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AVOID; a Phase III, Randomised Controlled Trial Using Indocyanine Green for the Prevention of Anastomotic Leakage in Colorectal Surgery

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Abstract

Introduction: Anastomotic leakage (AL) is one of the major complications after colorectal surgery. Compromised tissue perfusion at the anastomosis site increases the risk of AL. Several cohort studies have shown that indocyanine green (ICG) combined with fluorescent near-infrared imaging is a feasible and reproducible technique for real-time intraoperative imaging of tissue perfusion, leading to reduced leakage rates after colorectal resection. Unfortunately, these studies were not randomised. Therefore, we propose a randomised controlled trial to assess the value of ICG-guided surgery in reducing AL after colorectal surgery.

Methods and analysis: A multicentre, randomised controlled clinical trial will be conducted to assess the benefit of ICG-guided surgery in preventing AL. A total of 978 patients scheduled for colorectal surgery will be included. Patients will be randomised between the Fluorescence Guided Bowel Anastomosis (FGBA) group and the Conventional Bowel Anastomosis (CBA) group. The primary endpoint is clinically relevant AL (defined as requiring active therapeutic intervention or re-operation) within 90 days after surgery. Among the secondary endpoints are 30-day clinically relevant AL, all-cause postoperative complications, all-cause and AL related mortality, surgical and non-surgical reinterventions, total surgical time, length of hospital stay, and all-cause and AL related readmittance.

Ethics and dissemination: This protocol has been approved by the Medical Ethical Committee Leiden-Den Haag-Delft (METC-LDD) and is registered at ClinicalTrials. The results of this study will be reported through peer-reviewed publications and conference presentations.

Trial registration numbers: NCT04712032

Keywords: bowel perfusion, near infrared fluorescence, indocyanine green, colorectal surgery, colorectal cancer, inflammatory bowel disease

Article Summary

Strengths and limitations of this study

1. This study is a multicentre randomised controlled trial
2. AL is a major complication with huge impact on patient's life
3. A clinically relevant endpoint will be used as the primary endpoint
4. Quantification of fluorescence-guided bowel perfusion with indocyanine green would be a preferable addition, however its clinical correlation is unclear at this point

Introduction

Anastomotic leakage (AL) is a major complication after colorectal surgery, accounting for considerable morbidity and mortality.[1-6] The incidence of AL in colorectal surgery ranges from 2.4 to 11% in colon cases and up to 23.3% in rectal cancer surgery.[4-15] The occurrence of AL often has a multifactorial cause, including risk factors such as tumour location, level of anastomosis, male gender, high ASA score, comorbidities, smoking, obesity and (neoadjuvant) radiotherapy.[3 4 6 11 13 14 16]

Most risk factors for AL can no longer be changed at the time of surgery. Therefore, it is important to focus on the few factors that can be influenced, such as compromised tissue perfusion at the anastomosis site. It has been reported that this factor significantly increases the risk of AL.[17-19] Perfusion is commonly assessed by palpating the mesenteric arterial pulsations, inspection of the bowel colour, and bleeding at the anastomosis sides. Other intraoperative tests to prove the integrity of the anastomosis are the air leak test and inspection of the resection doughnuts.[20] Though useful, these clinical assessments have proven to have a low predictive value for AL which emphasises the urge for a better diagnostic test.[21]

A promising diagnostic tool is intraoperative near-infrared (NIR) fluorescence imaging. This technique combines a fluorescent contrast agent, e.g. indocyanine green (ICG), and a dedicated NIR imaging system.[22] The intravenous injection of ICG has proven to be a feasible and reproducible application for real-time perfusion assessment.[23-25] ICG was introduced by Fox et al. in 1957 and is currently used for a variety of diagnostic indications.[26] Diluted and intravenously injected ICG, with a peak emission at 820 nm, is

invisible for the naked eye and will therefore not interfere with the surgical field.[27] Moreover, it is cleared quickly by the liver and has low toxicity.[28]

Several cohort studies have investigated the benefit of NIR fluorescence imaging with ICG for intraoperative assessment of bowel perfusion. Some of these studies have shown that this technique enables clear visualisation of bowel perfusion within minutes after intravenous injection of ICG, resulting in reduced leakage rates and hospital stay.[29-32] Moreover, several systematic reviews support this promising results concerning the prevention of AL. On the other hand, Kin et al. have shown no benefit by using ICG in preventing AL.[33] Major drawbacks of these studies are that they were not randomised and did not use clinically relevant AL as the primary endpoint. Therefore, we propose AVOID: '***A****nastomotic leakage and* ***V****alue* ***O****f* ***I****ndocyanine green in* ***D****ecreasing leakage rates*', a randomised controlled trial to investigate the benefit of intraoperative imaging with ICG for the reduction of AL rate in colorectal surgery.

METHODS AND ANALYSIS

Primary aim

The main objective of this study is to assess if ICG-guided perfusion assessment will result in a reduction of the AL rate within 90 days after surgery. ICG-guided perfusion assessment will be an adjunct to conventional laparoscopic imaging versus conventional laparoscopic imaging alone.

Hypothesis

It is hypothesised that intraoperative assessment of bowel perfusion using NIR fluorescence imaging with ICG will lower the incidence of clinically relevant AL within 90 days after colorectal resection.

Study design

In this multicentre randomised controlled trial, patients will be allocated to two groups: the Fluorescence Guided Bowel Anastomosis group (FGBA) or the Conventional Bowel Anastomosis group (CBA). Patients in the FGBA group will receive at least one dose of 5 milligram ICG, up to a maximum of 3 doses, to assess bowel perfusion. Patients in the CBA group will not receive any study related interventions and will be treated according to standard of care. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the procedure.

Setting

This study will take place in at least two academic hospitals and multiple large teaching hospitals in the Netherlands. More centres will be added during the course of the study.

Participants

All patients scheduled for laparoscopic or robotic-assisted colorectal surgery (malignant and benign indications) with primary anastomosis will be screened for eligibility during multidisciplinary team meetings and, when eligible for participation, informed about the study by their attending physician. It will be emphasized that a patient can withdraw from the study at any given moment without having to offer any reason. The fundamental concepts outlined in the Declaration of Helsinki will be followed during the execution of the trial.[34]

Sample size calculation

The power analysis was performed based on Dutch national AL percentages, derived from the Dutch ColoRectal Audit (DCRA).[35] It is hypothesized that the use of ICG will decrease the AL rate in colorectal surgery from 7 to 3%. With a significance of 0.0492 (adjusted for the interim analysis using the O'Brien-Flemming approach), power of 80%, drop-out of 5% and a control-intervention ratio of 1:1, a sample size of 978 (489:489) patients is needed.[36]

Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria: aged 18 years and above, scheduled for laparoscopic or robotic-assisted colorectal resection with primary anastomosis, able to communicate in the Dutch language and willing to comply with the study restrictions, and signed informed consent prior to any study-mandated procedure.

Exclusion criteria

A potential patient who meets any of the following criteria will be excluded from participation in this study: known allergy or history of adverse reaction to ICG, iodine or iodine dyes, severe liver or kidney insufficiency, hyperthyroidism or a benign thyroid tumour, pregnant or breastfeeding women, scheduled for emergency surgery, palliative surgery or terminally ill, scheduled for a defunctioning stoma, taking phenobarbital, phenylbutazone, primidone, phenytoin, haloperidol, nitrofurantoin, and probenecid, or any other condition that the investigator considers to be potentially jeopardizing the patients well-being or the study objectives (following a detailed medical history and physical examination).

Randomisation

After inclusion in the study (i.e., after written informed consent is obtained), patients will be randomised to the FGBA or the CBA group. Randomisation will be performed online via Castor EDC (Castor, Amsterdam, the Netherlands) with variable block sizes and stratified by institute. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the surgical procedure.

Intervention

Patients in the CBA group will undergo laparoscopic or robotic colorectal resection according to standard of care using conventional methods to assess the integrity and viability of the anastomosis. Patients in the FGBA group will undergo the same standard of care surgical procedure as patients in the CBA group; however, in addition to the conventional methods, NIR fluorescence imaging with ICG will be performed to assess the bowel perfusion at the anastomosis side. This technique will be performed as follows (Figure 1): after dissection of the vascular branch, the preferred level of anastomoses (proximally and distally) will be highlighted by a stitch or diathermic mark in the adjacent mesocolon or mesorectum. Then, 5 mg ICG (2.5 mg/ml, Diagnostic Green, Aschheim, Germany), followed by 10 ml saline flush, will be injected intravenously by the anaesthesiologist. Within a few minutes, the anastomotic microvascularisation of both bowel ends will be assessed using the Olympus Medical Imaging Video System and Laparoscope (Olympus, Leiderdorp, the Netherlands) or Da Vinci Firefly (Intuitive Inc., Sunnyvale, CA, United States of America). The level of resection and subsequent anastomosis may be changed accordingly (with the mesocolic stitch serving as the baseline). During the procedure, the ICG injection (5 mg) may be repeated for a second or third time with a 15 minute wash-out period between each

administration. Repeated doses may be applicable when, for example, both anastomosis sides do not fit into the optical field, or when perfusion seems compromised after anastomosis finalisation. All injections, including the reason(s) for repeated injection(s), time of administration and consequences of administration, will be documented in the case report form (CRF).

Outcome measures

Primary outcome

The primary outcome is the rate of clinically relevant AL within 90 days after surgery. This will be compared between the FGBA group using ICG for perfusion assessment and the standard of care surgery, CBA group. The definition of clinically relevant AL is derived from the definition of Rahbari et al.[37] Grade B (requiring active therapeutic intervention but manageable without re-operation) and C AL (requiring re-operation) will be considered clinically relevant.

Secondary outcomes

1. 30-day clinically relevant AL
2. 30- and 90-day all-cause postoperative complications
3. 30- and 90-day mortality; all-cause and AL related
4. 30- and 90-day reinterventions; surgical and non-surgical
5. Total surgical time of primary surgery
6. Postoperative length of hospital stay; primary stay and readmittance within 90 days
7. Readmittance; all-cause and AL related

Data collection

A CRF will be filled in during surgery by trained local research staff. This CRF captures baseline characteristics, basic surgical data and study specific data. For patients in the FGBA group it will be documented whether the resection margins have been adjusted and, if so, which margin (distal or proximal margin) and the extent of adjustment in centimetres. In addition, in case of a non-planned defunctioning stoma, it will be recorded whether ICG-guidance contributed to this decision. All clinical data will be prospectively registered via an electronic CRF (eCRF) in a digital database of Castor EDC.

