Indocyanine Green Fluorescence in Laparoscopic Cholecystectomy: An Easy Procedure to Prevent Big Troubles

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Abbreviations:

1. Abstract

1.1. Background: Laparoscopic Cholecystectomy (LC) has replaced the open technique due to its advantages in terms of Length of Stay (LOS), postoperative pain and surgical site infection rate, increasing however the number of biliary tract lesions. Indocyanine Green Fluorescence (ICG) is used for a better intraoperative identification of the anatomical structures and showed similar results compared to the intraoperative cholangiography in reducing biliary lesions, with the advantage of a shorter time of operation.

1.2. Methods: A series of 126 LC was analyzed in order to define whether a better identification of the biliary tree by ICG may reduce the time of operation, the conversion rate and the incidence of biliary lesions. From January to December 2019, 109 elective cholecystectomies and 17 urgent cholecystectomies were performed by different operators; 101 patients were administered with intravenous ICG at least 2 hours before surgery.

1.3. Results: Operation time ranged between 35 and 150 minutes, considering all the 126 patients, between 35 and 130 minutes for the patients operated with ICG technique and without associated pathologies (101 patients). The conversion rate on 126 cases was 2.4%, 1.1% if we consider ICG patients only. The complications rate resulted 6.3% on 126 patients: 2 patients (1.6%) presented minor lesions of the biliary tract conservatively treated, 3% had other complications; no major lesions of the biliary tract were observed. In ICG cholecystectomies, only 1 biliary lesion was detected (1%). The LOS was calculated excluding 7 patients who had associated surgery: it was 1.9 days versus 1.86 days considering the ICG group only.

1.4. Conclusions: The use of ICG during LC allows a better intraoperative visualization of the biliary tree anatomy and seems to reduce the incidence of biliary tract lesions or at least reduces their severity. Furthermore, we observed a decreased conversion rate, while the hospital stay seemed not to be affected by the use of ICG.

2. Introduction

Laparoscopic Cholecystectomy (LC) has replaced the open technique sharply showing its advantages in terms of hospital stay, postoperative pain and surgical site infection incidence. On the other hand, especially at the beginning of the laparoscopic era, the laparoscopic approach increased the biliary tract iatrogenic lesions (0.3-0.5% vs 0.1%) [1-3]. Only the improved surgeons’ laparoscopic skills, the standardization of the procedure and the introduction of the SAGES’ Critical View of Safety reduced the incidence of biliary tract lesions [4].
Indocyanine green is a sterile, anionic, water-soluble but relatively hydrophobic, tricarbocyanine molecule with a molecular mass of 776 Daltons [6] (Figure 2). ICG dye was developed for near infra-red (NIR) photography by the Kodak research laboratories in 1955 and was approved for clinical use in 1959 by the FDA [5-6]. The usual dose for standard clinical use (0.1–0.5 mg/ml/kg) is well below the toxicity level [5-7, 9]. Indocyanine green fluorescence (ICG) has been used for a better intraoperative identification of the biliary tract and showed similar results compared to the classic intraoperative cholangiography in reducing biliary tract lesions, with the advantage of a shorter time of operation [5-9].

This retrospective study investigates the possible advantages of the ICG method in relation to time of operation, conversion rate to open surgery and postoperative complications rate in both the elective and urgent cholecystectomy procedures.

![Figure 1: Near-infrared fluorescent cholangiography (NIRF-C)](image)

![Figure 2: Tricarbocyanine molecule](image)

### 3. Materials and Methods

A series of 126 LC from a single surgical center was retrospectively analyzed in order to define whether a better identification of the biliary tree by ICG may have reduced the incidence of iatrogenic biliary lesions, the conversion to open surgery rate, the time of surgery and the Length of Stay (LOS).

The factors affecting the qualitative and quantitative visualization of the fluorescence such as obesity, inflammation, timing of administration of the vital dye were also analyzed.

