ORIGINAL ARTICLE

Combined use of intraoperative ultrasound and indocyanine green fluorescence imaging to detect liver metastases from colorectal cancer

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Abstract

Objectives: Surgical excision is the standard strategy for managing liver metastases from colorectal carcinoma. The achievement of negative (R0) margins is a major determinant of disease-free survival in these patients. Current imaging techniques are of limited value in achieving this goal. A new approach to the intraoperative detection of colorectal liver metastatic tissue based on the emission of indocyanine green (ICG) fluorescence was evaluated.

Methods: A total of 25 consecutive patients with liver metastases from primary colorectal cancers who were eligible for liver resection received a bolus of ICG (0.5 mg/kg body weight) 24 h before surgery. During surgery, ICG fluorescence, which accumulates around lesions as a result of defective biliary clearance, was detected with a near-infrared camera system, the Photodynamic Eye (PDE). Numbers of lesions detected by, respectively, PDE + ICG, intraoperative ultrasound (IOUS) and preoperative computed tomography (CT) were recorded.

Results: The near-infrared camera plus ICG revealed a total of 77 metastatic liver nodules. Preoperative CT demonstrated 45 (58.4%) and IOUS showed 55 (71.4%). Preoperative CT and IOUS alone were inferior to the combined use of PDE + ICG and IOUS in the detection of lesions of ≤3 mm in size.

Conclusions: This experience suggests that PDE + ICG, combined with IOUS, may represent a safe and effective tool for ensuring the complete surgical eradication of liver metastases from colorectal cancer.

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Introduction

Colorectal carcinoma (CRC) is one of the most commonly occurring tumours throughout the world.[1] The prognosis in CRC has improved, mainly as a result of the integrated use of oncological and surgical therapies, but the development of liver metastases is still a frequent event in the natural history of the disease.[2,3] In a significant percentage of patients, the liver is the sole site of seeding.[3,4] The development and progression of hepatic metastases have been widely studied in the field of surgery.[5] Left untreated,

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these lesions are associated with a median survival of about 5 months.[3–5] In the 1980s, Adson and van Heerden demonstrated that liver metastases could be potentially cured by liver resection,[6] and subsequently surgery became the reference-standard treatment for these lesions.[7] However, although surgery did appear to offer the best chance of cure, only about 20% of patients with liver metastases were eligible for resection.[8] The percentage is not much higher today, although recent studies show that resectability rates can be increased with the use of new technologies, drugs and surgical procedures.[9–13] The contraindications for liver resection defined in the 1980s are no longer valid: patients with more than three metastatic nodules or nodules of >5 cm in diameter can now be safely treated with surgical resection. Recent studies indicate

that liver surgery, including extensive resection, is associated with mortality rates of 1–2%.[14] Survival rates have also improved and several large studies have shown disease-free survival rates of around 45%.[15–22]

There is widespread agreement that one single variable has a very significant impact on survival in this setting: the achievement of negative (R0) resection margins.[23–26] This requires the complete removal of all tumour so that microscopic examination of the margins shows no neoplastic cells. The presence of residual foci of tumour cells, whether they are macroscopic or microscopic, is associated with tumour recurrence and lower survival rates.[24] The importance of radical tumour removal is undeniable, although the optimal dimensions of the tumour-free margin are still a topic of debate.[24]

This is a complicated problem because small nodules can be located at varying distances from larger metastatic lesions, and the resolution offered by current imaging techniques may not be sufficient to detect them. Computed tomography (CT), magnetic resonance imaging and ultrasonography (US) are the mainstays of liver tumour detection and staging.[27,28] All have a high degree of accuracy and the preoperative use of these imaging techniques is useful in planning a liver resection. However, they can easily miss lesions with diameters of ≤ 3 mm and they provide still pictures only, which are of limited value as a navigation tool.

Intraoperative US (IOUS) is currently regarded as the reference-standard method of guiding liver resection. It offers the undeniable advantage of real-time visualization, but it also has several shortcomings: (i) it cannot detect lesions of ≤ 3 mm in size; (ii) its accuracy is highly dependent on the skill and experience of the operator; (iii) the images it provides are two- rather than three-dimensional, and (iv) there is a superficial blind area about 1 cm under the liver surface, which is especially problematic in colorectal liver metastases as these are mostly located on the surface of the liver parenchyma. The introduction of acoustic contrast enhancement is an appealing evolution, but the contrast effect is too short-lived to be of real value as a navigation tool during standard liver resection, which can take 2–6 h.