Data validation and management

Patient data will be registered coded and analysed by comparing the FGBA group with the CBA group. Only the local investigators will have access to local source data after informed consent is given. The research group from Leiden University Medical Centre (LUMC) will have access to all coded data in the Castor EDC database.

Study timeline

Patients will be included in the study from July 2020, starting in the LUMC, and with an anticipated last inclusion in the final quarter of 2022. In addition, it is expected that patients can be enrolled in at least 7 additional hospitals in the first year. There is no maximum for the number of centres or the number of inclusions per centre.

Statistical analysis

The most recent version of SPSS (IBM, Armonk, New York, USA) will be used for statistical analysis. Categorical variables of the FGBA and CBA group will be compared by the Chi-Square test. Numerical variables will be compared by the independent sample T-test or the

Mann-Whitney U test, depending on distribution. All p-values will be 2-sided. A p-value of less than 0.0492 will indicate a statistically significant difference. All data will be analysed on an intention-to-treat principle and, when applicable, on a per protocol analysis.

The primary outcome measure, clinically relevant AL within 90 days after surgery, will be compared using the Mantel-Haenszel test, stratified by centre.

An interim analysis will be conducted after 489 patients have been randomised and reached the last day of follow-up (day 90). This interim analysis will aim at stopping the study for futility, if the conditional power for the primary endpoint (clinically relevant AL within 90 days after surgery) with the planned sample size, based on the observed results at the interim analysis, using the original settings of null and alternative hypothesis, is less than 10%.

If this interim analysis shows efficacy based on the primary endpoint with a nominal alpha level of 0.0054, the study will be stopped as well. Already included patients will be followed until the last follow-up moment.

Data monitoring

The study will be monitored for quality and regulatory compliance, by study-independent LUMC staff. Monitoring frequency will be at least annually, but may be increased depending on findings.

Adverse events

All adverse events related to indocyanine green will be reported. Furthermore, all events that are serious adverse events will be registered in the online Dutch database, toetsingonline.nl, and in the eCRF of Castor EDC.

ETHICS AND DISSEMINATION

The study was approved by the certified Medical Ethics Review Committee Leiden, Den Haag, Delft (METC-LDD) on 11 November 2019 under identifier P19.079, and feasibility declarations as required by Dutch law, were obtained for the remaining hospitals. The protocol's current version (2.0) is dated 26 March 2020. The first patient was recruited on 2 July 2020 in LUMC. Six centres are currently enrolling patients. Protocol amendments will first be reviewed by the METC-LDD and after approval be shared with the participating centres for local feasibility declarations.

A manuscript with the results of this study will be published in a peer-reviewed journal. Moreover, the results will be shared via conference presentations.

AUTHOR CONTRIBUTIONS

RM, RF, OB, JB, EM, JB, DH and AV all contributed to the study concept and design. HP was responsible for the statistical analysis plan and the sample size calculation. RM, RF and OB prepared the manuscript. DH and AV supervised the manuscript preparation. All authors and members of the AVOID study group reviewed the manuscript before submission.

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COMPETING INTERESTS STATEMENT

AV and LS are members of the Diagnostic Green advisory board. All other authors declare to have no competing interest concerning this work.

REFERENCES

1. Buchs NC, Gervaz P, Secic M, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *Int J Colorectal Dis* 2008;23(3):265-70. doi: 10.1007/s00384-007-0399-3
2. Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. *Best Pract Res Clin Gastroenterol* 2004;18(5):865-80. doi: 10.1016/j.bpg.2004.06.026
3. Bakker IS, Grossmann I, Henneman D, et al. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg* 2014;101(4):424-32; discussion 32. doi: 10.1002/bjs.9395
4. Walker KG, Bell SW, Rickard MJ, et al. Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer. *Ann Surg* 2004;240(2):255-9.
5. Isbister WH. Anastomotic leak in colorectal surgery: a single surgeon's experience. *ANZ J Surg* 2001;71(9):516-20.
6. Kang CY, Halabi WJ, Chaudhry OO, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. *JAMA Surg* 2013;148(1):65-71. doi: 10.1001/2013.jamasurg.2
7. Platell C, Barwood N, Dorfmann G, et al. The incidence of anastomotic leaks in patients undergoing colorectal surgery. *Colorectal Dis* 2007;9(1):71-9. doi: 10.1111/j.1463-1318.2006.01002.x
8. Vignali A, Fazio VW, Lavery IC, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. *J Am Coll Surg* 1997;185(2):105-13.
9. Single-stage treatment for malignant left-sided colonic obstruction: a prospective randomized clinical trial comparing subtotal colectomy with segmental resection following intraoperative irrigation. The SCOTIA Study Group. Subtotal Colectomy versus On-table Irrigation and Anastomosis. *Br J Surg* 1995;82(12):1622-7.
10. Kockerling F, Rose J, Schneider C, et al. Laparoscopic colorectal anastomosis: risk of postoperative leakage. Results of a multicenter study. Laparoscopic Colorectal Surgery Study Group (LCSSG). *Surg Endosc* 1999;13(7):639-44.
11. Frasson M, Flor-Lorente B, Rodriguez JL, et al. Risk Factors for Anastomotic Leak After Colon Resection for Cancer: Multivariate Analysis and Nomogram From a Multicentric, Prospective, National Study With 3193 Patients. *Ann Surg* 2015;262(2):321-30. doi: 10.1097/SLA.0000000000000973
12. Senagore A, Lane FR, Lee E, et al. Bioabsorbable staple line reinforcement in restorative proctectomy and anterior resection: a randomized study. *Dis Colon Rectum* 2014;57(3):324-30. doi: 10.1097/DCR.0000000000000065
13. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients. *Ann Surg* 2004;240(2):260-8.
14. Yeh CY, Changchien CR, Wang JY, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg* 2005;241(1):9-13.
15. Hyman N, Manchester TL, Osler T, et al. Anastomotic leaks after intestinal anastomosis: it's later than you think. *Ann Surg* 2007;245(2):254-8. doi: 10.1097/01.sla.0000225083.27182.85
16. Snijders HS, Henneman D, van Leersum NL, et al. Anastomotic leakage as an outcome measure for quality of colorectal cancer surgery. *BMJ Qual Saf* 2013;22(9):759-67. doi: 10.1136/bmjqs-2012-001644
17. Vignali A, Gianotti L, Braga M, et al. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. *Dis Colon Rectum* 2000;43(1):76-82.
18. Kologlu M, Yorganci K, Renda N, et al. Effect of local and remote ischemia-reperfusion injury on healing of colonic anastomoses. *Surgery* 2000;128(1):99-104. doi: 10.1067/msy.2000.107414

19. Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension as a predictor of colonic anastomotic healing. *Dis Colon Rectum* 1987;30(11):867-71.
20. Hirst NA, Tiernan JP, Millner PA, et al. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis* 2014;16(2):95-109. doi: 10.1111/codi.12411 [published Online First: 2013/09/03]
21. Karliczek A, Harlaar NJ, Zeebregts CJ, et al. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. *Int J Colorectal Dis* 2009;24(5):569-76. doi: 10.1007/s00384-009-0658-6 [published Online First: 2009/02/18]
22. van Manen L, Handgraaf HJM, Diana M, et al. A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *J Surg Oncol* 2018;118(2):283-300. doi: 10.1002/jso.25105 [published Online First: 2018/06/26]
23. Jafari MD, Wexner SD, Martz JE, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. *J Am Coll Surg* 2015;220(1):82-92 e1. doi: 10.1016/j.jamcollsurg.2014.09.015
24. van den Hoven P, Ooms S, van Manen L, et al. A systematic review of the use of near-infrared fluorescence imaging in patients with peripheral artery disease. *J Vasc Surg* 2019;70(1):286-97 e1. doi: 10.1016/j.jvs.2018.11.023 [published Online First: 2019/06/25]
25. Griffiths M, Chae MP, Rozen WM. Indocyanine green-based fluorescent angiography in breast reconstruction. *Gland Surg* 2016;5(2):133-49. doi: 10.3978/j.issn.2227-684X.2016.02.01 [published Online First: 2016/04/06]
26. Fox IJ, Brooker LG, Heseltine DW, et al. A tricarbocyanine dye for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturation. *Proc Staff Meet Mayo Clin* 1957;32(18):478-84. [published Online First: 1957/09/04]
27. Sauda K, Imasaka T, Ishibashi N. Determination of protein in human serum by high-performance liquid chromatography with semiconductor laser fluorometric detection. *Anal Chem* 1986;58(13):2649-53.
28. Landsman ML, Kwant G, Mook GA, et al. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol* 1976;40(4):575-83. doi: 10.1152/jappl.1976.40.4.575
29. Kudszus S, Roesel C, Schachtrupp A, et al. Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. *Langenbecks Arch Surg* 2010;395(8):1025-30. doi: 10.1007/s00423-010-0699-x
30. Boni L, Fingerhut A, Marzorati A, et al. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. *Surg Endosc* 2017;31(4):1836-40. doi: 10.1007/s00464-016-5181-6
31. Kim JC, Lee JL, Yoon YS, et al. Utility of indocyanine-green fluorescent imaging during robot-assisted sphincter-saving surgery on rectal cancer patients. *Int J Med Robot* 2016;12(4):710-17. doi: 10.1002/rcs.1710
32. Jafari MD, Lee KH, Halabi WJ, et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surg Endosc* 2013;27(8):3003-8. doi: 10.1007/s00464-013-2832-8
33. Kin C, Vo H, Welton L, et al. Equivocal effect of intraoperative fluorescence angiography on colorectal anastomotic leaks. *Dis Colon Rectum* 2015;58(6):582-7. doi: 10.1097/DCR.0000000000000320
34. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310(20):2191-4. doi: 10.1001/jama.2013.281053 [published Online First: 2013/10/22]
35. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. *Control Clin Trials* 1990;11(2):116-28. doi: 10.1016/0197-2456(90)90005-m [published Online First: 1990/04/01]

36. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979;35(3):549-56.
37. Rahbari NN, Weitz J, Hohenberger W, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery* 2010;147(3):339-51. doi: 10.1016/j.surg.2009.10.012 [published Online First: 2009/12/17]

