STUDY PROTOCOL

Defining the ideal Indocyanine Green dose for fluorescence cholangiography during laparoscopic cholecystectomy

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PRINCIPAL INVESTIGATOR STATEMENT:

The Principal Investigator will be responsible to ensure the study is conducted in accordance with the protocol, Good Clinical Practice (GCP) and principles of Declaration of Helsinki.

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ABBREVIATIONS

ICG	Indocyanine green
IV	Intravenous
FDA	Food and Drug Administration
NIR	Near-infrared
FI	Fluorescence Intensity
LB	Liver Background
SBR	Signal to background ratio
ROI	Region of interest
CRF	Case report form

BACKGROUND AND RATIONAL

Injuries of the biliary tract are the most scaring complication ant the main concern of general surgeons performing cholecystectomies. Anatomical variation of the extrahepatic biliary tree, associated with inflammatory change and surgeon inexperience recognizing the anatomy are the most common reasons explaining this potentially-fatal complication. To date, the incidence of bile duct injury during laparoscopic cholecystectomy is estimated at approximately 0.3. Over the last several years, indocyanine green (ICG)-fluorescent cholangiography has been consistently shown to increase the visualization and identification of extrahepatic biliary structures. Indocyanine-green is a sterile, anionic, water soluble but relatively hydrophobic, tricarbocyanine molecule, which was approved for clinical use in 1959 by the Food and Drug Administration. After intravenous injection, ICG rapidly bounds to plasma protein, especially lipoproteins, with minimal leakage into the interstitium. This dye has no known metabolites. The standard dose commonly administered in clinical practice is 0.1-0.5 mg/ml/kg, which is well below the toxicity level. It contains sodium iodide and should be used with caution in patients who have a history of allergy to iodides. ICG becomes fluorescent when excited either by a laser beam, or by near-infrared light at about 820 nm and longer wave lengths. The fluorescence released by ICG can be detected using specifically designated scopes and cameras. A randomized clinical trial including more than 600 patients, showed that ICG fluorescent cholangiography is statistically superior to standard light cholecystectomy in identifying extrahepatic biliary structures before and after starting the dissection. Increased body mass index and level of inflammation were associated with reduced detection for most structures.

To date, standard dose and timing of IV ICG administration for fluorescent cholangiography have not been defined. The recommended dose of IV ICG for other clinical indications is 0.1-0.5 mg/Kg. According to literature, about 0.1-0.5 mg/kg of ICG are administered 3-12 hours before surgery to perform fluorescence cholangiography. The Department of Surgery of the Policlinico Hospital uses a dose of 0.1 mg/kg of ICG at least 3 hours before surgery. This dose allows high fluorescence intensity of biliary structures, but it is always associated with fluorescence hepatic background with consequent reduction of visualization. Indeed, the above dosage has been identified for different reasons other than fluorescence guided surgery (i.e. liver function tests, ophthalmic angiography, cardiac output study). Recently, some studies tried to assess the correct dose and timing to perform ICG fluorescent cholangiography. These studies include two or more groups of patients who received a standard high dose of ICG (5 – 10 mg) at different time points (0.5-24 hours before surgery). And all these studies conclude that the defined ICG dose (5 – 10 mg) should be administered 3-10 hours before surgery. The first limit of this study is to assess a standard ICG dose for all patients, as in order to achieve high fluorescence intensity of biliary structures with no fluorescence hepatic background, a weight-based dosing has to be defined. Furthermore, for routine clinical practice, administration of ICG several hours before surgery is unpractical, since most of

the patients who undergo laparoscopic cholecystectomy are admitted the same day of surgery, so usually there are less than 3 hours from admission to surgery.

The purpose of this study is to define the ideal weight-base range of ICG dose (mg/Kg) to perform fluorescent cholangiography during laparoscopic cholecystectomy.

OBJECTIVES OF THE STUDY

Primary objective

The primary objective is to define the ideal range of ICG dose (mg/kg) that should be administered intravenously in order to perform fluorescence cholangiography during laparoscopic cholecystectomy. The ideal dose is defined by the combination of evidence of high fluorescence intensity of biliary structures and absence of fluorescence of the surrounding liver parenchyma.