From January to December 2019, 109 elective LC and 17 urgent LC were performed by different operators. Seven of these patients had an associated surgical disease (e.g. incisional hernia, umbilical hernia, ovarian cyst, uterine fibroid).

71 patients (56%) were female and 55 were male (44%) with a median age of 53.71 ± 12.90. 21 of them (16.7%) had associated cardiovascular, pulmonary and metabolic comorbidities, in 9 patients (7.1%) a body mass index (BMI) greater than 30 was observed (Table 1).

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
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<tr>
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<td>&gt;30</td>
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<tr>
<td>Other</td>
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<td>1,6%</td>
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<tr>
<td>Surgical indication</td>
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<tr>
<td>Symptomatic biliary lithiasis</td>
<td>17</td>
<td>13%</td>
</tr>
<tr>
<td>Chronic cholecystitis</td>
<td>109</td>
<td>87%</td>
</tr>
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</table>

LC Laparoscopic cholecystectomy; BMI Body mass index; yrs years

Inclusion criteria were symptomatic cholelithiasis, chronic cholecystitis, acute cholecystitis (as documented by abdominal sonography or computed tomography) without suspicion of bile duct stones, patients operable by laparoscopy and who agreed to study. The main exclusion criteria were the presence of hepatic failure, a history of bile duct surgery, ongoing pregnancy or breastfeeding and allergy to ICG. Patients with a history of cholecystitis and prior abdominal surgery were not excluded. All patients included
in the study signed a written informed consent before surgery. LC was performed by using French approach and four-port technique. In 101 (80.1%) patients a Near-Infrared Fluorescent Cholangiography (NIRF-C) was performed (Figure 1).

In our experience 25 mg ICG (VERDYE 5 mg/ml Diagnostic Green Aschheim-Dornach, Germany) diluted with saline solution were administered intravenously at least 2 hours before surgery. Intrahepatic administration of green indocyanine was used in one patient.

ICG is a fluorophore that responds to NIR irradiation, absorbing light from 790 to 805 nm and re-emitting it with an excitation wavelength of 835 nm. Administered intravenously, ICG binds to plasma proteins and becomes confined to the intravascular space. Then it is exclusively metabolized by the liver and excreted via the biliary system from 15 to 20 minutes after administration [5, 8, 9]. These properties, combined with the absence of any native biological fluorescence within these wavelengths, render ICG an ideal agent for acquiring high-quality biliary-tract images [9, 10].

The fluorescence released by ICG can be detected using specifically designated scopes and cameras. For all procedures, an Image 1 S imaging system with OPAL1 technology for NIR/ICG imaging (KARL STORZ SE & Co KG, Tuttlingen, Germany) was used. During LC, alternating exposure from xenon white light (STD mode) to NIR light (ICG mode) was used to identify biliary structures before, during, and after dissection.

The fluorescent imaging system consisted of a xenon light source (D-Light P) that could emit both near-infrared (>780 nm) and white light, a NIR transmitting telescope and a full HD 3-chip fluorescence imaging camera head, capable of capturing both white-light and NIR images. A foot pedal allows the surgeon a rapid conversion between fluorescent and white light.

NIRF-C was performed at three defined time point during LC: (i) following exposure of Calot’s triangle (Figure 3), prior to any dissection; (ii) after partial dissection of Calot’s triangle; (iii) after complete dissection of Calot’s triangle, according to the “Critical View of Safety” method (Figure 4, 5).