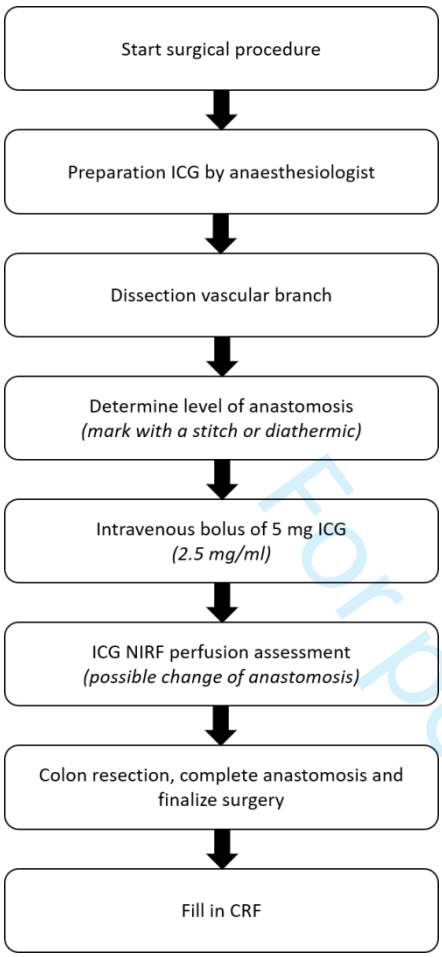

Figure 1 Surgical flowchart

ICG indocyanine green, *NIRF* Near-infrared, *CRF* case report form

Appendix A: List of members of the AVOID study group

Academic committee

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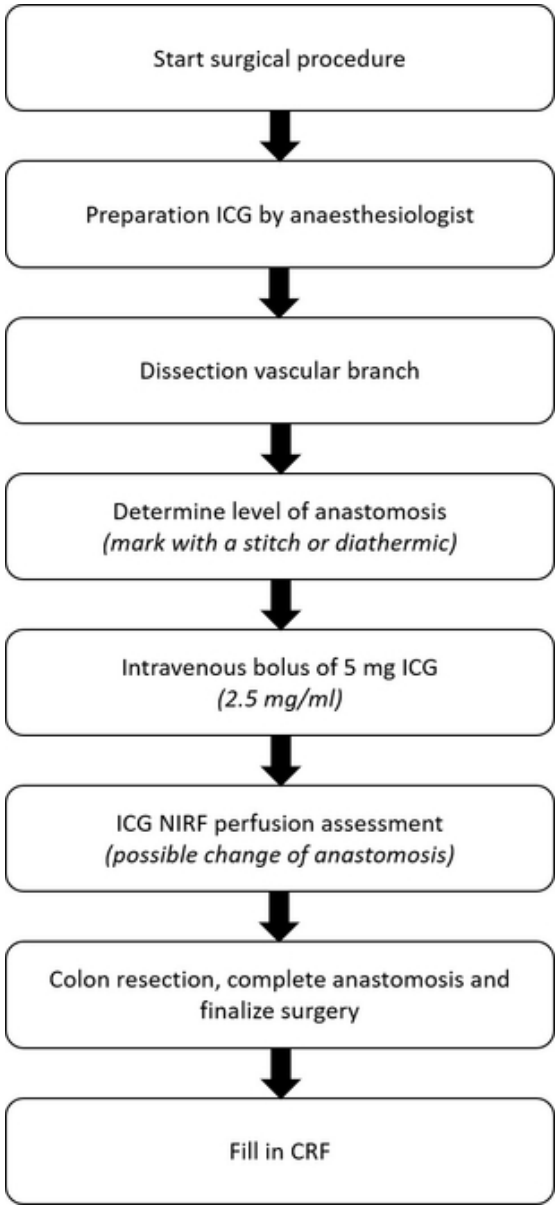

Figure 1 Surgical flowchart
ICG indocyanine green, NIRF Near-infrared, CRF case report form

12x28mm (600 x 600 DPI)

Reporting checklist for protocol of a clinical trial.

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		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	2

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	5-11
Protocol version	#3	Date and version identifier	12
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
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	12
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	10-11

		groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
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	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5-6
Objectives	#7	Specific objectives or hypotheses	5
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)	5-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries	6

		where data will be collected. Reference to where list of study sites can be obtained	
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)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
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)	n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8-9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg,	9

		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	
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)	8
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	10-11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	8

		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	
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	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6-8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a surgeons are always unblinded
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	10
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	n/a only 1 intervention moment
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	10-11
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	10-11
Statistics: additional	#20b	Methods for any additional analyses (eg,	10-11

analyses		subgroup and adjusted analyses)	
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)	10-11
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	n/a
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	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	#23	Frequency and procedures for auditing trial	11

		conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
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)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
6Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and	12-13

		each study site	
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	10
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	n/a
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	12
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and	n/a model consent in fully in Dutch and

		authorised surrogates	will therefore not be shared
Biological specimens	#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

Notes:

- 11b: n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
- 11c: n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
- 17b: n/a surgeons are always unblinded
- 18b: n/a only 1 intervention moment
- 32: n/a model consent in fully in Dutch and will therefore not be shared The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 09. March 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

AVOID; a Phase III, Randomised Controlled Trial Using Indocyanine Green for the Prevention of Anastomotic Leakage in Colorectal Surgery

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AVOID; a Phase III, Randomised Controlled Trial Using Indocyanine Green for the Prevention of Anastomotic Leakage in Colorectal Surgery

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Abstract

Introduction: Anastomotic leakage (AL) is one of the major complications after colorectal surgery. Compromised tissue perfusion at the anastomosis site increases the risk of AL. Several cohort studies have shown that indocyanine green (ICG) combined with fluorescent near-infrared imaging is a feasible and reproducible technique for real-time intraoperative imaging of tissue perfusion, leading to reduced leakage rates after colorectal resection. Unfortunately, these studies were not randomised. Therefore, we propose a randomised controlled trial to assess the value of ICG-guided surgery in reducing AL after colorectal surgery.

Methods and analysis: A multicentre, randomised controlled clinical trial will be conducted to assess the benefit of ICG-guided surgery in preventing AL. A total of 978 patients scheduled for colorectal surgery will be included. Patients will be randomised between the Fluorescence Guided Bowel Anastomosis (FGBA) group and the Conventional Bowel Anastomosis (CBA) group. The primary endpoint is clinically relevant AL (defined as requiring active therapeutic intervention or re-operation) within 90 days after surgery. Among the secondary endpoints are 30-day clinically relevant AL, all-cause postoperative complications, all-cause and AL related mortality, surgical and non-surgical reinterventions, total surgical time, length of hospital stay, and all-cause and AL related readmittance.

Ethics and dissemination: This protocol has been approved by the Medical Ethical Committee Leiden-Den Haag-Delft (METC-LDD) and is registered at ClinicalTrials.gov and trialregister.nl. The results of this study will be reported through peer-reviewed publications and conference presentations.

Trial registration numbers: NCT04712032 and NL7502

Keywords: bowel perfusion, near infrared fluorescence, indocyanine green, colorectal surgery, colorectal cancer, inflammatory bowel disease

Article Summary

Strengths and limitations of this study

1. This study is a multicentre randomised controlled trial
2. AL is a major complication with huge impact on patient's life
3. A clinically relevant endpoint will be used as the primary endpoint
4. Quantification of fluorescence-guided bowel perfusion with indocyanine green would be a preferable addition, however its clinical correlation is unclear at this point

Introduction

Anastomotic leakage (AL) is a major complication after colorectal surgery, accounting for considerable morbidity and mortality.[1-6] The incidence of AL in colorectal surgery ranges from 2.4 to 11% in colon cases and up to 23.3% in rectal cancer surgery.[4-15] The occurrence of AL often has a multifactorial cause, including risk factors such as tumour location, level of anastomosis, male gender, high ASA score, comorbidities, smoking, obesity and (neoadjuvant) radiotherapy.[3 4 6 11 13 14 16]

Most risk factors for AL can no longer be changed at the time of surgery. Therefore, it is important to focus on the few factors that can be influenced, such as compromised tissue perfusion at the anastomosis site. It has been reported that this factor significantly increases the risk of AL.[17-19] Perfusion is commonly assessed by palpating the mesenteric arterial pulsations, inspection of the bowel colour, and bleeding at the anastomosis sides. Other intraoperative tests to prove the integrity of the anastomosis are the air leak test and inspection of the resection doughnuts.[20] Though useful, these clinical assessments have proven to have a low predictive value for AL which emphasises the urge for a better diagnostic test.[21]

A promising diagnostic tool is intraoperative near-infrared (NIR) fluorescence imaging. This technique combines a fluorescent contrast agent, e.g. indocyanine green (ICG), and a dedicated NIR imaging system.[22] The intravenous injection of ICG has proven to be a feasible and reproducible application for real-time perfusion assessment.[23-25] ICG was introduced by Fox et al. in 1957 and is currently used for a variety of diagnostic indications.[26] Diluted and intravenously injected ICG, with a peak emission at 820 nm, is

invisible for the naked eye and will therefore not interfere with the surgical field.[27] Moreover, it is cleared quickly by the liver and has low toxicity.[28]

Several cohort studies have investigated the benefit of NIR fluorescence imaging with ICG for intraoperative assessment of bowel perfusion. Some of these studies have shown that this technique enables clear visualisation of bowel perfusion within minutes after intravenous injection of ICG, resulting in reduced leakage rates and hospital stay.[29-32] Moreover, several systematic reviews support this promising results concerning the prevention of AL [33 34]. This has already led to the start of two randomised controlled trials (ICG-COLORAL; NCT03602677 and InTACT trial; ISCRN 13334746) which are currently recruiting patients. On the other hand, Kin et al. have shown no benefit by using ICG in preventing AL.[35] Major drawbacks of these cohort studies are that they were not randomised and did not use clinically relevant AL as the primary endpoint. Therefore, we propose AVOID: '***A****nastomotic leakage and* ***V****alue* ***O****f* ***I****ndocyanine green in* ***D****ecreasing leakage rates*', a randomised controlled trial to investigate the benefit of intraoperative imaging with ICG for the reduction of AL rate in colorectal surgery.

METHODS AND ANALYSIS

Primary aim

The main objective of this study is to assess if ICG-guided perfusion assessment will result in a reduction of the AL rate within 90 days after surgery. ICG-guided perfusion assessment will be an adjunct to conventional laparoscopic imaging versus conventional laparoscopic imaging alone.