Recently, a new approach based on the emission of indocyanine green (ICG) fluorescence has been used to improve the intraoperative detection of neoplastic tissue.[29,30] The aim of the present study was to test the hypothesis that this system could be used synergistically with IOUS to detect metastatic liver lesions in patients with CRC. Previous studies have shown that the combined use of both techniques can overcome the limitations of each and identify a substantial number of lesions that are not revealed by IOUS alone.[29]

Materials and methods
Patient characteristics
From August 2011 to June 2012, 25 patients (Table 1) with liver metastases from primary CRC were enrolled at the IRCCS (Istituto di Recovero e Cura a Carattere Scientifico) San Matteo

Table 1 Characteristics of patients included in the study ($n = 25$)

Patient characteristics		
Age, years, median (range)		61.5 (42–87)
Gender, n	Male	17
	Female	8
Preoperative chemotherapy, n	Yes	13
	No	12
Lesions ($n = 78$)		
	Synchronous	38
	Metachronous	40

General Hospital in Pavia. All had been previously evaluated by the hospital's multidisciplinary liver team and deemed eligible for resection with curative intent. The decisions were based on imaging findings (multidetector-row CT and contrast-enhanced US) and histological examination of US-guided percutaneous biopsies of a sample liver lesion. Patients were excluded if enrolment interviews revealed evidence of allergies to iodine or ICG. Written informed consent was obtained from each study participant.

Administration of ICG and fluorescence imaging
At 24 h prior to surgery, each patient was given an i.v. dose of ICG (0.5 mg/kg body weight). After 24 h, normal liver parenchyma will have washed out most of the ICG. The dye is retained as a fluorescent rim that is found in stromal tissue in the transition area between tumour and normal liver tissue in all liver metastases. During surgery, such fluorescence was detected with a near-infrared (NIR) camera system, the Photodynamic Eye (PDE) (Hamamatsu Photonics KK, Hamamatsu, Japan), which activates the ICG fluorescence with a series of diodes that emit light at a wavelength of 760 nm while cutting off emissions below 820 nm. The emissions are detected with a charge-coupled device. For intraoperative use, the unit was wrapped in a disposable, sterile drape, and the real-time video output of the system was displayed on a monitor next to that of the US scanner.

Surgical procedure and intraoperative lesion detection
After standard transverse laparotomy, the liver was freed from the surrounding tissues by dissection of the ligaments and vena cava. The liver hilum was dissected and circled with a tape for an eventual Pringle manoeuvre. The surgeon then performed a standard IOUS examination and PDE assessment of ICG fluorescence (PDE + ICG). The surgical procedure was then performed under dual US and PDE guidance. The parenchyma was dissected using a Cavitron ultrasonic surgical aspirator and the Kelly clamp crush technique. The resection planes were examined with IOUS and PDE + ICG during and after resection to ensure that there was no residual neoplastic tissue. Blood vessels and bile ducts of >5 mm

Figure 1 In computed tomography (a) and intraoperative ultrasound (b), findings appear to be unremarkable. (c) Near-infrared imaging using the Photodynamic Eye demonstrates a nodule

in diameter were ligated; smaller vessels were sealed with a harmonic scalpel.

Evaluation of imaging

In each case, preoperative and intraoperative imaging data were subjected to post hoc review (Fig. 1). For each method used (CT, IOUS and PDE + ICG fluorescence), the total number of nodules detected was recorded, as were the numbers of nodules that were >3 mm or ≤ 3 mm in diameter (a diameter of 3 mm is commonly regarded as a reasonable detection threshold in both CT and US).

Statistical analysis

Data were analysed using GraphPad Prism Version 6.00 for OsX (GraphPad Software, Inc., La Jolla, CA, USA). Differences were assessed with Kruskal–Wallis non-parametric analysis of variance (ANOVA) and Dunn's multiple comparisons test. A P-value of <0.05 was considered to indicate statistical significance.

Results

Outcome of surgery

All patients recovered satisfactorily from surgery and had uneventful postoperative courses. As expected, transient alterations were observed in liver function parameters, but in all cases values returned to the preoperative baseline range by postoperative day 3. Postoperative complications included pleural effusion, fever and a slight delay in the return of bowel function. Mean ± standard deviation intraoperative blood loss was 300 ± 200 ml. Mean hospital stay was 6 days.