4. Results

Operation time ranged between 35 and 150 minutes (average 92.5), considering all the 126 patients. Examining only the patients operated with ICG technique (ICG-LC) (101 patients), the operation time falls in a range between 35 and 130 minutes (average 82.5). We were able to identify the biliary anatomy in all cases (100% sensitivity), especially the cystic duct-common bile duct junction (CD-J), irrespectively of whether the tissues were normal or inflamed (Figure 6a, b). Before the Calot dissection we identified the cystic duct (CD) in 98% of cases and the common bile duct (CBD) in 70% of cases (Figure 7a, b); on the contrary the CD and the CBD were identified after the dissection respectively in 100% and in 96% of cases (Figure 8a, b).
There were no adverse reactions to the ICG injection. The conversion to open surgery rate on 126 cases was 2.4% (3 patients), which dropped to 1.1% (1 patient) if we consider ICG operated patients only. The postoperative hospital stay was calculated excluding the 7 patients who had associated surgery: considering 116 patients, the postoperative hospital stay was 1.9 days, while in the ICG group (108) the postoperative hospital stay resulted in 1.86 days (Table 2).

<table>
<thead>
<tr>
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<th>ALL-LC</th>
<th>ICG-LC</th>
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<tbody>
<tr>
<td>Operative time</td>
<td>35-150 min</td>
<td>35-130 min</td>
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<tr>
<td>ICG dose</td>
<td></td>
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<tr>
<td>Visualization before dissection (ICG-LC)</td>
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<tr>
<td>CD identification</td>
<td>98%</td>
<td></td>
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<tr>
<td>CBD identification</td>
<td>70%</td>
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<tr>
<td>Visualization after dissection (ICG-LC)</td>
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<tr>
<td>CD identification</td>
<td>100%</td>
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<tr>
<td>CBD identification</td>
<td>96%</td>
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<tr>
<td>Postoperative Complications (ALL-LC)</td>
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<tr>
<td>Minor lesions of the biliary tract rate (ALL-LC)</td>
<td>2 (1,6%)</td>
<td>1 (1%)</td>
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<tr>
<td>Minor lesions of the biliary tract (ICG-LC)</td>
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<tr>
<td>Postoperative bleeding (ALL-LC)</td>
<td>4 (3,2%)</td>
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<tr>
<td>Small Bowel perforation (ALL-LC)</td>
<td>1 (0,8%)</td>
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<tr>
<td>Non-surgical complications (ALL-LC)</td>
<td>4 (3,2%)</td>
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<tr>
<td>Conversion to open surgery</td>
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<tr>
<td>ALL-LC</td>
<td>3 (2,4%)</td>
<td>1 (1%)</td>
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<tr>
<td>ICG-LC</td>
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<tr>
<td>Postoperative hospital stay</td>
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<tr>
<td>ALL-LC</td>
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<td>ICG-LC</td>
<td>1,86 days</td>
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ALL-LC Laparoscopic cholecystectomy n=126; ICG-LC Indocyanine green-laparoscopic cholecystectomy n=101 CD Cystic duct; CBD Common bile duct; min minutes
The overall complications rate, considering both ICG and non-ICG group, resulted in 6.3% of 126 patients: 2 patients (1.8%) presented minor lesions of the biliary tract (type A according to the Strasberg classification), 4 patients (3%) had non-surgical complications including 1 postoperative fever, 4 postoperative bleedings conservatively treated, 1 reoperation for small bowel perforation; no major lesions of the biliary tract were observed.

The two cases of lesions of biliary tract lesions consisted of bile leakage from cystic duct and were successfully treated by ERCP and bile stenting. Considering only ICG cholecystectomies, only 1 lesion of the biliary tree was detected with an incidence of 1% (Table 2).

5. Discussion

Bile Duct Injury (BDI) is a rare but serious complication of Laparoscopic Cholecystectomy (LC) with an incidence between 0.4% and 0.7% [1-3], and recently reported to be as high as 1.3% [11], generally due to misinterpretation of biliary tract anatomy [4].