Hypothesis

It is hypothesised that intraoperative assessment of bowel perfusion using NIR fluorescence imaging with ICG will lower the incidence of clinically relevant AL within 90 days after colorectal resection.

Study design

In this multicentre randomised controlled trial, patients will be allocated to two groups: the Fluorescence Guided Bowel Anastomosis group (FGBA) or the Conventional Bowel Anastomosis group (CBA). Patients in the FGBA group will receive at least one dose of 5 milligram ICG, up to a maximum of 3 doses, to assess bowel perfusion. Patients in the CBA group will not receive any study related interventions and will be treated according to standard of care. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the procedure.

Setting

This national study will take place in multiple academic and large teaching hospitals in the Netherlands. More Dutch hospitals will be added during the course of the study.

Participants

All patients scheduled for laparoscopic or robotic-assisted colorectal surgery (malignant and benign indications) with primary anastomosis will be screened for eligibility during multidisciplinary team meetings and, when eligible for participation, informed about the study by their attending physician. It will be emphasized that a patient can withdraw from the study at any given moment without having to offer any reason. The fundamental

concepts outlined in the Declaration of Helsinki will be followed during the execution of the trial.[36]

Sample size calculation

The power analysis was performed based on Dutch national AL percentages, derived from the Dutch ColoRectal Audit (DCRA).[37] It is hypothesized that the use of ICG will decrease the AL rate in colorectal surgery from 7 to 3%. With a significance of 0.0492 (adjusted for the interim analysis using the O'Brien-Flemming approach), power of 80%, drop-out of 5% and a control-intervention ratio of 1:1, a sample size of 978 (489:489) patients is needed.[38]

Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria: aged 18 years and above, scheduled for laparoscopic or robotic-assisted colorectal resection with primary anastomosis, able to communicate in the Dutch language and willing to comply with the study restrictions, and signed informed consent prior to any study-mandated procedure.

Exclusion criteria

A potential patient who meets any of the following criteria will be excluded from participation in this study: known allergy or history of adverse reaction to ICG, iodine or iodine dyes, severe liver or kidney insufficiency, hyperthyroidism or a benign thyroid tumour, pregnant or breastfeeding women, scheduled for emergency surgery, palliative surgery or terminally ill, scheduled for a defunctioning stoma, taking phenobarbital, phenylbutazone, primidone, phenytoin, haloperidol, nitrofurantoin, and probenecid, or any other condition that the investigator considers to be potentially jeopardizing the patients

well-being or the study objectives (following a detailed medical history and physical examination).

Randomisation

After inclusion in the study (i.e., after written informed consent is obtained), patients will be randomised to the FGBA or the CBA group. Randomisation will be performed online via Castor EDC (Castor, Amsterdam, the Netherlands) with variable block sizes and stratified by institute. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the surgical procedure.

Intervention

Patients in the CBA group will undergo laparoscopic or robotic colorectal resection according to standard of care using conventional methods to assess the integrity and viability of the anastomosis. Patients in the FGBA group will undergo the same standard of care surgical procedure as patients in the CBA group; however, in addition to the conventional methods, NIR fluorescence imaging with ICG will be performed to assess the bowel perfusion at the anastomosis side. All surgeries, in both arms, will be performed by an attending surgeon. NIR fluorescence imaging with ICG will be performed as follows (Figure 1): after dissection of the vascular branch, the preferred level of anastomoses (proximally and distally) will be highlighted by a stitch or diathermic mark in the adjacent mesocolon or mesorectum. Then, 5 mg ICG (2.5 mg/ml, Diagnostic Green, Aschheim, Germany), followed by 10 ml saline flush, will be injected intravenously by the anaesthesiologist. Within a few minutes, the anastomotic microvascularisation of both bowel ends will be assessed using the Olympus Medical Imaging Video System and Laparoscope (Olympus, Leiderdorp, the Netherlands) or Da Vinci Firefly (Intuitive Inc.,

Sunnyvale, CA, United States of America). The level of resection and subsequent anastomosis may be changed accordingly (with the mesocolic stitch serving as the baseline). During the procedure, the ICG injection (5 mg) may be repeated for a second or third time with a 15 minute wash-out period between each administration. Repeated doses may be applicable when, for example, both anastomosis sides do not fit into the optical field, or when perfusion seems compromised after anastomosis finalisation. All injections, including the reason(s) for repeated injection(s), and the consequences of administration, will be documented in the case report form (CRF).

The 90-day follow-up is a standard of care follow-up moment in all participating hospitals. It will be done either by phone, by videoconference or in person, according to standard of care in the participating hospital. Patients who, for any reason, do not visit the hospital 90 days after resection, will be contacted by phone and asked for any postoperative complications or reinterventions.

Outcome measures

Primary outcome

The primary outcome is the rate of clinically relevant AL within 90 days after surgery. This will be compared between the FGBA group using ICG for perfusion assessment and the standard of care surgery, CBA group. The definition of clinically relevant AL is derived from the definition of Rahbari et al.[39] Grade B (requiring active therapeutic intervention but manageable without re-operation) and C AL (requiring re-operation) will be considered clinically relevant. The assessment of AL will be based on the evaluation of clinical features and subsequent CT scan at the judgment of the attending surgeon. No routine CT scans will be performed for AL assessment.

Secondary outcomes

1. 30-day clinically relevant AL
2. 30- and 90-day all-cause postoperative complications
3. 30- and 90-day mortality; all-cause and AL related
4. 30- and 90-day reinterventions; surgical and non-surgical
5. Total surgical time of primary surgery
6. Postoperative length of hospital stay; primary stay and readmittance within 90 days
7. Readmittance; all-cause and AL related

Training

Prior to their first inclusion, surgeons and other involved hospital staff of the participating center will be trained during a site initiation visit by the principal investigator or one of the coordinating investigators. If needed, training with the Olympus Medical Imaging Video System and Laparoscope or Da Vinci Firefly will be provided by either Olympus or Intuitive. Surgeons are invited to observe surgical procedures, using NIR fluorescence imaging with ICG for intraoperative assessment of bowel perfusion, in the LUMC. One of the coordinating investigators, with a broad experience in fluorescence-guided surgery, will assist all participating surgeons during their first number of cases to ensure standardization of the technique.

Data collection

A CRF will be filled in during surgery by trained local research staff. This CRF captures baseline characteristics, basic surgical data and study specific data. For patients in the FGBA group it will be documented whether the resection margins have been adjusted and, if so, which margin (distal or proximal margin) and the extent of adjustment in centimetres. In

addition, in case of a non-planned defunctioning stoma, it will be recorded whether ICG-guidance contributed to this decision. All clinical data will be prospectively registered via an electronic CRF (eCRF) in a digital database of Castor EDC.

Data validation and management

Patient data will be registered coded and analysed by comparing the FGBA group with the CBA group. Only the local investigators will have access to local source data after informed consent is given. The research group from Leiden University Medical Centre (LUMC) will have access to all coded data in the Castor EDC database.

Study timeline

Patients have been included in the study from July 2020, starting in the LUMC. As per August 1st 2021, 352 patients were included in 6 different hospitals. With a mean inclusion rate of 40 patients per month the anticipated last inclusion will be in the final quarter of 2022. There is no maximum for the number of centres nor the number of inclusions per centre.

Statistical analysis

The most recent version of SPSS (IBM, Armonk, New York, USA) will be used for statistical analysis. Categorical variables of the FGBA and CBA group will be compared by the Chi-Square test. Numerical variables will be compared by the independent sample T-test or the Mann-Whitney U test, depending on distribution. All p-values will be 2-sided. A p-value of less than 0.0492 will indicate a statistically significant difference. All data will be analysed on an intention-to-treat principle and, when applicable, on a per protocol analysis.

The primary outcome measure, clinically relevant AL within 90 days after surgery, will be compared using the Mantel-Haenszel test, stratified by centre.

An interim analysis will be conducted after 489 patients have been randomised and reached the last day of follow-up (day 90). This interim analysis will aim at stopping the study for futility, if the conditional power for the primary endpoint (clinically relevant AL within 90 days after surgery) with the planned sample size, based on the observed results at the interim analysis, using the original settings of null and alternative hypothesis, is less than 10%.

If this interim analysis shows efficacy based on the primary endpoint with a nominal alpha level of 0.0054, the study will be stopped as well. Already included patients will be followed until the last follow-up moment.

Sub-group analysis will be conducted by separately assessing patients with 1. colon and rectal resections, 2. left and right sided resections, 3. malignant and benign pathology and 4. laparoscopic and robotic-assisted surgery.

Data monitoring

The study will be monitored for quality and regulatory compliance, by study-independent LUMC staff. Monitoring frequency will be at least annually, but may be increased depending on findings.

Adverse events

All adverse events related to indocyanine green will be reported. Furthermore, all events that are serious adverse events will be registered in the online Dutch database, toetsingonline.nl, and in the eCRF of Castor EDC.

Patient and Public Involvement

Patients or public were neither involved in the development of the research questions and outcome measures nor the planning of the study design. Patients are not involved in the recruitment or conduct of the study. Results of the study will be published in peer-reviewed journals, no other information of the results of the study are provided to the patients. Patients will not take part in assessment regarding possible burden of the interventions of this study.

EXPECTED LIMITATIONS AND DIFFICULTIES

Intraoperative fluorescence assessment of bowel perfusion is currently a subjective tool. This will most likely influence our results as over 30 different surgeons will interpret the fluorescence output. Quantification of the NIR fluorescence signal would improve standardized assessment of tissue perfusion.

Using different NIR platforms (the Olympus Medical Imaging Video System and Laparoscope, and the Da Vinci Firefly) will have some influence on our results as well. Nevertheless, both systems are optimized for the detection of ICG, we therefore think its effect on our study results is minimal.

AL after colorectal surgery is a multifactorial complication. It is unclear which percentage of AL is solely based on compromised perfusion. It is especially questionable if compromised perfusion plays a role in late AL (> 7 days after surgery).

ETHICS AND DISSEMINATION

The study was approved by the certified Medical Ethics Review Committee Leiden, Den Haag, Delft (METC-LDD) on 11 November 2019 under identifier P19.079, and feasibility

declarations as required by Dutch law, were obtained for the remaining hospitals. The protocol's current version (2.0) is dated 26 March 2020. The first patient was recruited on 2 July 2020 in LUMC. Six centres are currently enrolling patients. Protocol amendments will first be reviewed by the METC-LDD and after approval be shared with the participating centres for local feasibility declarations.

