Fluorescence cholangiography for laparosc cholecyst: how, when, and why?A single-center preliminary study

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ABSTRACT

Introduction: Bile duct injuries avoidance is a key goal of biliary surgery. In this prospective study we evaluate safety and feasibility of ICG fluorescent cholangiography during laparoscopic cholecystectomy (LC) focusing on the optimization of timing and dose administration.

MaterialsandMethods: From February to December 2022 fifty-four LC were performed with fluorescence imaging in our surgical department. 2.5mg ICG were administered intravenously between 5h and 24h before surgery. Near-infrared fluorescent cholangiography (NIRF-C) was performed ~~with Elevision™ IR Platform (Medtronic, Mansfield, MA, USA)~~. Adequate fluorescence was evaluated by comparing agent accumulation in gallbladder and extrahepatic duct and background of liver parenchyma.

Results: Biliary anatomy was identified in all cases. Median time of ICG administration was 12h previous surgery and 3 groups of patients were identified: group A receiving ICG 5h-9h before surgery, group B 10h-14h before, group C 15h-24h before. Peak contrast was gained in group B, with minimal fluorescence of liver parenchyma and more intense visibility of biliary tract. Intraoperative cholangiogram was unnecessary in all cases.

Conclusion: Fluorescent cholangiography during LC is safe and feasible overcoming the limits of other techniques available. 2.5 mg ICG administered 12-15 h before surgery ~~using Elevision™ IR Platform~~ produces optimal outcomes for NIR fluorescent cholangiography.

Key words: laparoscopic cholecystectomy, fluorescent cholangiography, indocyanine green, fluorescence

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INTRODUCTION

Cholecystectomy is the most common operation performed worldwide by general surgeons. Between 750,000 and 1,000,000 cholecystectomies are carried out in the U.S. annually [1] representing one of the first operations performed by young surgeons during their training, 80% laparoscopically [2]. Although laparoscopy has certain advantages over open cholecystectomy, concern has been expressed regarding the potential for bile duct injuries (BDI). BDI are the most common serious complications of cholecystectomy frequently resulting in additional surgeries, long recovery time, and diminution of quality of life [3, 4].

Several methods have been described to identify the structures in Calot's triangle in order to reduce biliary complications including intraoperative cholangiogram (IOC) by Mirizzi [5] and critical view of safety (CVS) by Strasberg [6].

Since its introduction, in the era of open cholecystectomy the IOC has been routinely used to detect common bile duct stones (CBDS) and prevent BDI. However, the routine method is currently controversial. A growing number of surgeons advocate a selective approach, believing that IOC leads to prolonged operating times, costs, exposure to ionizing radiation, and cystic catheterization itself increases the risk of BDI.

In 1955 the Kodak research laboratories developed the ICG dye for near infra-red (NIR) photography and in 1959 the FDA approved its clinical use [7, 8]. Recently, indocyanine green (ICG)-enhanced fluorescence was introduced in laparoscopic surgery to improve the view and provide detailed anatomical information during operation [7]. In 2009 Ishizawa et al. [9] first described intraoperative fluorescence cholangiography.

ICG is an amphiphilic, tricarbocyanine iodide dye (mass = 751.4 Da) that is reconstituted in aqueous solution of pH 6.5 for intravenous injection in patients [10]. Intravascularly, the compound binds to plasma proteins and protein-bound emits light with a peak wavelength of around 830 nm when illuminated with near-infrared light. While ICG is rapidly excreted via the bile duct, it is most natural to apply ICG intraoperatively to aid bile examination and operation. Biliary excretion of ICG starts within minutes after intravenous injection, peaking within 2 hours and continuing for as long as 20 hours [11, 12].

Whereas several studies have shown its feasibility, dosing and timing for practical use have not been optimized. The present study aims to demonstrate the safety and feasibility of ICG fluorescent cholangiography focusing on the optimization of timing and dose administration.