Secondary objectives

The secondary objectives are:

- To defining the best timing for IV ICG administration. The timing will be defined according to the dose identified
- To defining the best dilution of ICG, according to the dose identified.

DESIGN OF THE STUDY

Study design: prospective cohort study

The study will be divided into two parts:

Part one

1. **Bisection Method**

The first part aims at defining the correct range of ICG dose through the bisection method ($[x_0+x_1]/2$): starting from the current ICG dose, 0.1 mg/Kg, as "x_1", and assuming 0 mg/kg as "x0". Thus, the first dose administered to the first patient will be 0.1 mg/kg. The second dose (for the second patient) will be calculated according to results of fluorescence intensity (see "methods: measure of fluorescence intensity") of the previous patients, applying the aforementioned method. Even if the is no previous literature published on ICG dose calculation with this method, we assume that no more than 15 patients will be enrolled. Indeed, after applying the method for 15 times, the number (ICG dose, mg/Kg) will be unchanged for the first three decimal places.

ICG will be always administered one hour before surgery. Indeed, for this part of the study, the

timing of ICG administration is the fixed parameter of the method. The specific time (one hour before surgery) was chosen as it allows all patients to be included in the study, even if admitted to the department only one hour before scheduled surgery.

At the end of this this step, the four ICG dose with best FI measurements will be chosen to be tested in the second step of part one of the study.

2. **Parallel Dose Comparison**

After identification of the four ICG dosage with the best FI parameters, a total of 16 patients will receive one of the doses (4 patients for each group). The meadians of FI parameters (FI of biliary structures and of liver background) will be compared to define the limits of the optimal range of ICG dose.

Thus, a total of 31 patients will be included at the end of part one of the study.

Once the range of ICG dose will be defined, this step allows identification of the best ICG dilution, that will be established according to the range of ICG dose identified. Indeed, as the expected range of ICG dose is low, the dilution would be high in order to have precise IV administration of the dye.

Second Part

The second part aims at confirming the range of ICG dose identified by testing it in 50 consecutive cholecystectomies. Intraoperative evaluation of ICG dose will be performed as described below.
Values of all patients included in the second step obtained by ImageJ programme will be used to calculate the medians of fluorescence intensity parameters. Then, these data will be analysed.
This step allows defining if the chosen time is suitable to perform the fluorescence cholangiography.

Methods: Measure of Fluorescence Intensity

ICG intensity will be evaluated with defined setting of NIR (Near-infrared) contrast and NIR brightness of the SynergyID System for all patients. NIR contrast and NIR brightness will be set before the beginning of the study and will be kept unchanged throughout the whole study.
In order to define ICG intensity, the scope will be about 5-10 cm far away the structures (biliary structures and liver).

The ideal ICG dose will be evaluated by measuring the fluorescence intensity of biliary structures and liver parenchyma.
The evaluation will be performed:
- On cystic duct and on common bile duct if visible, in order to define the fluorescence intensity
- On liver parenchyma: on the left and right side of the infundibulum (next to the cystic duct), in order to define the liver background.

Two evaluation will be made for both biliary structure and liver:

- Subjective evaluation by the operating surgeon and ranked as: 1(absent), 2 (poor) 3 (medium), 4 (high).
  Surgeons will be blind to administered ICG dose during the first part of the study.

  Fluorescence intensity results will be combined to obtain the signal to background ratio (SBR) for both the cystic duct and the common bile duct with the following formula:

  SBR = Target FI / Liver background FI

  With "target" as the cystic duct or the common bile duct. According to the rank used, the minimum SBR achievable is 0.25, while the maximum was 4.

- Objective evaluation: performed using Fiji software(*) on selected pictures captured on recorded surgical procedures
  Signal to background contrast (for biliary structures (CD, CBD) and liver) will be calculated with Kono et al. approach, using FI range of 0-510, with the following formula:

  SBR = (Target FI - Liver background FI)/ 510

  According to the formula, the minimum SBR achievable is -1, while the maximum was 1.

The range of ICG dose will be defined by the best balance between the highest fluorescence intensity of biliary structure associated with the lowest liver parenchyma background achievable.
Data obtained by objective evaluation (through the Fiji software) will be used to define the signal to background ratio (SBR) = mean signal of biliary structures fluorescence intensity / mean signal of liver background to strengthen data.