This study was prospectively registered at the Netherlands trial register (NL7502) and after the first inclusion registered at clinicaltrials.gov (NCT04712032). A manuscript with the results of this study will be published in a peer-reviewed journal. Moreover, the results will be shared via conference presentations.

AUTHOR CONTRIBUTIONS

RM, RF, OB, JB, EM, JM, KB, AV and DH all contributed to the study concept and design. HP was responsible for the statistical analysis plan and the sample size calculation. RM, RF and OB prepared the manuscript. JM, AV and DH supervised the manuscript preparation. All authors and members of the AVOID study group reviewed the manuscript before submission.

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COMPETING INTERESTS STATEMENT

AV and LS are members of the Diagnostic Green advisory board. All other authors declare to have no competing interest concerning this work.

REFERENCES

1. Buchs NC, Gervaz P, Secic M, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *Int J Colorectal Dis* 2008;23(3):265-70. doi: 10.1007/s00384-007-0399-3
2. Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. *Best Pract Res Clin Gastroenterol* 2004;18(5):865-80. doi: 10.1016/j.bpg.2004.06.026
3. Bakker IS, Grossmann I, Henneman D, et al. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg* 2014;101(4):424-32; discussion 32. doi: 10.1002/bjs.9395
4. Walker KG, Bell SW, Rickard MJ, et al. Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer. *Ann Surg* 2004;240(2):255-9.
5. Isbister WH. Anastomotic leak in colorectal surgery: a single surgeon's experience. *ANZ J Surg* 2001;71(9):516-20.
6. Kang CY, Halabi WJ, Chaudhry OO, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. *JAMA Surg* 2013;148(1):65-71. doi: 10.1001/2013.jamasurg.2
7. Platell C, Barwood N, Dorfmann G, et al. The incidence of anastomotic leaks in patients undergoing colorectal surgery. *Colorectal Dis* 2007;9(1):71-9. doi: 10.1111/j.1463-1318.2006.01002.x
8. Vignali A, Fazio VW, Lavery IC, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. *J Am Coll Surg* 1997;185(2):105-13.
9. Single-stage treatment for malignant left-sided colonic obstruction: a prospective randomized clinical trial comparing subtotal colectomy with segmental resection following intraoperative irrigation. The SCOTIA Study Group. Subtotal Colectomy versus On-table Irrigation and Anastomosis. *Br J Surg* 1995;82(12):1622-7.
10. Kockerling F, Rose J, Schneider C, et al. Laparoscopic colorectal anastomosis: risk of postoperative leakage. Results of a multicenter study. Laparoscopic Colorectal Surgery Study Group (LCSSG). *Surg Endosc* 1999;13(7):639-44.
11. Frasson M, Flor-Lorente B, Rodriguez JL, et al. Risk Factors for Anastomotic Leak After Colon Resection for Cancer: Multivariate Analysis and Nomogram From a Multicentric, Prospective, National Study With 3193 Patients. *Ann Surg* 2015;262(2):321-30. doi: 10.1097/SLA.0000000000000973
12. Senagore A, Lane FR, Lee E, et al. Bioabsorbable staple line reinforcement in restorative proctectomy and anterior resection: a randomized study. *Dis Colon Rectum* 2014;57(3):324-30. doi: 10.1097/DCR.0000000000000065
13. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients. *Ann Surg* 2004;240(2):260-8.
14. Yeh CY, Changchien CR, Wang JY, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg* 2005;241(1):9-13.
15. Hyman N, Manchester TL, Osler T, et al. Anastomotic leaks after intestinal anastomosis: it's later than you think. *Ann Surg* 2007;245(2):254-8. doi: 10.1097/01.sla.0000225083.27182.85
16. Snijders HS, Henneman D, van Leersum NL, et al. Anastomotic leakage as an outcome measure for quality of colorectal cancer surgery. *BMJ Qual Saf* 2013;22(9):759-67. doi: 10.1136/bmjqs-2012-001644
17. Vignali A, Gianotti L, Braga M, et al. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. *Dis Colon Rectum* 2000;43(1):76-82.
18. Kologlu M, Yorganci K, Renda N, et al. Effect of local and remote ischemia-reperfusion injury on healing of colonic anastomoses. *Surgery* 2000;128(1):99-104. doi: 10.1067/msy.2000.107414

19. Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension as a predictor of colonic anastomotic healing. *Dis Colon Rectum* 1987;30(11):867-71.
20. Hirst NA, Tiernan JP, Millner PA, et al. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis* 2014;16(2):95-109. doi: 10.1111/codi.12411 [published Online First: 2013/09/03]
21. Karliczek A, Harlaar NJ, Zeebregts CJ, et al. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. *Int J Colorectal Dis* 2009;24(5):569-76. doi: 10.1007/s00384-009-0658-6 [published Online First: 2009/02/18]
22. van Manen L, Handgraaf HJM, Diana M, et al. A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *Journal of surgical oncology* 2018;118(2):283-300. doi: 10.1002/jso.25105 [published Online First: 2018/06/26]
23. Jafari MD, Wexner SD, Martz JE, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. *J Am Coll Surg* 2015;220(1):82-92 e1. doi: 10.1016/j.jamcollsurg.2014.09.015
24. van den Hoven P, Ooms S, van Manen L, et al. A systematic review of the use of near-infrared fluorescence imaging in patients with peripheral artery disease. *J Vasc Surg* 2019;70(1):286-97 e1. doi: 10.1016/j.jvs.2018.11.023 [published Online First: 2019/06/25]
25. Griffiths M, Chae MP, Rozen WM. Indocyanine green-based fluorescent angiography in breast reconstruction. *Gland Surg* 2016;5(2):133-49. doi: 10.3978/j.issn.2227-684X.2016.02.01 [published Online First: 2016/04/06]
26. Fox IJ, Brooker LG, Heseltine DW, et al. A tricarbocyanine dye for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturation. *Proc Staff Meet Mayo Clin* 1957;32(18):478-84. [published Online First: 1957/09/04]
27. Sauda K, Imasaka T, Ishibashi N. Determination of protein in human serum by high-performance liquid chromatography with semiconductor laser fluorometric detection. *Anal Chem* 1986;58(13):2649-53.
28. Landsman ML, Kwant G, Mook GA, et al. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol* 1976;40(4):575-83. doi: 10.1152/jappl.1976.40.4.575
29. Kudszus S, Roesel C, Schachtrupp A, et al. Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. *Langenbecks Arch Surg* 2010;395(8):1025-30. doi: 10.1007/s00423-010-0699-x
30. Boni L, Fingerhut A, Marzorati A, et al. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. *Surg Endosc* 2017;31(4):1836-40. doi: 10.1007/s00464-016-5181-6
31. Kim JC, Lee JL, Yoon YS, et al. Utility of indocyanine-green fluorescent imaging during robot-assisted sphincter-saving surgery on rectal cancer patients. *Int J Med Robot* 2016;12(4):710-17. doi: 10.1002/rcs.1710
32. Jafari MD, Lee KH, Halabi WJ, et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surg Endosc* 2013;27(8):3003-8. doi: 10.1007/s00464-013-2832-8
33. Song M, Liu J, Xia D, et al. Assessment of intraoperative use of indocyanine green fluorescence imaging on the incidence of anastomotic leakage after rectal cancer surgery: a PRISMA-compliant systematic review and meta-analysis. *Tech Coloproctol* 2021;25(1):49-58. doi: 10.1007/s10151-020-02335-1 [published Online First: 2020/09/05]
34. Chan DKH, Lee SKF, Ang JJ. Indocyanine green fluorescence angiography decreases the risk of colorectal anastomotic leakage: Systematic review and meta-analysis. *Surgery* 2020;168(6):1128-37. doi: 10.1016/j.surg.2020.08.024 [published Online First: 2020/10/05]
35. Kin C, Vo H, Welton L, et al. Equivocal effect of intraoperative fluorescence angiography on colorectal anastomotic leaks. *Dis Colon Rectum* 2015;58(6):582-7. doi: 10.1097/DCR.0000000000000320

36. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310(20):2191-4. doi: 10.1001/jama.2013.281053 [published Online First: 2013/10/22]
37. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. *Control Clin Trials* 1990;11(2):116-28. doi: 10.1016/0197-2456(90)90005-m [published Online First: 1990/04/01]
38. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979;35(3):549-56.
39. Rahbari NN, Weitz J, Hohenberger W, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery* 2010;147(3):339-51. doi: 10.1016/j.surg.2009.10.012 [published Online First: 2009/12/17]

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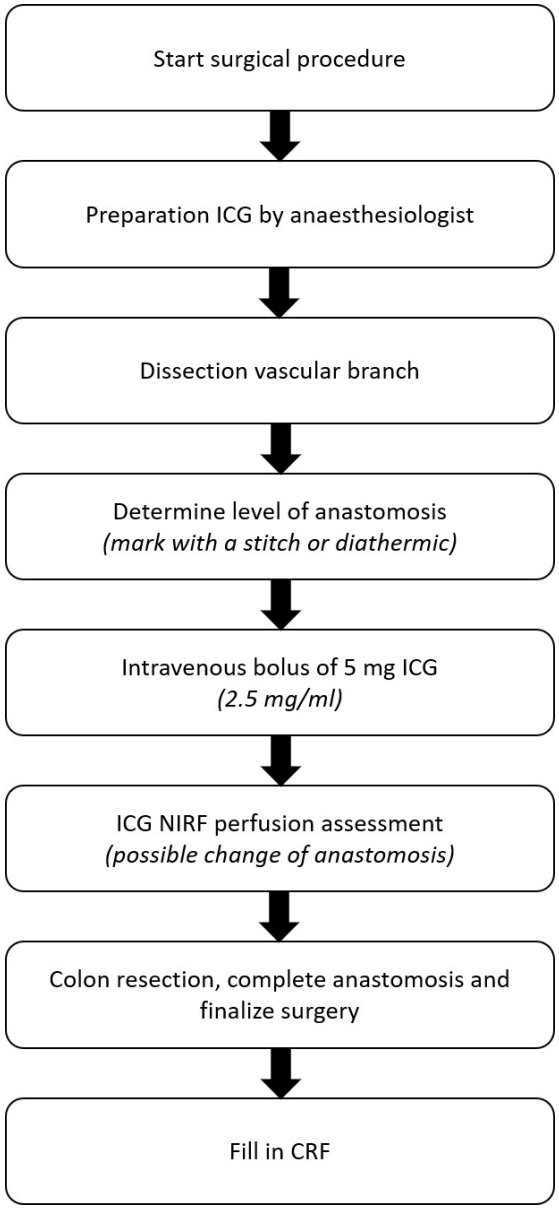

Figure 1 Surgical flowchart
ICG indocyanine green, NIRF Near-infrared, CRF case report form

13x28mm (1200 x 1200 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.

