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'Comparison between the accuracy of positive and negative indocyanine green staining in guiding laparoscopic anatomical liver resection: a protocol for a randomized controlled trial

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ABSTRACT

Introduction

Knowledge of the clinical liver anatomy has evolved with advanced imaging modalities and laparoscopic surgery. Therefore, precise anatomical resection knowledge has become the standard treatment for primary and secondary liver cancer. Segmentectomy, a parenchymal-preserving approach, is regarded as an option for anatomical resections in patients with impaired liver. Indocyanine green (ICG) staining is a promising method for understanding the anatomical borders of the liver segments. There are two methods of ICG staining (positive and negative), and the superiority of one of both approaches has not been determined to date.

Methods and analysis

In this regard, a comparison between the accuracy of positive and negative ICG staining in guiding laparoscopic anatomical liver resection is planned in this study. Possible candidates are patients with liver malignant tumors in whom laparoscopic mono- or subsegmentectomy is planned. Fifty patients ≥ 18years of age will be prospectively allocated into the following two groups with 25 patients in each: Group A, ICG-negative staining group, and Group B, ICG-positive staining group. The optimal dose of ICG for positive staining will be determined during the preparation phase. To assess the ability of the ICG fluorescence guidance in anatomical resection, the primary endpoint is the success rate of ICG staining, which consists of a subjective optical scoring based on three components: superficial demarcation in the liver surface, visualization of the parenchymal borders, and consistency with the preoperative three-dimensional (3D) simulation. The

secondary endpoints are the evaluation of short-term surgical outcomes and recurrence-free survival at one year.

ARTICLE SUMMARY

Strengths and limitations of this study

- This study aims to compare the accuracy of ICG fluorescence navigation between the two staining methods in the context of performing precise laparoscopic liver resection and demarcation of the intersegmental/sectional planes.
- The study will help to further disseminate ICG fluorescence navigation in liver surgery worldwide.
- The oncological outcomes of the ICG fluorescence-guided liver resection can be investigated in future clinical trials.
- Surgical trainees will be increasingly able to use ICG fluorescence navigation when the staining technique is standardized.
- As a limitation, the staining technique is operator-dependent; therefore, a definitive conclusion could not be made only from this single-center trial.

ETHICS AND DISSEMINATION

The study has been approved by Ageo Central General Hospital Clinical Research Ethical Committee (No: 1044) and it carried out following the Helsinki Declaration (2013 revision).Informed consent will be taken from the patients before participating. The findings will be disseminated through peer-reviewed publications, scientific meetings, and conferences

TRIAL REGISTRATION NUMBER

This study has been registered in the UMIN Clinical Trials Registry (UMIN000049815).To see the study protocol and regulations, visit the registration website:

https://center6.umin.ac.jp/cgi-bin/ctr/ctr_view_reg.cgi?recptno=R000056739.

INTRODUCTION

Since the previous three international consensus meetings (Louisville, Iwate, and Southampton)(1-3),laparoscopic liver resection (LLR) as a treatment for the chronic liver disease has developed considerably worldwide. Understanding clinical liver anatomy has gained increasing attention with the advancement of three-dimensional (3D) simulation software. The emergence of indocyanine green (ICG) fluorescence and high-quality magnified view of laparoscopic imaging has also contributed to the knowledge of clinical liver anatomy to a large extent. Therefore, more precise liver resections, such as anatomical liver resections (ALR), have recently been more commonly performed based on liver inflow and outflow. Currently, ALR is accepted as a standard therapy for liver cancer because of its oncologic effectiveness. However, even if we do not consider the long-term efficiency of ALR, the watershed of the segments/sections is easy to transect because of the sparse vessels in the intersegmental/sectional planes. Besides, leaving fewer ischemic areas in the remnant liver is considered reasonable after ALR.

In 1985, Makuuchi reported a new concept of small ALR (i.e., segmentectomy in Brisbane terminology 2000), which applies well to the therapeutic principle in the Asia Pacific region, where most hepatic malignancies are hepatocellular carcinoma arising in the impaired liver (4). Thus, a small ALR was established based on the parenchyma-sparing principle in mal-distributed patient groups. Since the 1990s, when laparoscopic surgery has developed noticeably, anatomical knowledge of the internal and external liver has gradually increased owing to its magnified and unique caudal/dorsal view. Clinical questions regarding the landmarks for the segmental borders and approach for the tumor-bearing portal pedicles were discussed during the 32nd meeting of the Japanese Society of Hepato-

Biliary-Pancreatic Surgery (JSHBPS) held in Japan in 2021, and the new terminology for the small ALR was described by updating the Brisbane 2000 terminology(5-7)(Table 1).

Terminology	Definition
Anatomical liver resection	Complete removal of the liver parenchyma confined within the responsible portal territory.
Segmentectomy	The complete removal of a territory (territories) of the third-order portal venous branches of a Couinaud segment.
Sub-segmentectomy	The removal of the liver parenchyma within the portal territory (territories) of less than a Couinaud's segment. These are also defined as cone units, and their areas can be intra-operatively assessed by using ischemic demarcation, ICG (negative/ positive) staining, or both.
A sub-segment	An anatomical portion of a Couinaud segment, which is defined as a cone unit or cone units, based on sub-segmental inflow. This concept particularly adapts to Sg 8 (ventral and dorsal), Sg 4 (basal and apical), and Sg 1 (Spiegel, caudate process, and paracaval).
Segment 4	Redefined as consisting of two sub-segments: Sg 4a (apical) and 4b (basal). Sg 4a is defined as the cranial anatomical portion of Sg 4 according to the third-order portal territories, and Sg 4b is the caudal anatomical portion of Sg 4.
Segment 9	Sg 9 definition of the Brisbane 2000 terminology is abandoned, and caudate lobe is redefined based on portal ramifications instead of spatial recognition.
Segment 1	Classified into three parts as follows, i) the Spiegel lobe, ii) the paracaval portion, and iii) the caudate process.

Table 1: The Tokyo 2020 terminology of liver anatomy and resections: Updates of the Brisbane 2000 system

ICG fluorescence imaging is considered helpful for the real-time identification of segmental boundaries during liver parenchymal transection in LLR, achieving the concept of anatomical parenchyma-sparing resection (8).To assess the real-time hemodynamics, ICG fluorescence has not yet been matched in the field of intraoperative imaging modality

owing to its unique excretion(through bile juice) characteristic and deep penetration of approximately 1cm. However, the optimal usage (i.e., the dose and timing for multiple uses) has not yet been clarified according to its various uses in each report. Two methods of ICG staining have been reported based on its administration routes: positive and negative staining (9). Although Wakabayashi T et al. addressed the optimal dose and timing of ICG application for positive and negative staining (10), the superiority of either staining method has not been determined to date (Figure 1).

It is of utmost importance to accurately dissect the anatomical boundaries between the tumor-bearing liver segment and adjacent segments in the case of ALR with ICG fluorescence guidance. Funamizu N et al. reported a positive correlation between the estimated and actual liver volumes after the ICG negative staining approach (11). ICG-negative staining can precisely delineate the anatomical borders during resection, maintaining both radical resection and sufficient healthy parenchyma. Additionally, Chiow AKH et al. reported preferable clarity of ICG fluorescence guidance in the two approaches of staining in robotic ALR (12). However, the results depended only on subjective assessment, and the outcomes were never statistically compared between the two staining approaches.

This study aims to compare the accuracy of liver segmentation using positive and negative staining during LLR to achieve precise ALR, such as segmentectomy, based on preoperative planning. Furthermore, future research can be conducted on the long-term outcomes of precise ALR.

METHODS AND ANALYSIS

Study design

This prospective study is a randomized controlled superiority clinical trial on patients with malignant liver lesions who will undergo segmentectomy using ICG fluorescence imaging navigation. This study will be conducted at the Ageo Central General Hospital (Saitama, Japan), a referral center for LLR in Japan.

Pilot trial

A small-scale pilot study will be performed on six patients (12% of the sample size of the main study) to determine the appropriate dose of ICG-positive staining.

Hypothesis

We hypothesize that there is a statistical difference between the success rate of staining and short-term outcomes of the positive and negative ICG staining approaches in performing precise LLR. Theoretically, ICG-negative staining is a more solid approach for liver segmentation than ICG-positive staining. To perform ICG-negative staining, the Glissonean approach advocated by Prof. Takasaki (13) is reasonable because the inflow of tumor-bearing areas is completely blocked before liver transection. This concept is based on a non-touch isolation technique for malignant tumors. However, to our knowledge, no

available literature has specifically examined the potential benefit of negative staining compared to positive staining in laparoscopic segmentectomy.