The use of intraoperative cholangiography is not routinely used, due to several problems (increased operating time, technical difficulties, etc.). Indocyanine Green (ICG) Near-Infrared Fluorescent Cholangiography (NIRF-C) is a non-invasive technique and provides real-time biliary images during surgery, which may improve the safety of laparoscopic cholecystectomy when associated with careful and meticulous dissection of the Calot’s triangle, achieving the so-called “critical view of safety” [4-6]. NIRF-C enables to perform a sort of “virtual” cholangiography at the very start of the procedure, allowing the surgeon to identify either the normal anatomy or possible anatomic variations in normal settings or in potentially dangerous situations (i.e., the presence of inflammatory tissue), areas to be respected until the dissection allows a better identification of the different structures [7-10, 12].

Several factors influence the fluorescence intensity during NIRF-C with ICG. Important are patient factors like obesity and inflammation, i.e. cholecystitis. These factors may increase thickening of tissue around biliary tree and decrease the fluorescence signal intensity. Although fluorescence imaging has a better tissue penetration than white light, due to its emission in the near-infrared wavelength zone, penetration is limited by such factors [10].

In several studies, patients with cholecystitis were included. Zrowback et al. [13] appreciated the NIRF technology most while dissecting the most inflamed gallbladders. Conversely other colleagues argue that cholecystitis was more frequently present in patients in whom the biliary tree could not be visualized, than in patients in whom imaging was clear [9, 14]. In patients with cholecystitis Liu et al. report a significant difference in favor of NIRF imaging about the better visualization of the CD compared to white light [15]. Also the visceral obesity, namely the amount of intra-abdominal adipose tissue, could influence ICG imaging. Unfortunately, there are few studies, examining this factor and to date we have no significant conclusions about it. Interestingly, one study [16] reported improved visualization rates for the CD-J in patients with lower BMI (Body Mass Index), while another study reported no differences [17] with increasing BMI. In effect it seems that more intra-abdominal adipose tissue results in a decreased penetration of NIR light and thus decreased visualization of biliary tract structures.

Other studies claim that BMI has a negative influence on the visibility of the structures using NIRF-C [18, 19]. It is even suggested that also a more common inflammatory response is present in these patients [19]. The great advantage of fluorescence cholangiography is that it is used gradually during surgical dissection and sooner or later during this dissection NIRF-C will lead to the confirmation of the anatomical structures. The presence of fatty tissue usually requires more dissection and, often, later visualization of anatomical structures. The earlier or later recognition may influence the surgeon’s satisfaction with the NIRF technique.

The differences between studies may also depend on the ICG concentration used because patients with a higher bodyweight probably need of a different concentration instead of a fixed and not a weight-adjusted dose.

Therefore, it seems that ICG fluorescence is useful also when the thickness of overlying tissue limits the penetration depth. In these cases, in fact the image will be obtained later, after relatively more dissection has been performed. The surgeon should be aware of this, but with this in mind, can use the technique to enhance recognition of the essential structures [18-20].

There is not a well-defined “optimal time” and several studies reported a wide range of time in whom ICG administration can be performed.

Verbeek et al. [21] investigated the timing of ICG administration and found that administration 24 h prior to surgery results in a significantly better signal to background ratio, mainly due to a lesser powerful fluorescence coming from the liver. But this approach has evident and practical problems: in elective surgery and in acute cholecystitis often it’s not feasible the injection of ICG 24h before surgery. Moreover, they tested only 30 min versus 24h before surgery so that the optimal dosage could stay between these two timing. Kono et al. [22] and Booger et al. [23] also conclude that earlier administration results in better visualization. Tsutsui et al. [24] investigated the optimum timing of ICG administration in order to obtain strong fluorescence intensity of the biliary tract and weak fluorescence intensity of the liver. The timing of ICG administration was set immediately before surgery and at 3, 6, 9, 12, 15, 18, and 24h before surgery. The authors conclude the optimal timing of ICG administration for fluorescent cholangiography during laparoscopic cholecystectomy was 15h before surgery.

In conclusion, administration closer to the time of surgery can result in a more prominent background signal from the liver. There-
fore, administration as early as possible (but not earlier than 24h) should be considered.