MATERIALS AND METHODS

From February 15 to December 15, 2022 fiftyty-four consecutive elective laparoscopic cholecystectomies were performed with fluorescence imaging in our surgical department. Data regarding patients' characteristics, intraoperative findings and postoperative outcomes were collected and entered in a prospective database. The study was conducted retrospectively and in accordance with the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all participants. Exclusion criteria were biliary obstruction, serum bilirubin>30 μmol/L, cirrhosis.

All patients were studied preoperatively with blood tests and evaluation of cholestasis indices, abdominal US, and in case of suspicion of stones in the CBD, a magnetic resonance cholangiopancreatography (MRCP) was always performed. If bile duct stones were present, endoscopic retrograde cholangiopancreatography (ERCP) was performed before surgery.

The operations were carried out by both a senior surgeon and residents assisted by a tutor. Laparoscopic cholecystectomy was performed by four ports using the French position. For intraoperative fluorescent cholangiography, 2.5 mg ICG (ICG, Diagnostic Green GmbH, Germany) was administered intravenously between 5h and 24h before surgery. In most of cases near-infrared fluorescent cholangiography (NIRF-C) was performed by using the Elevision™ IR Platform (Medtronic, Mansfield, MA, USA) that enables superimposition of fluorescence images on full-color images. The Visionsense Iridium system delivers NIR fluorescence HD visualization and it represents the unique support of simultaneous NIR and white light imaging as well as the ability to overlay NIR images onto white light images ("extended reality"). This system, CE (Conformite Europeenne) marked and available for clinical use in Europe and the United States, allows for qualitative and quantitative measurement of IR intensity with a translation of images from the camera control unit to a 1920×1080 pixels high-resolution display monitor mounted on a video cart [13].

In 8 cases the RUBINA™ system by KARL STORZ SE & CO. KG, Tuttlingen, Germany was used. This system is composed of OPAL1® NIR/ICG, a camera head for ICG-NIRF imaging in combination with POWER LED RUBINA™ and TIPCAM®1 RUBINA™, a high-resolution laparoscope with two distally integrated video chips for ICG-NIRF imaging.

With both the Elevision™ IR Platform and the RUBINA™ components through the control panel it is possible to obtain single-touch recording and snapshots, scalable viewing images with enhanced image quality. Monochromatic, fusion and color images were used to evaluate the fluorescence intensities.

The primary endpoint was the evaluation of adequate fluorescence gained through the agent accumulation in the extrahepatic duct and the fluorescence absence in the liver parenchyma. The visibility of both cystic duct and CBD was scheduled as well as the presence of liver hyperfluorescence.

Secondary endpoints included peri- and post-operative complications and 30-day mortality.

Statistical analysis was performed using a computer software program (SPSS Version 25.0.0.2; SPSS Chicago, IL, USA for MacOS High Sierra ver.10.13.4, Apple Inc. 1983-2018 Cupertino, CA, USA). A chi-square test was applied to analyse categorical data. Results are presented as two-tailed values and considered statistically significant if $p<0.05$.

RESULTS

Preoperative and intraoperative data are summarized in Table 1.

Every patient received the same dosage of ICG corresponding to 2.5 mg administered intravenously. The first step in all cases was the identification of the main anatomical landmarks: the hepatocystic triangle (Figg 1a-b), the cystic plate, the umbilical fissure, and the Rouviere's sulcus on the under surface of the right hepatic lobe, running to the right of the liver's hilum (Figg 2a-b). NIRF-C was performed prior and after partial and complete dissection of Calot's triangle, according to the CVS method. As a result, we identified the biliary anatomy in all cases.

Intra- and postoperative outcomes are summarized in Table 2.

The median time of administration was 11h previous surgery (min 5h - max 24 h). However, we distinguished a group of 22 patients (group A) which received ICG between 5h and 9h before surgery, a group of 22 patients (group B) receiving fluorescence between 10h and 14h before surgery, and a group of 10 patients (group C) receiving ICG between 15h and 24h before surgery. We then compared images obtained during operations and observed that the fluorescence intensity of liver parenchyma resulted minimal in the groups B and C compared to the group A (Figg. 3a-b), leading to a stronger contrast between liver, and biliary tract. The peak of the contrast was gained in the group B (Figg. 4a-b), with a minimal fluorescence of liver parenchyma and a more intense visibility of both cystic duct and CBD. In the group C liver parenchyma and bile duct are both low fluorescent with consequently low visibility of bile duct Figg. 5a-b). Therefore, a statistically significant difference was reported for liver parenchyma interference in the comparison of groups A vs B and B vs C whereas in CBD visualization between groups B and C.