Target population

Patients with primary or metastatic liver tumors planned for mono- and subsegmentectomy from February2023 to December 2025will be candidates for this clinical trial. The following inclusion and exclusion criteria are created to unify the selection of patients in this study. The inclusion criteria are as follows: male or female patients with solitary primary or metastatic liver tumors, aged ≥18 years, scheduled for elective LLR, preserved liver function, ability to understand the nature of the study, and willingness to join and provide voluntary written consent. The liver functional reserve will be evaluated by serum biochemical tests (albumin level, total bilirubin level, and prothrombin time) and ICG retention rate at 15 min (ICG-15R). The severity of the liver disease will be assessed based on Child-Pugh stages and liver damage classification defined by the Liver Cancer Study Group of Japan (14). Preserved liver function is defined as an ICG-15R less than 30% and a Child-Pugh classification A or B. The exclusion criteria are as follows: repeat liver resection, multiple tumors, concomitant resection of other organs, severe liver or renal insufficiency, ICG hypersensitivity, pregnancy or breastfeeding, and inability to understand the nature of the study or refuse it. The schematic representation of the algorithm for this project, which has been designed with close consideration of the SPIRIT guidelines (15,16), is shown in Figure 2.

Sample size calculation

The sample size was calculated using the EpiCalc 2000 software (Gilman & Myatt, 1998)(17).

$$n_1 = (z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \frac{P_1(1-P_1) + P2(1-P_2)}{(P_1 - P_2)^2}$$

Where α = 0.05, (1-β) = 0.95.

Although no previous data are available in the literature to compare the negative and positive ICG staining, we decided to use the data reported by Chiow AKH et al. (12). Thus, P1 (percentage of cases that had clear demarcation with positive ICG) = 50% and P2 (percentage of cases that had clear demarcation with negative ICG) = 92.5% are set in the power calculation. Consequently, the minimum required sample size is 25 in each group, with a total of 50 patients. Investigators may enroll more participants to avoid a significant decrease in the study power caused by attrition bias.

Randomization and blinding

A randomized controlled superiority trial will be performed at the Ageo Central General Hospital. Fifty patients will be randomly assigned (1:1) to receive either positive or negative ICG staining. The minimization method will be used for the randomization dividing the participants into two groups. In addition, ICG-15R will be used as a parameter to equalize the background liver function to minimize the intergroup bias. Tumor etiology will be also equalized between the groups. Allocation concealment will be performed until the patients are enrolled and assigned to the operation.

Intervention and surgical procedures

The preoperative routine test and planning for the patient have been described elsewhere (18).ICG-R15 tests will be conducted two weeks before surgery to assess patients' hepatic reserve using an ICG dose of 0.5 mg/kg. Three-dimensional (3D) vascular simulation models are constructed by a specific workstation (ZIOSTATION 2, Ziosoft Inc., Tokyo, Japan), depending on the multi detector slice computed tomography (CT). Surgical planning is fashioned in line with the "cone unit" theory instead of Couinaud's stratification. Furthermore, preoperative volumetry will measure the total liver volume (TLV) and estimated liver resection volume (ELRV). To examine the accuracy of LLR, the actual liver volume (ALRV) will be calculated by dividing the actual liver resection mass (g) by standardized liver density (1.05g/mL)(11,19). Finally, the discrepancy between the ELRV and ALRV will be calculated as |ELRV-ALRV|/TLV×100 (%).We will use the 1688 Advanced Imaging Modalities Platform (Stryker Co., MI, USA) as the laparoscopic near-infrared camera throughout the designated study period. The extra-hepatic (extra-fascial) Glissonean approach will be used in all patients involved in this study to encircle the target Glissonean pedicle supplying the tumor following the preoperative simulation. Liver parenchyma division will be performed using a Cavitron Ultrasonic Surgical Aspirator (CUSA, ValleyLab, CO, USA). During the extra-hepatic Glissonean approach, the 3D simulation model will be repeatedly referred to on a screen to ensure that the targeted pedicle tree is addressed.

Operative procedure for group A

During the early phase of surgery, the extra-hepatic (extra-fascial) Glissonean approach will be performed to encircle the target Glissonean pedicle, feeding the tumorous area, corresponding precisely to the preoperative simulation (Figure 3). To avoid postoperative bile leakage, it is essential to transect towards the liver parenchyma instead of the Glissonean sheath using the Cavitron Ultrasonic Surgical Aspirator (CUSA, ValleyLab, CO, USA). During the extra-hepatic Glissonean approach, the 3D simulation model will be repeatedly referred to on a primary screen to correct the pedicle tree. When identified, the target pedicle will be clamped using an endoscopic bulldog to make the diseased area completely ischemic. Sequentially, inflow blockage will be confirmed using laparoscopic intraoperative ultrasonography with Doppler mode. Since the staining is irreversible after ICG injection, 0.15mL/kg ultrasound contrast medium (SONAZOID, Daiichi-Sankyo, Tokyo, Japan) will be systematically injected before ICG injection. If the target area is adequately cyanosed, 0.5 mg/body ICG will be intravenously injected in the ICG-negative staining method. The demarcation line appears as a border between the color-coded and non-color-coded areas,—marked on the liver surface. In the deeper parenchyma, the intersegmental plane can also be coded by the ICG fluorescence emission, which corresponds to the course of transection. The 1688 Advanced Imaging Modalities Platform will be used for the near-infrared camera system in all cases. This system has an overlay mode that enables the user to superimpose an ICG fluorescence image to a white-light image. This mode facilitates precise parenchymal transection according to the border between the color-coded and non-color-coded areas. Liver transection will be performed using the CUSA and other energy devices.

Operative procedure for group B

On the contrary, in ICG positive-staining, ICG will be directly injected into the portal branches responsible for resected territories or surrounding territories to visualize the clear demarcation planes (Figure 4). The portal branches of the tumor-bearing liver segments will be targeted and punctured under ultrasound guidance with an 18- or 21- gauge spinal or percutaneous transhepatic cholangio-drainage needle introduced through the abdominal wall. The needle hole will assist the direction of the needle in a dedicated laparoscopic ultrasound probe (provided by BK Medical, Herlev, Denmark). Subsequently, a small volume of ICG (1 mL of 0.025 mg/mL) will be slowly injected into the portal branch to avoid the risk of ICG retrograde flow into the neighboring segments with undesired staining without clamping the hepatic artery. Liver transection will be performed using CUSA and other energy devices.

Primary endpoint

To determine the ability of the ICG fluorescence guidance in anatomical resection, the primary endpoint will be the success rate of ICG staining, which consists of a subjective optical scoring (SOS) based on three components: superficial demarcation in the liver surface, visualization of the parenchymal borders, and consistency with the preoperative 3D simulation. It is also subjective to estimate the resection margin and shape of the specimen in comparison with the pre-and postoperative 3D simulations of the liver.

Secondary endpoints

The secondary endpoints will be the short-term surgical outcomes, such as the operative time, blood loss, and complication rates. Recurrence-free survival at 1-year will also be addressed. The patients will be followed up at the outpatient clinic after surgery every three months with regular laboratory and radiological assessments using CT and magnetic resonance imaging (MRI).

Data collection

The data will be collected in four phases: pilot, preoperative, operative, and postoperative (Table 2). In each phase, specific information will be collected for assessment. All the phases will be digitally recorded and reviewed by the authors.

Assessment		
Preoperative (Within 14 days)	**operative**	**postoperative**
Participation & eligibility	primary end point (subjective three components)	Tri-phasic liver CT scan with volumetry at POD 1
patient factors*	operative time	hospital stay
informed consent	blood loss	complications
blood investigations	pathology	early period until POD 90
LFT, Albumin	specimen weight	late period until POM 12
PT, PT-INR, APTT	surgical margin	CT scan / MRI in every 3 months
ICG-15R	tumor size	blood investigations
Child Pugh score	final diagnosis	tumor markers
Triphasic liver CT scan with volumetry and MRI		
Tumor markers**		

Table 2: Schedule of participation, investigation and assessment, preoperative findings and 12-month follow-up

LFT: Liver function test, PT: prothrombin time, PTT: activated partial thromboplasin time,ICG-15R: indocyanine green retention test at 15 minutes.

*including age, sex, body mass index (BMI), American Society of Anesthesiologist (ASA) physical status, underlying liver co-morbidities, chronic hepatitis status, and preoperative chemotherapy.

**includes: CEA, CA19-9, AFP, and PIVKA-II.

Phase 0 (pilot study)

To determine the best dose of ICG to be administered for positive staining, a preliminary study of six patients will be performed as the first step. The initial trial dose will be 0.025 mg/ml, and the first patient will be administered1 mL of this dose. Each successive patient will receive one extra milliliter of ICG at the same dose until sufficient positive staining is achieved.