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

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		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	2

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	5-11
Protocol version	#3	Date and version identifier	12
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
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	12
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	10-11

		groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
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	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5-6
Objectives	#7	Specific objectives or hypotheses	5
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)	5-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries	6

		where data will be collected. Reference to where list of study sites can be obtained	
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)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
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)	n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8-9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg,	9

		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	
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)	8
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	10-11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	8

		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	
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	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6-8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a surgeons are always unblinded
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	10
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	n/a only 1 intervention moment
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	10-11
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	10-11
Statistics: additional	#20b	Methods for any additional analyses (eg,	10-11

analyses		subgroup and adjusted analyses)	
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)	10-11
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	n/a
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	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	#23	Frequency and procedures for auditing trial	11

		conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
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)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
6Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and	12-13

		each study site	
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	10
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	n/a
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	12
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and	n/a model consent in fully in Dutch and

		authorised surrogates	will therefore not be shared
Biological specimens	#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

Notes:

- 11b: n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
- 11c: n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
- 17b: n/a surgeons are always unblinded
- 18b: n/a only 1 intervention moment
- 32: n/a model consent in fully in Dutch and will therefore not be shared The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 09. March 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

AVOID; a Phase III, Randomised Controlled Trial Using Indocyanine Green for the Prevention of Anastomotic Leakage in Colorectal Surgery

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AVOID; a Phase III, Randomised Controlled Trial Using Indocyanine Green for the Prevention of Anastomotic Leakage in Colorectal Surgery

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Abstract

Introduction: Anastomotic leakage (AL) is one of the major complications after colorectal surgery. Compromised tissue perfusion at the anastomosis site increases the risk of AL. Several cohort studies have shown that indocyanine green (ICG) combined with fluorescent near-infrared imaging is a feasible and reproducible technique for real-time intraoperative imaging of tissue perfusion, leading to reduced leakage rates after colorectal resection. Unfortunately, these studies were not randomised. Therefore, we propose a randomised controlled trial to assess the value of ICG-guided surgery in reducing AL after colorectal surgery.

Methods and analysis: A multicentre, randomised controlled clinical trial will be conducted to assess the benefit of ICG-guided surgery in preventing AL. A total of 978 patients scheduled for colorectal surgery will be included. Patients will be randomised between the Fluorescence Guided Bowel Anastomosis (FGBA) group and the Conventional Bowel Anastomosis (CBA) group. The primary endpoint is clinically relevant AL (defined as requiring active therapeutic intervention or re-operation) within 90 days after surgery. Among the secondary endpoints are 30-day clinically relevant AL, all-cause postoperative complications, all-cause and AL related mortality, surgical and non-surgical reinterventions, total surgical time, length of hospital stay, and all-cause and AL related readmittance.

Ethics and dissemination: This protocol has been approved by the Medical Ethical Committee Leiden-Den Haag-Delft (METC-LDD) and is registered at ClinicalTrials.gov and trialregister.nl. The results of this study will be reported through peer-reviewed publications and conference presentations.

Trial registration numbers: NCT04712032 and NL7502

Keywords: bowel perfusion, near infrared fluorescence, indocyanine green, colorectal surgery, colorectal cancer, inflammatory bowel disease

Article Summary

Strengths and limitations of this study

1. This study is a multicentre randomised controlled trial
2. AL is a major complication with huge impact on patient's life
3. A clinically relevant endpoint will be used as the primary endpoint
4. Quantification of fluorescence-guided bowel perfusion with indocyanine green would be a preferable addition, however its clinical correlation is unclear at this point

Introduction

Anastomotic leakage (AL) is a major complication after colorectal surgery, accounting for considerable morbidity and mortality.[1-6] The incidence of AL in colorectal surgery ranges from 2.4 to 11% in colon cases and up to 23.3% in rectal cancer surgery.[4-15] The occurrence of AL often has a multifactorial cause, including risk factors such as tumour location, level of anastomosis, male gender, high ASA score, comorbidities, smoking, obesity and (neoadjuvant) radiotherapy.[3 4 6 11 13 14 16]

Most risk factors for AL can no longer be changed at the time of surgery. Therefore, it is important to focus on the few factors that can be influenced, such as compromised tissue perfusion at the anastomosis site. It has been reported that this factor significantly increases the risk of AL.[17-19] Perfusion is commonly assessed by palpating the mesenteric arterial pulsations, inspection of the bowel colour, and bleeding at the anastomosis sides. Other intraoperative tests to prove the integrity of the anastomosis are the air leak test and inspection of the resection doughnuts.[20] Though useful, these clinical assessments have proven to have a low predictive value for AL which emphasises the urge for a better diagnostic test.[21]

A promising diagnostic tool is intraoperative near-infrared (NIR) fluorescence imaging. This technique combines a fluorescent contrast agent, e.g. indocyanine green (ICG), and a dedicated NIR imaging system.[22] The intravenous injection of ICG has proven to be a feasible and reproducible application for real-time perfusion assessment.[23-25] ICG was introduced by Fox et al. in 1957 and is currently used for a variety of diagnostic indications.[26] Diluted and intravenously injected ICG, with a peak emission at 820 nm, is

invisible for the naked eye and will therefore not interfere with the surgical field.[27] Moreover, it is cleared quickly by the liver and has low toxicity.[28]

Several cohort studies have investigated the benefit of NIR fluorescence imaging with ICG for intraoperative assessment of bowel perfusion. Some of these studies have shown that this technique enables clear visualisation of bowel perfusion within minutes after intravenous injection of ICG, resulting in reduced leakage rates and hospital stay.[29-32] Moreover, several systematic reviews support this promising results concerning the prevention of AL [33 34]. This has already led to the start of two randomised controlled trials (ICG-COLORAL; NCT03602677 and InTACT trial; ISCRN 13334746) which are currently recruiting patients. On the other hand, Kin et al. have shown no benefit by using ICG in preventing AL.[35] Major drawbacks of these cohort studies are that they were not randomised and did not use clinically relevant AL as the primary endpoint. Therefore, we propose AVOID: '***A****nastomotic leakage and* ***V****alue* ***O****f* ***I****ndocyanine green in* ***D****ecreasing leakage rates*', a randomised controlled trial to investigate the benefit of intraoperative imaging with ICG for the reduction of AL rate in colorectal surgery.

METHODS AND ANALYSIS

Primary aim

The main objective of this study is to assess if ICG-guided perfusion assessment will result in a reduction of the AL rate within 90 days after surgery. ICG-guided perfusion assessment will be an adjunct to conventional laparoscopic imaging versus conventional laparoscopic imaging alone.

Hypothesis

It is hypothesised that intraoperative assessment of bowel perfusion using NIR fluorescence imaging with ICG will lower the incidence of clinically relevant AL within 90 days after colorectal resection.

Study design

In this multicentre randomised controlled trial, patients will be allocated to two groups: the Fluorescence Guided Bowel Anastomosis group (FGBA) or the Conventional Bowel Anastomosis group (CBA). Patients in the FGBA group will receive at least one dose of 5 milligram ICG, up to a maximum of 3 doses, to assess bowel perfusion. Patients in the CBA group will not receive any study related interventions and will be treated according to standard of care. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the procedure.

Setting

This national study will take place in multiple academic and large teaching hospitals in the Netherlands. More Dutch hospitals will be added during the course of the study.

Participants

All patients scheduled for laparoscopic or robotic-assisted colorectal surgery (malignant and benign indications) with primary anastomosis will be screened for eligibility during multidisciplinary team meetings and, when eligible for participation, informed about the study by their attending physician. It will be emphasized that a patient can withdraw from the study at any given moment without having to offer any reason. The fundamental

concepts outlined in the Declaration of Helsinki will be followed during the execution of the trial.[36]

Sample size calculation

The power analysis was performed based on Dutch national AL percentages, derived from the Dutch ColoRectal Audit (DCRA).[37] It is hypothesized that the use of ICG will decrease the AL rate in colorectal surgery from 7 to 3%. With a significance of 0.0492 (adjusted for the interim analysis using the O'Brien-Flemming approach), power of 80%, drop-out of 5% and a control-intervention ratio of 1:1, a sample size of 978 (489:489) patients is needed.[38]

Inclusion criteria

In order to be eligible to participate in this study, a patient must meet all of the following criteria: aged 18 years and above, scheduled for laparoscopic or robotic-assisted colorectal resection with primary anastomosis, able to communicate in the Dutch language and willing to comply with the study restrictions, and signed informed consent prior to any study-mandated procedure.

Exclusion criteria

A potential patient who meets any of the following criteria will be excluded from participation in this study: known allergy or history of adverse reaction to ICG, iodine or iodine dyes, severe liver or kidney insufficiency, hyperthyroidism or a benign thyroid tumour, pregnant or breastfeeding women, scheduled for emergency surgery, palliative surgery or terminally ill, scheduled for a defunctioning stoma, taking phenobarbital, phenylbutazone, primidone, phenytoin, haloperidol, nitrofurantoin, and probenecid, or any other condition that the investigator considers to be potentially jeopardizing the patients

well-being or the study objectives (following a detailed medical history and physical examination).

Randomisation

After inclusion in the study (i.e., after written informed consent is obtained), patients will be randomised to the FGBA or the CBA group. Randomisation will be performed online via Castor EDC (Castor, Amsterdam, the Netherlands) with variable block sizes and stratified by institute. The allocated treatment result is not blinded for the surgeon performing the procedure. Patients will be unblinded after the surgical procedure.