As concerns the exact dose and concentration of ICG to be given to patients, most authors use 0.2–0.5 mg/kg body weight [5, 25]. Morita et al. [26] used 2.5 mg but did not state the dilution or the volume. Boni et al. [25] reported 5 ml of 0.3–0.4 mg/ml/kg provided adequate concentration in the bile hence an adequate visualization of the biliary tree. Boogerd et al. [23] compared a dose of 5 and 10 mg and advise to use the lower dose of 5 mg. Visualization rates of the biliary structures using NIRF imaging techniques with ICG appear to be equally good for either 2.5 mg fixed dosage or 0.05 mg per kg dosage of ICG [10, 22]. Some authors, in systematic reviews, reported the ICG dosage between 2.5 mg in a single IV administration to 0.5 mg/kg [20, 27]. Zarrinpar et al. reported that the best visualization of biliary ducts was obtained with 0.25 mg/kg of ICG, administered at least 45 min before the NIRF view [28]. In our experience, 25 mg ICG was administered approximately two hours before surgery, with good visualization rates of biliary tree.

The EURO-FIGS registry confirms the large differences using ICG fluorescence in several European centers, particularly about the dose and the interval timing between ICG administration and intraoperative imaging [29]. The weakness of NIRF-C is the need to inject a fluorophore, the impossibility to detect retained stones, and the background liver fluorescence signal.

As previously stated, a wide disparity in NIRF-C protocols has been pointed out in review articles [20, 27], particularly in terms of dosing and timing of ICG administration. The optimization of those two major controllable factors influences the noise liver signal and the image quality and, consequently the performance of NIRF-C. Other non-controllable factors include pathology (inflammatory status) [15], BMI [19], and sensitivity of the NIR device.

The injection of ICG directly into the gallbladder permits to overcome the drawback of liver noise fluorescence and this is particularly interesting in case of cholecystitis, managed with percutaneous drainage. Alternatively, it is possible to simply puncture the gallbladder intraoperatively, but there is some risk of dye spillage, which could impair the visibility by contaminating the operative field. The direct injection is relevant especially in case of cholecystitis, considering, as reported in the literature [15, 30], that inflammation affects negatively the visualization of biliary anatomy. And direct gallbladder fluorophore injection also improves significantly the visualization of the plane between the gallbladder and the liver bed. It has also the advantage to quickly detect any accessory bile ducts arising from the gallbladder bed, usually missed with a standard light view [30].

Only in one patient of our study we used intracholecystic administration of ICG.

This technology may also be considered as an important teaching tool for laparoscopic surgery for training surgeons [27, 31]. Roy et al. reported that medical students and surgery residents identified more precisely the cystic duct through near-infrared light when compared to white light [32]. In the experience of Pesce et al. [27] the NIRF-C received positive feedback from surgery residents with a good perceived benefit. It is noteworthy that the residents considered the NIRF-C a useful tool for intra-operative visualization of the biliary tree and in facilitating Calot’s dissection reducing the risk of BDI.

There are also some technical tips that can be used in different circumstances like the reflux maneuver that lifts up the bile from the CBD towards the CD, in order to “illuminate” it when it’s inflamed or the patient has high BMI, or the compression maneuver to compress the fatty tissue in favor of fluorescence of biliary tract (Figure 9a, b) or, at last, the liver bed evaluation during the proper cholecystectomy time just to evaluate the presence of Luschka ducts.

In conclusion the use of ICG during laparoscopic cholecystectomy allows a better intraoperative visualization of the anatomy of the biliary tree and seems to reduce the incidence or at least the severity of iatrogenic biliary lesions. Moreover, we observed a decreased conversion rate, while the hospital stay seemed not to be affected by the use of ICG.

In conclusion it seems evident that the constant use of NIR fluorescence can lead to a safer procedure, especially in case of unfavorable technical conditions and/or less experienced surgeons.
References


