DOTIG CLINICAL TRIAL (NCT05419947)
STATISTICAL ANALYSIS

Study promoter	**Castilla y Institute of Health Sciences Studies Foundation (IECSCYL)-Institute of Biomedical Research of Salamanca (IBSAL)**
EUDRA CT number	2022-000904-36
Promoter's Protocol Code	IBS-DOTIG-ECM-2202
Title	Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy.
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1. **Working hypothesis. There are differences in the visualisation rates of extrahepatic biliary structures as a function of drug dose and time of administration.**

To test the hypothesis, we created four intervention groups combining two doses (fixed dose of 2.5 mg and weight-adjusted dose ratio of 0.05 mg/kg body weight) and two administration times (15-30 minutes preoperatively and >3 hours preoperatively). The primary endpoints are dichotomous and polytomous qualitative variables.

Before analysing the main and secondary objectives, the groups (G1, G2, G3, G4) and their baseline characteristics should be compared in order to demonstrate that they are homogeneous and the randomisation has been correct. To do this, the following variables collected in the CRDe should be compared:

- Sex (male/female)
- Age (years)
- BMI (kg/m2)
- ASA classification (ASA I/II/III/IV/IV/V)
- Previous abdominal surgeries (NO/SI). In the YES group, break down the variables biliary/liver/pancreas/intestinal/other).
- Previous liver disease (NO/SI). In the SI group, break down the variables: fatty liver disease, hepatitis, liver cirrhosis.
- Previous biliary examinations (NO/SI). In the SI group, break down the ERCP/CTPH variables.
- Previous biliary prostheses (NO/YES)
- Previous acute cholecystitis (NO/SI).
- Previous acute cholangitis (NO/SI).

- Previous gallbladder drainage (NO/SI). In the SI group, break down the variables percutaneous cholecystostomy/endoscopic cholecystostomy.
- Indication for surgery (biliary colic/acute cholecystitis/choledocholithiasis/acute cholangitis/acute lithiasic pancreatitis/bladder polyps/bladder adenomyomatosis).
- Type of surgery (elective/early/deferred surgery). Probably in this variable we do not have a very similar N, because most of them, at least in our centre, were elective.
- Grade of cholecystitis (1/2/3). In case of early indication/delayed urgency.
- Imaging system (Olympus/Karl-Storz/Stryker)
- Type of optics (30 degrees/ 0 degrees).

2. Main objective. To analyse whether there are differences between different doses (single dose and weight-adjusted dose) and different VI administration intervals in the visualisation rates of extrahepatic biliary structures.

Carry out a comparative analysis between the different groups (G1/G2/G3/G4) and the following qualitative variables:

- Identification of biliary structures prior to dissection of the hepatocystic triangle.

o Identification of cystic duct (yes/no).
o Identification of common bile duct (Y/N).
o Identification of the junction of the cystic duct with the common bile duct (Y/N).

o Identification of the junction of the cystic duct with the gall bladder (Y/N).
o Identification of the common hepatic duct (Y/N).
o Identification of anatomical variables (yes/no).

- Identification of biliary structures after dissection of the hepatocystic triangle.

o Identification of cystic duct (yes/no).
o Identification of common bile duct (Y/N).
o Identification of the junction of the cystic duct with the common bile duct (Y/N).

o Identification of the junction of the cystic duct with the gall bladder (yes/no).

o Identification of the common hepatic duct (Y/N).
o Identification of anatomical variables (yes/no.).

- Degree of identification of biliary structures prior to dissection of the hepatocystic triangle

  1=slightly
  2=sufficient
  3=quite a lot
  4=good
  5=excellent

- Degree of identification of biliary structures after dissection of the hepatocystic triangle:

  1=slightly
  2=sufficient
  3=quite a lot
  4=good
  5=excellent

- Extent to which fluorescence cholangiography was perceived to be useful for surgery

  Not useful
  Moderately useful
  Very useful

- Extent to which the fluorescence of the liver background (contrast between liver and ducts) was perceived as disturbing.

  0=absence of disturbance
  1=slightly disturbed
  2=disturbed visualisation, but cystic-choledochal junction was clearly visible before dissection
  3= visualisation disturbed and the cystic-choledochal junction was visible only after dissection
  4= very disturbed: it was impossible to visualise the biliary structures correctly.

3. **Secondary objectives.**

In these secondary objectives, we tried to analyse predictors of the quality of the fluorescence technique. To this end, we have found numerous factors in the literature that could influence cholangiography. We want to show whether there are differences between the factors listed below and the different working groups.

a) To analyse the influence of body mass index on FC outcomes during CL.

In this section, we could recode the qualitative variable BMI into the different BMI subgroups defined by the World Health Organisation or use normal weight/overweight as the cut-off point.

Clasificación	IMC (Kg/m²)
Normal	18.5 - 24.9
Sobrepeso	25 - 29.9
Obesidad grado I	30 - 34.9
Obesidad grado II	35 - 39.9
Obesidad grado III	Más de 40

We could also analyse it as a quantitative variable and see if there is a correlation between them.

b) To analyse the influence of the type of biliary pathology requiring surgery and previous liver disease on the results of FC during LC.

Analyse whether there are differences between "Previous liver disease (NO/SI). In the SI group, break down the variables: fatty liver disease, hepatitis, liver cirrhosis". And the main variables shown.

c) To analyse the influence of the type of surgery (elective/early/ delayed surgery) on the results of FC during LC.

To analyse whether there are differences between the variables "type of surgery" and "indication for surgery".

d) To analyse the influence of previous gallbladder or bile duct instrumentation on the results of FC during LC.

To analyse whether there are differences between the variables "previous biliary explorations", "previous biliary prostheses" and "previous gallbladder drainage". Both separately and united in a single variable.

e) To analyse the influence of different laparoscopic imaging systems on the results of FC during LC.

To analyse whether there are differences between the variable "image system" and the main target variables.

f) To analyse the rate of intraoperative complications related to CF during LC.

To analyse intraoperative complication rates in the series.

g) To analyse the rate of postoperative complications related to CF during LC.

To analyse the postoperative complication rate for the series overall and for each type of Clavien-Dindo classification (consecutive variable).

Also to analyse separately the rate of bile duct injury and the characteristics of bile duct injuries (consecutive variables).

Analyse in-hospital and 30-day mortality rates.

h) To analyse the impact of FC during LC on general surgeons and/or surgeons specialised in hepatobiliopancreatic pathology and their subjective assessment of the procedure.

To analyse this secondary objective, we should analyse globally the primary variable detailed above "Extent to which fluorescence cholangiography was perceived to be useful for surgery". We should also analyse it individually in each group.

i) Analyse the rate and characteristics of late adverse reactions and AEs (separate Excel).
j) To analyse the rate of anatomical variables (for this purpose we only analysed the variables (identification of biliary anatomical variables prior to dissection and the variable identification of biliary anatomical variables after dissection) globally.
k) To analyse the influence of the technique on surgical time. To do so, we analysed the median complete surgical time (variable surgical time).

Note: in the study protocol we have put "Finally, all the statistical information obtained about the relevance of each variable in the study could be used to apply classification tools to the individuals in the study (decision trees, logistic regression, ROC curves)". I think this is a very interesting aspect but I need to talk about it and I need you to explain this section to me