Intraoperative fluorescence imaging : clinical translation of targeted and non-targeted tracers

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CHAPTER 4

Identification of malignant tumors in the liver

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ABSTRACT

To date, surgery is the only curative treatment option for patients with resectable metastases in the liver and is regarded as standard-of-care. However, recurrence rates remain high, which might partly be explained by the inability of conventional technologies to detect small lesions. Near-infrared fluorescence imaging is able to detect lesions as small as one millimeter, but only if they are localized on or several millimeters below the liver surface. Several studies reported the identification of otherwise undetectable liver tumors using near-infrared fluorescence imaging. Research in the coming years will have to determine if this technology is truly beneficial for patients requiring resection of hepatic metastases.

INTRODUCTION

Prognosis and survival of patients with cancer deteriorates dramatically when metastases are present. Most metastases in the liver originate from colorectal cancer. 14.5% of colorectal cancer patients have liver metastases at the moment of diagnosis and 15.2% will develop liver metastases within 5 years after curative resection of the primary tumor [1]. Metastases are the leading cause of cancer-related death, with no survivors at 5 years if left untreated [2]. Besides advancements in radiotherapy, (neoadjuvant) chemotherapy and preoperative imaging, improvements in surgical techniques have led to improved prognosis and survival rate. In the past decades, hepatic metastasectomy has evolved from a high-risk procedure to a more commonly performed curative treatment. Surgical resection is currently the only potential curative therapy and is regarded as standard-of-care, but is only possible in patients with sufficient functional liver reserve, without unresectable extrahepatic disease and when liver metastases can be resected with a tumor-free margin. Unfortunately, only 10 to 20% of patients are potential candidates for curative resection. Even with strict patient selection, intrahepatic recurrence rates after hepatic metastasectomy remain high, ranging from 11% to 28%, of which 78% occurs within 1 year [3, 4]. Factors influencing the recurrence rate of colorectal cancer liver metastases are positive resection margins, extrahepatic disease, node-positive primary tumor, disease-free interval from primary tumor to metastases less than 12 months, more than one hepatic tumor, cases where the largest hepatic tumor is more than 5 centimeters, and a serum carcinoembryonic antigen level of more than 200 ng/ml [5]. The high recurrence rate could partly be explained by inability of available technologies to detect the presence of micrometastases during liver metastasectomy. In addition, positive resection margins (R1 and R2) are still a major issue, with reported rates ranging from 11% to 23% [5, 6]. In the preoperative setting, imaging technologies such as ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) are used for surgical planning. However, these technologies are less suitable for intraoperative use and are unable to provide real-time surgical guidance. In addition, technologies available during surgery, such as intraoperative ultrasonography (IOUS), visual inspection and palpation by the surgeon, are less suitable for the detection of small lesions [7]. Recently, the use of near-infrared (NIR) fluorescence imaging has emerged as an additional technique for real-time detection of small and superficial hepatic metastases [8]. This chapter focuses on the development and current applications of NIR fluorescence imaging in identifying metastases in the liver.

Conventional technologies for identification of metastases in the liver

Several imaging technologies can be used to detect hepatic abnormalities, though sensitivity and specificity can vary greatly. Among these technologies are ultrasound, CT, MRI, fluorodeoxyglucose positron emission tomography (FDG-PET) and FDG-PET/CT. CT is considered to be the preferred technology for staging of liver disease in the majority of hospitals, because it provides good coverage of the liver and, if necessary, the abdomen and thorax in the same scan [9]. However, CT is relatively expensive and requires ionizing radiation. In addition, lesions only several millimeters small are frequently missed [7]. MRI has a higher detection rate of lesions smaller than 10 millimeters compared to CT, with a sensitivity of 60.2% and 47.3% respectively [10]. However, for both modalities, this is still unacceptably low. Diagnostic accuracy of FDG-PET is strongly affected by neoadjuvant chemotherapy; treatment resulted in a drop in sensitivity from 81.3% to 54.5% [11].