Intervention

Patients in the CBA group will undergo laparoscopic or robotic colorectal resection according to standard of care using conventional methods to assess the integrity and viability of the anastomosis. Patients in the FGBA group will undergo the same standard of care surgical procedure as patients in the CBA group; however, in addition to the conventional methods, NIR fluorescence imaging with ICG will be performed to assess the bowel perfusion at the anastomosis side. All surgeries, in both arms, will be performed by an attending surgeon. NIR fluorescence imaging with ICG will be performed as follows (Figure 1): after dissection of the vascular branch, the preferred level of anastomoses (proximally and distally) will be highlighted by a stitch or diathermic mark in the adjacent mesocolon or mesorectum. Then, 5 mg ICG (2.5 mg/ml, Diagnostic Green, Aschheim, Germany), followed by 10 ml saline flush, will be injected intravenously by the anaesthesiologist. Within a few minutes, the anastomotic microvascularisation of both bowel ends will be assessed using the Olympus Medical Imaging Video System and Laparoscope (Olympus, Leiderdorp, the Netherlands) or Da Vinci Firefly (Intuitive Inc.,

Sunnyvale, CA, United States of America). The green overlay setting of these systems will be used for perfusion assessment. The level of resection and subsequent anastomosis may be changed accordingly (with the mesocolic stitch serving as the baseline). During the procedure, the ICG injection (5 mg) may be repeated for a second or third time with a 15 minute wash-out period between each administration. Repeated doses may be applicable when, for example, both anastomosis sides do not fit into the optical field, or when perfusion seems compromised after anastomosis finalisation. All injections, including the reason(s) for repeated injection(s), and the consequences of administration, will be documented in the case report form (CRF).

The 90-day follow-up is a standard of care follow-up moment in all participating hospitals. It will be done either by phone, by videoconference or in person, according to standard of care in the participating hospital. Patients who, for any reason, do not visit the hospital 90 days after resection, will be contacted by phone and asked for any postoperative complications or reinterventions.

Outcome measures

Primary outcome

The primary outcome is the rate of clinically relevant AL within 90 days after surgery. This will be compared between the FGBA group using ICG for perfusion assessment and the standard of care surgery, CBA group. The definition of clinically relevant AL is derived from the definition of Rahbari et al.[39] Grade B (requiring active therapeutic intervention but manageable without re-operation) and C AL (requiring re-operation) will be considered clinically relevant. There is no central study protocol for the detection of AL. No routine CT scans will be performed for AL assessment. Post-operative blood tests, radiologic

assessment and subsequent assessment of AL will be based on local protocols and the judgement of the local surgical team.

Secondary outcomes

1. 30-day clinically relevant AL
2. 30- and 90-day all-cause postoperative complications
3. 30- and 90-day mortality; all-cause and AL related
4. 30- and 90-day reinterventions; surgical and non-surgical
5. Total surgical time of primary surgery
6. Postoperative length of hospital stay; primary stay and readmittance within 90 days
7. Readmittance; all-cause and AL related

Training

Prior to their first inclusion, surgeons and other involved hospital staff of the participating center will be trained during a site initiation visit by the principal investigator or one of the coordinating investigators. If needed, training with the Olympus Medical Imaging Video System and Laparoscope or Da Vinci Firefly will be provided by either Olympus or Intuitive. Surgeons are invited to observe surgical procedures, using NIR fluorescence imaging with ICG for intraoperative assessment of bowel perfusion, in the LUMC. One of the coordinating investigators, with a broad experience in fluorescence-guided surgery, will assist all participating surgeons during their first number of cases to ensure standardization of the technique.

This study is performed in collaboration with Olympus. In order to keep the study data as homogenous as possible, the use of camera system has been limited to the Olympus Medical Imaging Video System and the Da Vinci Firefly in case of robotic-assisted surgery.**Data collection**

A CRF will be filled in during surgery by trained local research staff. This CRF captures baseline characteristics, basic surgical data and study specific data. For patients in the FGBA group it will be documented whether the resection margins have been adjusted and, if so, which margin (distal or proximal margin) and the extent of adjustment in centimetres. In addition, in case of a non-planned defunctioning stoma, it will be recorded whether ICG-guidance contributed to this decision. All clinical data will be prospectively registered via an electronic CRF (eCRF) in a digital database of Castor EDC. We will not transfer or collect imaging data (video or pictures) for postoperative analysis.

Data validation and management

Patient data will be registered coded and analysed by comparing the FGBA group with the CBA group. Only the local investigators will have access to local source data after informed consent is given. The research group from Leiden University Medical Centre (LUMC) will have access to all coded data in the Castor EDC database.

Study timeline

Patients have been included in the study from July 2020, starting in the LUMC. As per August 1st 2021, 352 patients were included in 6 different hospitals. With a mean inclusion rate of 40 patients per month the anticipated last inclusion will be in the final quarter of

2022. There is no maximum for the number of centres nor the number of inclusions per centre.

Statistical analysis

The most recent version of SPSS (IBM, Armonk, New York, USA) will be used for statistical analysis. Categorical variables of the FGBA and CBA group will be compared by the Chi-Square test. Numerical variables will be compared by the independent sample T-test or the Mann-Whitney U test, depending on distribution. All p-values will be 2-sided. A p-value of less than 0.0492 will indicate a statistically significant difference. All data will be analysed on an intention-to-treat principle and, when applicable, on a per protocol analysis.

The primary outcome measure, clinically relevant AL within 90 days after surgery, will be compared using the Mantel-Haenszel test, stratified by centre.

An interim analysis will be conducted after 489 patients have been randomised and reached the last day of follow-up (day 90). This interim analysis will aim at stopping the study for futility, if the conditional power for the primary endpoint (clinically relevant AL within 90 days after surgery) with the planned sample size, based on the observed results at the interim analysis, using the original settings of null and alternative hypothesis, is less than 10%.

If this interim analysis shows efficacy based on the primary endpoint with a nominal alpha level of 0.0054, the study will be stopped as well. Already included patients will be followed until the last follow-up moment.

Sub-group analysis will be conducted by separately assessing patients with 1. colon and rectal resections, 2. left and right sided resections, 3. malignant and benign pathology and 4. laparoscopic and robotic-assisted surgery.

Data monitoring

The study will be monitored for quality and regulatory compliance, by study-independent LUMC staff. Monitoring frequency will be at least annually, but may be increased depending on findings.

Adverse events

All adverse events related to indocyanine green will be reported. Furthermore, all events that are serious adverse events will be registered in the online Dutch database, toetsingonline.nl, and in the eCRF of Castor EDC.

Patient and Public Involvement

Patients or public were neither involved in the development of the research questions and outcome measures nor the planning of the study design. Patients are not involved in the recruitment or conduct of the study. Results of the study will be published in peer-reviewed journals, no other information of the results of the study are provided to the patients. Patients will not take part in assessment regarding possible burden of the interventions of this study.

EXPECTED LIMITATIONS AND DIFFICULTIES

Intraoperative fluorescence assessment of bowel perfusion is currently a subjective tool. This will most likely influence our results as over 30 different surgeons will interpret the

fluorescence output. Quantification of the NIR fluorescence signal would improve standardized assessment of tissue perfusion.

Using different NIR platforms (the Olympus Medical Imaging Video System and Laparoscope, and the Da Vinci Firefly) will have some influence on our results as well. Nevertheless, both systems are optimized for the detection of ICG, we therefore think its effect on our study results is minimal.

AL after colorectal surgery is a multifactorial complication. It is unclear which percentage of AL is solely based on compromised perfusion. It is especially questionable if compromised perfusion plays a role in late AL (> 7 days after surgery).

ETHICS AND DISSEMINATION

The study was approved by the certified Medical Ethics Review Committee Leiden, Den Haag, Delft (METC-LDD) on 11 November 2019 under identifier P19.079, and feasibility declarations as required by Dutch law, were obtained for the remaining hospitals. The protocol's current version (2.0) is dated 26 March 2020. The first patient was recruited on 2 July 2020 in LUMC. Six centres are currently enrolling patients. Protocol amendments will first be reviewed by the METC-LDD and after approval be shared with the participating centres for local feasibility declarations.

This study was prospectively registered at the Netherlands trial register (NL7502) and after the first inclusion registered at clinicaltrials.gov (NCT04712032). A manuscript with the results of this study will be published in a peer-reviewed journal. Moreover, the results will be shared via conference presentations.

AUTHOR CONTRIBUTIONS

RM, RF, OB, JB, EM, JM, KB, AV and DH all contributed to the study concept and design. HP was responsible for the statistical analysis plan and the sample size calculation. RM, RF and OB prepared the manuscript. JM, AV and DH supervised the manuscript preparation. All authors and members of the AVOID study group reviewed the manuscript before submission.

FUNDING STATEMENT

This research is funded by an Olympus Support Grant (2019-03-0077). The funder will have no role in the conduct of the study; collection, management, analysis and interpretation of the data; and decision to submit the manuscript for publication.

COMPETING INTERESTS STATEMENT

AV and LS are members of the Diagnostic Green advisory board. All other authors declare to have no competing interest concerning this work.