Intraoperatively, CVS was obtained in all cases as a preliminary step to the cystic duct and cystic artery clippage and division. There were no adverse reactions to the ICG injection and no intraoperative or postoperative complications. IOC was unnecessary in all cases.

DISCUSSION

Avoidance of BDI is a key goal of biliary surgery. A safe cholecystectomy cannot be performed without identifying some anatomical landmarks and regardless of the new technologies available this remains an essential step. The hepatocystic triangle should always be exposed before any dissection begins (Fig. 1a). Moreover, all the dissection during the LC should be done ventral and cephalad to the line joining the roof of Rouviere's sulcus and base of segment 4 (Fig. 2a-b) [14]. The concept of the "CVS" was introduced in order to reduce the damage resulting from the uncorrect recognition of the main structures. Today CVS is widely used, requiring three goals: first, the hepatocystic triangle must be cleared of fat and fibrous tissue; secondly the lowest part of the gallbladder must be separated from the cystic plate; third, two structures, and only two, should be seen entering the gallbladder. Only when these three criteria are met, CVS has been obtained [15].

Various methods have been described for intraoperative assessment of biliary anatomy.

IOC is one of the most commonly adopted and many surgeons perform it systematically. However, while IOC has been proven to be helpful in identifying biliary anatomy, literature supports that the dissection of the Calot's essential to perform the IOC exposes in any case to the risk of type D injury of the CBD (Strasberg classification) because of misinterpretation of the anatomy [16, 17]. Furthermore, the need for dissection before IOC may be technically difficult in acute or chronic inflammatory disease, together with cannulation of a short, thin, or tortuous cystic duct [18]. Radiation exposure, higher costs, and prolonged operative times are other disadvantages that lead to IOC being discontinued as a routine imaging modality [19, 20]. For all these reasons, the role of the IOC is no longer considered to prevent BDI but only to diagnose it once it has occurred.

Laparoscopic ultrasound (LUS) during cholecystectomy has been described as an alternative non-invasive study of the biliary tract, with an excellent ability to detect common bile duct stones and identify anatomy [21]. Compared to IOC, it results cheaper, faster and non-irradiating. However, LUS has some important disadvantages which make it available only in dedicated centers: it is operator-dependent, and requires a greater learning curve and specific instrumentation.

In recent years, an increasing number of publications have described the principal advantages of ICG that seem to overcome the limits of CVS, IOC, and LUS. The strengths of fluorescence include high signal to noise ratio (SNR): only the target, not background, is visible because separate wavelengths are used for illumination and recording; high sensitivity: extremely small concentrations can often be made visible; low toxicity with a LD50 (lethal dosage) of 50 to 80 mg/kg for animals. Furthermore, it is cheap, easy to use and not time consuming [7-10, 19]. The optical instrumentation and computing needed are quite simple, requiring only three pieces of equipment: a laparoscopic tower, a specific camera, and a specific laparoscope. There

are no added personnel requirements or costs, and no prolongation of the surgery, unlike intraoperative cholangiogram. Dip et al compared the average procedural cost for FC versus IOC over 43 laparoscopic cholecystectomies resulting in the former being vastly less expensive (mean cost=$14 vs. $778, p<0.001) [22]. Last but not least, FC is incisionless and no preliminary dissection is needed to obtain images.

Although several investigations indicate that the ability of FC to visualize the critical junction is equal to that of X-ray cholangiography [23-26], we cannot fail to describe some of the most important limitations of this technique. First, FC does not allow for the detection of bile duct stones due to the inability of near-infrared light to penetrate the structures. Therefore, while FC is useful to detect gallstones impacted in the cystic duct, it would be difficult to visualize small CBD stones as defects on fluorescence images because of the presence of fluorescing bile around the stones [12]. For that reason, the combination of a preoperative MRCP and/or standard IOC is mandatory for patients with a likelihood of CBD stones. Moreover, due to the limited penetration depth of approximately 1 cm of light in the NIR spectrum, structures beneath a layer of periductal fat, especially in obese patients, are more difficult to identify using NIR fluorescence imaging [27, 28].

