by semi-quantitative analysis representing false-negative results in 2 patients. One PM located in the omentum and 2 PM located on the peritoneum of the flank were also not hyperfluorescent at clinical evaluation. Among the mucinous tumors, the sensitivity of ICG-FI was 0% with a cut-off TBR value of 1.3.

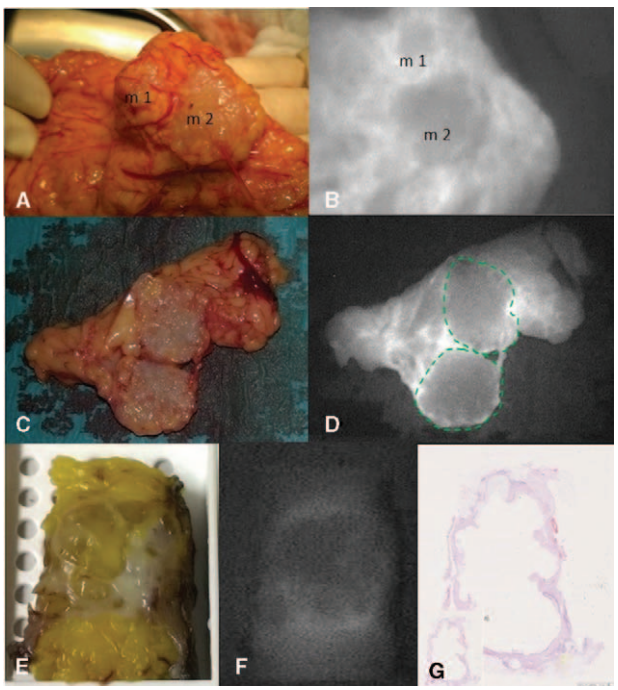

FIGURE 2. Mucinous malignant nodules shown before resection in standard white light A and by fluorescence imaging B, after resection in standard white light C and by fluorescence imaging D. Mucinous nodule in cassette before paraffin embedding under white light E. The same nodule under fluorescence imaging showing a hyperfluorescence at periphery and hypofluorescence in the center F. Pathology image showing the presence of cancer cells at the periphery of the nodule while mucin accumulates in the center E–G.

TABLE 3. Fluorescence (Tumor-to-Background Ratio) by Peritoneal Nodule Status Among Patients With Nonmucinous Adenocarcinoma (n = 33) and Mucinous Adenocarcinoma (n = 13) (PCI score ≤17)

		Peritoneal Nodules Status		
		Malignant	**Benign**	**Total**
Fluorescence	Positive (ratio ≥1.3)	21	0	21
	Negative (ratio <1.3)	3	9	12
Nonmucinous nodules	Total	24	9	33
Fluorescence	Positive (ratio ≥1.3)	0	0	0
	Negative (ratio <1.3)	12	1	13
Mucinous nodules	Total	12	1	13

Additional Metastases Detected Only by Fluorescence Imaging

In 4 out of 14 patients (29%), the surgery was modified by intraoperative IR-FI that allowed the detection of additional PM that were not found using visualization and palpation (Table 4). In 2 patients, this led to an increase of the PCI score by 1 point. In the first patient with a PCI > 17 and 50 clinically detected and clearly hyperfluorescent nodules at intraoperative exploration, 4 additional nodules, undetected under white light, were demonstrated with IR-FI (Fig. 3A). Of note, these hyperfluoresecent nodules remained undetectable by attentive and localized palpation, orientated according to FI detection. In the second patient, an increased uptake was detected on the anterior parietal peritoneum. Attentive surgical exploration, orientated by the FI, showed that it corresponded to a retroperitoneal epigastric nodule. In the third patient, in whom obesity hampered the surgical exploration of the abdominal cavity, FI allowed detection of a hyperfluorescent nodule in the left parietocolic gutter. Attentive surgical exploration, orientated by FI findings, allowed detection of a small palpable nodule, visible under white light (Fig. 3B–C). In the fourth case, FI demonstrated residual hyperfluorescent tissue at the margin of the planned surgery. Therefore, the hyperfluorescent tissue was resected and marked for its hyperfluorescence. This remaining hyperfluorescent area was thought to be related to a macroscopic steatonecrosis aspect due to a previous surgery but was confirmed to be malignant at final histopathological analyses while the suspected resected mass in the surgical specimen was benign.

DISCUSSION

In the treatment of PC from colorectal origin, the efficacy of CS remains dramatically limited by the poor sensitivity of preoperative imaging and the limited accuracy of surgical exploration for identifying small peritoneal metastases. This represents a major obstacle for obtaining optimal results, as incomplete resection invariably leads to peritoneal recurrence. Therefore, the development of new modalities, such as image-guided surgery, to increase the sensitivity of intraoperative detection of small subclinical peritoneal nodules would represent a major advance.