All these preoperative imaging modalities have great value for planning surgical procedures, but the hands and eyes of the surgeon combined with IOUS are still the most important tools during surgery. Hata et al. analyzed retrospectively a prospectively collected and recorded database of intraoperative detected colorectal liver metastases [12]. A total of 270 new metastases were detected in 183 consecutive patients. Intraoperative palpation and/or visual inspection detected 77% (207/270) of the new lesions. IOUS also showed to be of great value. 12% (33/270) of the new detected metastases was detected solely by IOUS. The remaining 11% (30/270) were found in removed tissue. Most of the newly found metastases were located on or less than 1 centimeter underneath the liver surface. Although this obviously shows the benefit of IOUS, palpation and visual inspection, small tumors are still missed by all detection methods. Nomura et al. histologically examined resected liver specimen and concluded that IOUS misses 25% of liver tumors smaller than 5 millimeters [7]. The same rate applies to palpation and visual inspection, although, sensitivity may vary greatly, e.g. due to the experience of surgeons and size and depth of tumors. In conclusion, there is a strong need for technologies which can intraoperatively detect small and superficial metastases in the liver. During minimally invasive liver resections, surgeons are deprived of tactile information and their visibility is hampered, increasing the need for additional imaging modalities.

Intraoperative NIR fluorescence imaging

Near-infrared (NIR) fluorescence imaging is a promising technique that can be used to identify tumors and vital structures during surgery [13]. It uses light with wavelengths between 700-900 nanometers, which is invisible to the naked eye. Advantages of NIR light include high tissue penetration and low autofluorescence.

NIR fluorescence does not alter the surgical field, as it is invisible. It is safe to use as no ionizing radiation is used, and no tissue contact is needed. As NIR fluorescence images can be acquired in real-time, it allows the surgeon to operate under direct image-guidance.

Several NIR fluorescence imaging systems are already commercially available for both open and laparoscopic surgery [14, 15]. The demand for minimally invasive liver resection increases due to exponential growth of patients eligible for hepatic metastasectomies. Laparoscopic liver resection shows a more favorable outcome than open liver resection with regard to complications, hospital stay and blood loss [16]. However, minimally invasive surgery also limits visualization and palpability of the liver surface. NIR fluorescence imaging could therefore contribute to this field of surgery by providing additional information. Several NIR fluorescence imaging systems can simultaneously acquire the NIR fluorescence signal and color video signal, enabling a real-time overlay of NIR fluorescence signal and enhance anatomical orientation. Ishizawa et al. were the first to demonstrate the safety and convenience of a prototype fluorescent imaging system during laparoscopic hepatectomy in a patient with hepatocellular carcinoma (HCC) and underlying chronic hepatitis C [17]. Laparoscopic NIR fluorescence imaging facilitated visual inspection by clearly delineating the tumor on the liver surface. Tummers at al. showed that laparoscopic NIR fluorescence imaging identified additional uveal melanoma metastases in the liver in 2 out of 3 patients [18].

NIR fluorescent contrast agents

NIR fluorescence imaging of metastases in the liver can be challenging due to hepatic uptake and clearance of many fluorescent agents, resulting in fluorescent liver tissue and, hence, invisible metastases due to high background fluorescence. Furthermore, liver tissue absorbs a higher proportion of NIR fluorescent light compared to other human tissues, such as colon and breast, resulting in a lower signal [19]. Fluorophores emitting fluorescence with longer wavelengths achieve deeper tissue penetration. However, wavelengths longer than 900 nm suffer from more absorption by water. An ideal fluorescent agent for hepatic metastases should be tumor-specific, cause low background fluorescence by being cleared renally, and emit light within the NIR fluorescence window (700-900 nanometers). To date, no such fluorescent agent is available for clinical use. Methylene blue (MB) and indocyanine green (ICG) are currently the only suitable NIR fluorescence agent approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). MB is cleared simultaneously by liver and kidneys, while ICG is cleared solely by liver. Hepatic metastases are visualized by non-tumor-targeted dyes when this dye is cleared by healthy hepatocytes, while it retains in cancerous tissue. The fluorescent properties of MB make it a less favorable fluorophore; its peak emitted fluorescence wavelength is 700 nanometers and therefore