Another limit may be the background fluorescence from the liver; however, this can be improved by precise timing of ICG injection preoperatively [26]. Dosage and timing of intravenous ICG administration to ensure reliable images are still debated with current studies ranging from 2.5 to 20 mg for dose, and from 24 hours prior to the operation to immediately after induction of anaesthesia for timing [28, 29].

According to the *Consensus Guidelines for the Use of Fluorescence Imaging in Hepatobiliary Surgery* [30] ICG administered intravenously up to 24 hours before cholecystectomy has been shown to improve the fluorescence ratio between the biliary tree and the liver parenchyma.

Verbeek et al. [26] reported that a dosage of 10 mg administered 24 h before surgery appears to produce optimal results for NIR fluorescent cholangiography during both open and laparoscopic surgery recommending a prolonged interval between ICG administration and intraoperative bile duct imaging. Similarly, in 2017 Tsutsui et al. [31] examined eight timings with a fixed maximum dosage (25 mg/body) in a total of 72 patients underwent LC: immediately before surgery and at 3, 6, 9, 12, 15, 18, and 24 h before surgery. Visibility of the gallbladder and bile ducts was classified into three categories (grades A, B, and C) based on the degree of visibility in contrast to the liver. The luminance intensity ratio was found to be optimal in the 15-h group. More recently Chen et al. [32] published an interesting clinical trial designed in two parts. In the first part, including patients with T tubes for more than 1 month, after the patient was injected with ICG, bile was collected at 10 time points to explore the change and trends of bile fluorescence intensity. In the second part 4 groups of patients were injected with 10 mg ICG at 8, 10, 12 and 14 h prior to surgery. The optimal effect of FC was gained by performing 10 mg ICG, from 10 h to 12 h prior to surgery. However several studies support earlier administration times, according to some trials even after intubation [33-35]. These conflicting opinions could be due to some confounding factors.

First, many reports concluding for earlier administration fail to investigate all prior dosing times (e.g. from 12 to 24h before surgery).

The second drawback is the lack of standardization of fluorescence imaging systems [36]. Starting with the Novadaq SPY system, which was the first to be approved in 2005, several new molecular tracers have been developed in recent years. Quality of these instruments is assessed through specific criteria, including: i) a high sensitivity for nanomolecules with quantitative measurements; ii) real-time superimposition of white light and fluorescence images; iii) possibility of application to open surgery; iv) uptake of multiple fluorophores.

Kono et al. compared 5 different fluorescence laparoscopic imaging systems for FC: the Hamamatsu Photonics and the Shinko Optical laparoscope, the fluorescence imaging system of Olympus Medical Systems, the Karl Storz HD fluorescence laparoscope, and the fluorescence imaging system of Novadaq. The study concluded that the contrast of ICG was significantly different among all the used laparoscopic imaging systems [12].

In our experience we have deliberately chosen to use Elevision™ IR Platform (Medtronic, Minneapolis, MN, USA) for most cases and as it meets most of the qualities required for an ideal fluoroscope providing qualitative and quantitative IR fluorescence imaging by measuring the relative fluorescence signal intensity in both open and laparoscopic procedures empowering surgeons with real-time information [13]. Compared to other systems it offers high-definition for both fluorescence and visible light imaging with different modalities available (Figg. 6a-d). The system consists of a laser with an excitation wavelength of 805 nm that can be activated to detect absolute and relative IR signal intensity (quantitative evaluation) of a selected area on the screen. The relative intensity is computed relative to a Base Point that usually was automatically set on the brightest area on screen.

Postprocedural quantitative reassessment of fluorescence intensity obtained by image fusion mode allowed to establish the ICG administration time more precisely obtaining an objective measurement of signal intensity and the relevant contrast between liver parenchyma and the biliary tree.