Detection of metastatic liver nodules

Figure 2 reports the findings of this study. After the postoperative histopathological examination, one of the lesions identified only by PDE + ICG was considered benign. Only one malignant lesion showed positive microscopic resection margins.

The use of concomitant IOUS and PDE significantly improved the detection of liver metastases in comparison with preoperative
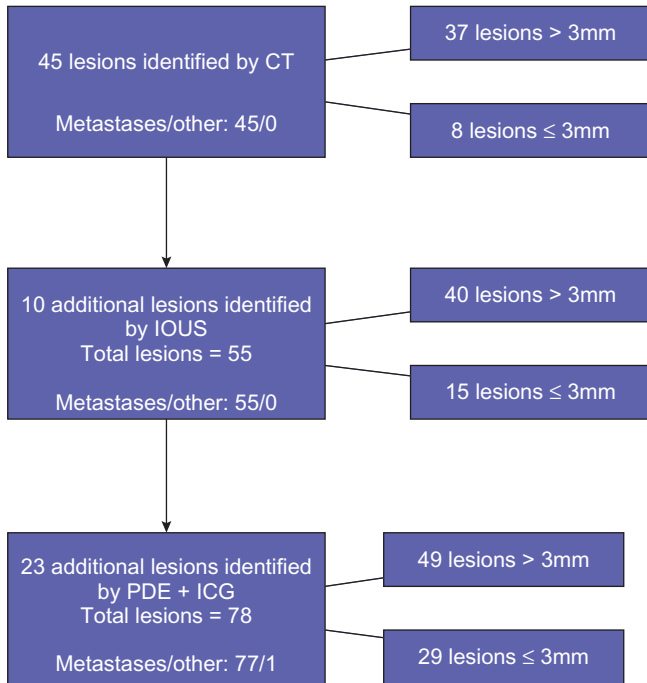
Figure 2 Stratification of findings among the cases. Only one event was deemed a false positive. CT, computed tomography; IOUS, intra-operative ultrasound; PDE, Photodynamic Eye; ICG, indocyanine green

CT ($P = 0.0029$). The use of IOUS alone, however, did not improve detection significantly in comparison with preoperative CT ($P = 0.3905$) (Fig. 3).

The lesions identified were divided into two groups according to size, consisting of lesions of >3 mm and lesions of ≤3 mm in diameter.

No differences emerged between preoperative CT, IOUS and IOUS + PDE in the detection of lesions of >3 mm ($P > 0.9999$) (Fig. 4). Intraoperative US is limited to the detection of lesions of >3 mm in size.

Differences among the three imaging methods emerged in the detection of lesions of ≤3 mm. The use of concomitant IOUS and PDE was most successful, detecting 29 lesions in comparison with 15 detected by IOUS alone (IOUS mean rank: 33.88; IOUS + PDE mean rank: 46.48; $P = 0.0328$) and nine detected by preoperative CT alone (CT mean rank: 22.00; IOUS + PDE mean rank: 46.48; $P < 0.0001$) There was no significant difference in numbers detected between CT and IOUS (CT mean rank: 22.00; IOUS mean rank: 33.88; $P = 0.0708$) (Fig. 5).

Discussion

The present study provides preliminary clinical evidence indicating that the intraoperative detection of liver metastases, particularly of very small lesions, which are frequently missed with
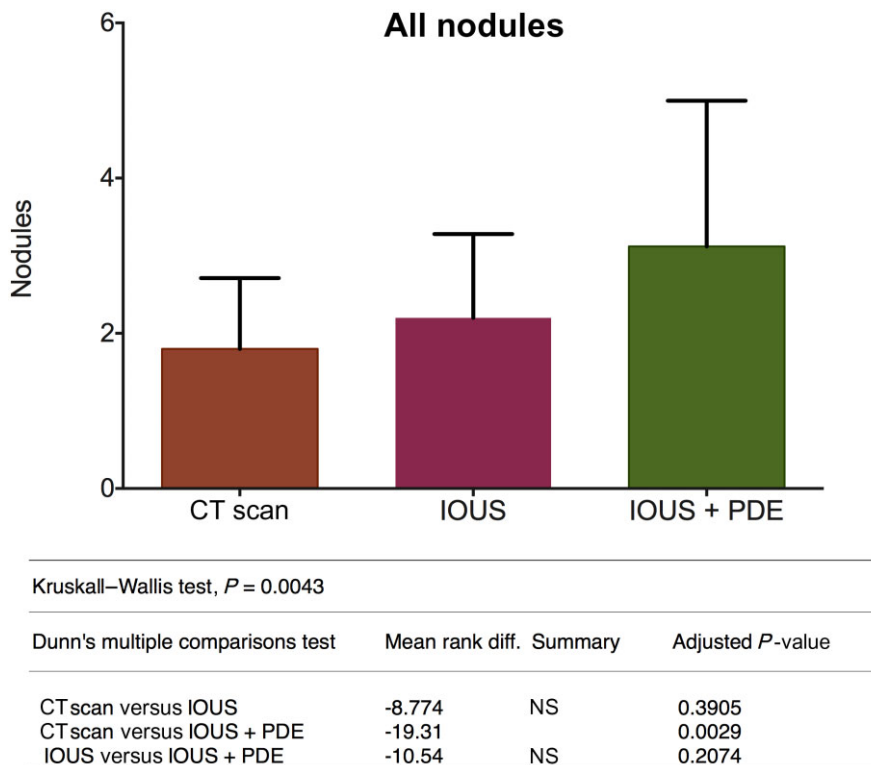

Figure 3 Overall evaluation of all observed nodules. CT, computed tomography; IOUS, intraoperative ultrasound; PDE, Photodynamic Eye; NS, not significant
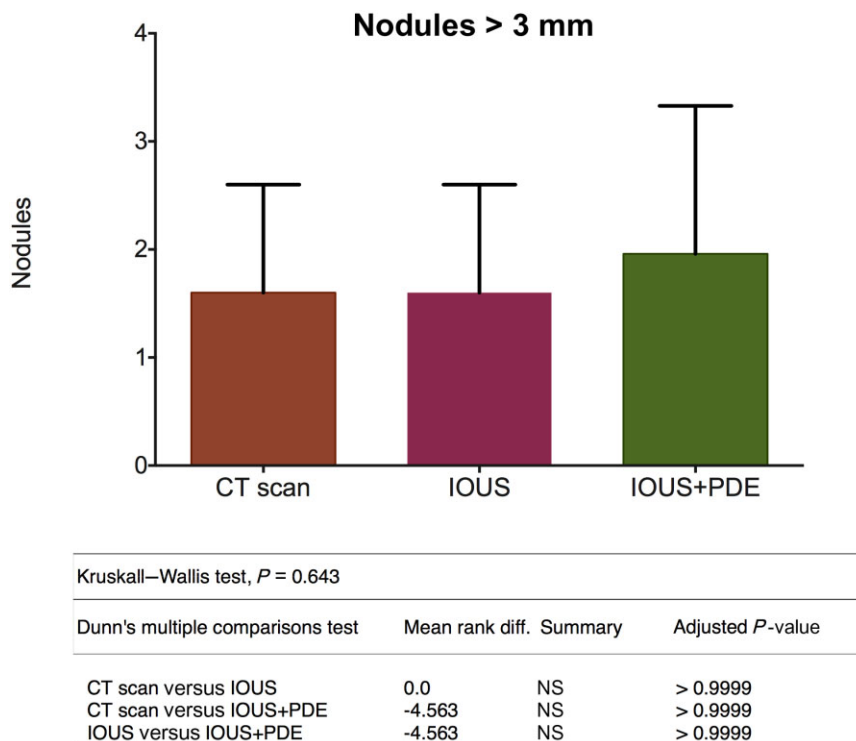
Nodules > 3 mm

Kruskall–Wallis test, $P = 0.643$			
Dunn's multiple comparisons test	Mean rank diff.	Summary	Adjusted P-value
CT scan versus IOUS	0.0	NS	> 0.9999
CT scan versus IOUS+PDE	-4.563	NS	> 0.9999
IOUS versus IOUS+PDE	-4.563	NS	> 0.9999