Phase 1 (preoperative period)

The databases will be extracted from patient charts, which include the following baseline characteristics: age, sex, body mass index (BMI), American Society of Anesthesiologist (ASA) physical status, underlying liver comorbidities, chronic hepatitis status, preoperative chemotherapy, tumor size and location, volumetric and biochemical laboratory investigations including liver function test, ICG-15R, and tumor markers.

Phase 2 (operative period)

This stage contains anesthetic, technical (surgical), and pathological parameters. The anesthetic parameters include the ICG dose, route, and anaphylactic reactions, if any. The surgical parameters include the SOS components, blood loss, operative time, and intraoperative complications. The pathological parameters include the histopathological diagnosis, largest tumor size, margin status (R0≥1mm), and weight(20,21).

Phase 3 (postoperative period)

The postoperative period will focus on early and late complications. It will be graded according to the extended Clavien–Dindo classification of surgical complications, published by the Japan Clinical Oncology Group, which describes the original criteria of the Clavien–Dindo classification more specifically (22).The first follow-up visits will be conducted two weeks after hospital discharge and every three months after that. Follow-up assessment will be performed by adapting routine blood tests, including liver function tests, coagulation function tests, tumor markers, and abdominal CT and MRI.

Study Timeline

Data will be collected between February 2023 and December 2025, and statistical analysis will be completed after December 2026. Participants will be officially informed about the study during their preoperative visit to our clinic; therefore, they will have an extended period to choose to participate. Possible complications will be evaluated 12 months after the surgery. The outline of enrollment, interventions, and follow-up assessments are described in Table 2.

Data monitoring

The data will be monitored by frequently checking whether the study is being carried out safely by the proposed algorithm and whether the information is precisely collected. The following items will be reviewed every three months: informed consent (obtained and signed), participant retention, study implementation system, security, data, and the progression in the process.

Statistical analysis of outcome measures

Data will be analyzed using IBM SPSS Statistics for Windows version 28 (IBM Corp., Armonk, N.Y., USA). The general characteristics of the participants will be summarized using descriptive statistics. The chi-squared test will be used to analyze categorical data. ANCOVA and logistic regression tests will be used to test the hypothesis and compare the groups using the baseline values as covariates; the choice of the test will depend on the type of response variables. Statistical significance will be set at $p < 0.05$. Interim succinct will not be included in this project.

Safety analysis

The safety endpoint of this study is the incidence of adverse events. A chart will be prepared to determine the endpoints. A two-sided 95% confidence interval will be calculated to estimate the proportion of adverse events.

Patient and public involvement

There is no intention to select or specify any patient or citizen to participate in the planning of this study.

ETHICS AND DISSEMINATION

Is there any scientific and clinical value in conducting this study?

The ICG fluorescence imaging system plays a significant role in laparoscopic liver surgery because of the illustration of transection surfaces during parenchymal resection. We aim to evaluate the efficacy and safety of performing sub/mono-segmentectomy, using the two techniques of the ICG-staining imaging system, by assessing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help determine the staining technique that can achieve precise resection and fewer complications. Theoretically, this is expected to reflect the improvement in outcomes and patient safety positively. This study is the first to compare the accuracy of these two staining procedures. We believe that the results will point towards the method for performing precise laparoscopic liver segmentectomy and subsegmentectomy. This study is expected to establish a milestone for the indications of each staining method to achieve the best outcomes and broaden our scientific experience in laparoscopic liver surgery.

Ethical approval

The study has been approved by the Ageo Central General Hospital Clinical Research Ethical Committee (approval number: 1044) and will be carried out in accordance with the Helsinki Declaration (2013 revision) (23). If any adjustment must be made during the study process, information will be sent to the Ageo Central General Hospital Clinical Research Ethical Committee.

Participants' rights, safety, and disadvantages

All authors and contributors involved in the study are committed to maintaining each patient's privacy. No identifying factors will be divulged in the study. Very little information that is only relevant to the case will be included, but without risking the exposure of patients' identities. We will assign an identification code for each subject in the study to ease access to all data and documents.

Foreseeable disadvantages (burdens and risks)

To date, ICG administration is not known to cause many serious side effects (24).However, anaphylactic reactions may occur in a few patients. Our patients will be followed-up for adverse events and pre-examined for any health conditions that might precipitate or aggravate any resulting complications. We will inform the patients before the procedure about the possible side effects and management plans once they develop. They will also be informed about the need to postpone or cancel the procedure and surgery if any contraindications or complications arise.

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Author contributions

All authors played a significant role in preparing the study outlines and design. Dr. Ebaa Ababneh significantly contributed to the statistical process. All authors have crucially contributed to the review and final approval of the manuscript.

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Competing interest statement

None declared

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Figure legends

Figure 1: summary of practical doses and timing of injection.

ICGR15: indocyanine green retention test after 15 minutes, HCC: hepatocellular carcinoma, CRLM: colorectal liver metastasis.
* Passed on experience, florescence technology and patient conditions.

Figure 2: Project Algorithm

This scheme provides the algorithm of this project and has been designed with close consideration of the SPIRIT guidelines.

Figure 3: Indocyanine green negative staining for colorectal liver metastasis in segment 5 and 6.

(a) Dissection between the Laennec's capsule and Glissonean sheath and identification of right posterior and anterior Glissonean pedicles.

(b) Dissection continuing ahead liver parenchyma and clamping the Glissoneanpedicle5 and 6 with applying bulldog forceps.

(c) After the administration of indocyanine greeninto peripheral vein, the demarcation line is identified.

(d) The demarcation line is marked along the ICG fluorescent border.

(e) Parenchymal transection is completed along the watershed between ICG coded and non-coded areas.

Figure 4: Indocyanine green positive staining for colorectal liver metastasis in segment 7.

(a) Identification and puncture of portal venous branch 7 (P7) under the guidance of intraoperative ultrasound.

(b) Injection of the ultrasound contrast medium (SONAZOID, Daiichi-Sankyo, Tokyo, Japan) to the target portal vein.

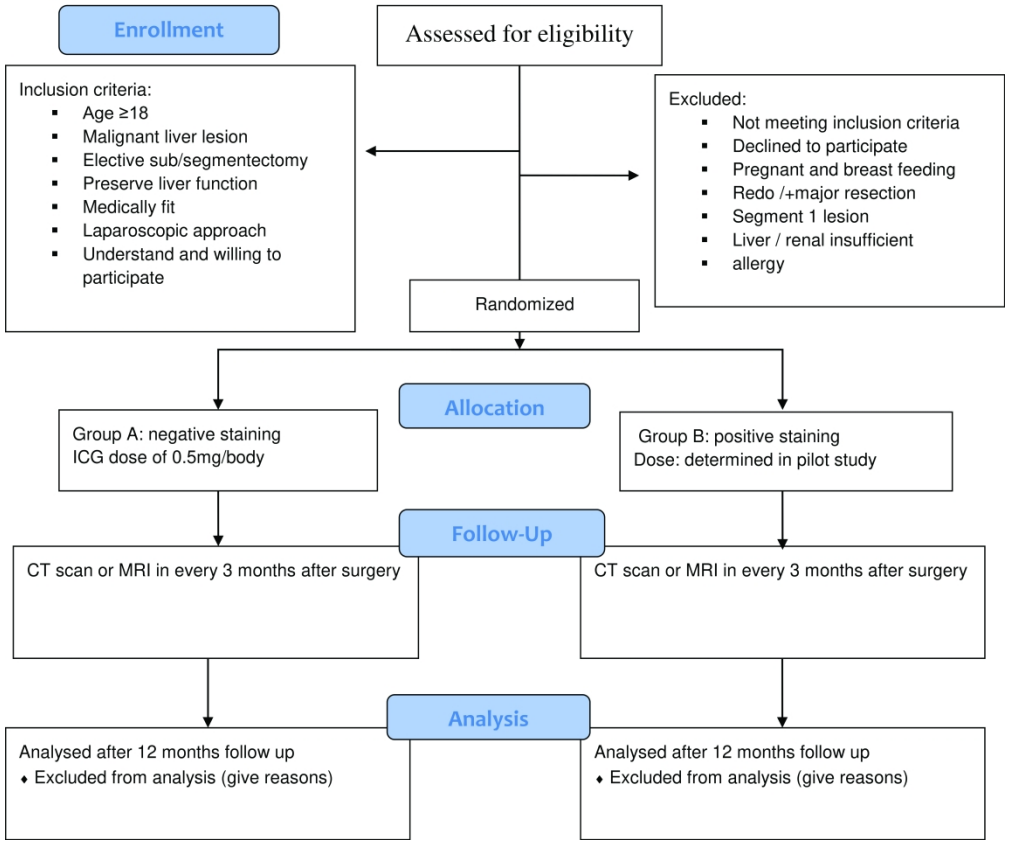
(c) Identification and transection of the demarcation line between segment 7 and the adjacent segments.