Similarly to Elevision™ IR Platform, also RUBINA™ system (KARL STORZ SE & CO. KG, Tuttlingen, Germany) used for 8 cases in our experience, has an high-definition camera simultaneously capturing white light and fluorescence images. The software in the camera control unit merges the two images in real time, delivering them as an image overlay. This feature allowed us to carry on preliminary dissection of the Calot' structures under fluorescence guidance with minimum impairment of vision quality.

With our systems we found that the optimal vision given by the fluorescence contrast between the liver on the one hand and the gallbladder, cystic duct and CBD on the other was obtained with an administration of 2.5 mg and a timing between 12 and 15 hours before the intervention.

Finally, an important issue that we aimed to emphasize is the role of FC in surgery residency programs. In our trial LCs were safely performed by residents in nearly two thirds of patients (38/54) including more complex cases. No significant differences were reported compared to surgeries realised by experienced surgeons, either for the operative time or for the

complications, which were absent in all cases. Based on our experience we have found that this may be due in part to the facilitation of the trainees in recognizing the structures already before starting the dissection, thus making them more confident and safer. On the other hand, it represents a stronger motivation for the teachers who have a quick approach and a clear means of communication that allows them to give indications based on bright images and not on random directions. Teaching LC is a challenging task because it is difficult to explain the correct site of dissection when the anatomical structures are hidden with fat and scar tissue while looking at a monitor with a regular xenon light source. As well demonstrated in the more recent literature, ICG may be a useful tool to teach LC in the future and it can act as a communication platform for the attending surgeon to guide residents [16, 19, 37]. Previously, Pesce et al. [38] reported the experience of 50 fluorescence-assisted elective LCs, half of which were performed by trainees. The study included among the results, a survey reserved for residents through which it was possible to express a judgement about the utility of FC in surgical performance by using a Likert scale. All of them strongly agreed with the fluorescence's benefits for surgical training and felt it facilitated Calot's dissection. Furthermore, over 90% of them considered it easy to carry out. In another trial Rungsakulkij N et al. [39] described how during the without-FC phase the resident group reported a misidentification rate of the biliary structures significantly greater than the experienced surgeons. The intraoperative vision magnification obtained by using the FC is the best representation of how technological innovation applied to surgical anatomy could represent a new keystone in the training process for the present and the future.

~~However, the current study has some limitations due to the small number of the enrolled patients, making it impossible to perform a comparative statistical analysis.~~

CONCLUSIONS

Fluorescent cholangiography during laparoscopic cholecystectomy is safe and feasible~~. In consideration of the risks and benefits of this method, we could affirm that NIR-ICG currently represents a key tool to avoid damage to the biliary tract. This is even more evident when compared with the other techniques available which either require longer operative times, or higher costs or in some cases offer less accuracy adding themselves the risk of injuries. The dose and dosing time of ICG are basic factors that could affect the performance of high-quality fluorescence imaging.~~ According to our experience, a dosage of 2.5 mg administered 12-15 h before laparoscopic surgery produces the optimal outcomes for NIR fluorescent cholangiography. ~~The Elevision™ IR Platform, might overcome the limits of all other fluorescence systems offering unique advantages to surgeons by allowing a quantitative and not only qualitative IR evaluation, through a superimposition of fluorescence images on full-color images and different modalities: white light, green overlay, gray scale and intensity.~~

More studies with larger samples are essential to establish objective parameters in order to optimize an increasingly promising technique for the present and for the future.