subject to higher tissue autofluorescence, more light absorption and less tissue penetration capacity. No study showed the capability of MB to identify metastases in the liver. The peak emitted fluorescence wavelength of ICG is 810 nanometers, making it a better candidate for NIR fluorescence imaging. In addition, since ICG is cleared exclusively by the liver, it results in stronger signal in the liver compared to MB. Extensive medical experience with ICG already exists, due to its use in a broad spectrum of other clinical applications, e.g. assessing coronary artery bypass graft patency, retinal angiography and liver function. Since ICG contains iodine, iodine allergy and thyrotoxicosis are contraindications for its use. ICG is not metabolized, is cleared exclusively by the liver and does not undergo enterohepatic recirculation, making it an ideal candidate for detecting liver dysfunction [20].

Tumor-targeting dyes

Both ICG and MB are non-targeted dyes and the chemical structures do not readily allow conjugation to tissue-specific ligands. The lack of other clinically available NIR fluorescent agents is a considerable limitation, since ICG is only capable of imaging metastases inside the liver; extrahepatic metastases will not show any fluorescent signal. In contrast, tumor-targeted dyes do have this property and offer therefore great advantages. NIR fluorescence imaging of metastases in the liver is challenging due to hepatic uptake and clearance of many fluorescent dyes. This may result in unfavorable tumor-to-liver ratio (TLR). For identification, tumorous tissue has to be more fluorescent than its background. Several academic and commercial parties are currently developing tumor-targeting dyes. Integrin $\alpha_V\beta_3$ is a potential target, as it shows overexpression in various cancer types, such as colorectal, ovarian and breast cancer, but low expression in hepatocytes [21]. Several integrin $\alpha_V\beta_3$ targeting agents have been studied, such as IntegriSense680 (Perkin Elmer, Waltham, Massachusetts), targeting integrin $\alpha_V\beta_3$. Hutteman et al. demonstrated the feasibility for detecting colorectal metastases using IntegriSense680 in a syngeneic rat model [18]. Another potential target is matrix metalloproteinase 2 (MMP-2), which is associated with metastatic capacity of colorectal cancer. By using the NIR fluorescence probe CY5.5-C6 in mice with induced colorectal cancer, tumors with increased expression of MMP-2 were successfully imaged [22]. Another excellent target for colorectal cancer is epidermal growth factor receptor (EGFR). Cetuximab, a human antibody binding specifically to EGFR, coupled with CY5.5 (cetuximab-CY5.5), resulted in the accurate detection of tumors [23]. Much more tumor-targeted dyes are currently under development and reviewed by Luo et al. [21]. However, as fluorescent dyes are excreted by liver and kidneys, this may result in high background signal. Therefore, optimal TLR should be studied for each new dye. Tumor-targeted dyes can potentially contribute during liver metastasectomy by visualizing not only intrahepatic, but, in contrast to non-targeted dyes, also extrahepatic tumors.

Dose and timing of ICG administration

After intravenous injection, ICG is absorbed by hepatocytes and eventually excreted into the bile. Therefore, the first period after intravenous administration results in highly fluorescent liver tissue, i.e. unfavorable TLR. Timing of administration before surgery is therefore crucial to reach an optimal TLR. Fluorescent intensity of the liver strongly decreases after 24 hours, although liver dysfunction may influence this rate [24]. In patients with an unfavorable ICG retention rate, e.g. in cirrhosis, steatosis and after chemotherapy, the fluorescence signal of noncancerous liver parenchyma is higher, which makes it more challenging to obtain an adequate TLR [25]. To determine optimal dose and timing of ICG administration, van der Vorst et al. performed a preclinical study with syngeneic rats with colorectal liver metastases [24]. The highest TLR was achieved 72 hours after intravenous injection. No significant effects were observed regarding doses, although a trend favoring 0.25 milligram per kilogram body weight (extrapolated to humans) was shown. Ishizwa et al. suggested that the interval between ICG administration and surgery should be longer than at least 2 days to obtain an optimal TLR, especially in patients with advanced cirrhosiss [25]. However, as in most other studies, the used dose was 0.5 milligram per kilogram body weight. In a clinical trial, administration 24 hours and 48 hours before surgery as well as doses of 10 milligrams and 20 milligrams ICG all showed sufficient TLR and no significant difference in TLR [13]. A dose of 10 milligrams ICG administered 24 hours prior to surgery is therefore advised.