Figure(1) summary of practical doses and timing of injection

Purpose	ICG R15
Liver segmentation	Positive staining dose: from o.o25 to 12.5 mg / body .*Mainly 0.25 ml Negative staining dose: from 0.025 to 25 mg/body.* Mainly 2.5 ml
Tumor detection	HCC: 0.5 mg/ kg between 7 to 14 days before operation CRLM : 0.5 mg/ kg between 3-7 days before operation Or 2.5 mg / body 24 hours before operation

ICGR15: indocyanine green retention test after 15 minutes, HCC: hepatocellular carcinoma , CRLM : colorectal liver metastasis.

* Passed on experience, florescence technology and patient conditions.



The following scheme provides the algorithm of this project and has been designed with close consideration of the SPIRIT guideline guidelines.

552x487mm (300 x 300 DPI)

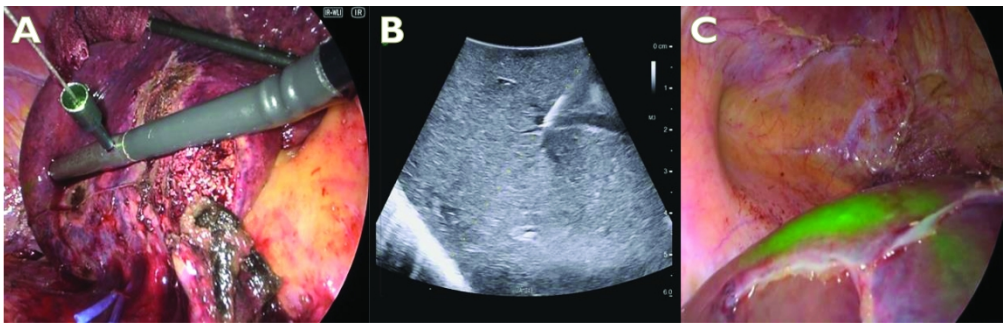


Figure (3) positive indocyanine staining for colorectal liver metastasis segment 7.
(a) Identification of glassonian pedicle using intraoperative ultrasound.
(b) Injected the indocyanine green to the pertaining pedicles.
(c) demarcation line of liver segment 7.

833x263mm (300 x 300 DPI)

Figure (4) negative indocyanine staining for colorectal liver metastasis segment 5 and 6.
(a) Dissection the porta hepatis and identification right posterior and anterior glassonian pedicles.
(b) Continue dissection intra-hepatically and identification glassonian 5 and 6 and applying bulldogs
(c) injection indocyanine green and mark the resection surface.

833x266mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-JeriĆ K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	21
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21
Roles and	#5b	Name and contact information for the trial sponsor	21

responsibilities: sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12,13, 15,16
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	12,13,14

Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	17,18
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	17,18
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13,14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16,17
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a	

emergency unblinding		participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	20
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse	19

		events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8,15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	17
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	3, 17
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14,16
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical	18

reproducible research		code	
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

'Comparing the accuracy of positive and negative indocyanine green staining in guiding laparoscopic anatomical liver resection: protocol for a randomized controlled trial

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Primary Subject Heading:	Surgery
Secondary Subject Heading:	Oncology
Keywords:	Hepatobiliary surgery < SURGERY, Hepatobiliary disease < GASTROENTEROLOGY, ONCOLOGY

'Comparing the accuracy of positive and negative indocyanine green staining in guiding laparoscopic anatomical liver resection: protocol for a randomized controlled trial'

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Word count: 2,995

Keywords: Positive staining, Negative staining, Indocyanine green, Laparoscopic anatomical liver resection, Glissonean pedicle.

ABSTRACT

Introduction

Knowledge of the clinical liver anatomy has evolved with advanced imaging modalities and laparoscopic surgery. Therefore, precise anatomical resection knowledge has become the standard treatment for primary and secondary liver cancer. Segmentectomy, a parenchymal-preserving approach, is regarded as an option for anatomical resections in patients with impaired liver. Indocyanine green (ICG) staining is a promising method for understanding the anatomical borders of the liver segments. There are two methods of ICG staining (positive and negative), and the superiority of either approach has not been determined to date.

Methods and analysis

This is a prospective randomized controlled superiority clinical trial performed in a single center tertiary hospital in Japan. A comparison between the accuracy of positive and negative ICG staining in guiding laparoscopic anatomical liver resection is planned in this study. Possible candidates are patients with liver malignant tumors in whom laparoscopic mono- or subsegmentectomy is planned. Fifty patients will be prospectively allocated into the following two groups: Group A, ICG-negative staining group, and Group B, ICG-positive staining group. The optimal dose of ICG for positive staining will be determined during the preparation phase. To assess the ability of the ICG fluorescence guidance in anatomical resection, the primary endpoint is the success rate of ICG staining, which consists of a subjective optical scoring based on three components: superficial demarcation in the liver surface, visualization of the parenchymal borders, and consistency with the

preoperative three-dimensional (3D) simulation. The secondary endpoints are the evaluation of short-term surgical outcomes and recurrence-free survival.

Ethics and Dissemination

The study was approved by Ageo Central General Hospital Clinical Research Ethical Committee (No: 1044) and it carried out following the Helsinki Declaration (2013 revision). Informed consent will be taken from the patients before participating. The findings will be disseminated through peer-reviewed publications, scientific meetings, and conferences.

TRIAL REGISTRATION NUMBER

This study has been registered in the UMIN Clinical Trials Registry (UMIN000049815).To see the study protocol and regulations, visit the registration website:

https://center6.umin.ac.jp/cgi-bin/ctr/ctr_view_reg.cgi?recptno=R000056739.

ARTICLE SUMMARY

Strengths and limitations of this study

• Performing a pilot study prior to the clinical trial helps to more accurately determine the appropriate dose of indocyanine green.

• As a limitation, the staining technique is operator-dependent; therefore, a definitive conclusion cannot be drawn solely from this single-center trial.

• The blinded randomized nature of the trial will reduce bias resulting from subjective assessments by the operators.

• The study is conducted at a single center; therefore, this might limit the generalizability of the results.

• Our study population will consist of patients with malignant liver tumors; hence, the results might not be applicable to other pathological conditions.

INTRODUCTION

Since the previous three international consensus meetings (Louisville, Iwate, and Southampton)(1-3),laparoscopic liver resection (LLR) as a treatment for the chronic liver disease has developed considerably worldwide. Understanding clinical liver anatomy has gained increasing attention with the advancement of three-dimensional (3D) simulation software. The emergence of indocyanine green (ICG) fluorescence and high-quality magnified view of laparoscopic imaging has also contributed to the knowledge of clinical liver anatomy to a large extent. Therefore, more precise liver resections, such as anatomical liver resections (ALR), have recently been more commonly performed based on liver inflow and outflow. Currently, ALR is accepted as a standard therapy for liver cancer because of its oncologic effectiveness. However, even if we do not consider the long-term efficiency of ALR, the watershed of the segments/sections is easy to transect because of the sparse vessels in the intersegmental/sectional planes. Besides, leaving fewer ischemic areas in the remnant liver is considered reasonable after ALR.

In 1985, Makuuchi reported a new concept of small ALR (i.e., segmentectomy in Brisbane terminology 2000), which applies well to the therapeutic principle in the Asia Pacific region, where most hepatic malignancies are hepatocellular carcinoma arising in the impaired liver (4). Thus, a small ALR was established based on the parenchyma-sparing principle in mal-distributed patient groups. Since the 1990s, when laparoscopic surgery has developed noticeably, anatomical knowledge of the internal and external liver has gradually increased owing to its magnified and unique caudal/dorsal view. Clinical questions regarding the landmarks for the segmental borders and approach for the tumor-bearing portal pedicles were discussed during the 32nd meeting of the Japanese Society of Hepato-

Biliary-Pancreatic Surgery (JSHBPS) held in Japan in 2021, and the new terminology for the small ALR was described by updating the Brisbane 2000 terminology (5-7)(Table 1).

Terminology	Definition
Anatomical liver resection	Complete removal of the liver parenchyma confined within the responsible portal territory.
Segmentectomy	The complete removal of a territory (territories) of the third-order portal venous branches of a Couinaud segment.
Sub-segmentectomy	The removal of the liver parenchyma within the portal territory (territories) of less than a Couinaud's segment. These are also defined as cone units, and their areas can be intra-operatively assessed by using ischemic demarcation, ICG (negative/ positive) staining, or both.
A sub-segment	An anatomical portion of a Couinaud segment, which is defined as a cone unit or cone units, based on sub-segmental inflow. This concept particularly adapts to Sg 8 (ventral and dorsal), Sg 4 (basal and apical), and Sg 1 (Spiegel, caudate process, and paracaval).
Segment 4	Redefined as consisting of two sub-segments: Sg 4a (apical) and 4b (basal). Sg 4a is defined as the cranial anatomical portion of Sg 4 according to the third-order portal territories, and Sg 4b is the caudal anatomical portion of Sg 4.
Segment 9	Sg 9 definition of the Brisbane 2000 terminology is abandoned, and caudate lobe is redefined based on portal ramifications instead of spatial recognition.
Segment 1	Classified into three parts as follows, i) the Spiegel lobe, ii) the paracaval portion, and iii) the caudate process.