*The Fiji software is an image processing and analysis programme that calculates pixel intensity value of a selected area on the aforementioned anatomical structures through a defined algorithm. These areas are called region of interest (ROI) and will be selected manually.
The image (with the ROIs) will be scanned in a single pass and a running count of the number of pixels found at each intensity value is kept. This will be then used to construct a suitable histogram. The pixel intensity will be clustered around separated values corresponding to the different ROIs. Values obtained will show fluorescence intensity of the different areas and will be compared according to the ICG dose administered.

SELECTION CRITERIA & STUDY POPULATION

Inclusion criteria

All participants will require the following Patient eligibility criteria:

- age ≥ 18 years old
- indication for cholecystectomy (gallstones disease; adenomyomatosis)
- ability to understand and follow study procedures
- having provided signed consent.

Exclusion criteria

- known allergy to iodides
- coagulopathy
- pre-existing liver disease
- pregnancy
- acute cholecystitis
- BMI ≥ 40.

STATISTICS

Power analysis will not performed before beginning the study, as similar studies have never been published.
The Shapiro-Wilk test will be used to check if continuous variables follow normal distribution. Data will be presented as the mean ± standard error of mean (SEM) or median according to data distribution.

In case of normal distribution, the Kruskal-Wallis will be performed for between-group analysis, with Dunn's test for pairwise comparisons. In case of non-normal distribution, one-way Brown-Forsythe ANOVA test will be used for between-group analysis, with Dunnett's T3 for pairwise comparisons. A p-value of <0.05 will be considered statistically significant. Statistical analysis will be performed by statistician using GraphPad Prism (https://www.graphpad.com/updates/prism-843-release-notes).

DATA HANDLING & RECORDING

The collection of personal patient information will be limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected.
Only study personnel will collect data. Hard copy documents will be retained for the duration of the study until data entry. All hard copy documents will be kept in a locked cabinet. Data entry will be completed in the Excel secure database (password protected), which will cover all the created

CRFs. Only de-identified data will be used for data analysis. All hard copy documents will be stored for a maximum of 10 years after completion of the study and then they will be shredded upon Sponsor approval.

The Principal Investigator will be responsible for data handling and keeping before, during and after the present study.

Data recorded

As reported by the clinical report form, the following data will be collected:

Patient's characteristics

- Age
- Sex
- BMI
- ASA classification
- Comorbidities
- Chronic medications
- Indication for surgery
    - Cholelithiasis
    - Adenomyomatosis
- Preoperative imaging technique
    - US
    - MRCP
    - ERCP
- Previous acute cholecystitis

Preoperative data

- Hospitalization days before surgery (nights)
- ICG dilution
- ICG dose
    - mg/Kg
    - ml administered
- Timing of ICG administration: minutes between administration and first look at biliary structure and liver parenchyma (for subjective and objective evaluation)

Intraoperative characteristics

- Adhesions

- Subjective evaluation of fluorescence intensity
    - Biliary structures: cystic duct and common bile duct
    - Liver parenchyma
- Subjective evaluation of fluorescence intensity
    - Biliary structures: cystic duct and common bile duct
    - Liver parenchyma
    - Signal to background ratio
- Duration of surgery
- Intraoperative complications

Postoperative outcomes

- Hospitalization days after surgery (nights)
- Follow-up visit
    - Y/N
- Post-operative complications (30 days)

Data Monitoring

The Principal Investigator will be responsible to ensure the study is conducted in accordance with the protocol, Good Clinical Practice (GCP), applicable regulatory requirements, and that the data recorded is valid. To achieve this objective, the study will be continuously monitored and reviewed on a monthly basis by the study team.
Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently protocol, with GCP, and with applicable regulatory requirement(s).

ADMINISTRATIVE ASPECTS

This study will be conducted in accordance with the protocol, Good Clinical Practice (GCP) [ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996 Directive 91/507/EEC; D.M. 15.7.1997], and principles of Declaration of Helsinki. By signing this protocol, the Principal Investigator will be responsible to ensure that the study will be conducted in accordance with Good Clinical Practice and principles of Declaration of Helsinki.

Data Publication

Study results will be published within 1 year after the end of the study.

FUNDING

This research will not receive any specific grant from funding agencies in the public or commercial sectors.

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