Indocyanine green fluorescence imaging in the surgical management of liver cancers: Current facts and future implications


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Summary Imaging detection of liver cancers and identification of the bile ducts during surgery, based on the fluorescence properties of indocyanine green, has recently been developed in liver surgery. The principle of this imaging technique relies on the intravenous administration of indocyanine green before surgery and the illumination of the surface of the liver by an infrared camera that simultaneously induces and collects the fluorescence. Detection by fluorescence is based on the contrast between the (fluorescent) tumoral or peri-tumoral tissues and the healthy (non-fluorescent) liver. Results suggest that indocyanine green fluorescence imaging is capable of identification of new liver cancers and enables the characterization of known hepatic lesions in real time during liver resection. The purpose of this paper is to present the fundamental principles of fluorescence imaging detection, to describe successively the practical and technical aspects of its use and the appearance of hepatic lesions in fluorescence, and to expose the diagnostic and therapeutic perspectives of this innovative imaging technique in liver surgery.

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Introduction

During the last couple of years, medical imaging research has been rejuvenated by the development of lesion detection with fluorescence. Several medico-surgical specialties (dermatology [1], pulmonology [2], urology [3], neurosurgery [4], gastro-intestinal surgery [5], as well as plastic and reconstructive surgery [6]) now use fluorescence imaging techniques to diagnose cancer or premalignant lesions, and/or lymph nodes draining a tumor. The principle behind this imaging technique relies on the administration of
a fluorescent marker capable of specifically targeting "fluorescent" cancer cells; this has opened one of the most promising fields of tumor detection. Indocyanine green (IG) is a soluble dye, which emits a fluorescent light when illuminated by an infrared laser source. In hepatic surgery, IG clearance is used to evaluate hepatic function before surgical procedures [7–9], but the application of the fluorescent properties of IG is just beginning. The first application of this imaging technique in hepatic surgery was reported by Ishizawa et al. in 2009 [10].

The goal of this update was to report the experience of several Asian teams with this innovating intra-operative imaging technique in hepatic surgery, and, after presenting the concept and the technical bases of fluorescence imaging, to summarize the practical and technical aspects of its use, to describe the features of the images that can be obtained, to review the pathophysiologic mechanisms implicated, and finally to outline the limitations of its use in hepatic surgery.

Concept

Indocyanine green is a fluorescent dye that has been used in ophthalmology to visualize the retinal and choroid vasculization for more than 20 years [11]. Its metabolic characteristics (intravascular confinement enhanced by fixation to plasma proteins and rapid hepatic excretion into bile), the spectral properties of this molecule, and the development of new imaging systems have extended the fields of application of IG fluorescence to several other surgical specialties (cardiac surgery [12], neurosurgery [13], plastic and reconstructive surgery [6]). In oncologic surgery, IG fluorescence has been tested to detect kidney tumors [3], and has been used for detection of sentinel lymph nodes in breast cancer [14], malignant melanoma [15] and gastro-intestinal cancers [16–18].

In hepatic surgery, this dye has been used to evaluate hepatic function and outline hepatocytology strategies for oncologic resections [19,20], and for living donor hepatic transplantation [21]. More recently, teams from Asia have improved imaging techniques based on IG fluorescence, allowing the visualization of bile ducts [22–24], as well as hepatocellular carcinoma (HCC) and hepatic metastases during liver surgery [10,25]. Basically, IG is administered intravenously 12 to 48 hours before surgery and lights up the liver surface when illuminated with a near-infrared source. After injection, IG is rapidly taken up by tumoral and non-tumoral hepatocytes. In the presence of normal or subnormal hepatic function and in the absence of bilary obstruction, IG is excreted in the bile and disappears from healthy liver parenchyma within a few hours [26]. On the other hand, IG remains fixed in tumoral hepatocytes and in pathological areas of the liver, particularly around non-hepatocellular tumors. Thanks to particular features of the camera, the fluorescent light emitted by IG allows detection of hepatocellular (tumor fluorescence) and non-hepatocellular (peri-tumoral fluorescence) tumors.

Indocyanine green

Indocyanine green is a hydro soluble molecule that fixes rapidly and intensely to plasma proteins after intravenous injection. Also, 98–99% of IG molecules link to proteins with a large proportion becoming fixed to high molecular weight proteins such as albumin. The mode of elimination is exclusively hepatobiliary. IG is selectively taken up by the hepatocytes and excreted in bile via an active transport system. IG is not metabolized and does not enter the enterohepatic circulation. Consequently, the disappearance rate of IG from plasma to bile reflects the excretory function of the liver [27].