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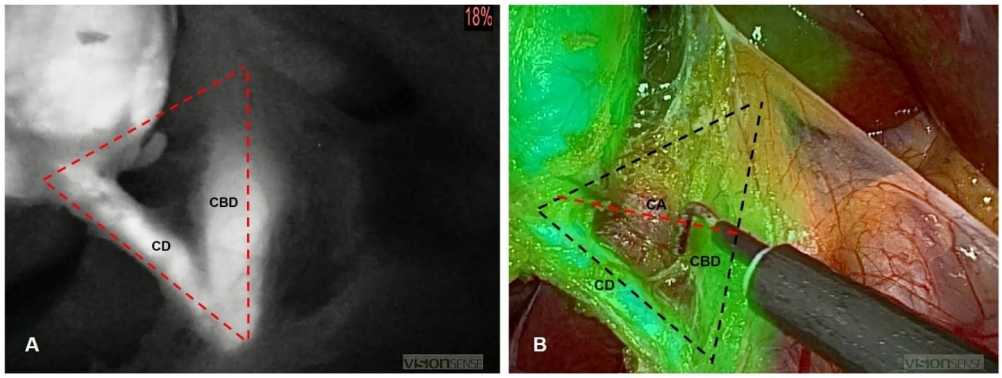

Fig. 1a. The hepatocystic triangle is placed on the right side of the porta hepatis on the under surface of the liver. The boundaries of this triangle are: common bile duct (CBD) on the medial side, cystic duct (CD) caudally, and liver under surface cranially. Inside are located the cystic artery, part of the right hepatic artery, lymphatics, and fibro-fatty connective tissue.
Fig. 1b. The hepatocystic triangle is different from Calot's triangle where the cephalic border is represented by the cystic artery (CA) rather than the surface of the liver.

131x51mm (300 x 300 DPI)

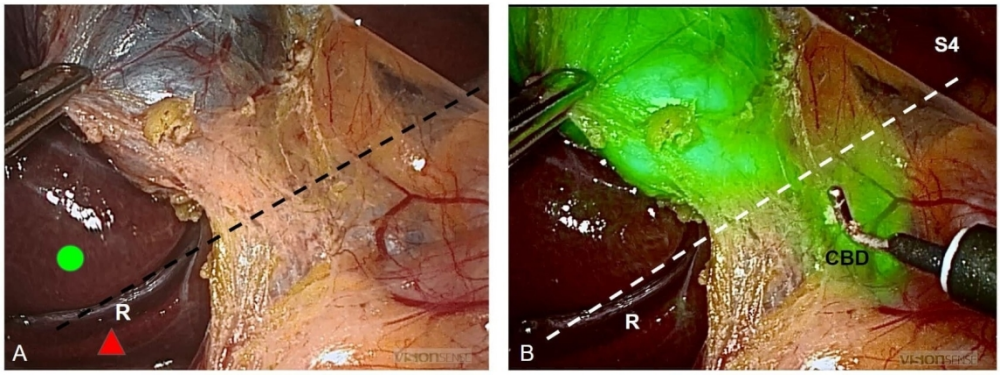

Fig. 2a. The Rouviere's sulcus (R) placed on the under surface of the right hepatic lobe up to the porta hepatis usually contains the right portal pedicle. For a safe LC it is mandatory to perform the dissection cephalad and ventral to the line joining the roof of Rouviere's sulcus and base of segment 4 (S4)
Fig. 2b. The importance of Rouviere's sulcus appears even more evident in the images obtained with fluorescence. Common bile duct (CBD) lies below this line.

143x54mm (300 x 300 DPI)

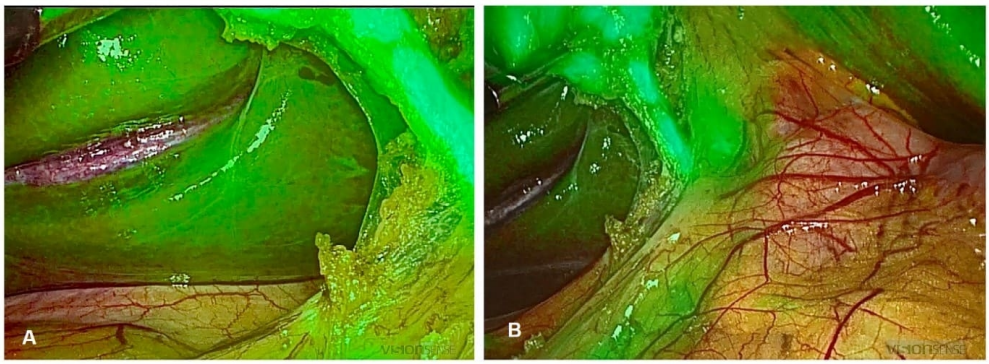

Fig. 3a. The group A received ICG between 5h and 9h before surgery: liver parenchyma and cystic duct are both highly fluorescent with consequently low contrast.
Fig. 3b. Hyperfluorescence of the liver background interferes with biliary tract fluorescence.