Table 1: The Tokyo 2020 terminology of liver anatomy and resections: Updates of the Brisbane 2000 system

ICG fluorescence imaging is considered helpful for the real-time identification of segmental boundaries during liver parenchymal transection in LLR, achieving the concept of anatomical parenchyma-sparing resection(8).To assess the real-time hemodynamics, ICG fluorescence has not yet been matched in the field of intraoperative imaging modality

owing to its unique excretion(through bile juice) characteristic and deep penetration of approximately 1cm. However, the optimal usage (i.e., the dose and timing for multiple uses) has not yet been clarified according to its various uses in each report. Two methods of ICG staining have been reported based on its administration routes: positive and negative staining (9). Although Wakabayashi T et al. addressed the optimal dose and timing of ICG application for positive and negative staining (10), the superiority of either staining method has not been determined to date (Figure 1).

It is of utmost importance to accurately dissect the anatomical boundaries between the tumor-bearing liver segment and adjacent segments in the case of ALR with ICG fluorescence guidance. Funamizu N et al. reported a positive correlation between the estimated and actual liver volumes after the ICG negative staining approach (11). ICG-negative staining can precisely delineate the anatomical borders during resection, maintaining both radical resection and sufficient healthy parenchyma. Additionally, Chiow AKH et al. reported preferable clarity of ICG fluorescence guidance in the two approaches of staining in robotic ALR (12). However, the results depended only on subjective assessment, and the outcomes were never statistically compared between the two staining approaches.

This study aims to compare the accuracy of liver segmentation using positive and negative staining during LLR to achieve precise ALR, such as segmentectomy, based on preoperative planning. Furthermore, future research can be conducted on the long-term outcomes of precise ALR.

METHODS AND ANALYSIS

Study design

This prospective study is a randomized controlled superiority clinical trial on patients with malignant liver lesions who will undergo segmentectomy using ICG fluorescence imaging navigation. This study will be conducted at the Ageo Central General Hospital (Saitama, Japan), a referral center for LLR in Japan.

Pilot trial

A small-scale pilot study will be performed on six patients (12% of the sample size of the main study) to determine the appropriate dose of ICG-positive staining.

Hypothesis

We hypothesize that there is a statistical difference between the success rate of staining and short-term outcomes of the positive and negative ICG staining approaches in performing precise LLR. Theoretically, ICG-negative staining is a more solid approach for liver segmentation than ICG-positive staining. To perform ICG-negative staining, the Glissonean approach advocated by Prof. Takasaki (13) is reasonable because the inflow of tumor-bearing areas is completely blocked before liver transection. This concept is based on a non-touch isolation technique for malignant tumors. However, to our knowledge, no

available literature has specifically examined the potential benefit of negative staining compared to positive staining in laparoscopic segmentectomy.

Target population

Patients with primary or metastatic liver tumors planned for mono- and subsegmentectomy from February2023 to December 2025will be candidates for this clinical trial. The following inclusion and exclusion criteria are created to unify the selection of patients in this study. The inclusion criteria are as follows: male or female patients with solitary primary or metastatic liver tumors, aged ≥18 years, scheduled for elective LLR, preserved liver function, ability to understand the nature of the study, and willingness to join and provide voluntary written consent. The liver functional reserve will be evaluated by serum biochemical tests (albumin level, total bilirubin level, and prothrombin time) and ICG retention rate at 15 min (ICG-15R). The severity of the liver disease will be assessed based on Child-Pugh stages and liver damage classification defined by the Liver Cancer Study Group of Japan (14). Preserved liver function is defined as an ICG-15R less than 30% and a Child-Pugh classification A or B. The exclusion criteria are as follows: repeat liver resection, multiple tumors, concomitant resection of other organs, severe liver or renal insufficiency, ICG hypersensitivity, pregnancy or breastfeeding, and inability to understand the nature of the study or refuse it. The schematic representation of the algorithm for this project, which has been designed with close consideration of the SPIRIT guidelines (15, 16), is shown in supplemental file 1

.

Sample size calculation

The sample size was calculated using the EpiCalc 2000 software (Gilman & Myatt, 1998)(17).

$$n_1 = (z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \frac{P_1(1-P_1) + P2(1-P_2)}{(P_1 - P_2)^2}$$

Where α = 0.05, (1-β) = 0.95.

Although no previous data are available in the literature to compare the negative and positive ICG staining, we decided to use the data reported by Chiow AKH et al. (12). Thus, P1 (percentage of cases that had clear demarcation with positive ICG) = 50% (6 out of 12) and P2 (percentage of cases that had clear demarcation with negative ICG) = 92.5% (37 out of 40) are set in the power calculation. Consequently, the minimum required sample size is 25 in each group, with a total of 50 patients. Investigators may enroll more participants to avoid a significant decrease in the study power caused by attrition bias.

Randomization and blinding

A randomized controlled superiority trial will be performed at the Ageo Central General Hospital. Fifty patients will be randomly assigned (1:1) to receive either positive or negative ICG staining. The minimization method will be used for the randomization dividing the participants into two groups. In addition, ICG-15R will be used as a parameter to equalize the background liver function to minimize the intergroup bias. Tumor etiology will be also equalized between the groups. Allocation concealment will be performed until the patients are enrolled and assigned to the operation.

Intervention and surgical procedures

The preoperative routine test and planning for the patient have been described elsewhere (18).ICG-R15 tests will be conducted two weeks before surgery to assess patients' hepatic reserve using an ICG dose of 0.5 mg/kg. Three-dimensional (3D) vascular simulation models are constructed by a specific workstation (ZIOSTATION 2, Ziosoft Inc., Tokyo, Japan), depending on the multidetector slice computed tomography (CT). Surgical planning is fashioned in line with the "cone unit" theory instead of Couinaud's stratification. Furthermore, preoperative volumetry will measure the total liver volume (TLV) and estimated liver resection volume (ELRV). To examine the accuracy of LLR, the actual liver volume (ALRV) will be calculated by dividing the actual liver resection mass (g) by standardized liver density (1.05g/mL)(11,19). Finally, the discrepancy between the ELRV and ALRV will be calculated as |ELRV-ALRV|/TLV×100 (%). We will use the 1688 Advanced Imaging Modalities Platform (Stryker Co., MI, USA) as the laparoscopic near-infrared camera throughout the designated study period. The extra-hepatic (extra-fascial) Glissonean approach will be used in all patients involved in this study to encircle the target Glissonean pedicle supplying the tumor following the preoperative simulation. Liver parenchyma division will be performed using a Cavitron Ultrasonic Surgical Aspirator (CUSA, ValleyLab, CO, USA). During the extra-hepatic Glissonean approach, the 3D simulation model will be repeatedly referred to on a screen to ensure that the targeted pedicle tree is addressed.

Operative procedure for group A

During the early phase of surgery, the extra-hepatic (extra-fascial) Glissonean approach will be performed to encircle the target Glissonean pedicle, feeding the tumorous area, corresponding precisely to the preoperative simulation (Figure 2). To avoid postoperative bile leakage, it is essential to transect towards the liver parenchyma instead of the Glissonean sheath using the Cavitron Ultrasonic Surgical Aspirator (CUSA, ValleyLab, CO, USA). During the extra-hepatic Glissonean approach, the 3D simulation model will be repeatedly referred to on a primary screen to correct the pedicle tree. When identified, the target pedicle will be clamped using an endoscopic bulldog to make the diseased area completely ischemic. Sequentially, inflow blockage will be confirmed using laparoscopic intraoperative ultrasonography with Doppler mode. Since the staining is irreversible after ICG injection, 0.15mL/kg ultrasound contrast medium (SONAZOID, Daiichi-Sankyo, Tokyo, Japan) will be systematically injected before ICG injection. If the target area is adequately cyanosed, 0.5 mg/body ICG will be intravenously injected in the ICG-negative staining method. The demarcation line appears as a border between the color-coded and non-color-coded areas, marked on the liver surface. In the deeper parenchyma, the intersegmental plane can also be coded by the ICG fluorescence emission, which corresponds to the course of transection. The 1688 Advanced Imaging Modalities Platform will be used for the near-infrared camera system in all cases. This system has an overlay mode that enables the user to superimpose an ICG fluorescence image to a white-light image. This mode facilitates precise parenchymal transection according to the border between the color-coded and non-color-coded areas. Liver transection will be performed using the CUSA and other energy devices.