Schematically, all molecules can take on different levels of electronic energy. In its basal state, molecular energy is minimal. Under the action of light, depending on wavelengths, absorbed light can raise the level of energy. As the molecule returns to its basal state, light is emitted with a wavelength superior to the light responsible for the excitation. The difference between excitation and emission wavelengths is exploited thanks to cameras equipped with interferential filters to obtain the images.

When the IG-protein complex is excited by a light source with a wavelength between 750 and 810 nm, fluorescent light with a wavelength of approximately 830 nm (wavelength situated in the infrared spectrum) [28] is emitted. The human eye cannot see these wavelengths.

Fluorescent light is largely attenuated by hemoglobin and water as it traverses biological tissues. Hemoglobin strongly attenuates all wavelengths less than 700 nm (which corresponds in fact to the entire visible spectrum excepting deep red). Water is transparent in visible and near-infrared light but attenuates wavelengths over 900 nm. There is therefore a "window" of wavelengths at the limit between deep red and near infrared (700–900 nm) where tissue transparency is maximal. This is one of the reasons why IG fluorescence can be detected in the near-infrared zone from as deep as 10 mm from the surface of tissues.

In France, the only fluorescent molecules approved for injection in man are fluorescein and IG and both can be administered intravenously. Indocyanine green has an advantage in that is absorbs light in the near infrared range (while the spectrum of fluorescein is in the range of visible light) and can be detected much deeper in tissues. Although IG seems to be better tolerated than fluorescein, several cases of allergy (hives, nausea and anaphylactic shock) have been reported in ophthalmology. Some of these cases have been attributed to the iodide contained in certain preparations of IG. In case of recognized allergy to iodine, it is necessary to use special preparations of IG that do not contain iodine [29].

Technique

Apparatus

The device used is not specifically designed for intraoperative use, but the camera can be equipped with a sterile cover that allows the operator to manipulate the apparatus under sterile conditions. A portable imaging system provides real time quantitative fluorescent imaging (Fig. 1). This imaging system includes an infrared camera and amplifier. The camera simultaneously provides the functions of fluorescence excitation with a laser (LED emitting an infrared radiance) diffused over the operative field and fluorescence image acquisition is ensured by a captor (CCD), which filters the light so that only near infrared wavelengths can be seen. In practice, the camera and cable do not need to be sterilized. The length of the cable allows the screen and amplifier to be placed sufficiently far away so that a non-sterile person can hold the infrared camera above the sterile operative field. New imaging systems have been developed recently,
measuring the fluorescence in the near infrared spectrum while the area under investigation is illuminated with white light.

**Utilization**

In Asia, the indications for liver resection and the type of hepatectomy are determined according to a flow chart that takes into account the presence or absence of controlled ascites, total bilirubin, and IG clearance at 15 minutes [19]. This test is performed three days before surgery on average (1 to 7 days for HCC and 1 to 14 days for metastases) [10,30]. IG is injected intravenously at a dosage of 0.25 to 0.5 mg/kg, depending on the team. The exact optimal dosage remains to be defined. The investigation is performed by placing the infrared camera above the liver. The light in the operation room should be decreased as much as possible to improve the contrast of images seen on the screen.

The contrast between fluorescent (tumor) and non-fluorescent (non-tumoral parenchyma) areas depends closely on the interval between the time of IG injection and the moment when the fluorescence is measured, as well as on the liver function of the patient. IG should be injected 12 to 48 hours before the operation, thus allowing a "wash-out" of IG from normally functioning liver to occur, i.e. the lesions become fluorescent while the fluorescence of the non-tumoral parenchyma is completely washed out (and therefore non-fluorescent). Of note, the greater the impairment of liver function (particularly in the case of preoperative portal embolization), the more intense and persistent is the parenchymal fluorescence [31].

**Cost and future perspectives**

A fluorescence detection apparatus costs between 45,000 and 50,000 euros in France. It is sold by a Japanese firm, Hamamatsu Photonics, and distributed by Pulsion Medical Systems in France and in Europe. At the present time, most clinical studies, especially in the domain of plastic and reconstructive surgery, use the apparatus commercialized by Hamamatsu Photonics (PDE™, Hamamatsu Photonics, Hamamatsu, Japan). Other systems available include the SPY™ (Novadaq Technologies, Concord, Canada) and the Fluobeam™ systems (Fluoptics, Grenoble, France).