117x43mm (300 x 300 DPI)

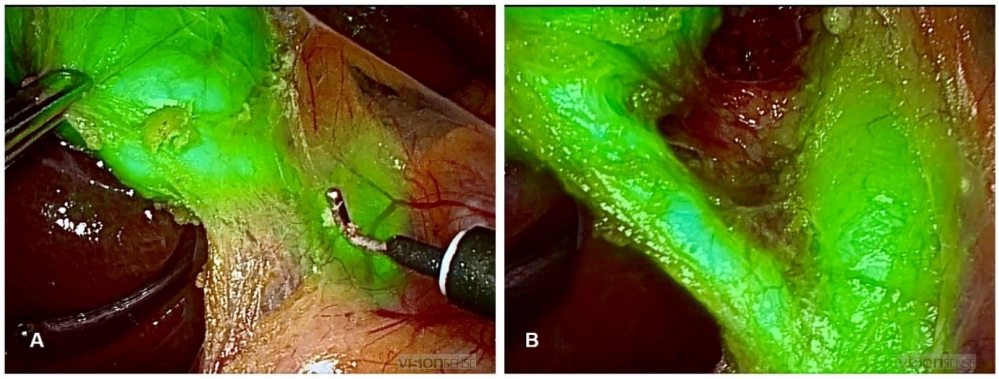

Fig. 4a.The group B received ICG between 10h and 14h before surgery. Only gallbladder and bile duct are highly fluorescent, and a strong contrast with liver parenchyma is gained.
Fig. 4b. At the end of dissection, the cyst duct and its insertion into the common biliary duct are clearly seen.

117x44mm (300 x 300 DPI)

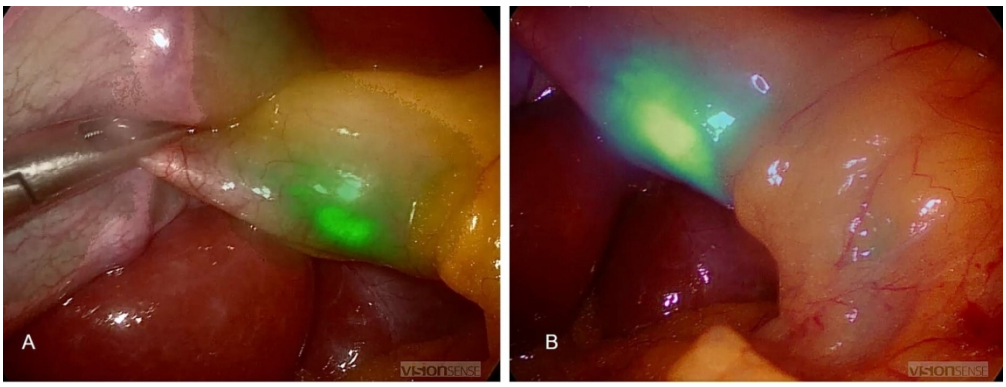

Fig. 5a. The group C received ICG between 15h and 24h before surgery. Liver parenchyma and gallbladder with bile duct are both low fluorescent with consequently low visibility of bile duct.
Fig. 5b. The recognition of biliary structures decreases resulting in suboptimal.