Figure 4 Stratification of the nodules by size shows no difference in findings of tumours of >3 mm. CT, computed tomography; IOUS, intraoperative ultrasound; PDE, Photodynamic Eye; NS, not significant
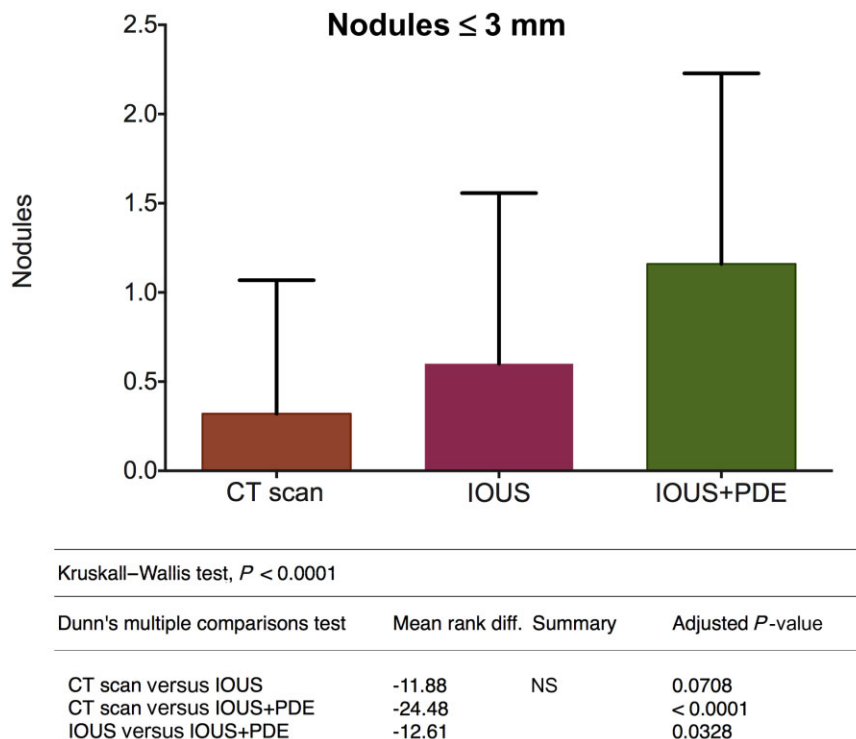
Nodules ≤ 3 mm

Kruskall–Wallis test, $P < 0.0001$			
Dunn's multiple comparisons test	Mean rank diff.	Summary	Adjusted P-value
CT scan versus IOUS	-11.88	NS	0.0708
CT scan versus IOUS+PDE	-24.48		< 0.0001
IOUS versus IOUS+PDE	-12.61		0.0328

Figure 5 Stratification of the nodules by size shows that the combined use of intraoperative ultrasound and the Photodynamic Eye allows a significant advantage in the detection of nodules of ≤3 mm. CT, computed tomography; IOUS, intraoperative ultrasound; PDE, Photodynamic Eye; NS, not significant
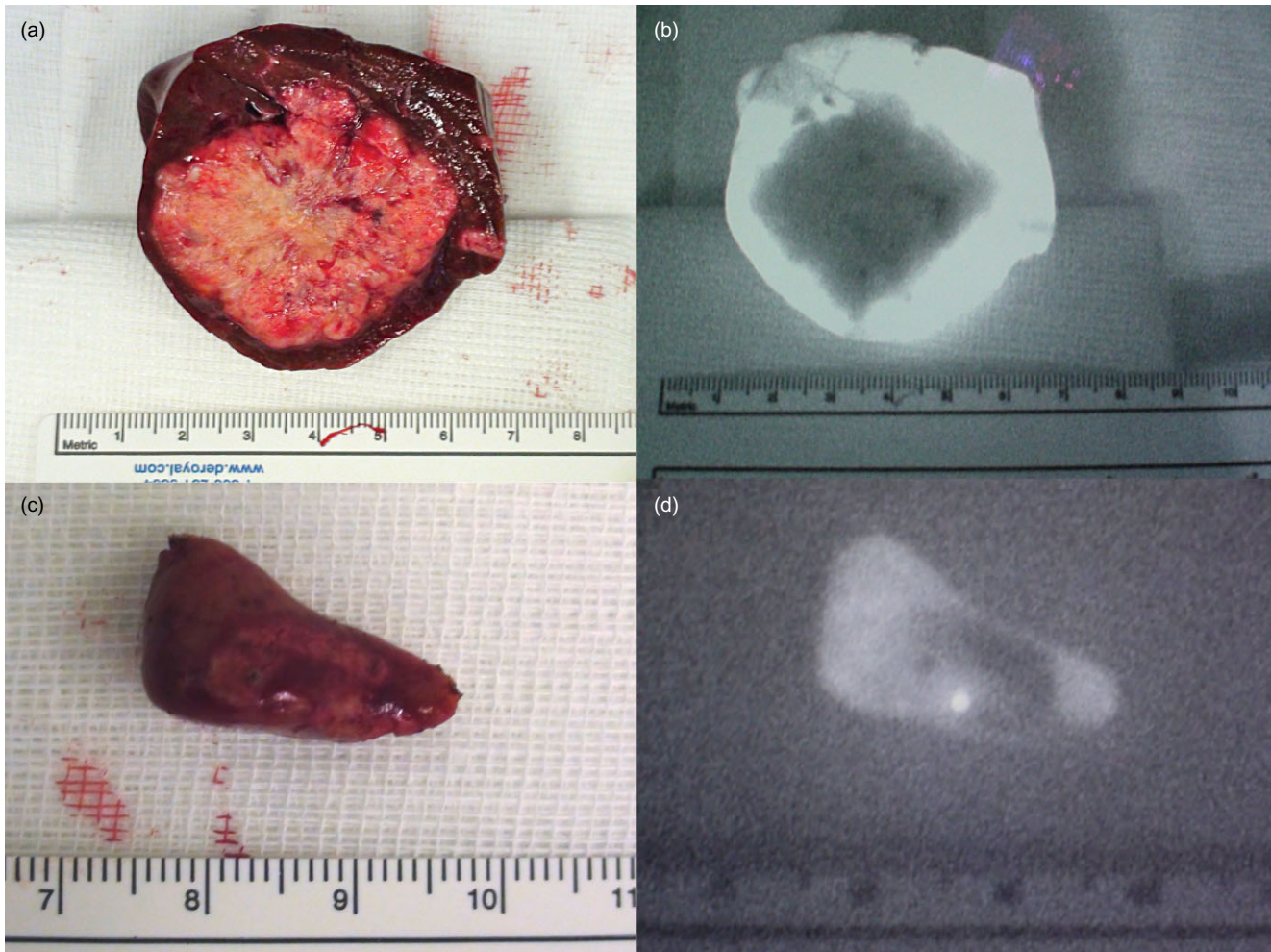
Figure 6 The figure is a proof of concept demonstrated by resection specimens and associated images obtained using the Photodynamic Eye (PDE) with indocyanine green (ICG) staining. (a, b) When PDE + ICG is used to detect large nodules, it allows a safe resection in which the rim represents the limit of resection. A parenchyma-sparing resection is safer with this technique. (c, d) The PDE + ICG technique is particularly suited to demonstrating small nodules that may be missed by computed tomography and intraoperative ultrasound. The technique can be used to change the staging of the disease and possibly the therapeutic approach

conventional imaging modalities, can be substantially improved with the use of ICG fluorescence detection (Fig. 6). The PDE + ICG system provides detailed, real-time video imaging that serves as an excellent guide throughout the liver resection procedure. This approach can be used alone or in combination with traditional IOUS, thereby exploiting the strong points of each method: US provides high sensitivity for the examination of inner regions of the liver and PDE + ICG provides a higher-resolution capture of the outermost regions. The detection of green fluorescence heralds the presence of neoplastic tissue.

The use of IOUS and PDE in combination can be very useful in achieving R0 surgery. Surgeons naturally make every attempt to identify and remove all macroscopically detectable neoplastic tissue. If there is macroscopic evidence of residual cancer at the end of surgery, it is generally agreed that the procedure should be

regarded as a technical failure that can be expected to have a detrimental impact on both disease-free and overall survival. In most cases, however, this outcome is not the result of a medical error. Frequently, the disease manifestation is more advanced or complicated than it appeared on preoperative imaging findings. This reflects the intrinsic limits of currently available technology. The surgeon may find an unexpected area of infiltration or a major blood vessel or bile duct in close proximity to the lesion, which precludes wider dissection. Because of its infiltrating nature, the metastatic nodule will be irregularly shaped and thus the placement of each portion of the resection line potentially involves making a choice between the risk for immediate organ damage and the risks associated with a non-radical tumour resection. In some cases, it is simply impossible to eliminate all nests of viable cancer cells. In the present group's experience, NIR imaging

was useful to define the surgical margin. Imaging by NIR was used not only before, but also after liver resection: in this way, all the metastatic tissue was removed, with an instant revision of the resection. In a single case (one of 77), pathology showed an R1 resection. Even in this resection, NIR imaging did not show any remaining fluorescence, but the section was close to a major vessel that could not be sacrificed.

The present findings suggest that the use of a combination of PDE + ICG and IOUS in these settings could increase the accuracy of intraoperative exploration and dramatically improve the chances of complete tumour eradication. Further studies will be necessary to confirm these conclusions in a larger population.

Conflicts of interest
None declared.

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