Lastly, other applications of this new imaging technique are under investigation in laparoscopic and robotic surgery.

**Detection of liver tumors**

**Aspect of fluorescent lesions**

The fluorescent aspect of HCC is variable (Figs. 2–4) [10,25]. Well-differentiated HCC are seen as intense and homogeneous fluorescent spots whereas moderately differentiated HCC are seen as partial and heterogeneous areas. Poorly differentiated HCC and liver colorectal (adenocarcinoma) metastases are not visible in fluorescence and the characteristic of these lesions is a fluorescent ring corresponding to the peri-tumoral parenchyma surrounding an empty zone corresponding to the lesion.

**Figure 1.** Fluorescence imaging system. A. The imaging system includes a camera and amplifier. B. The camera simultaneously induces the excitation of fluorescence thanks to a laser (LED emitting an infrared radiance) and acquires fluorescence images ensured by a captor (CCD) which filters the light so that only near infrared wavelengths can be seen. C. Intra-operative use of fluorescence imaging. Photos published courtesy of Pr. Norhiro Kokudo, and photo 1C courtesy of Dr Takeaki Ishizawa [30].
Pathophysiological mechanisms

The pathophysiological mechanisms underlying the fluorescent anomalies are not totally elucidated but could possibly implicate hepatocyte-to-bile duct secretion anomalies [32]. It has been well established that IG selectively taken up by hepatocytes via two membrane transport systems, OATP1B3 (transporter belonging to the family of OATP or organic anion transporters) and NTCP (Na+/Taurocholate Co-transporting Polypeptide). The molecule is then excreted into the bile via other membrane transporters located on the membranes of the biliary canalicules (MRP2 or multidrug-resistance-associated protein 2) [33]. The fluorescence obtained in the case of well-differentiated HCC could be related to MRP2 canalicular transporter anomalies, which lead to intracellular accumulation of IG. This hypothesis implies that IG is captured by tumor cells but cannot be excreted correctly in the biliary canalicules.

Conversely, in poorly differentiated HCC and colorectal metastases, the lack of fluorescence could be interpreted as the absence of capture of the molecule by the tumor cells. Here again, the mechanism responsible for the peri-tumoral fluorescence is not known but could imply impaired hepatocyte biliary secretion in immature hepatocytes [34]. In a recently published Dutch study [34], the authors observed fluorescence in the cytoplasm and outside cells expressing the CK7 marker with a microscope. The presence of CK7+ cells was mostly detected at the periphery of liver metastases. These cells expressing the CK7 marker can be identified as progenitor cells (dual hepatocyte and biliary potentiality) and attests to the presence of a ductal reaction associated with liver metastases. In addition, a decrease in membrane transporter expression leading to decreased excretion of several molecules, including IG, has also been observed.

The rationale behind peri-tumoral fluorescence reposes on the hypothesis that IG accumulation in the CK7+ cells found in the peripheral ductular reaction of liver metastases is largely responsible for this peri-tumoral fluorescence.

Value of the technique

Tumor localization

Intra-operative fluorescence could improve detection of small superficial lesions and help to determine the nature of...
Figure 3. Fluorescent imaging on hepatectomy specimens. A. Total and homogeneous fluorescence imaging (well-differentiated HCC). B. Partial fluorescence imaging (moderately differentiated HCC). C. Peri-tumoral fluorescence (fluorescent ring, poorly differentiated HCC). D. Peri-tumoral fluorescence (colorectal liver metastasis). Figures courtesy of Dr Takeaki Ishizawa [30].

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Figure 4. Schematic aspect of liver lesion seen in fluorescence.
these lesions, especially when clinical examination, preoperative imaging (CT, MRI), and intra-operative investigations (inspection, liver palpation and ultrasound) are inconclusive. Currently, fluorescence findings should be compared with those obtained by inspection, liver palpation and ultrasound, in order to determine the existence of supplementary lesions.

The limits of this technique today are related to the sensitivity of the camera and the function of the underlying parenchyma. In certain patients with cirrhosis, present-day cameras occasionally detect several hot spots corresponding to dysplastic and/or regeneration nodules rather than to authentical HCC. In the cirrhotic liver, thanks to the intensity of the fluorescence, this type of camera is especially useful to detect one or more peripheral satellite nodules around the main nodule. This technique is useful in the "healthy liver", particularly during the treatment of colorectal liver metastases, and has an impact on therapy in 5% to 8% of cases [31]. Another value of this technique is to visualize extra-hepatic metastases of HCC (sub-diaphragmatic nodules, peritoneal carcinomatosis and lymph-nodes), but here again the mechanism behind this extra-hepatic fluorescence is unknown and comparative studies (fluorescence vs. CT and PET-CT scans) are necessary to evaluate the true mechanism of action [35].

The most innovative concept consists of detection of micrometastases by multislice illumination of the operative specimen [36]. This type of investigation does not require any complementary techniques (occult metastases are usually detected by immunohistology). This technique could also allow intra-operative analysis of the quality of surgical excision, by distinguishing between R0 and R1 excisions.

Surgery of liver tumors

Based on the same principle as methylene blue tattooing of the liver segment to be resected (puncture of the portal branch of the segment and injection of methylene blue under ultrasound guidance), intra-operative fluorescence allows outlining of the segment(s) to be resected, in order to perform a formal liver resection (Fig. 5) [37]. During laparoscopic hepatectomies, difficulties in delimiting the territory to resect may arise because the abilities to inspect and palpate are limited compared with conventional open surgery [38]: this is also true for repeat hepatectomy where the presence of adhesions on the surface of the liver make it difficult to visualize the tattooed territory or the limits of ischemia. The technique of IG injection is also useful in performing liver resections for biliary lesions that cause localized biliary stasis. Strong fluorescence can be detected in the stagnant biliary zone [39].

In sum, the most pertinent indications of the use of IG fluorescence in the management of malignant liver tumors include:

• detection of small superficial nodules;
• identification and characterization of HCC and colorectal liver metastases, distinguishing between these lesions and benign lesions.

At the present time, there is not enough available data to justify the use of this technique to detect extra-hepatic nodules or to find hepatic lesions that disappear under chemotherapy.

In the future, this technique should potentially be useful for laparoscopic resections, not only to identify the lesions but also to determine the resection margins, which are

Figure 5. Formal segment VI resection. A. Early images after ultrasound-guided puncture and intraportal injection (in portal branch of segment VI) of indocyanine green. B. Resection of segment VI. Note the presence of fluorescent parenchyma in segment VI in the plane of division.
often difficult to determine during atypical resection via laparoscopy.

Limitations
The main limitation of this imaging technique is low sensitivity for the detection of deep nodules and the false positive results seen in the cirrhotic liver. These limitations are anatomical, but also related to the physical properties of IG because of the attenuation of fluorescence as the proportion of light reaching the camera decreases as it traverses tissues. Thus only superficial or fluorescent structures less than 10 mm deep can be visualized. Ishizawa et al. reported a detection rate of 65% (37/57 lesions) in a series of 26 patients with a total of 57 lesions (41 HCC and 16 metastases) [10, 30]. Lesions not identified by fluorescence were smaller (11 mm vs. 18 mm) and deeper (10 mm vs. 2 mm) than those visible under fluorescence. Intra-operative ultrasound allowed the detection of 33 HCC nodules and 16 metastases (86% detection rate). Nonetheless, among the eight HCC undetected by ultrasound, three nodules were found by fluorescence (sub-capsular located 1–2 mm below the surface of the liver).

Moreover, it is important to recognize that false positive findings have been reported with this technique. Benign lesions such as regenerative nodules or biliary hamartomas can generate hyperfluorescence, indistinguishable from that produced by malignant lesions. Approximately 40 to 50% of lesions visualized by fluorescence in HCC resection specimens were false positives [10, 25].

Lastly, there are no studies that allow confirmation of the absence of tumor cells in the hyperfluorescent halo found on the periphery of metastases; fluorescence measurement therefore does not allow affirmation of the R0 character of resection.

Conclusions and perspectives
Indocyanine green fluorescence imaging is a promising technique in liver surgery. This technique provides additional information in the localization and diagnosis of the more superficial lesions. The anatomical limits (loss of diagnostic sensitivity for deep nodules) of this imaging require further optimization to improve the results. Additional knowledge on hepatobiliary transport systems and pathophysiologic mechanisms should allow further progress with this technique. In the future, this new imaging technique and navigation tool should allow detection of subclinical lesions and increase the safety and precision of hepatocellular carcinoma.

ESSENTIAL POINTS
- Imaging based on the fluorescent properties of indocyanine green allows simultaneous visualization of biliary canals and hepatic segments, as well as liver cancer, during liver surgery.

Disclosure of interest
The authors declare that they have no conflicts of interest concerning this article.

References
Reference List