NIR fluorescence imaging of metastases in the liver

NIR fluorescence imaging of metastases in the liver started after the incidental finding that hepatocellular carcinoma (HCC) shows a very strong fluorescent signal in patients who have been given ICG several days prior to surgery as a routine preoperative liver function test. In a subsequent study by Gotoh et al., all primary HCCs in ten patients were identified as bright NIR fluorescent lesions and could be removed completely [26]. In addition, four new HCC nodules that were not detected by any preoperative examinations or IOUS were detected due to the use of NIR fluorescence imaging. Harada et al. were the first to demonstrate the feasibility during hepatectomy in a patient with colorectal hepatic metastasis [27]. Several other clinical studies followed, describing the detection of both HCC and metastatic liver cancer (Table 1) [13, 18, 25, 27-32]. A total of 167 patients with colorectal or pancreatic liver metastases have been included in eight studies. In these patients, ICG has been shown to accumulate as a rim around the tumor (Figure 1 and 2). This pattern differs from primary hepatic cancers, e.g. HCC, where ICG shows total or partial fluorescence of the tumor [13]. Since ICG is removed from circulation exclusively by the liver, the rim pattern is influenced by clearance by the liver and biliary

drainage. Ishizawa et al. showed microscopically that fluorescence did not exist in metastatic tissue itself, but in surrounding noncancerous liver tissue compressed by the tumor [25]. Compression by metastases not only leads to obstructed bile canaliculi, but also to changes in the liver parenchyma due to inflammation, ductular transformation and increased presence of immature hepatocytes [33]. Compared to well-differentiated hepatocytes, immature hepatocytes display less expression of organic anion transporters [34]. Multidrug resistance P-glycoprotein 2 (MDR2), an organic anion transporter in the hepatocyte canalicular membrane, is essential for the transport of certain hydrophobic organic anions such as ICG and thus for its excretion. In its absence, ICG excretion is reduced by 90% [35]. The rim pattern of fluorescence can therefore be explained by the fact that ICG can be transported from circulation into immature hepatocytes, but is retained intracellular and not cleared into the bile canaliculi. The rim pattern is specific for malignant lesions. Van der Vorst et al. could differentiate 25 benign lesions (8 hemangiomas, 13 cysts, and 4 bile duct hamartomas) from malignant lesions by a lack of a fluorescent signal rim (Figure 4). False-negative results have not been reported so far, resulting in a sensitivity of 100% on resected tissue *ex vivo*. However, several studies describe a combined total of 14 false-positive lesions, among which 4 large regenerative nodules and 1 bile duct proliferation [25, 30-32, 36]. The incidence and characteristics of false-positive lesions should be clarified in larger study populations.

One of the great challenges in NIR fluorescence imaging is still its limited capability to penetrate human tissue. None of the above described studies reported the ability to detect metastases more than 8 millimeters below the liver capsule. For colorectal liver metastases, however, this technique is very useful, as colorectal liver metastases are mostly located on the surface of liver parenchyma. Deeper localized metastases also show a fluorescent rim after resection and sectioning (Figure 2) [37], but detection of these tumors still needs conventional technologies. Although this is a great limitation, NIR fluorescence imaging also has major advantages. Conventional imaging technologies easily miss superficial liver metastases smaller than 10 millimeters [7, 10]. In NIR fluorescence imaging, the bright signal enables surgeons to detect lesions as small as one millimeter in real-time (Figure 3) [7, 13]. Indeed, additional, otherwise undetectable metastases were identified in 7 studies [13, 25, 26, 30-32, 36]. Van der Vorst et al. report identification of otherwise undetectable liver metastases in 5 of 40 patients (12.5%, 95% CI = 5.0-26.6) [13]. Combining contrast-enhanced IOUS and NIR fluorescence imaging together with CT and MRI, Uchiyama et al. improved diagnostic sensitivity in 32 consecutive patients from 88.5% to 98.1% ((p=0.05).(30) Yokoyama et al. showed the potential of using NIR fluorescence imaging by screening the hepatic surface during pancreatic surgery with curative intent [32]. In 49 patients without preoperative detected hepatic metastases, 13 abnormal fluorescent lesions were detected without any apparent tumor, and 8 of them contained micrometastases. Within 6 months after surgery, 10 patients with abnormal fluorescence developed

hepatic metastases, versus 1 of 36 patients in the fluorescence negative group, resulting in a positive predictive value of 77% and a negative predictive value of 97%. This outcome could possibly help surgeons select patients for either curative or palliative treatment.

Other applications of NIR fluorescence imaging during hepatectomy

Besides tumor demarcation, excretion of ICG into the bile can be used for real-time cholangiography of the biliary anatomy. Iatrogenic damage of bile ducts is a major issue in liver surgery. Bile leakage after hepatic resection is associated with high risk for liver failure and postoperative mortality [36]. During cholangiography, the feasibility is shown by Ishizwa et al. [38]. Potentially, bile leakage could also be detected during resection of hepatic metastasis, though no study reports the feasibility. Another novel and intraoperative technique is the use of ICG for image-guided liver segment identification for anatomical hepatic resection. Aoki et al. performed a study in 35 patients who underwent hepatectomy for hepatic malignancy [39]. After administration of 5 milligrams ICG into the portal vein, stained subsegments and segments of the liver were identified in 33 of 35 patients. Both cholangiography and identification of liver segments are very usable during hepatic metastectomy and are described in other chapters.

CONCLUSIONS

Surgical resection is currently the only potentially curative therapy and standard-of-care for metastases in the liver of selected patients. The high recurrence rate demands a technology which is able to detect lesions that are otherwise undetected by current visualization methods. Current available literature suggests an important complementary role for intraoperative NIR fluorescence imaging in the detection of metastatic and primary tumors in the liver. It enables the identification of otherwise undetectable metastases as small as one millimeter and localized on or up to 8 millimeters below the liver surface. Although several studies show promising results, larger clinical trials are required to truly validate the benefit for patients requiring hepatic metastasectomy. In addition, optimization of NIR fluorescent contrast agents and imaging systems is necessary before NIR fluorescence imaging becomes standard-of-care.

Figure 1 In vivo near-infrared fluorescence imaging of a hepatic metastasis using the Mini-FLARE system (Beth Israel Deaconess Hospital, Boston, USA). A characteristic rim of fluorescence around the lesion is shown.

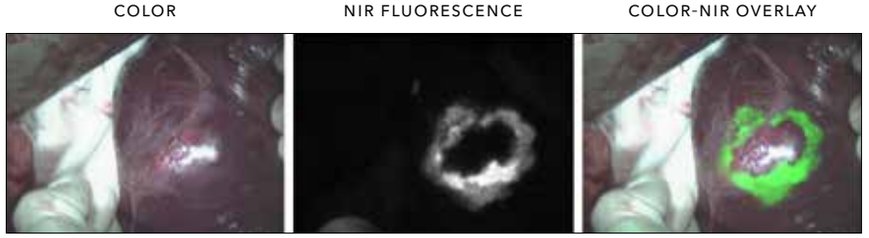

Figure 2 *Ex vivo* near-infrared fluorescence imaging of resected and sliced lesion at pathology department using the FLARE imaging system (Beth Israel Deaconess Hospital, Boston, USA).

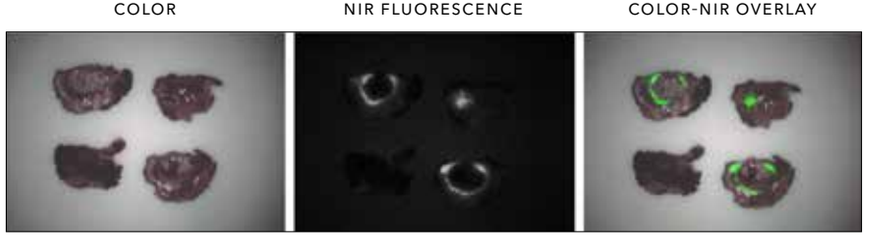

Figure 3 Small, superficial, otherwise occult metastases are identified by near-infrared fluorescence imaging (arrow).

Figure 4 Benign lesions (arrow) can be differentiated from malignant lesions by a lack of a near-infrared fluorescent rim around the lesion.

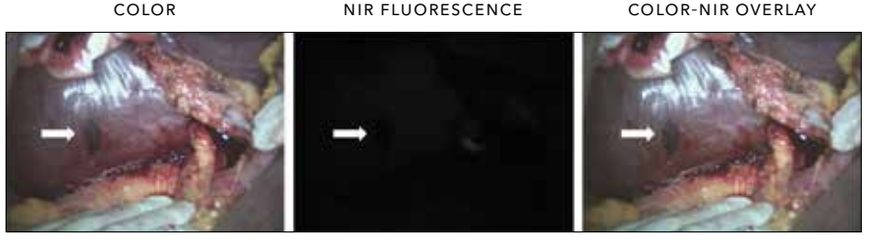

Table 1 List of studies using near-infrared fluorescence imaging in surgery for hepatic metastases.

First author, year	Number of patients	Pre-operative diagnosis	Imaging system	Dose of ICG	Injection site	Time between injection and imaging	Intraoperative IR (tumors)	Additional metastases identified	False-positive lesions	Smallest tumor size
Harada, 2009	3	ICC (n=2); CLM (n=1)	PDE	0.5 mg/kg	i.v.	4 days (1,2) & 2 days (3)	3/3	-	0	20 mm
Ishizawa, 2009	49*	HCC (n=37); CLM (n=12)	PDE	0.5 mg/kg	i.v.	1-7 days for HCC & 1-14 days for CLM	21/41 HCCs & 16/16 CLM **	+	5	2 mm
Kasuya, 2010	1	CLM	PDE	500 µl mixed with ethanol	Locally	NA	NA	-	0	3 mm
Uchiyama, 2010	32	CLM	PDE	0.5 mg/kg	i.v.	<2 weeks	NA	+	2	4 mm
Yokoyama, 2011	49	PCM	PDE	25 mg	i.v.	1 day	NA	+	5	1.5 mm
Ishizuka, 2012	7	CLM	PDE	0.1 ml/kg	NA	NA	26/26	+	1	NA
Peloso, 2012	25	CLM	PDE	0.5 mg/kg	i.v.	24 h	NA/77	+	1	3 mm
van der Vorst, 2013	40	CLM	Mini-FLARE	10 and 20 mg	i.v.	24 and 48 h	71/97	+	0	1 mm
Tummers, 2015	3	UMM	Karl Storz Fluorescence laparoscope	10 mg	i.v.	24 h	NA	+	0	1 mm

HCC=Hepatocellular carcinoma; CLM=Colorectal Liver Metastases; ICC=Intrahepatic cholangiocarcinoma; PCM=pancreatic cancer metastasis; PDE=Photo Dynamic Eye; FLARE= Fluorescence-Assisted Resection and Exploration; NA=not available; IR=identification rate; i.v.=intravenous; UMM: uveal melanoma metastases.
*) 26 of 49 patients (20 with HCC and 6 with CLM) underwent near-infrared fluorescence imaging during surgery;
**) Identification rate of 26 patients who were examined with near-infrared fluorescence imaging during surgery.

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