Until now, only a few clinical studies have analyzed the potential role of FI for the staging of PC. FI has been evaluated in PC from ovarian cancer, using folate conjugated to fluorescein isothiocyanate (folate-FITC) for targeting FR-α[16] or ICG,[17,18] but has not been tested in PC from colorectal origin. ICG represents a good candidate for FI in a clinical setting. The ability of ICG to facilitate tumor visualization has been demonstrated in hepatocellular carcinoma and LM of CRC origin.[11,12,19] The underlying mechanism for preferential uptake of ICG in tumor tissues is not

TABLE 4. Characteristics of Peritoneal Metastases Detected Only by Near-infrared Fluorescence Imaging

Obs	Patient	Nodule Location	Fluorescence	Histopathology	Size (mm)
1–4	1	Left parietal peritoneum	Hyper	AC	2
5	2	Right parietal peritoneum	Hyper	AC	6
6	3	Left parietocolic gutter	Hyper	AC	2
7	4	Right inguinal fossae peritoneum (margin resection)	Hyper	AC	NA

AC indicates adenocarcinoma; NA, not available; Obs, observations.

fully understood. It has been hypothesized that ICG accumulates in tissues with leaky capillaries.[20] Due to the fact that it has both lipophilic and hydrophilic properties, ICG is 98% protein-bound in vivo. ICG molecules have been shown to bind to albumin and serum globulins such as alpha1-lipoprotein (a high density lipoprotein).[21] Unlike free ICG, highly protein-bound ICG behaves like a macromolecule in circulation and often demonstrates enhanced permeability and retention effects within tumoral tissue, secondary to immature and leaky tumor vessels that result from tumor-induced angiogenesis.[22] For clinical use, ICG has been demonstrated to be safe and currently, it is the only contrast agent approved for several indications in surgery by the Food and Drug Administration and European Medicines Agency.

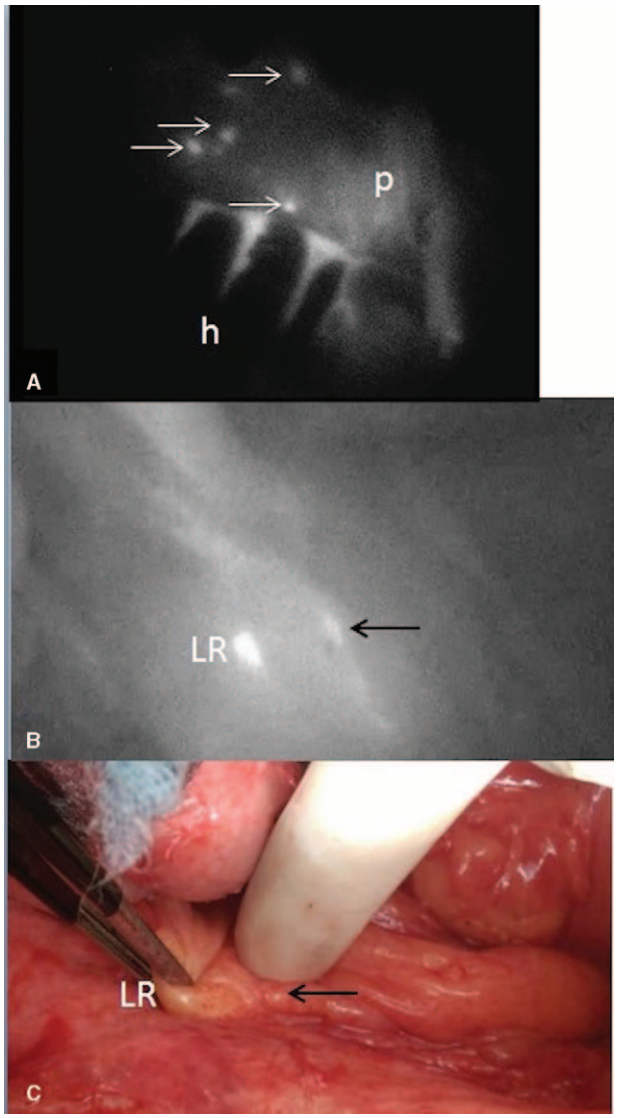

FIGURE 3. Fluorescence imaging of nodules that were undetected under white light or by palpation A. Identification of a nodule by indocyanine green fluorescence imaging in another patient B followed by attentive surgical exploration, orientated by FI findings, that allowed detection of a small palpable nodule, visible under white light C.

The primary objective of our study was to verify whether FI after ICG injection could detect PM that were identified on preoperative imaging and/or during surgical exploration. Although the accumulation of ICG has been reported in the periphery of LM from CRC after ICG IV injection more than 24 hours before surgery,[11,19] this was not verified in our study with PM. PM were not detected when ICG was injected 24 hours before surgery. Accordingly, our approach was modified and ICG injected intraoperatively.

After intraoperative IV ICG injection, FI allowed detection of PM from nonmucinous colorectal adenocarcinoma. This study represents the first proof-of-concept report on the use of FI in this area. For determination of the sensitivity of intraoperative FI, to reduce the potential bias related to subjective visual inspection, we only considered as positive the PM that were markedly hyperfluorescent. Accordingly, in the group of patients with nonmucinous CRC, intraoperative FI was able to detect 87.5% of the PM previously visualized on preoperative imaging and/or at surgical exploration. PM were clinically hyperfluorescent at visual FI exploration and were hyperfluorescent at semiquantitative analysis. Only 3 false-negative results were observed in 2 patients. The FI exploration of the abdominal cavity was limited in several areas where physiological ICG accumulation was high, such as at the liver surface and the visceral peritoneum. This hampered the detection of small PM in regions adjacent to the organs that preferentially accumulate ICG, such as in the liver. This underlines the need for further research designed to precisely define the pharmacokinetic distribution and clearance of ICG and to determine the optimal timing that allows surgeons to distinguish between abnormal ICG accumulation in cancer tissue and physiological ICG retention in nontumor tissues or organs. In our study, intraoperative detection of hyperfluorescent nodules varied from 5 minutes to 40 minutes, but ex vivo FI confirmed that all resected nodules remained hyperfluorescent up to 120 minutes after ICG injection. We did not observe a wash out of tumoral nodules during intraoperative FI. However, we observed that small tumoral nodules that were hyperfluorescent during in vivo FI were found not hyperfluorescent on ex vivo FI probably due to the delay and ICG wash out. Hence, the hyperfluorescence of small nodules during FI suggests an underlying tumoral hypervascularization.

In our experience, FI exploration was also poorly informative in patients with advanced PC. In these cases, the duration of surgery, the difficulty of selectively measuring the fluorescence in regions massively invaded by tumor, and the need for extensive peritoneal resection dramatically limited the contribution of FI. However, the

majority of patients that we evaluated had limited disease, with a median PCI score of 8, and, therefore, the data obtained could serve as validation of the technique. Moreover, it has been recently shown that CS only provides a significant benefit in patients with a PCI < 17,[23] suggesting that ICG-FI may contribute in the particular subgroup of patients with PM that will benefit the most from this treatment.

In contrast with nonmucinous CRC, ICG-based FI was poorly sensitive for detection of PM in patients with mucinous tumors. In our series, the sensitivity of ICG-FI for the detection of PM in these cases was 0%. This is consistent with the correlation between the presence of mucinous material and the absence of vascularized tissue within tumor nodules and is similar to the observation that fixation of other tumor markers, such as glucose in Fluoro-Deoxy-Glucose-Positron Emission Tomography (FDG-PET) imaging, is limited in this subgroup.[24,25]

The secondary objectives of this study were to evaluate whether ICG-FI could identify additional subclinical metastases that were not detected pre- or intraoperatively, and provide more precise delineation of the fluorescent versus nonfluorescent regions to improve the safety of the surgical margins.

In the present study, ICG-FI allowed detection of additional PM, that were not revealed during standard work-up and surgery, in 3 patients (21.4%). All of these metastases (4 in 1 patient and 1 in 2 patients) were found in the abdominal flank regions and paracolic gutters. Interestingly, in 1 patient a retroperitoneal node was only revealed with the use of FI. Therefore, the addition of ICG-FI led to a modification of the surgical technique and improvement of the radicality of CS in 30% of the patients evaluated in this cohort. Intraoperative use of FI for margin delineation modified the surgery in 1 patient. In the other patients, the distinction between the fluorescent nodules and surrounding tissue was poor due to the limited image definition of the camera system and the absence of correlated colorized images, limiting, under the current technical conditions, the contribution of ICG-FI for this application.

In conclusion, these first results indicate that PM from non-mucinous cancer are accurately identified by FI after intraoperative injection of ICG, serving as a proof-of-concept to validate this technique and to further explore its value for improving the radicality of CS in this indication. We observed that intraoperative ICG-FI may allow detection of additional subclinical malignant peritoneal nodules, directly contributing to the completeness of the surgical resection. At this point, further investigations are required to better characterize the optimal timing for improving the sensitivity of the technique and to evaluate its real impact on long-term results. From this perspective, the ease of use, tolerability, and availability of ICG make it a good candidate for the design of a larger clinical trial using this probe for intraoperative FI.

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