Operative procedure for group B

On the contrary, in ICG positive-staining, ICG will be directly injected into the portal branches responsible for resected territories or surrounding territories to visualize the clear demarcation planes (Figure 3). The portal branches of the tumor-bearing liver segments will be targeted and punctured under ultrasound guidance with an 18- or 21- gauge spinal or percutaneous transhepatic cholangio-drainage needle introduced through the abdominal wall. The needle hole will assist the direction of the needle in a dedicated laparoscopic ultrasound probe (provided by BK Medical, Herlev, Denmark). Subsequently, a small volume of ICG (1 mL of 0.025 mg/mL) will be slowly injected into the portal branch to avoid the risk of ICG retrograde flow into the neighboring segments with undesired staining without clamping the hepatic artery. Liver transection will be performed using CUSA and other energy devices.

Primary endpoint

To determine the ability of the ICG fluorescence guidance in anatomical resection, the primary endpoint will be the success rate of ICG staining, which consists of a subjective optical scoring (SOS) based on three components: superficial demarcation in the liver surface, visualization of the parenchymal borders, and consistency with the preoperative 3D simulation. Each criterion is scored on a scale of 0-2 (max 6 points). We will compare the scores between two groups (Group A and Group B) using a t-test to determine if there are significant differences in the effectiveness of the intervention. It is also subjective to estimate the resection margin and shape of the specimen in comparison with the pre-and postoperative 3D simulations of the liver.

Secondary endpoints

The secondary endpoints will be the short-term surgical outcomes, such as the operative time, blood loss, and complication rates. Recurrence-free survival at 1-year will also be addressed. The patients will be followed up at the outpatient clinic after surgery every three months with regular laboratory and radiological assessments using CT and magnetic resonance imaging (MRI).

Data collection

The data will be collected in four phases: pilot, preoperative, operative, and postoperative (Table 2). In each phase, specific information will be collected for assessment. All the phases will be digitally recorded and reviewed by the authors.

Assessment		
Preoperative (Within 14 days)	**operative**	**postoperative**
Participation & eligibility	primary end point (subjective three components)	Tri-phasic liver CT scan with volumetry at POD 1
patient factors*	operative time	hospital stay
informed consent	blood loss	complications
blood investigations	pathology	early period until POD 90
LFT, Albumin	specimen weight	late period until POM 12
PT, PT-INR, APTT	surgical margin	CT scan / MRI in every 3 months
ICG-15R	tumor size	blood investigations
Child Pugh score	final diagnosis	tumor markers
Triphasic liver CT scan with volumetry and MRI		
Tumor markers**		

Table 2: Schedule of participation, investigation and assessment, preoperative findings and 12-month follow-up

LFT: Liver function test, PT: prothrombin time, PTT: activated partial thromboplasin time,ICG-15R: indocyanine green retention test at 15 minutes.

*including age, sex, body mass index (BMI), American Society of Anesthesiologist (ASA) physical status, underlying liver co-morbidities, chronic hepatitis status, and preoperative chemotherapy.

**includes: CEA, CA19-9, AFP, and PIVKA-II.

Phase 0 (pilot study)

To determine the best dose of ICG to be administered for positive staining, a preliminary study of six patients will be performed as the first step. The initial trial dose will be 0.025 mg/ml, and the first patient will be administered 1 mL of this dose. Each successive patient will receive one extra milliliter of ICG at the same dose until sufficient positive staining is achieved. Since positive staining can potentially lead to over-staining due to ICG reperfusion, we have imposed a maximum limit of 3ml for the ICG injection to minimize the impact of over-staining.

Phase 1 (preoperative period)

The databases will be extracted from patient charts, which include the following baseline characteristics: age, sex, body mass index (BMI), American Society of Anesthesiologist (ASA) physical status, underlying liver comorbidities, chronic hepatitis status, preoperative chemotherapy, tumor size and location, volumetric and biochemical laboratory investigations including liver function test, ICG-15R, and tumor markers.

Phase 2 (operative period)

This stage contains anesthetic, technical (surgical), and pathological parameters. The anesthetic parameters include the ICG dose, route, and anaphylactic reactions, if any. The ICG dose will be determined in accordance with the findings from Phase 0. The surgical parameters include the SOS components, blood loss, operative time, and intraoperative complications .The pathological parameters include the histopathological diagnosis, largest tumor size, margin status (R0≥1mm), and weight(20,21).

Phase 3 (postoperative period)

The postoperative period will focus on early and late complications. It will be graded according to the extended Clavien–Dindo classification of surgical complications, published by the Japan Clinical Oncology Group, which describes the original criteria of the Clavien–Dindo classification more specifically (22).The first follow-up visits will be conducted two weeks after hospital discharge and every three months after that. Follow-up assessment will be performed by adapting routine blood tests, including liver function tests, coagulation function tests, tumor markers, and abdominal CT and MRI.

Study Timeline

Data will be collected between February 2023 and December 2025, and statistical analysis will be completed after December 2026. Participants will be officially informed about the study during their preoperative visit to our clinic; therefore, they will have an extended period to choose to participate. Possible complications will be evaluated 12 months after

the surgery. The outline of enrollment, interventions, and follow-up assessments are described in Table 2.

Data monitoring

The data will be monitored by frequently checking whether the study is being carried out safely by the proposed algorithm and whether the information is precisely collected. The following items will be reviewed every three months: informed consent (obtained and signed), participant retention, study implementation system, security, data, and the progression in the process.

Statistical analysis of outcome measures

Data will be analyzed using IBM SPSS Statistics for Windows version 29 (IBM Corp., Armonk, N.Y., USA). The general characteristics of the participants will be summarized using descriptive statistics. The chi-squared test will be used to analyze categorical data. T test will be used to analyze continuous data. ANOVA and logistic regression tests will be used to test the hypothesis and compare the groups using the baseline values as covariates; the choice of the test will depend on the type of response variables. To compare recurrence-free survival, Kaplan-Meier curves will be plotted, and a log-rank test will be performed. Statistical significance will be set at $p < 0.05$. Interim succinct will not be included in this project.

Safety analysis

The safety endpoint of this study is the incidence of adverse events. A chart will be prepared to determine the endpoints. A two-sided 95% confidence interval will be calculated to estimate the proportion of adverse events.

Patient and public involvement

There is no intention to select or specify any patient or citizen to participate in the planning of this study.

ETHICS AND DISSEMINATION

Is there any scientific and clinical value in conducting this study?

The ICG fluorescence imaging system plays a significant role in laparoscopic liver surgery because of the illustration of transection surfaces during parenchymal resection. We aim to evaluate the efficacy and safety of performing sub/mono-segmentectomy, using the two techniques of the ICG-staining imaging system, by assessing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help determine the staining technique that can achieve precise resection and fewer complications. Theoretically, this is expected to reflect the improvement in outcomes and patient safety positively. This study is the first to compare the accuracy of these two staining procedures. We believe that the results will point towards the method for performing precise laparoscopic liver segmentectomy and subsegmentectomy. The present

study is expected to establish a milestone for the indications of each staining method to achieve the best outcomes and broaden our scientific experience in laparoscopic liver surgery. To enhance objectivity influenced by staining techniques, future trials may be necessary to determine the appropriate dosage for each staining, taking into consideration the variations among near-infrared camera settings.

Ethical approval

The study has been approved by the Ageo Central General Hospital Clinical Research Ethical Committee (approval number: 1044) and will be carried out in accordance with the Helsinki Declaration (2013 revision) (23). If any adjustment must be made during the study process, information will be sent to the Ageo Central General Hospital Clinical Research Ethical Committee. Informed consent is to be obtained from all participating patients. This ensures that all participants involved in the study will receive comprehensive information about the study's objectives, procedures, potential risks, and benefits before their participation. Ethical considerations and strict adherence to informed consent protocols are of paramount importance to safeguard the rights and well-being of the participants throughout the research process. The requirements for participation includes age 18 years or older, preserved liver function, and willingness to be included in the study (An example of the participant consent form can be found in the supplemental file 2).

Participants' rights, safety, and disadvantages

All authors and contributors involved in the study are committed to maintaining each patient's privacy. No identifying factors will be divulged in the study. Very little information that is only relevant to the case will be included, but without risking the exposure of patients' identities. We will assign an identification code for each subject in the study to ease access to all data and documents.

Foreseeable disadvantages (burdens and risks)

To date, ICG administration is not known to cause many serious side effects (24).However, anaphylactic reactions may occur in a few patients. Our patients will be followed-up for adverse events and pre-examined for any health conditions that might precipitate or aggravate any resulting complications. We will inform the patients before the procedure about the possible side effects and management plans once they develop. They will also be informed about the need to postpone or cancel the procedure and surgery if any contraindications or complications arise.

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Author contributions

Malek Alomari has contributed to the conception and design of the work, acquisition of data, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Taiga Wakabayashi has contributed to the conception and design of the work, analysis and interpretation of data, revising the work critically for important intellectual function, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Marco Colella has contributed to the conception and design of the work, analysis and interpretation of data, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Kohei Mishima has contributed to the conception and design of the work, acquisition of data, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Yoshiki Fujiyama has contributed to the conception and design of the work, acquisition of data, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of

the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ebaa Ababneh has contributed to the conception and design of the work, significantly contributed to the analysis and interpretation of data for the work, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Go Wakabayashi has contributed to the conception and design of the work, acquisition of data, drafting the work and revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Competing interest statement

None declared

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Figure legends

Figure 1: summary of practical doses and timing of injection.

ICGR15: indocyanine green retention test after 15 minutes, HCC: hepatocellular carcinoma, CRLM: colorectal liver metastasis.
* Passed on experience, florescence technology and patient conditions.

Figure 2: Indocyanine green negative staining for colorectal liver metastasis in segment 5 and 6.

(a) Dissection between the Laennec's capsule and Glissonean sheath and identification of right posterior and anterior Glissonean pedicles.

(b) Dissection continuing ahead liver parenchyma and clamping the Glissoneanpedicle5 and 6 with applying bulldog forceps.

(c) After the administration of indocyanine green into peripheral vein, the demarcation line is identified.

(d) The demarcation line is marked along the ICG fluorescent border.

(e) Parenchymal transection is completed along the watershed between ICG coded and non-coded areas.

Figure 3: Indocyanine green positive staining for colorectal liver metastasis in segment 7.

(a) Identification and puncture of portal venous branch 7 (P7) under the guidance of intraoperative ultrasound.

(b) Injection of the ultrasound contrast medium (SONAZOID, Daiichi-Sankyo, Tokyo, Japan) to the target portal vein.

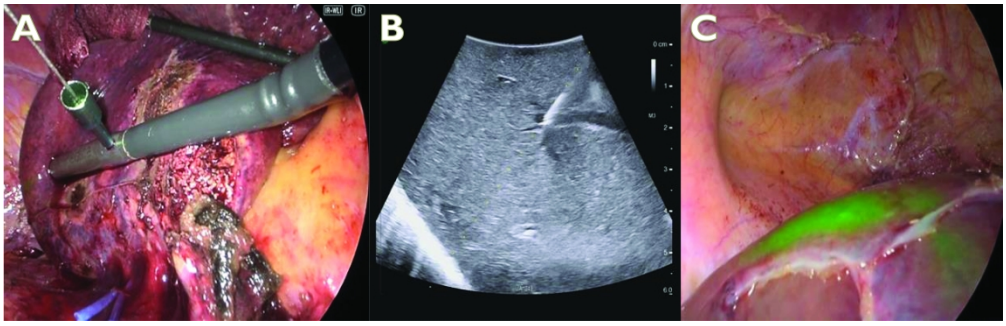
(c) Identification and transection of the demarcation line between segment 7 and the adjacent segments.

Figure(1) summary of practical doses and timing of injection

Purpose	ICG R15
Liver segmentation	Positive staining dose: from o.o25 to 12.5 mg / body .*Mainly 0.25 ml Negative staining dose: from 0.025 to 25 mg/body.* Mainly 2.5 ml
Tumor detection	HCC: 0.5 mg/ kg between 7 to 14 days before operation CRLM : 0.5 mg/ kg between 3-7 days before operation Or 2.5 mg / body 24 hours before operation

ICGR15: indocyanine green retention test after 15 minutes, HCC: hepatocellular carcinoma , CRLM : colorectal liver metastasis.

* Passed on experience, florescence technology and patient conditions.

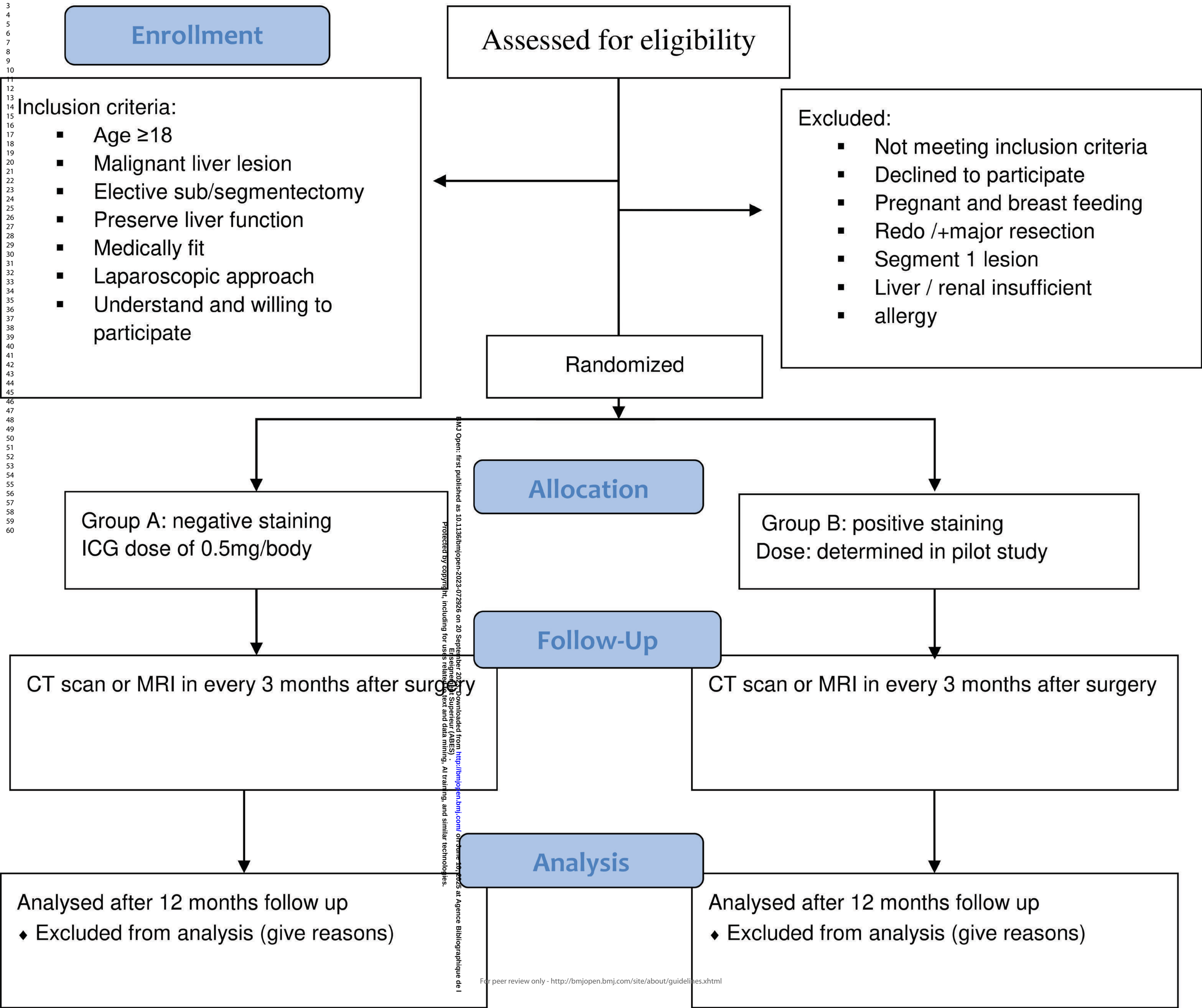


Indocyanine green negative staining for colorectal liver metastasis in segment 5 and 6.
(a) Dissection between the Laennec's capsule and Glissonean sheath and identification of right posterior and anterior Glissonean pedicles.
(b) Dissection continuing ahead liver parenchyma and clamping the Glissoneanpedicle5 and 6 with applying bulldog forceps.
(c) After the administration of indocyanine green into peripheral vein, the demarcation line is identified.
(d) The demarcation line is marked along the ICG fluorescent border.
(e) Parenchymal transection is completed along the watershed between ICG coded and non-coded areas.

833x263mm (300 x 300 DPI)

Indocyanine green positive staining for colorectal liver metastasis in segment 7.
(a) Identification and puncture of portal venous branch 7 (P7) under the guidance of intraoperative ultrasound.
(b) Injection of the ultrasound contrast medium (SONAZOID, Daiichi-Sankyo, Tokyo, Japan) to the target portal vein.
(c) Identification and transection of the demarcation line between segment 7 and the adjacent segments.

833x266mm (300 x 300 DPI)



August 12, 2023

Request for Participation and Cooperation in the Examination

[Trial Name: Prospective Randomized Comparative Trial on Indocyanine Green-Positive and Negative Staining in Laparoscopic liver subsegmentectomy and segmentectomy]

1. Purpose of the Trial

   The purpose of this trial is to investigate which method, either the positive staining method using a reagent called indocyanine green injected into the portal vein along the blood flow during anatomical liver resection or the negative staining method injecting the reagent into the systemic circulation through the arm or other blood vessels, provides a higher accuracy in determining the extent of the liver that should be resected.

2. Trial Method and Duration Subjects

   Patients who are scheduled to undergo laparoscopic subsegmentectomy or segmentectomy for primary or metastatic liver cancer at research institutions from the approval of the head of the institution until December 2024, and meet the following conditions:

   I. Individuals aged 18 years or older at the time of consent acquisition
   II. Individuals with preserved liver function
   III. Individuals who consent to participate

   Method: If the subjects meet the inclusion criteria, they will undergo surgery to

determine the extent of the liver to be resected using either the indocyanine green-positive staining method or the negative staining method during liver resection. Patients cannot choose between the positive and negative staining methods, and at present, it is not known which method is superior. However, there is generally no disadvantage to patients as a result of this choice.

We plan to recruit a total of 50 patients for this study.

3. Expected Effects and Risks

This study is a prospective registration study conducted within routine insurance medical care. Therefore, we consider the risk of participating in this study to be low. However, in the unlikely event of any health damage, the physicians will provide appropriate examination and treatment. Since this study uses already commercially available drugs within their indications, the treatment of health damage caused by these drugs will be covered by the patients' health insurance, similar to regular medical care. In the event that health damage eligible for compensation occurs, the patient will be able to claim compensation through the Pharmaceutical and Medical Device Act's compensation system for health damage caused by pharmaceutical products.

4. No Disadvantages for Not Agreeing to Participate in the Trial

It is entirely your decision whether or not to cooperate in this trial. Even if you choose not to participate, there will be no disadvantages whatsoever. You will receive the best available treatment using existing drugs and therapies, and there will be no disadvantages in your future treatments.

5. Ability to Withdraw Consent to Participate in the Trial at Any Time

After consenting to participate in this trial, or even during the course of participation, you have the right to withdraw your participation at any time.

6. Costs Related to the Trial

All medical procedures will be conducted within the scope of insurance coverage, so there will be no increase in personal financial burden as a result of participating in this trial.

7. Other Necessary Matters Regarding Protection of Human Rights

Your participation in this research study is voluntary, and your feelings and preferences will be respected. There is no need to worry about the disclosure of your name or privacy to external parties. If you have any questions or concerns regarding the study or medication, please feel free to raise them at any time. Furthermore, the confidentiality of your personal information, such as your name and medical condition, will be strictly protected.

8. Publication of Trial Results

The trial results may be presented and published in academic conferences, papers, etc., for the purpose of benefiting future treatments. However, we assure you once again that the confidentiality of your personal information, including your name, will be strictly maintained.

[Contact Information at Our Hospital]

Department of Surgery, Ageo Central General General Hospital,

Taiga Wakabayashi

TEL: 048-773-1111 (Main Line)

Physician or other staff who provided the explanation:

Consent Form

I have received an explanation regarding the trial named "Prospective Randomized Comparative Trial on Indocyanine Green-Positive and Negative Staining Methods in Laparoscopic Subsegmental Liver Resection" using the attached explanatory document, and I fully understand the methods, risks, handling of trial results, etc. Therefore, of my own free will, I consent to participate in the trial.

Please mark a check (✓) in the box to indicate your understanding for the following items (you may check orally):

☐ Purpose of the trial

☐ Trial method and duration

☐ Expected effects and risks

☐ No disadvantages for not agreeing to participate in the trial

☐ Ability to withdraw consent to participate at any time

☐ Costs related to the trial

☐ Other necessary matters regarding protection of human rights

☐ Publication of trial results

Date:

Signature:

Date of Explanation:

Signature of Explaining Physician:

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-JeriĆ K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	21
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21
Roles and	#5b	Name and contact information for the trial sponsor	21

responsibilities: sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12,13, 15,16
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	12,13,14

Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	17,18
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	17,18
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13,14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16,17
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
Blinding (masking):	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a	

emergency unblinding		participant's allocated intervention during the trial	
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	20
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	17
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	17
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse	19

		events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	8,15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	17
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	3, 17
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14,16
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy:	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical	18

reproducible research		code	
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

Correction: *Comparing the accuracy of positive and negative indocyanine green staining in guiding laparoscopic anatomical liver resection: protocol for a randomised controlled trial*

Alomari MAM, Wakabayashi T, Colella M, et al. Comparing the accuracy of positive and negative indocyanine green staining in guiding laparoscopic anatomical liver resection: protocol for a randomised controlled trial. *BMJ Open* 2023;**13**:e072926. doi:10.1136/bmjopen-2023-072926

This article was previously published with some errors.

Author name 'Kohei Mishima' has been spelled correctly.

The unit in figure 1 has been corrected to mg.

Purpose	ICG R15
Liver segmentation	Positive staining dose: from 0.025 to 12.5 mmǵbody .*Mainly 0.25 mg Negative staining dose: from 0.025 to 25 mg/body.* Mainly 2.5 mg
Tumor detection	HCC: 0.5 mg/ kg between 7 to 14 days before operation CRLM : 0.5 mg/ kg between 3-7 days before operation Or 2.5 mg / body 24 hours before operation

Figure 1 Summary of practical doses and timing of injection. *Passed on experience, florescence technology and patient conditions. CRLM, colorectal liver metastasis; HCC, hepatocellular carcinoma; ICGR15, indocyanine green retention test after 15 min.

Figure 2 and figure 3 have been swapped. The images in figure 2 show positive staining, while the images in figure 3 demonstrate negative staining.

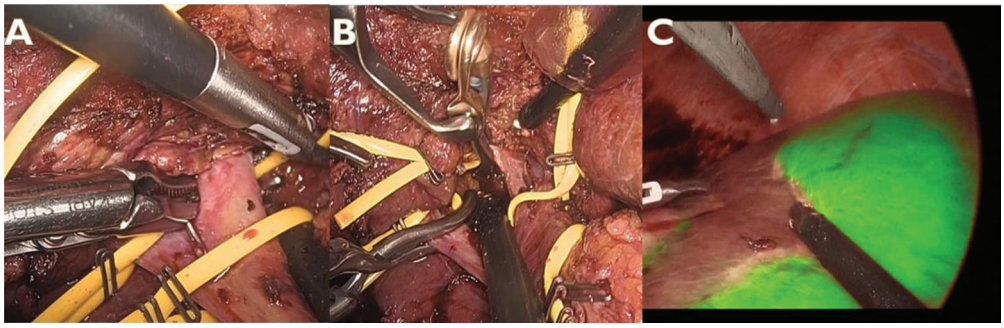


Figure 2 Indocyanine green (ICG) negative staining for colorectal liver metastasis in segment 5 and 6. (A) dissection between the Laennec'S capsule and Glissonean sheath and identification of right posterior and anterior Glissonean pedicles. (B) dissection continuing ahead liver parenchyma and clamping the Glissonean pedicle 5 and 6 with applying bulldog forceps. (C) after the administration of indocyanine green into peripheral vein, the demarcation line is identified.

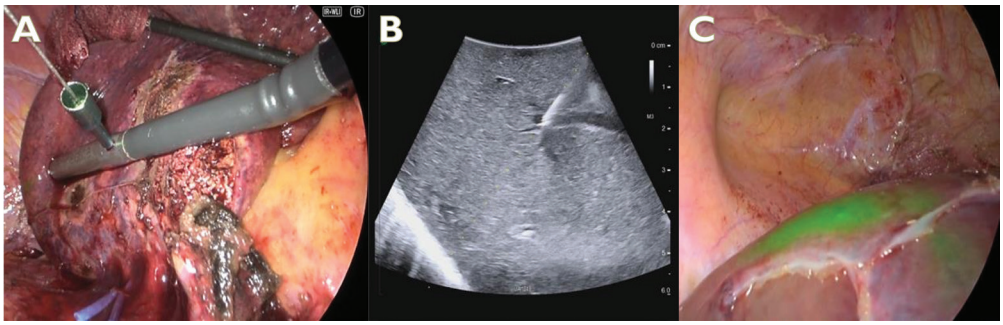


Figure 3 Indocyanine green positive staining for colorectal liver metastasis in segment 7. (A) identification and puncture of portal venous branch 7 (P7) under the guidance of intraoperative ultrasound. (B) ultrasound findings after puncturing the p7. (C) identification of the demarcation line between segment 7 and the adjacent segments after ICG injection into the p7.

BMJ Open 2023;**13**:e072926corr1. doi:10.1136/bmjopen-2023-072926corr1