156x59mm (300 x 300 DPI)

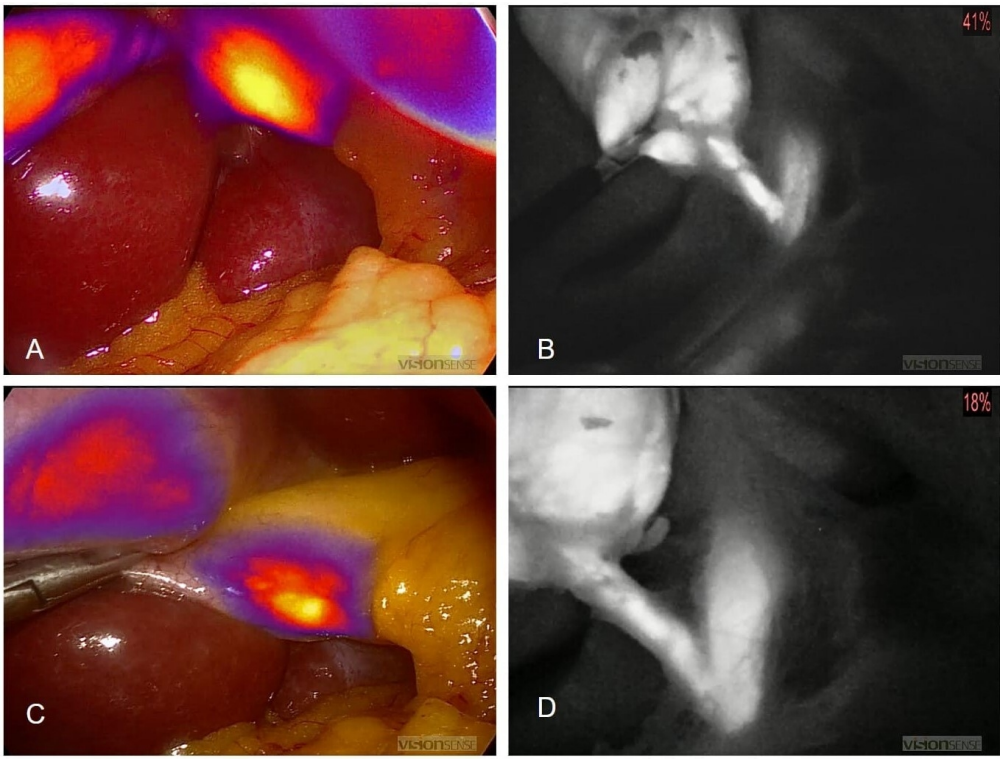

Fig.6. The Elevision™ IR Platform enables a superimposition of fluorescence images on full-color images and different modalities such as intensity map (a, c) and monochromatic (b, d).

114x86mm (300 x 300 DPI)

PREOPERATIVE DATA			
	Group A (5h-9h)	**Group B (10h-14h)**	**Group C (15h-24h)**
Patients (*n*)	22	22	10
Gender (M/F)	10/12	9/13	4/6
Age at surgery (*years*)	45.6	50.3	44
Liver function test* - normal - mild dysfunction - moderate dysfunction - severe dysfunction	 19 2 0 0	 18 4 0 0	 9 1 0 0
BMI, kg/m2 (*median*)	28.5	31.6	30.5
Preoperative study - Ultrasound - MRI	 22 16	 22 15	 9 4
Preoperative ERCP	0	1	3
Dose ICG ev, *mg*	2.5	2.5	2.5
Surgeon (resident/senior)	15/7	16/6	7/3

Table 1. Preoperative data.

BMI, Body Mass Index; MRI, Magnetic Resonance Imaging; ERCP, Endoscopic Retrograde Cholangiopancreatography.
*Based on Child-Pugh classification

SURGICAL OUTCOMES				
	Group A (5h-9h)	**Group B (10h-14h)**	**Group C (15h-24h)**	***p* value**
Hyperfluorescence liver background	15/22	2/22	0/10	A *vs* B ***p*<0.05** B *vs* C *p*>0.05 A *vs* C ***p*<0.05**
Cystic duct visualization	20/22	22/22	9/10	A *vs* B *p*>0.05 B *vs* C *p*>0.05 A *vs* C *p*>0.05
Common duct visualization	17/22	21/22	5/10	A *vs* B *p*>0.05 B *vs* C ***p*<0.05** A *vs* C *p*>0.05
Intraoperative complications	0	0	0	
Conversion to open surgery	0	0	0	
Postoperative complications	0	0	0	

Table 2. Intra- and postoperative outcomes.