

Intraoperative use of IndoCyaninE Green fluorescence imaging to prevent anastomotic leakage in colorectal surgery: Systematic Review, Meta-Analysis and Study Protocol for the ICEberG Trial

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1. ABSTRACT

Intraoperative use of IndoCyaninE Green fluorescence imaging to prevent anastomotic leakage in colorectal surgery: systematic review, meta-analysis and study protocol for the ICEberG trial

Aim: ICG fluorescence imaging has been increasingly considered as a potential tool to assess anastomosis perfusion. This study aims to validate its efficacy in reducing anastomotic leakage (AL) rate in colorectal surgery.

Method: A systematic review and a meta-analysis of studies comparing fluorescence imaging with standard care were conducted. Furthermore, a prospective randomised controlled trial (RCT) was proposed.

Results: 1302 patients from 5 studies were included. Fluorescence imaging significantly reduced AL risk in cancer surgery (OR:0.34; CI:0.16-0.74; P=0.006). Low AL rates were shown in rectal surgery (ICG:1.1% vs non-ICG:6.1%; P=0.02). There was no significant AL rate decrease when procedures for benign and malignant indication were combined. To date, there are no published RCTs, though 3 ongoing trials were identified.

Conclusions: Fluorescence imaging seems to reduce AL rate following colorectal surgery for cancer. However, large well-design RCTs are needed to provide evidence for its routine use. The proposed ICEberG trial would address this need.

Ús intraoperatori d'imatges per fluorescència amb verd d'indocianina per la prevenció de la fuita anastomòtica en cirurgia colorectal: revisió sistemàtica, meta-anàlisi i protocol per l'assaig clínic ICEberG

Objectiu: L'ús d'imatges per fluorescència amb verd d'indocianina s'està considerant cada cop més com una potencial eina per l'avaluació de la perfusió de l'anastomosi. L'objectiu d'aquest estudi és validar la seva eficàcia en la reducció de la taxa de fuita anastomòtica en la cirurgia colorectal.

Mètodes: S'ha realitzat una revisió sistemàtica i un meta-anàlisi dels estudis que comparaven l'ús d'imatges per fluorescència amb la pràctica habitual. A més, s'ha proposat un assaig controlat aleatori (ACA) prospectiu.

Resultats: 1302 pacients de 5 estudis van ser inclosos a l'anàlisi. L'ús d'imatges per fluorescència va reduir significativament el risc de fuita en cirurgia per càncer (OR:0.34; CI:0.16-0.74; P=0.006). Es va veure una baixa taxa de fuita anastomòtica en cirurgia de recte (ICG:1.1% vs no-ICG:6.1%; P=0.02). No es va trobar un descens significatiu en la taxa de fuita anastomòtica quan es van combinar procediments amb indicació benigna i maligna. Actualment no existeixen ACA publicats, tot i que s'han identificat 3 assaigs en curs.

Conclusions: L'ús d'imatges per fluorescència sembla reduir la taxa de fuita anastomòtica després de cirurgia colorectal per càncer. No obstant, es necessiten grans ACA que proporcionin evidència en l'ús d'aquesta tècnica a la pràctica habitual. El proposat ACA ICEberG abordaria aquesta necessitat.

2. BACKGROUND

Anastomotic leakage (AL) represents one of the most feared complications following colorectal surgery; it has been associated with increased postoperative morbidity and mortality rates. (1,2) Due to the lack of a standardized definition for AL, there is still variability in studies reporting this condition. (3) In 2010, an attempt to define AL in anterior rectal resections was made by the International Study Group of Rectal Cancer. The definition proposed for AL was “a defect of the intestinal wall at the anastomotic site leading to a communication between the intraluminal and extraluminal compartments” and they recommended considering a pelvic abscess in the proximity of the anastomosis as AL. In addition, different AL grades were established according to their clinical management: Grade A when no change in patients’ management was required; Grade B if active therapeutic intervention was needed without surgical intervention; Grade C when re-laparotomy/laparoscopy was performed. (4) Kulu et al. (5) proved this AL definition and severity grading to be a simple, easily applicable, and valid classification.

AL rate in colorectal surgery vary from 1% to 19% depending on the anatomic location of the anastomosis: ileocolic (1% to 8%); colocolic (2% to 3%); ileorectal (3% to 7%); colorectal or coloanal (5% to 19%). (3,6,7) In the Rectal Cancer Project of the Spanish Society of Surgeons, the rate of AL for rectal cancer surgery was 10%. (8) The reduction of AL rates by improving its prevention, diagnosis and management, continues being a challenge nowadays. Finding new techniques to reduce AL has been highlighted as a research priority by the Association of Coloproctology of Great Britain and Ireland (ACPGBI). (9)

Multiple conditions have been associated with a greater risk of AL: male sex, age, comorbidities, high American Society of Anaesthesiologists (ASA) score, malnutrition, obesity, smoking, immunosuppression, alcohol abuse, preoperative chemotherapy and radiotherapy, advance tumour stage, diverticulitis, low anastomoses, prolonged operative

time, inadequate anastomotic blood supply, blood loss or perioperative blood transfusion and intraoperative septic conditions appearance. (3,10–12) Adequate perfusion of the anastomosis is essential for an optimal healing and AL prevention. (13–15) Consequently, bowel ischemia detected intraoperatively may reduce the risk of AL.

Different intraoperative techniques have been proposed to assess anastomotic integrity and bowel viability in colorectal surgery. Traditionally, usual anastomotic assessment includes direct visualisation of the anastomosis, integrity of doughnuts assessment and the air leak test. Subjective signs indicating optimal anastomosis perfusion are evaluated including serosal - mucosal colour and/or bleeding at the cut edge of the bowel and/or palpable pulsations of mesenteric arteries. (12,16) However, surgeons' predictive accuracy of AL risk has been shown to be low and underestimated in the study of Karliczek et al. (17) They suggest the need of a reliable predictive test that could be used intraoperatively. Although there is no consensus on the use of air leak test in colorectal anastomosis, it is a widespread and well-established technique. It consists in filling the pelvis with warm saline solution covering the anastomosis, then air is insufflated on the rectum to identify any possible leak. (3) This is a mechanical test, without any insight in the physiology of AL.

Other experimental techniques assessing blood supply and/or integrity of anastomosis have been described. Some of them are intraoperative endoscopy, pulse oximetry, Doppler ultrasound and Doppler flowmetry, intramucosal pH measurement, visible light oxygen spectroscopy and near infrared oxygen spectroscopy. None of the mentioned techniques are routinely used, mainly because of its complexity and its high variability of the measurements. (16,18,19)

Fluorescence imaging with indocyanine green (ICG) has been increasingly considered as a potential intraoperative tool that could be used in routine practice to ensure adequate perfusion at the time of anastomosis formation. It allows surgeons to visualize bowel

microperfusion at a real-time being a fast technique which is easy to perform. Recent literature shows the potential benefit of fluorescence imaging with ICG in lowering AL rates by changing the surgical plan. (20–26) Although it has been proven to be a safe and feasible tool in colorectal surgery, (27–29) further research is needed to validate its efficacy in reducing AL rate. (1)

ICG is a sterile, water-soluble, trycarbocyanine compound dye that absorbs near-infrared light in the region of 800-810 nm and emits it at 830 nm. ICG is administered by intravenous injection (1.25 – 3.75 mg, depending on the patient weight) and it is rapidly bounded to albumin with minimal leakage into the interstitium. ICG is removed from the blood by the liver with a half-life of 3-5 minutes and then it is excreted via the bile within 10-15 minutes with no known metabolites. (30,31) Since 1956, when ICG was approved for clinical and research use in humans by the Food and Drug Administration (FDA), it has been used in different areas including plastic surgery, neurosurgery, ophthalmology, hepatobiliary and transplant surgery. The ICG applications with greater interest in colorectal surgery are anastomosis perfusion assessment and lymphatic road-mapping. Fluorescence imaging for bowel perfusion assessment can be used immediately before and/or after anastomosis formation once tissues are in their anatomic positions. (32) ICG clinical use has been proven to be very safety and rare cases of anaphylaxis have been described. (33) However, it should be used with caution in patients with iodine allergy (allergic reaction 1/300000). Moreover, manufacturers recommend a maximum daily dose of less than 2 mg/kg. (30,32,34)

There are different platforms available to perform fluorescence imaging with ICG. SPY Elite™ system from Novadaq® was designed to be used in open cases and it provides a numeric assessment of perfusion. In laparoscopic surgery, the PINPOINT™ from Novadaq® (Canada), IC-View® from Pulsion Medical Systems (Germany) and the D-Light from Storz® (Germany) can be used. For robotic surgery, FIREFLY™ system integrated in surgical

robotic platform da Vinci SI™ (USA) is available. In white light mode, these systems provide a standard laparoscopic view and it can be readily switched to near-infrared (NIR) mode, in which ICG fluorescence is visualised. Also, PINPOINT™ has a dual display mode allowing to superimpose green ICG fluorescence with white-light image. (30,35) These commercial available systems lack to provide a quantification of tissue perfusion. (1)

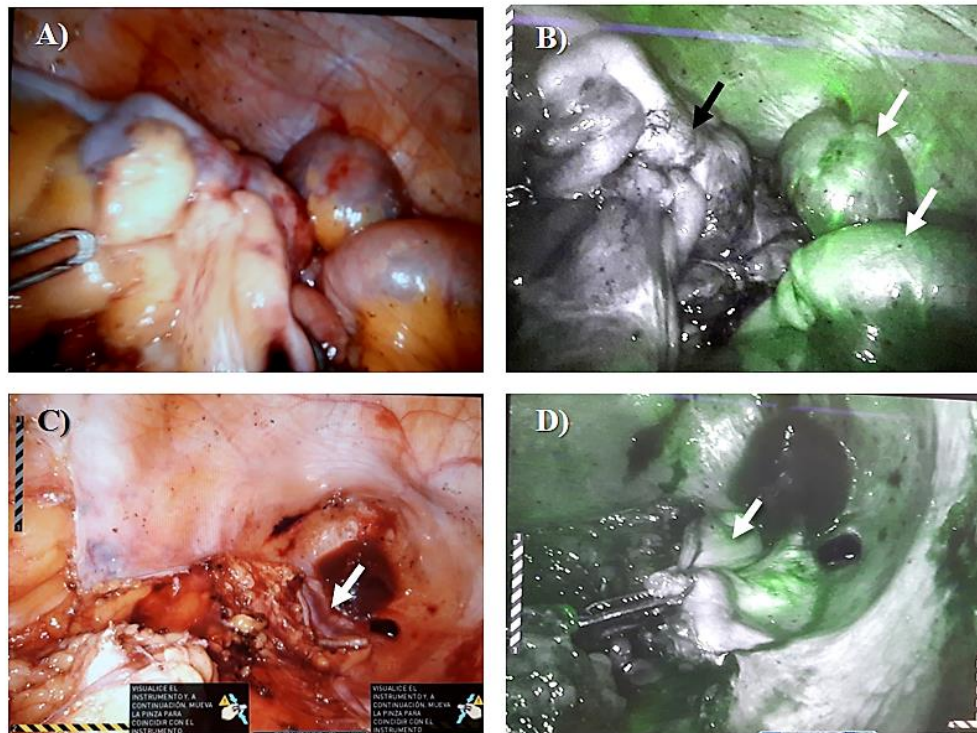


Figure 1: Bowel perfusion assessment with ICG fluorescence using FIREFLY system during a robotic sigmoid colectomy (“A” and “C” show bowel/rectum in standard white light mode; “B” and “D” show bowel/rectum perfusion assessed with fluorescence mode after ICG administration). “A” and “B” correspond to bowel perfusion assessment while planning transection point. In “B”, well perfused bowel is indicated by *white arrows* and non-perfused bowel is indicated by *black arrow*. “C” and “D” pictures correspond to rectal stump assessment (*white arrow*).

3. HYPOTHESIS AND OBJECTIVES

We hypothesized that anastomosis perfusion assessment using ICG fluorescence imaging may be associated with a low leak rate in those patients undergoing colorectal surgery.

This study aims to validate ICG fluorescence imaging efficacy in reducing the rate of AL in colorectal resections comparing with standard surgical care. As secondary aim, changes in surgical plan when anastomosis formation will be also considered.

4. SYSTEMATIC REVIEW AND META-ANALYSIS

4.1. Material and methods

A systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. (36)

Eligibility criteria

Studies that compared intraoperative use of ICG fluorescence imaging with standard care for the assessment of anastomosis perfusion or viability, were eligible for inclusion. Patients of any age undergoing colon or rectal resection with anastomosis were included, regardless of operative approach, urgency of surgery and surgical indication. The primary outcome measure was the AL rate with at least 30 days follow-up. Randomized Controlled Trials (RCTs), cohort studies, case-control studies and quasi-randomised studies were searched. Case reports were excluded. Studies using ICG fluorescence for other purposes different from perfusion assessment were excluded, as well as those studies based on animal models.

Search strategy

An electronic search was carried out using PubMed, Scopus, Web of Science, Google Scholar databases and the Cochrane Library. The reference list of identified systematic reviews and review articles were hand-searched for additional references. Furthermore, the register ClinicalTrials.gov was searched to identify ongoing trials. A combination of medical subject heading (MeSH) terms and keywords were searched: “indocyanine green”, “ICG”, “coloring agents”, “fluorescence”, “fluorescein angiography”, “fluorescent dyes”, “anastomotic leak”, “anastomotic leakage”, “anastomotic perfusion”, “anastomosis, surgical”, “bowel perfusion”, “blood supply”, “perfusion assessment”, “colorectal surgery”, “colon surgery”, “rectal surgery” “colorectal resection”, “bowel resection” using the Boolean operator “OR” for each concept. Each concept was combined with “AND”. The complete search strategy is shown in

the *Appendix*. No search limits were applied and all languages were included. The latest date of this search was 24th January 2017.

Study selection and data extraction

Studies were screened by title and abstract; then full-text was obtained for those studies identified as potentially eligible.

From each study, data were extracted on: study characteristics and year of publication, patient inclusion period, sample size, surgical indication, surgical management (operative approach, procedure and whether a change in surgical plan was made), fluorescence imaging system used and anastomotic leakage rate. Authors were contacted to provide additional information that was not available in the original studies; two authors could not be contacted or were not able to provide the requested data. (37,38)

Risk of bias assessment

The quality of the included studies was evaluated using the ROBINS-I risk of bias assessment tool for non-randomized studies of interventions. (39) Seven domains were covered including confounding and selection of participants into the study, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result.

Statistical analysis

Analyses were performed using Review Manager (RevMan) Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The odds ratios (OR) were calculated from the original data and were assessed as the summary statistic. Values were reported with 95% confidence intervals (CI). As there was a substantial level of heterogeneity expected across the included studies, Mantel-Haenszel (M-H) method and random-effects models were employed for quantitative statistical analysis of dichotomous variables. Statistical heterogeneity was assessed using I^2 test and by visual inspection of forest plots.

4.2. Results

Study selection

Results of literature search and selection process of eligible studies is presented on the PRISMA flow diagram (*Figure 2*). From the 518 studies identified by the search, full-text of 72 studies was evaluated. Finally, 5 non-randomised studies were included in the analysis. (37,38,40–42) To date, there are no published RCTs. On ClinicalTrial.gov search, 6 ongoing trials were identified, 3 of them were randomized studies that have a control group. (43–45)

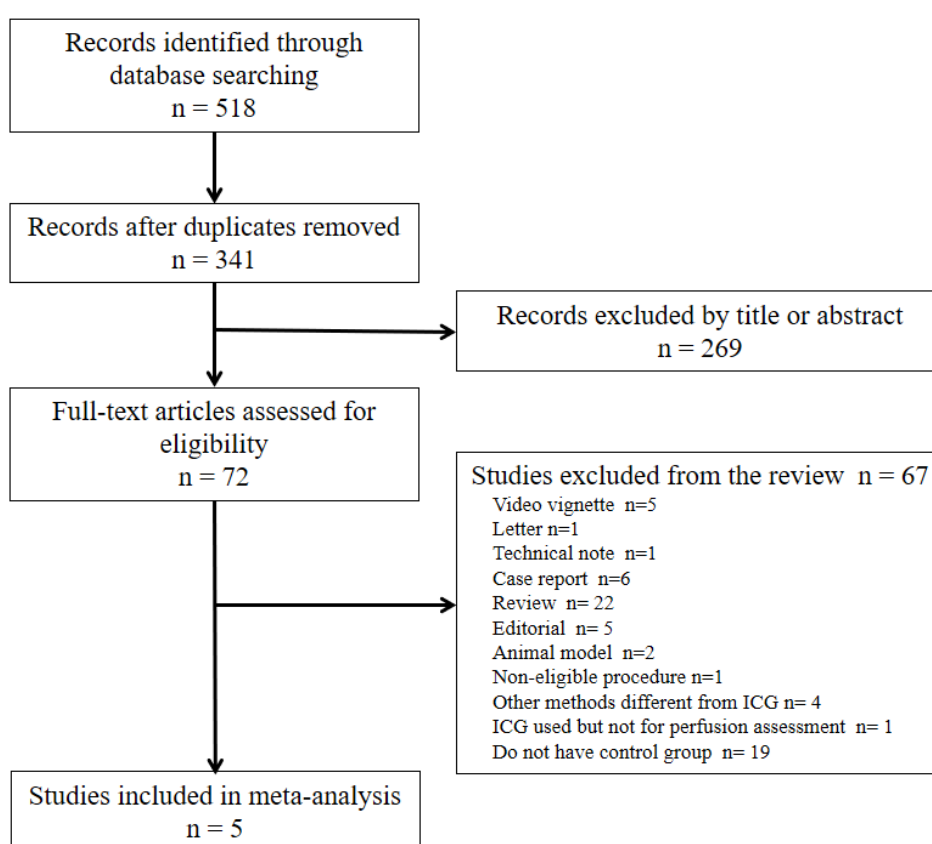


Figure 2: PRISMA flow chart of study selection process

Study characteristics

Characteristics of the analysed studies are reported in *Table 1*. The five studies included a total of 1302 adult patients. The sample size in the studies varied from 38 to 436. Most studies included elective rectal surgery and the commonest indication was cancer. Follow-up ranged from 1 month to more than 6 months.

Table 1: Characteristics of included studies

Reference	Country	Study interval	Design	Sample size (ICG : control group)	Age (range±SD) (Male %)	Gender (Male %)	Surgical procedure and approach	Surgical indication	ICG bolus i.v.: dose; before/ after anastomosis formation	ICG imaging system	Change surgical plan ICG group (%)	AL rate (%)
Kudszus et al. (37)	Germany	2003 - 2008 (<i>Historical control 1998 to 2003</i>)	SC; R	402 (201 : 201)	I: 69.0±21.8 C: 67.8 ±25.2	I: 42.2 C: 42.2	Laparoscopic and open right, left colectomies and LAR	Cancer	0.2-0.5 mg/kg (before ± after)	IC-View®	16.4%	ICG: 3.5 Control : 7.5
Kin et al. (38)	USA	2005 - 2012 (<i>ICG group after 2009</i>)	SC; R	346 (173 : 173)	I: 58.2± 13.2 C: 58.1±13.2	I: 54 C:54	Laparoscopic and open left colectomies and LAR	IBD, diverticular disease, cancer	3 ml (before)	SPY Imaging System	4.6%	ICG: 7.5 Control : 6.4
Jafari et al. (40)	USA	2011 - 2012	SC; R	38 (16 : 22)	I: 58 ± NR C: 63± NR	I: 75 C: 73	Robotic LAR	Cancer	6-8 mg (before)	FIREFLY	19%	ICG: 6 Control : 18
Kim et al. (41)	Korea	2010 - 2014 (<i>Control before 2013/ ICG group in or after 2013</i>)	SC; P	436 (123 : 313)	I: 57± 10 C: 58 ± 10	I: 59.3 C: 61.3	Robotic-assisted sphincter saving operations	Cancer	10 mg (before ± after)	FIREFLY	0%*	ICG: 0.8 Control : 5.4
Boni et al. (42)	Italy	2014 - 2015 (<i>Historical control October 2012 to September 2013</i>)	SC; R	80 (42 : 38)	I: 69±8 C: 67±7	I: 66.7 C: 57.9	Laparoscopic LAR with TME	Cancer	5 cc of 0.2 mg/kg (before and after)	D-Light from Storz®	4.7%	ICG: 0 Control : 5

SC: single centre; R: Retrospective non-randomised study; P: Prospective cohort study.

I: ICG group; C: Control group; NR: Not Reported; IBD: Inflammatory Bowel Disease; LAR: Low Anterior Resection; TME: Total Mesorectal Excision

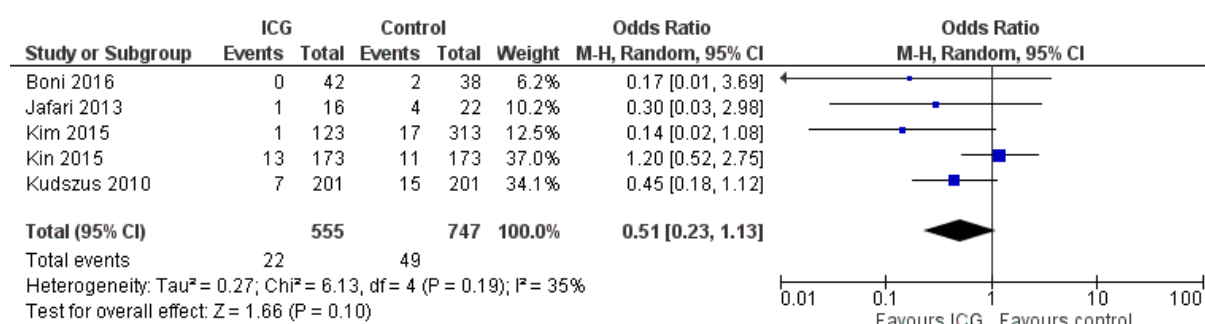
* 13 patients (10.6%) in the ICG group who were susceptible to anastomotic site ischaemia were further explored after anastomosis formation without any cases of revision and re-anastomosis.

Risk of bias assessment

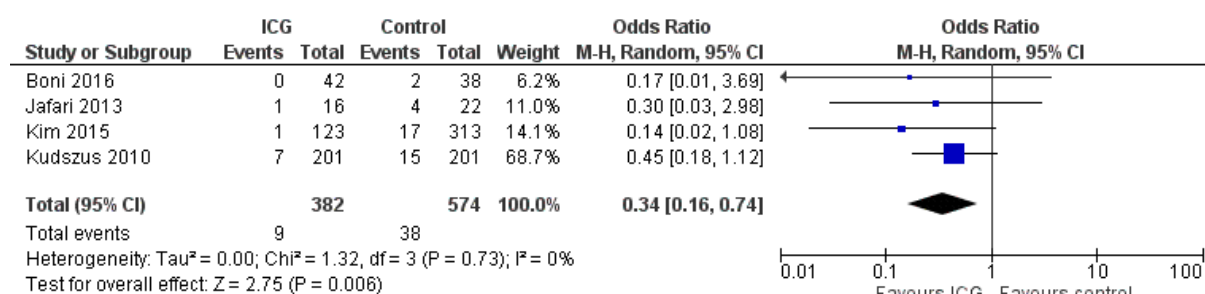
The five included studies for analysis were non-randomised studies of interventions due to the lack of published RCTs. Most of the studies were retrospective and in some cases, they included small sample sizes. For these reasons, results should be taken with caution. Overall bias risk was evaluated with ROBINS-I tool. (39) All the studies were at moderate risk of bias when assessed as non-randomised studies. See the *Appendix* for supporting information.

Outcome assessment

A



B



C

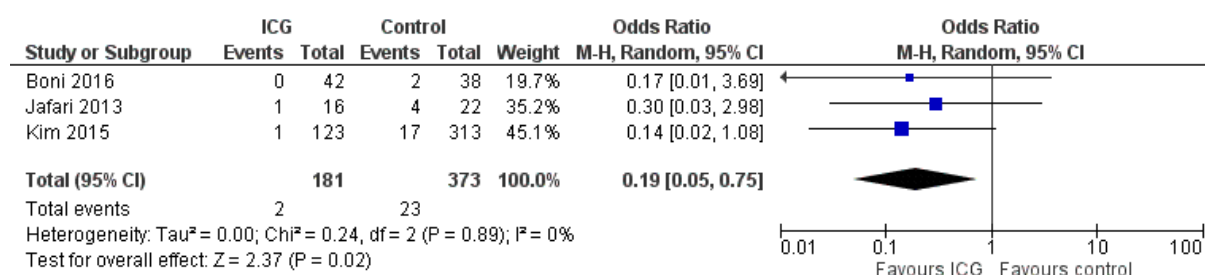


Figure 3: A) Forest plot showing odds ratio in AL following colorectal surgery in ICG group vs control group (non-ICG; standard care alone). B) Forest plot showing odds ratio in AL in patients undergoing any colorectal surgery for cancer indication in ICG group vs control. C) Forest plot showing odds ratio in AL in rectal cancer surgery in ICG group vs control. Mantel-Haenszel (M-H) method and random-effects model were used for meta-analysis. OR shown with 95 per cent confidence intervals.

The meta-analysis included 555 patients in the ICG group and 747 patients in the control group. Both groups included patients who underwent colorectal surgery for benign and malignant indication. The overall AL rate was 5.4%. There was no significant difference in AL rate with or without the use of ICG fluorescence (OR 0.51; 95% CI 0.23-1.13; P= 0.10) (*Figure 3A*). The I^2 value was 35%, which shows there was moderate heterogeneity.

Data from 956 cancer patients was obtained from four studies. (37,40–42) AL risk was significantly reduced when using ICG fluorescence imaging in patients undergoing any colorectal surgery for cancer (OR: 0.34; CI: 0.16-0.74; P=0.006; $I^2 = 0\%$) (*Figure 3B*). Rectal cancer surgery was assessed in 554 patients in three studies (40–42). ICG perfusion assessment in rectal surgery resulted in an 81% reduction in the odds of AL presentation (OR:0.19; 95% CI 0.05-0.75; P= 0.02; $I^2 = 0\%$) (*Figure 3C*), showing a low AL rate in comparison with standard care (1.1% vs 6.1% respectively).

A change in the planned anastomotic level was decided for 41 of the 555 patients in the ICG group (7.4%), due to hypoperfusion seen by ICG. Moreover, Kim et al. (41) reported 13 patients over 123 in the ICG group (10.6%) and Kudzus et al. (37) reported 5 patients over 201 (2.5%) in which further exploration with ICG after anastomosis formation helped to identify adequate perfusion despite clinical impression of malperfusion. None of those cases underwent additional resection or re-anastomosis.

4.3. Discussion

This systematic review and meta-analysis show that intraoperative use of ICG fluorescence imaging is a potential tool to reduce the AL risk following colorectal surgery for cancer. However, the inherent bias of the non-randomised studies included, should be taken into consideration when interpreting these findings.

Morbidity, mortality and costs generated by this postoperative complication, may be reduced with a decrease in AL rate. ICG fluorescence system did not seem to affect cost-effectiveness.

The initial burden of a NIR unit are 70.000€ and then the cost for ICG dye is 13€ per patient. (41) In contrast, AL represents 1.6 to 5 million euros of the annual direct healthcare costs in the UK and over 22.000€ per patient in the USA. (3) AL also increases the mortality risk (from 1.9% without AL to 15.9% with AL) and, the length of stay (from 7 days without AL to 23 days with AL). (11) In colorectal cancer surgery, AL has been associated with reduced long-term cancer specific survival and a greater risk for systemic and local recurrence. (46,47) However, this association remains unclear when referring to rectal surgery. (48)

Several studies have assessed the use of ICG fluorescence in colorectal surgery, but most of them correspond to case series with small sample sizes. Fluorescence imaging has been described in surgical procedures for benign and malignant indication and different operative approaches (22–25,49) including robotic colorectal surgery, (20,26) transanal rectal surgery, (27) and minimally invasive surgery. (50)

One of the limitations of this meta-analysis is the lack of randomisation in the studies included. In addition, four studies were retrospective (37,38,40,42) and results from ICG fluorescence group were compared with a control group from a different period of time. At present, there is no RCT published. Three ongoing RCTs were found on ClinicalTrial.gov register. AL rate is the primary outcome measure in the three studies, two of them with 30 days follow-up (43,44) and one of them with 2 months follow-up. (45) One of the RCTs would include low anterior resections for rectal cancer, (45) other would evaluate ICG use during rectal or left colectomies (benign and malignant disease) (44) and the last one includes robotic colorectal surgery for cancer, IBD or diverticular disease indication. (43)

Results of this study must be taken with caution due to the variability on AL definition, as well as differences in the length of follow-up, surgical technique and application of ICG on the experimental group. In all the included studies, ICG fluorescence was used before intestinal resection to plan transection level. In some cases, it was also used after the

anastomosis formation. (41,42) Moreover, the quantitative definition of an adequate or inadequate preanastomotic perfusion is not well defined, mainly because most of the actual imaging systems lack to quantify tissue perfusion. However, some experimental studies assessing fluorescence quantification in animal models have been published. (51) Additionally, Sherwinter et al. (27) used a fluorescence score in their study based on the sequence of fluorescence uptake and time of maximal excitation.

ICG fluorescence seems to help in identifying the need for a change in the surgical plan, extending resection margins or requiring revision and re-anastomosis. A change in the planned anastomotic level was decided in 7.4% (41 over 555 patients in the ICG group). Usually, a change is decided if a bowel hypoperfusion is detected by fluorescence, even if it had seemed well-perfused by visual examination. In contrast, ICG fluorescence can also help confirm a competent perfusion in those cases with a clinical impression of malperfusion, and therefore not to extent the resection margins further.

In the present meta-analysis, Kin et al. (38) was the only study that have reported a non-reduction in AL rate when using intraoperative fluorescence. However, this study presents some limitations that could have influenced results. Only proximal bowel perfusion was assessed, and therefore rectal stump perfusion was not confirmed. In contrast with the other studies, which only included patients undergoing surgery for cancer indication, this study also included patients with inflammatory bowel disease and diverticular disease.

Despite the limitation of the available studies, this systematic review and meta-analysis show that ICG fluorescence imaging is a promising tool that could change usual practice. It may reduce AL rate in patients undergoing colorectal resection for cancer indication. However, its use for benign indications is uncertain. There is a need of larger, well-designed RCTs to assess if AL rate can be reduced by incorporating ICG fluorescence imaging in routine colorectal surgery for benign or malignant indication.

5. STUDY PROTOCOL FOR THE ICEBERG TRIAL

5.1. Rationale for a RCT on ICG use in colorectal surgery

We propose a randomised controlled trial addressing the intraoperative use of ICG fluorescence imaging to reduce AL rate in patients undergoing colorectal surgery.

The following issues justify the need of a RCT:

1. There is no RCT published comparing ICG fluorescence imaging with standard care.
2. There are 3 ongoing trials. PILLAR III, is a multicentre RCT that is including patients undergoing low anterior resection for rectal or rectosigmoid neoplasm. (45) The other two are single centre RCT; one is including patients undergoing robotic surgery (43) and the other includes laparoscopic rectal resections and left colectomies, (44) both studies include surgeries for benign and malignant indications. Therefore, there is no RCT addressing ICG fluorescence use in routine practice for any condition (benign and malignant) under laparoscopic or robotic approach, in total, right or left colectomies and rectal resection procedures.
3. There is no current data assessing ICG dose, application, fluorescence diagnosis and management if a change in surgical plan is decided.

5.2. Study design

This is a prospective, multicentre, randomised controlled trial comparing intraoperative use of ICG fluorescence imaging with standard care alone in anastomosis formation.

5.3. Outcome measures

Primary outcome measure

AL rate within a 60 days follow-up. AL will be defined and graded as Rahbari et al. proposed in their study. (4) Pelvic abscess in the proximity of the anastomosis will also be considered as AL. Stricture of the anastomosis will not be considered as AL.

C-reactive protein (CRP) as well as procalcitonin (PCT) have been suggested as useful screening markers for early prediction of AL on postoperative days 3-5. (52) Their levels will be used to determine if a CT scan for AL detection is necessary.

Secondary outcome measure

1. Number of cases in which a change in the surgical plan is decided. For example: extension of the resection margins; colon/rectum preservation; protective ileostomy (avoided or performed).
2. Major postoperative complications defined by the Clavien-Dindo classification (53) grade III to V at 60 days of follow-up (see *Appendix* for more information).
3. Length of stay, including from day 0 (day of surgery) to day of discharge.
4. Fluorescence Reflection Score (on the experimental group) and Clinical Assessment Score (on both groups) for anastomosis evaluation. The Fluorescence Reflection Score corresponds to fluorescence reflection of the colon and rectum in the ICG fluorescence imaging arm. The Clinical Assessment Score is the evaluation of bowel appearance, the completeness of doughnuts and air leak test results in both arms. Following score has been adapted from the study of Sherwinter et al. (27):

Score	Clinical Assessment Score	Fluorescence Reflection Score
1	Dusky appearance and/or positive air leak test and/or non-integrity of doughnuts	No uptake
2	Pink appearance but no pulsatility of mesenteric vessels or no bleeding cut edges or alteration on bowel viability suspected	Hypofluorescent or patchy fluorescence comparing with other segments
3	Pink appearance, pulsatility of mesenteric vessels, bleeding cut edges, no alteration on bowel viability. Complete anastomotic doughnuts. Negative air leak test.	Isofluorescent to other segments

Table 2: Clinical Assessment Score and Fluorescence Reflection Score for anastomosis evaluation.

5.4. Eligibility criteria

Inclusion criteria

- Adult patients (≥ 18 years) undergoing colorectal resection with a primary anastomosis (including right or left colectomy, total colectomy, sigmoid and rectal resection) will be included. Surgeries under laparoscopic or robotic approach for benign or malignant indication will be eligible for inclusion.

Exclusion criteria

- Patients with known allergy or history of adverse reaction to iodine or indocyanine green
- Pregnant or breast-feeding patients
- Patients undergoing emergency surgery
- Patients undergoing colorectal resection without anastomosis
- Patients undergoing palliative surgery for terminal illness
- Not consenting patients

5.5. Site selection

This study is proposed by the general surgery department, colorectal surgery unit at *Hospital Universitari Vall d'Hebron*. The ICEberG trial is a multicentre study and it will be performed in European colorectal units. Moreover, it is opened to other international centres interested in joining the trial. Sites will be eligible to participate based on fluorescence imaging system availability at their units, case volumes and surgical experience.

5.6. Randomisation

After obtaining written consent from the patients, they will be randomised by a web-based method on a 1:1 basis. Stratified randomisation will be performed by the planned surgical procedure: total colectomy, right colectomy, left colectomy, sigmoid resection, and rectal resection.

5.7. Blinding

This trial is blinded to the patient. The trial design and the expected intervention on the experimental group do not enable to blind the surgeon performing the procedure.

5.8. Interventions: experimental and control arm

Standard care for AL detection will be performed in both arms and it will include:

- Visual examination of both segments (before and after anastomosis)
- Integrity of doughnuts assessment if applicable
- Air leak test (after anastomosis)

Intervention arm

The intervention group consists on using ICG fluorescence imaging intraoperatively. Preoperative bowel preparation, as well as planned surgical procedure will be performed according to surgeon's standard practice until time of transection point. A bolus of 0.1 – 0.3 mg/kg of indocyanine green will be injected intravenously by the anaesthetist. If laparoscopic approach, PINPOINT™ system will be used. If robotic approach, then FIREFLY™ will be the system used.

Prior to fluorescence assessment, surgeon will visually examine anastomosis segments as it would be done in standard care and will assign a score following the “Clinical Assessment Score” presented on *Table 2*. Then, fluorescence of both segments of the anastomosis will be assessed and a score will be assigned by the surgeon according to the “Fluorescence Reflection Score” on *Table 2* (1 no uptake to 3 maximal uptake). Once the anastomosis is done, “Clinical Assessment Score” and “Fluorescence Reflection Score” will be used again to assign a score after anastomosis performance. If a change on the surgical planned is decided, this will be noted on case report form.

Control arm – No intervention

In the control group, only standard care will be performed. Therefore, both segments will be visually examined using the “Clinical Assessment Score” before and after anastomosis formation. If a change on the surgical planned is decided, this will be noted on case report form.

5.9. Recruitment and trial timeline

Patients undergoing colorectal surgery will be screened for eligibility. If the patient meets inclusion criteria and does not meet exclusion criteria, the surgeon will explain trial details to the patient. An information sheet about ICEberG trial will be available for patients. If the patient accepts and signs the informed consent, it can be randomised into one of the two branches. Intervention or non-intervention will take place during the surgical procedure.

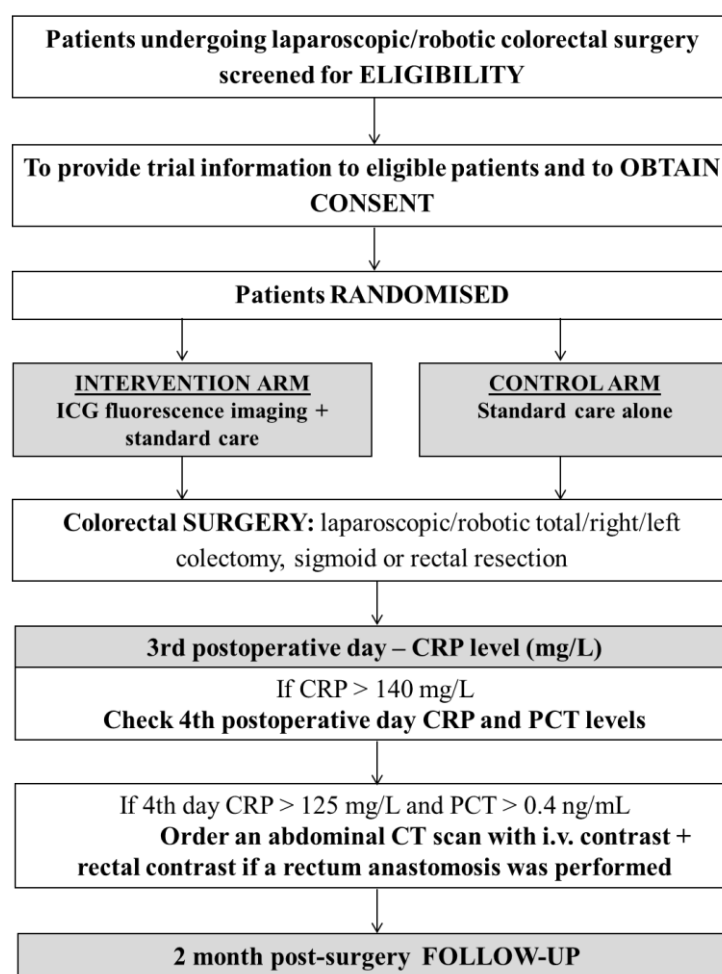


Figure 4: Trial flow chart

In order to detect AL postoperatively, all patients' CRP levels will be evaluated on the 3rd postoperative day. If CRP is over 140 mg/L, then 4th postoperative day CRP and PCT will be evaluated. If at 4th day CRP is over 125 and PCT is over 0.4 ng/mL, then a CT scan with intravenous contrast will be ordered, adding rectal contrast if a rectum anastomosis was performed.

Requirement of radiological or surgical management of an AL will be recorded, as well as major postoperative complications including death. Patients will be follow-up for at least two months.

The end of the study is the date of the last follow-up for the last patient included in the study.

5.10. Data management

Data collection will include preoperative, intraoperative and postoperative details. Data will be collected on predesigned paper forms (see *Appendix* – case report form) and then, it will be entered into an electronic database system.

5.11. Statistical methods

Sample size

In previously cohort studies in Europe, AL rate has ranged among 6% to 8% in colonic resections and among 10% to 12% for rectal resections. (7,8,54)

Assuming an average of AL following colorectal surgery of a 9% in the control group and a 3% in the experimental group based on published studies, accepting an alpha risk of 0.05 and a beta risk of 0.2, a total of 512 patients (256 patients each arm) are the minimum required for the study if a 10% of drop-out rate is assumed. The ARCSINUS approximation has been used.

Statistical analysis

Data analysis will be performed using R Foundation for Statistical Computing software (R version 3.3.1). For baseline characteristics, descriptive statistics will be used. Chi squared test

or Fisher's Exact test will be used for categorical variables, including AL rate. For continuous outcomes, Student's t test will be applied for normal distribution and Mann-Whitney U test for data that is not normally distributed. Univariate exploratory analysis and multiple logistic regression will be used to test risk factors associated with AL (see *Appendix* – case report form). $P < 0.05$ will be used as level of significance.

5.12. Ethical considerations and trial registration

The trial will be performed following the principles of the Declaration of Helsinki and Good Clinical Practice. (55) Before the start of the trial, protocol, information sheet and informed consent documents will be approved by the Ethical Committee at each participating centre. Recruitment will not start in any individual centre until local approval is obtained.

Only patients who accept and sign the informed consent will be included. Participation in the study is voluntary and patients can withdraw consent at any time.

The trial will be registered at the www.clinicaltrials.gov website.

6. CONCLUSIONS

Anastomotic leakage is still a common postoperative complication, which has been associated with greater morbidity and mortality rates. The findings of the systematic review and meta-analysis performed, suggest that ICG fluorescence imaging is a promising tool to prevent AL in patients undergoing colorectal anastomosis. In order to establish fluorescence imaging as part of routine colorectal surgery, randomised controlled trials are needed to provide evidence in its use. Therefore, ICEberG trial has been proposed in this work addressing this necessity.

Finally, European and international collaboration among surgeons is essential to run powered studies which allow to address this issue together and, consequently, to get better outcomes for our patients.

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APPENDIX

Intraoperative use of IndoCyaninE Green fluorescence imaging to prevent anastomotic leakage in colorectal surgery:

Systematic Review, Meta-Analysis and Study Protocol for the ICEberG Trial

- Abbreviations
- Search strategies (systematic review)
- Bias assessment
- ICEberG trial – Case report form
- Information sheet and informed consent for the ICEberG trial

1. ABBREVIATURES

AL – Anastomotic leakage

ICG – Indocyanine Green

RCTs – Randomised Controlled Trials

NIR – Near Infrared

FA – Fluorescence Angiography

ASA – American Society of Anaesthesiologists

ACPGBI – Association of Coloproctology of Great Britain and Ireland

FDA – Food and Drug Administration

OR – Odds Ratio

CI – Confidence Intervals

M-H – Mantel-Haenszel

IBD – Inflammatory bowel disease

LAR – Low anterior resection

TME – Total mesorectal excision

CT – Computerized tomography

CRP – C-reactive protein

PCT – Procalcitonin

2. SEARCH STRATEGIES

Search strategy, PubMed:

((("indocyanine green"[MeSH Terms] OR ("indocyanine"[All Fields] AND "green"[All Fields]) OR "indocyanine green"[All Fields]) OR ICG[All Fields] OR ("colouring agents"[All Fields] OR "coloring agents"[Pharmacological Action] OR "coloring agents"[MeSH Terms] OR ("coloring"[All Fields] AND "agents"[All Fields]) OR "coloring agents"[All Fields]) OR ("fluorescence"[MeSH Terms] OR "fluorescence"[All Fields]) OR ("fluorescein angiography"[MeSH Terms] OR ("fluorescein"[All Fields] AND "angiography"[All Fields]) OR "fluorescein angiography"[All Fields]) OR ("fluorescent dyes"[Pharmacological Action] OR "fluorescent dyes"[MeSH Terms] OR ("fluorescent"[All Fields] AND "dyes"[All Fields]) OR "fluorescent dyes"[All Fields])) AND ((("anastomotic leak"[MeSH Terms] OR ("anastomotic"[All Fields] AND "leak"[All Fields]) OR "anastomotic leak"[All Fields]) OR "anastomotic leakage"[All Fields] OR "anastomotic perfusion"[All Fields] OR ("anastomosis, surgical"[MeSH Terms] OR ("anastomosis"[All Fields] AND "surgical"[All Fields]) OR "surgical anastomosis"[All Fields] OR ("anastomosis"[All Fields] AND "surgical"[All Fields]) OR "anastomosis, surgical"[All Fields]) OR "bowel perfusion"[All Fields] OR ("blood supply"[Subheading] OR ("blood"[All Fields] AND "supply"[All Fields]) OR "blood supply"[All Fields]) OR "perfusion assessment"[All Fields]) AND ((("colorectal surgery"[MeSH Terms] OR ("colorectal"[All Fields] AND "surgery"[All Fields]) OR "colorectal surgery"[All Fields]) OR "colon surgery"[All Fields] OR "rectal surgery"[All Fields] OR "colorectal resection"[All Fields] OR "bowel resection"[All Fields]))

Search strategy, Scopus:

(ALL (("indocyanine green" OR icg OR "coloring agents" OR fluorescence OR "fluorescein angiography" OR "fluorescent dyes")) AND ALL (("anastomotic leak" OR "anastomotic leakage" OR "anastomotic perfusion" OR "anastomosis,surgical" OR "bowel

perfusion" OR "blood supply" OR "perfusion assessment")) AND ALL (("colorectal surgery" OR "colon surgery" OR "rectal surgery" OR "colorectal resection" OR "bowel resection")) – **All Fields**

Search strategy, Web of Science (Core Collection):

TOPIC: (("indocyanine green" OR icg OR "coloring agents" OR fluorescence OR "fluorescein angiography" OR "fluorescent dyes")) AND **TOPIC:** (("anastomotic leak" OR "anastomotic leakage" OR "anastomotic perfusion" OR "anastomosis,surgical" OR "bowel perfusion" OR "blood supply" OR "perfusion assessment")) AND **TOPIC:** (("colorectal surgery" OR "colon surgery" OR "rectal surgery" OR "colorectal resection" OR "bowel resection"))

Timespan: All years.

Search strategy, Google Scholar:

"indocyanine green" "anastomotic leakage" "colorectal surgery"

Search strategy, Cochrane Library:

("indocyanine green" OR icg OR "coloring agents" OR "fluorescence" OR "fluorescein angiography" OR "fluorescent dyes") AND ("anastomotic leak" OR "anastomotic leakage" OR "anastomotic perfusion" OR "anastomosis,surgical" OR "bowel perfusion" OR "blood supply" OR "perfusion assessment") AND ("colorectal surgery" OR "colon surgery" OR "rectal surgery" OR "colorectal resection" OR "bowel resection") – **Search all text**

Search strategy, ClinicalTrials.gov:

"indocyanine green" AND "colorectal"

"indocyanine green" AND "rectal"

3. BIAS ASSESSMENT WITH ROBINS-I TOOL¹

ROBINS-I tool (Stage II): For each study

	Confounding	Selection of participants	Classification of interventions	Deviations from intended interventions	Missing data	Measurement of outcomes	Bias in selection of reported result
Kudszus et al. (37)	Moderate	Low	Low	Low	Low	Moderate	Low
Kin et al. (38)	Moderate	Low	Low	Low	Low	Low	Moderate
Jafari et al. (40)	Moderate	Low	Low	Low	Low	Low	Low
Kim et al. (41)	Moderate	Low	Low	Low	Low	Moderate	Low
Boni et al. (42)	Moderate	Low	Low	Low	Low	Low	Low

ROBINS-I tool (Stage III): Overall risk of bias assessment

- Kudszus et al. (37) – Overall MODERATE
- Kin et al. (38) – Overall MODERATE
- Jafari et al. (40) – Overall MODERATE
- Kim et al. (41) – Overall MODERATE
- Boni et al. (42) – Overall MODERATE

¹ Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. Br Med J [Internet]. 2016;355:i4919. Available from: <http://www.bmj.com/lookup/doi/10.1136/bmj.i4919>

POSTOPERATIVE DATA				
3 rd postoperative day CRP level (mg/L)				
<i>If applicable: 4th postop day CRP level (mg/L)</i>				
<i>If applicable: 4th postop day PCT level (ng/mL)</i>				
CT scan findings	None	AL	Pelvic abscess	Other
Anastomotic leak (60 day follow-up) <i>If AL - Please record postoperative day of diagnosis</i>	No	Yes → Grade A / Grade B / Grade C		
Length of stay (days)				
60-day Clavien-Dindo grade	I	II	III	IV
Readmission (<i>if YES – indicate postoperative day</i>)			Yes	No
Reoperation (<i>if YES – indicate postoperative day</i>)			Yes	No
Postoperative pain control with NSAIDS			Yes	No
TNM after surgery (<i>If cancer indication</i>)				

(*) Variables associated with AL – for exploratory analysis on primary outcome measure

Clavien Dindo Classification

GRADE	DEFINITION
I	Any deviation from the normal postoperative course without the need for pharmacological (other than the “allowed therapeutic regimens”), surgical, endoscopic or radiological intervention. Allowed therapeutic regimens are: selected drugs (antiemetics, antipyretics, analgesics, diuretics and electrolyte replacement), physiotherapy and wound infections opened at the bedside but not treated with antibiotics.
II	Requiring pharmacological treatment with drugs beyond those allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
IIIa	Requiring surgical, endoscopic or radiological intervention, not under general anaesthetic.
IIIb	Requiring surgical, endoscopic or radiological intervention, under general anaesthetic.
IVa	Life-threatening complications requiring critical care management – single organ dysfunction, or neurological complications including brain haemorrhage and ischemic stroke (excluding transient ischemic attack).
IVb	Life-threatening complications requiring critical care management – multi-organ dysfunction.
V	Death of a patient

5. INFORMED CONSENT AND INFORMATION SHEET FOR THE ICEBERG TRIAL²

Modelo de consentimiento informado para el ensayo clínico ICEberG

Título del estudio: ICEberG trial: IndoCyaninE Green fluorescence imaging to prevent anastomotic leakage in colorectal surgery

Yo _____ (nombre y apellidos del participante)

He leído la hoja de información que se me ha entregado.

He podido hacer preguntas sobre el estudio.

He hablado con: _____ (nombre del investigador).

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

1. Cuando quiera
2. Sin tener que dar explicaciones
3. Sin que esto repercuta en mis cuidados médicos

Presto libremente mi conformidad para participar en el estudio.

Fecha y firma del participante

Fecha y firma del investigador

² These documents are in Spanish as required by the *Clinical Research Ethics Committee (CREC) of Hospital Universitari Vall d'Hebron*.

Hoja de información al paciente para el ensayo clínico ICEberG

Proyecto de investigación titulado *ICEberG trial: IndoCyaninE Green fluorescence imaging to prevent anastomotic leakage in colorectal surgery*

Estimado paciente,

Le solicitamos su participación en este proyecto de investigación cuyo objetivo principal es evaluar si el uso intraoperatorio de verde indocianina para la visualización directa de la vascularización colorrectal reduce la tasa de fuga de anastomosis.

Beneficios:

Es posible que de su participación en este estudio no obtenga un beneficio directo. Sin embargo, la evaluación de nuevas estrategias relacionadas con la prevención de la fuga anastomótica podría contribuir a una futura reducción de esta complicación postoperatoria.

Procedimientos del estudio:

En este estudio se pretenden comparar dos procedimientos. La asignación a uno de ellos vendrá determinada por el azar. Su médico no intervendrá en este proceso. Usted tendrá una probabilidad del 50% de recibir cada uno de los procedimientos contemplados en este estudio. El primer grupo contempla los procedimientos que se hacen usualmente en la práctica habitual durante la cirugía y en el segundo grupo, además de los procedimientos habituales se empleará el uso de verde de indocianina. Éste es un colorante que permite visualizar por fluorescencia la vascularización colorrectal y, por tanto, decidir cambios en el plan quirúrgico según precise para asegurar una anastomosis en zona viable con buena perfusión.

Molestias y posibles riesgos:

El verde de indocianina es un colorante seguro, de baja toxicidad y buena tolerancia. Se ha descrito algún caso de reacción anafiláctica en pacientes con alergia a la yodina o a contrastes iodados (1/300000).

Protección de datos personales:

De acuerdo con la Ley 15/1999 de Protección de Datos de Carácter Personal, los datos personales que se obtengan serán los necesarios para cubrir los fines del estudio. En ninguno de los informes del estudio aparecerá su nombre, y su identidad no será revelada a persona alguna salvo para cumplir con los fines del estudio, y en el caso de urgencia médica o requerimiento legal. Cualquier información de carácter personal que pueda ser identificable será conservada por métodos informáticos en condiciones de seguridad. Cualquier información de carácter personal que pueda ser identificable será conservada por métodos informáticos en condiciones de seguridad. El acceso a dicha información quedará restringido al personal del hospital, designado al efecto o a otro personal autorizado que estará obligado a mantener la confidencialidad de la información.

De acuerdo con la ley vigente, usted tiene derecho al acceso de sus datos personales; asimismo, y si está justificado, tiene derecho a su rectificación y cancelación. Si así lo desea, deberá solicitarlo al médico que le atiende en este estudio.

De acuerdo con la legislación vigente, tiene derecho a ser informado de los datos relevantes para su salud que se obtengan en el curso del estudio. Esta información se le comunicará si lo desea; en el caso de que prefiera no ser informado, su decisión se respetará.

Su participación en el estudio es totalmente voluntaria, y si decide no participar recibirá todos los cuidados médicos que necesite y la relación con el equipo médico que le atiende no se verá afectada.

Agradecimiento:

Le agradecemos el tiempo dedicado a la lectura de esta información. Por favor, tómese el tiempo necesario para leer toda la información y preguntar las dudas que le pudieran surgir. Si decide tomar parte en este estudio, deberá firmar el consentimiento informado conforme acepta su participación voluntaria en el ensayo clínico ICEberG.