FIGURE LEGENDS

Figure 1 Surgical flowchart

ICG indocyanine green, NIRF Near-infrared, CRF case report form

REFERENCES

1. Buchs NC, Gervaz P, Secic M, et al. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *Int J Colorectal Dis* 2008;23(3):265-70. doi: 10.1007/s00384-007-0399-3
2. Chambers WM, Mortensen NJ. Postoperative leakage and abscess formation after colorectal surgery. *Best Pract Res Clin Gastroenterol* 2004;18(5):865-80. doi: 10.1016/j.bpg.2004.06.026
3. Bakker IS, Grossmann I, Henneman D, et al. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg* 2014;101(4):424-32; discussion 32. doi: 10.1002/bjs.9395
4. Walker KG, Bell SW, Rickard MJ, et al. Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer. *Ann Surg* 2004;240(2):255-9.
5. Isbister WH. Anastomotic leak in colorectal surgery: a single surgeon's experience. *ANZ J Surg* 2001;71(9):516-20.
6. Kang CY, Halabi WJ, Chaudhry OO, et al. Risk factors for anastomotic leakage after anterior resection for rectal cancer. *JAMA Surg* 2013;148(1):65-71. doi: 10.1001/2013.jamasurg.2
7. Platell C, Barwood N, Dorfmann G, et al. The incidence of anastomotic leaks in patients undergoing colorectal surgery. *Colorectal Dis* 2007;9(1):71-9. doi: 10.1111/j.1463-1318.2006.01002.x
8. Vignali A, Fazio VW, Lavery IC, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. *J Am Coll Surg* 1997;185(2):105-13.
9. Single-stage treatment for malignant left-sided colonic obstruction: a prospective randomized clinical trial comparing subtotal colectomy with segmental resection following intraoperative irrigation. The SCOTIA Study Group. Subtotal Colectomy versus On-table Irrigation and Anastomosis. *Br J Surg* 1995;82(12):1622-7.
10. Kockerling F, Rose J, Schneider C, et al. Laparoscopic colorectal anastomosis: risk of postoperative leakage. Results of a multicenter study. Laparoscopic Colorectal Surgery Study Group (LCSSG). *Surg Endosc* 1999;13(7):639-44.
11. Frasson M, Flor-Lorente B, Rodriguez JL, et al. Risk Factors for Anastomotic Leak After Colon Resection for Cancer: Multivariate Analysis and Nomogram From a Multicentric, Prospective, National Study With 3193 Patients. *Ann Surg* 2015;262(2):321-30. doi: 10.1097/SLA.0000000000000973
12. Senagore A, Lane FR, Lee E, et al. Bioabsorbable staple line reinforcement in restorative proctectomy and anterior resection: a randomized study. *Dis Colon Rectum* 2014;57(3):324-30. doi: 10.1097/DCR.0000000000000065
13. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients. *Ann Surg* 2004;240(2):260-8.
14. Yeh CY, Changchien CR, Wang JY, et al. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg* 2005;241(1):9-13.
15. Hyman N, Manchester TL, Osler T, et al. Anastomotic leaks after intestinal anastomosis: it's later than you think. *Ann Surg* 2007;245(2):254-8. doi: 10.1097/01.sla.0000225083.27182.85
16. Snijders HS, Henneman D, van Leersum NL, et al. Anastomotic leakage as an outcome measure for quality of colorectal cancer surgery. *BMJ Qual Saf* 2013;22(9):759-67. doi: 10.1136/bmjqs-2012-001644
17. Vignali A, Gianotti L, Braga M, et al. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. *Dis Colon Rectum* 2000;43(1):76-82.
18. Kologlu M, Yorganci K, Renda N, et al. Effect of local and remote ischemia-reperfusion injury on healing of colonic anastomoses. *Surgery* 2000;128(1):99-104. doi: 10.1067/msy.2000.107414

19. Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension as a predictor of colonic anastomotic healing. *Dis Colon Rectum* 1987;30(11):867-71.
20. Hirst NA, Tiernan JP, Millner PA, et al. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis* 2014;16(2):95-109. doi: 10.1111/codi.12411 [published Online First: 2013/09/03]
21. Karliczek A, Harlaar NJ, Zeebregts CJ, et al. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. *Int J Colorectal Dis* 2009;24(5):569-76. doi: 10.1007/s00384-009-0658-6 [published Online First: 2009/02/18]
22. van Manen L, Handgraaf HJM, Diana M, et al. A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *Journal of surgical oncology* 2018;118(2):283-300. doi: 10.1002/jso.25105 [published Online First: 2018/06/26]
23. Jafari MD, Wexner SD, Martz JE, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. *J Am Coll Surg* 2015;220(1):82-92 e1. doi: 10.1016/j.jamcollsurg.2014.09.015
24. van den Hoven P, Ooms S, van Manen L, et al. A systematic review of the use of near-infrared fluorescence imaging in patients with peripheral artery disease. *J Vasc Surg* 2019;70(1):286-97 e1. doi: 10.1016/j.jvs.2018.11.023 [published Online First: 2019/06/25]
25. Griffiths M, Chae MP, Rozen WM. Indocyanine green-based fluorescent angiography in breast reconstruction. *Gland Surg* 2016;5(2):133-49. doi: 10.3978/j.issn.2227-684X.2016.02.01 [published Online First: 2016/04/06]
26. Fox IJ, Brooker LG, Heseltine DW, et al. A tricarbocyanine dye for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturation. *Proc Staff Meet Mayo Clin* 1957;32(18):478-84. [published Online First: 1957/09/04]
27. Sauda K, Imasaka T, Ishibashi N. Determination of protein in human serum by high-performance liquid chromatography with semiconductor laser fluorometric detection. *Anal Chem* 1986;58(13):2649-53.
28. Landsman ML, Kwant G, Mook GA, et al. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol* 1976;40(4):575-83. doi: 10.1152/jappl.1976.40.4.575
29. Kudszus S, Roesel C, Schachtrupp A, et al. Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. *Langenbecks Arch Surg* 2010;395(8):1025-30. doi: 10.1007/s00423-010-0699-x
30. Boni L, Fingerhut A, Marzorati A, et al. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. *Surg Endosc* 2017;31(4):1836-40. doi: 10.1007/s00464-016-5181-6
31. Kim JC, Lee JL, Yoon YS, et al. Utility of indocyanine-green fluorescent imaging during robot-assisted sphincter-saving surgery on rectal cancer patients. *Int J Med Robot* 2016;12(4):710-17. doi: 10.1002/rcs.1710
32. Jafari MD, Lee KH, Halabi WJ, et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surg Endosc* 2013;27(8):3003-8. doi: 10.1007/s00464-013-2832-8
33. Song M, Liu J, Xia D, et al. Assessment of intraoperative use of indocyanine green fluorescence imaging on the incidence of anastomotic leakage after rectal cancer surgery: a PRISMA-compliant systematic review and meta-analysis. *Tech Coloproctol* 2021;25(1):49-58. doi: 10.1007/s10151-020-02335-1 [published Online First: 2020/09/05]
34. Chan DKH, Lee SKF, Ang JJ. Indocyanine green fluorescence angiography decreases the risk of colorectal anastomotic leakage: Systematic review and meta-analysis. *Surgery* 2020;168(6):1128-37. doi: 10.1016/j.surg.2020.08.024 [published Online First: 2020/10/05]
35. Kin C, Vo H, Welton L, et al. Equivocal effect of intraoperative fluorescence angiography on colorectal anastomotic leaks. *Dis Colon Rectum* 2015;58(6):582-7. doi: 10.1097/DCR.0000000000000320

36. World Medical A. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310(20):2191-4. doi: 10.1001/jama.2013.281053 [published Online First: 2013/10/22]

37. Dupont WD, Plummer WD, Jr. Power and sample size calculations. A review and computer program. *Control Clin Trials* 1990;11(2):116-28. doi: 10.1016/0197-2456(90)90005-m [published Online First: 1990/04/01]

38. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics* 1979;35(3):549-56.

39. Rahbari NN, Weitz J, Hohenberger W, et al. Definition and grading of anastomotic leakage following anterior resection of the rectum: a proposal by the International Study Group of Rectal Cancer. *Surgery* 2010;147(3):339-51. doi: 10.1016/j.surg.2009.10.012 [published Online First: 2009/12/17]

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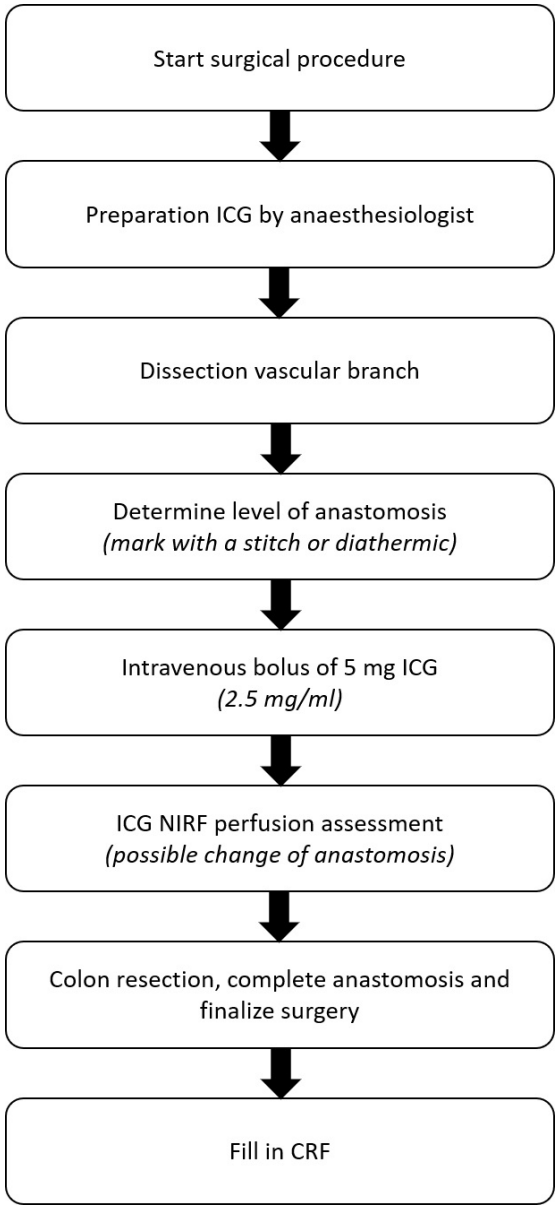

Figure 1 Surgical flowchart
ICG indocyanine green, NIRF Near-infrared, CRF case report form

13x28mm (1200 x 1200 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:

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		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	2

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	5-11
Protocol version	#3	Date and version identifier	12
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
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	12
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	10-11

		groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
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	4-5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5-6
Objectives	#7	Specific objectives or hypotheses	5
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)	5-11
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries	6

		where data will be collected. Reference to where list of study sites can be obtained	
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)	7
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
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)	n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8-9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg,	9

		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	
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)	8
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	10-11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	8

		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	
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	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6-8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a surgeons are always unblinded
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	10
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	n/a only 1 intervention moment
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	10-11
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	10-11
Statistics: additional	#20b	Methods for any additional analyses (eg,	10-11

analyses		subgroup and adjusted analyses)	
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)	10-11
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	n/a
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	11
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	#23	Frequency and procedures for auditing trial	11

		conduct, if any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
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)	12
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
6Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	10
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and	12-13

		each study site	
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	10
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	n/a
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	12
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and	n/a model consent in fully in Dutch and

		authorised surrogates	will therefore not be shared
Biological specimens	#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

Notes:

- 11b: n/a, patients can withdraw, but intervention will not be modified. Doses can not be changed.
- 11c: n/a there is only 1 intervention (during surgery) that a patient has to adhere to.
- 17b: n/a surgeons are always unblinded
- 18b: n/a only 1 intervention moment
- 32: n/a model consent in fully in Dutch and will therefore not be shared The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative Commons Attribution License CC-BY-NC. This checklist was completed on 09. March 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai