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ORIGINAL ARTICLE

Indocyanine green (ICG) fluorescence guided lymph node mapping for determination of resection margins in colon cancer – ISCAPE trial

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Abstract

Background Indocyanine green (ICG) lymphangiography for colon cancer has been regarded as a sentinel lymph node (LN) detection tool, but its repeatedly reported suboptimal sensitivity rates suggest that approach aiming to define locoregional lymphatic collector margins might be more efficient in guiding surgeon's decision making. Thus, present study was designed to determine if sensitivity of the latter approach is sufficient to guide resection margins' selection in colon cancer surgery.
Methods This is a prospective, single-centre, single-arm phase II interventional trial, including patients with histologically confirmed colon adenocarcinoma. ICG was injected subserosally in the beginning of operation, fluorescence was assessed 30 min after injection or later, when it became detectable. Primary endpoint was proportion of pN + patients in whom all metastatic lymph nodes were located within the area of fluorescence of lymphatics (AFL). Secondary endpoints included feasibility, safety, lymphatic spread patterns and proportion of resections modified based on ICG mapping.
Results Between 26 July 2022 and 27 February 2024, 101 patients underwent colectomies with intraoperative ICG lymphatic mapping. AFL was registered in all cases. Average lateral spread was 5.87 ± 3.20 proximally and 5.89 ± 2.54 cm distally. In two of 46 pN + cases affected LNs were discovered beyond AFL. ICG lymphatic mapping sensitivity was found to be 95.6%, which was beyond 0.960–0.990 interval, so null hypothesis was retained.
Conclusions In this trial, metastatic LNs were confined within ICG AFL in 95.6% of pN + cases. Although the predefined sensitivity threshold was not met, the result suggests potential for ICG mapping to guide resection margins in colon cancer surgery.

Keywords Indocyanine green (ICG) · Fluorescence lymphangiography · Fluorescence imaging · Lymphatic mapping · Colon cancer · Complete mesocolic excision (CME)

Introduction

Historically ICG lymphangiography for colon cancer (CC) was regarded as a sentinel LN detection (SLND) tool [1, 2]. The dye was injected near the tumour to map affected LNs (LNs). Multiple studies were conducted to investigate this method [3–7]. However, the accuracy was repeatedly reported as low. So, when two metanalyses were conducted in 2019, they both concluded that overall ICG performance for detection of LNs metastases was poor [1, 2].

Recent data suggest that ICG fluorescent image can show only patent lymphatics' anatomy. In cases of LN tissue replacement by cancer cells, LNs lose ability to filtrate lymph and become an obstacle to lymph flow. It also means that they become unable to uptake ICG [8, 9]. Mentioned specificity is supported by clinical data. For example,

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Kinoshita et al. in 2023 observed metastatic LNs both along ICG-stained and ICG-unstained vascular pedicles [10]. This feature makes ICG SLND concept invalid and suggests that another approach is needed.

ICG imaging of the whole locoregional lymphatic collector appears to be more beneficial. It was reported to help in identifying appropriate vascular pedicle [11, 12], guiding extension of resection plan [6, 7] and helping to increase LN harvest [13]. However, yet there is no data on accuracy of ICG lymphatic collector mapping.

Theoretically ICG visualization of locoregional lymphatic collector of the segment of the colon affect by a tumour provides a visual map of oncologically sufficient resection margins (RMs) both colonic and the colonic mesentery. Thus, present study was designed to test viability of this concept.

Methods

Present study (**ICG** fluore**SC**ence guided lymph node m**AP**ping for determination of r**E**section margins in colon cancer – ISCAPE) is a single centre interventional phase II trial with single group assignment aiming to investigate feasibility of defining colon RMs for CC with ICG by comparing lymphatic distribution of subserosally injected dye with actual spread of lymphatic metastases reported by pathologists after specimen examination. Trial's primary endpoint was proportion of pN + patients in which affected LNs were detected only within margins of ICG spread. Secondary endpoints were incidence of adverse events (AE) related to ICG lymphatic mapping (ICG LM), feasibility of ICG LM for CC, incidence of LN metastases outside conventional RMs (10 cm), CC lymphatic spread patterns and proportion of operations that were affected by ICG LM.

Eligible patients, who signed informed consent, were allocated for colonic resection with intraoperative ICG LM (Fig. 1). During pathological examination LNs were assessed for presence of metastases and location in relation to tumour and fluorescence margins (Fig. 2). The trial was registered at ClinicalTrials.gov NCT05468827 on 21.07.2022. Full study protocol was published earlier [14].

Patients

The study was conducted between 26 July 2022 and 27 February 2024 in N.N. Petrov NMRC of oncology in Saint Petersburg, Russia. Patients aged > 18 years were eligible if they had colon adenocarcinoma (T1-T4aN0-2bM0-1b), ECOG 0-2, clinical indication for elective resection and signed informed consent. Exclusion criteria included urgent surgery, adjacent organ invasion, carcinomatosis, hypersensitivity or adverse reactions to ICG or iodine, thyroid disorders, kidney or liver failure, or pregnancy. Eligibility criteria were defined in study protocol [14]. Eligible patients were allocated for colonic resection with intraoperative ICG LM. Analysis was performed after 101 ICG LM procedures.

Intervention

ICG solution was injected into colonic wall subserosally in the beginning of surgical procedures. ICG powder was diluted in sterile water to create 0.25% solution (Verdye™). The NIR mode of Olympus VISERA ELITE II was used to assess ICG fluorescence in cases of laparoscopic approach and laparotomy, in cases of robotics – Firefly Fluorescence Imaging of Da Vinci Xi system. Procedure specifications and specimen examination details were described in appendix S1 and S2 of study protocol [14].

Perioperative treatment

Preoperative investigations, treatment of co-morbidities, perioperative care and treatment for complications were provided according to local guidelines. Complications were registered according to Clavien–Dindo classification[15].

Sample size calculation

Considering its clinical significance, sensitivity was chosen as primary end point assessment method. It was assumed, that sensitivity of 99% would be sufficient to affect decision making on RMs. Sample size of 101 patients was calculated using Buderer method [16] with a confidence level (1-α) of 0.95 as a minimum of cases required to test accuracy of ICG lymphatic mapping for estimated sensitivity of 0.99 and precision of 0.03.

Statistical analysis

Primary endpoint was assessed using standard formula for sensitivity calculation. Secondary endpoints were assessed with descriptive statistics, parametric and non-parametric tests. Descriptive statistics and significance tests were performed with SPSS® software (IBM, Armonk, New York, USA). ITT (intention-to-treat) and PP (per-protocol) analysis of AE were performed to avoid underestimation of potential harms of ICG LM procedure.

Results

Patient enrollment

In total, 108 Patients signed an informed consent and underwent screening to participate in the trial. Demographic data are presented in Table 1 [17]. Among consented patients,

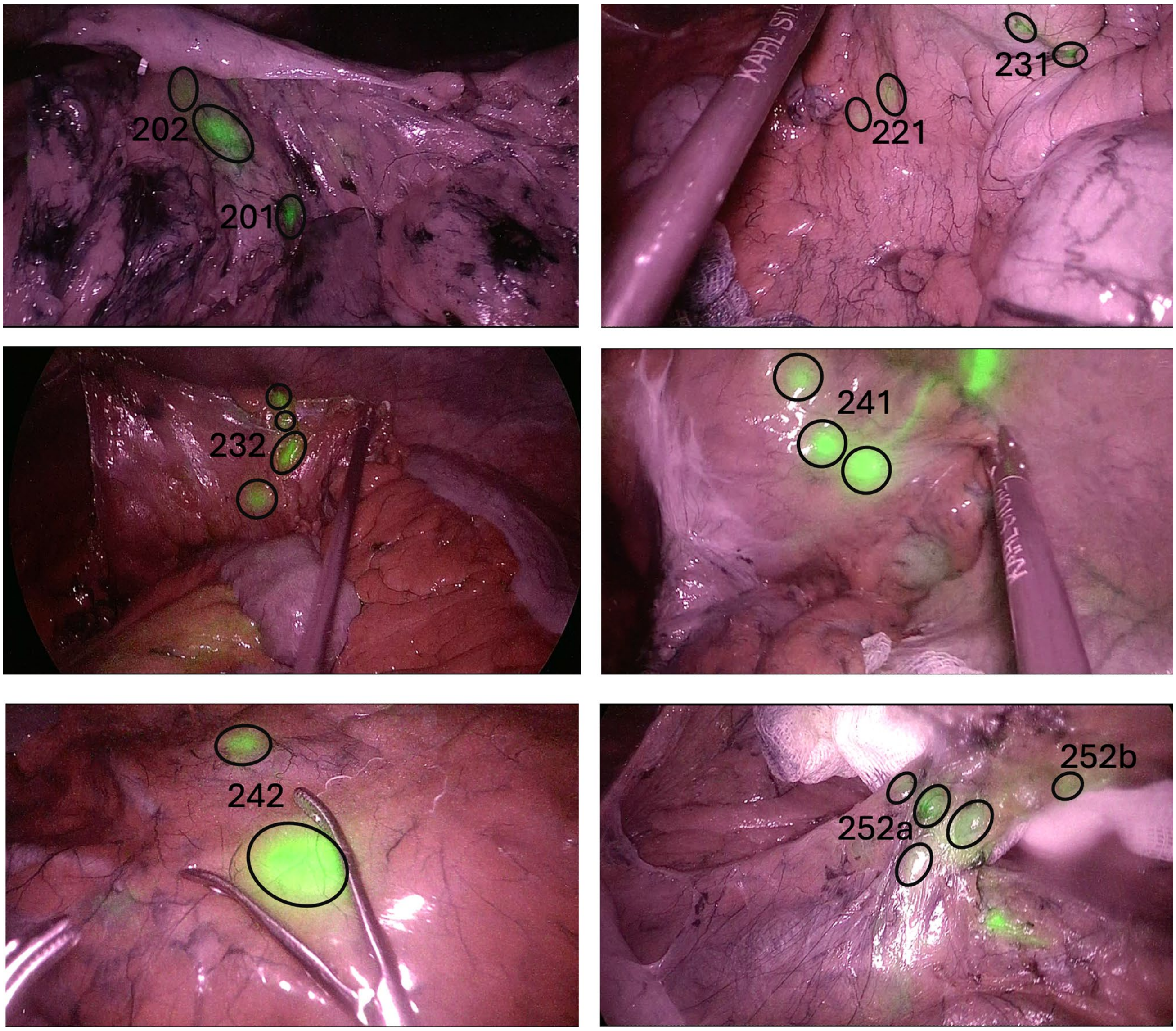

Fig. 1 Examples of fluorescence of LN groups recorded during surgical procedure. Legend: 201 – paracolic LNs (D1) of ileocolic artery, 202 – mesocolic LNs (D2) of ileocolic artery, 221 – D1 of middle colic artery, 231 – D1 of left colic artery, 232 – D2 of left colic artery, 241 – D1 of sigmoid arteries, 242 – D2 of sigmoid arteries, 252b D2 along inferior mesenteric artery (IMA) distal to the point of origin of left colic artery, 252a D2 along IMA proximal to the point of origin of left colic artery. Fluorescence of paracolic nodes and along vascular tie was recorded and depicted to ensure proper lymphatic node group and ICG spread marking after specimen removal

94.37% (101) underwent ICG LM procedure. Out of those, who did not receive it, one patient had atrial fibrillation on the day of surgery, in two cases NIR camera was unavailable and in four cases the number of peritoneal adhesions made visualisation of tumour impossible prior to mobilisation step. Data on all enrolled patients (108) was included into ITT analysis; PP analyses was performed using data on patients who underwent ICG LM (101).

Surgical procedures

Data on surgical procedures are presented in Table 2.

Eleven patients underwent high anterior instead of sigmoid resection to achieve sufficient distal clearance. One patient received proctocolectomy owing to familiar adenomatous polyposis. Two sigmoid resections included simultaneous procedures: removal of an abnormally looking ovary and resection of abdominal wall and left gonadal vessels, respectively.

There were three cases of intraoperative complications – all vessel injuries: ileocolic vein, small bowel mesentery vessels and right superior colic vein (Supplementary Table 1) In all situations vessels were clipped, no consequences recorded, no relation to ICG injection.

Fig. 2 Example of specimen marking (left to right – right hemicolectomy, splenic flexure resection, sigmoid resection). Legend: Tags are attached to the specimen according to Japanese classification of lymphatic node groups [31]. Segments of D1 collector are named according to Japanese Classification of pericolic lymphatic collector groups [25]. IO (ICG oral direction spread) and IA (ICG anal direction spread) marks are borders of D1 ICG spread visualized during surgical procedure. So, the area of ICG fluorescence is projected on lymphatic node groups harvested during surgery

Safety assessment

No ICG or mapping procedure-related AE were recorded during the study. Overall percentage of complications did not exceed average statistics of our centre – 33.33% (36 of 108).

Postoperative period was uneventful for 69.31% (72) of patients who underwent ICG LM and 28.57% (2) of subjects who could not receive the allocated procedure. Majority of registered AE were classified as Clavien–Dindo 1–2 and required medical treatment. Proportions of more severe AE were higher in the group of patients, where ICG LM was not performed. Complications, classified as Clavien–Dindo 4a happened in 2.97% (3 patients) in PP group and 28.57% (2 patients) in no ICG group. The only death was recorded in a patient who could not undergo ICG mapping due to multiple peritoneal adhesions. Data on severity of recorded AE are presented in Supplementary Table 1 and their range is specified in Supplementary Table 2.

ICG lymphatic mapping

In all mapping procedures area of fluorescence of lymphatics (AFL) was visualised. D1 collector presented identifiable fluorescence in all cases, but lymphatics of vascular tie could not be appreciated in one of the patients. Data on intraoperative fluorescence assessment are presented in Supplementary Table 3.

Time until appearance of AFL was recorded. In 12.87% (12 cases) AFL along vascular tie was appreciated within 30 min from injection, in 44.55% (45 cases) fluorescence took 30–45 min, in 28.71% (29 cases) fluorescence took 45–60 min and in 13.86% (14 cases) fluorescence took more than an hour to become detectable. Similar pattern was encountered for D1 fluorescence: less than 30 min – 6.93% (7 cases), 30–45 min – 48.51% (49 cases), 45–60 min – 29.70% (30 cases), more than 60 min – 31.25% (15 cases).

Mentioned time gaps resulted in fluorescence assessment at various steps of surgical procedure. Thus, only laparoscopic assessment was performed in 37.62% (38 cases) of cases. In 9.90% (10 cases), despite presence of fluorescence on laparoscopy, proper mesentery assessment could not be completed, and margins of ICG spread were also assessed during minilaparotomy before specimen removal. And in 48.51% (49 cases), fluorescence could not be appreciated up until minilaparotomy step. In 3.96% of cases, assessment was performed during open surgery (two laparotomies and two conversions from laparoscopy to laparotomy).

D1 fluorescence was present in all cases. It was registered strictly within margins of initial resection plan in 98.02% (99 patients). In two cases, one splenic flexure tumour and one sigmoid tumour, fluorescence was seen beyond planned RMs. In both cases, RMs were extended, but no metastatic LN were found (pN0).

Distance of ICG lateral spread within D1 lymphatic collector was a subject of significant variability with no obvious connection to location of primary tumour or direction of lymphatic flow. Data on distances of ICG D1 lymphatic spread according to pathological reports are presented in Table 3. Average distance of ICG spread in proximal direction was 5.87 ± 3.20 cm (1.50–23.00 cm), in distal direction – 5.89 ± 2.54 cm (2.00–15.00 cm).

Table 1 Demographics

Age	63.99 ± 10.29 (35–88) years
Sex	Male 49.07% (53/108)
	Female 50.93% (55/108)
Body mass index	27.2 ± 4.9 (20.9–41.3)
Tumour location	Caecum 5.56% (6/108)
	Ascending colon 17.59% (19/108)
	Hepatic flexure 3.70% (4/108)
	Proximal portion of transverse colon 2.78% (3/108)
	Middle third of transverse colon 2.78% (3/108)
	Distal portion of transverse colon 4.63% (5/108)
	Splenic flexure 4.63% (5/108)
	Descending colon 12.04% (13/108)
	Sigmoid colon 46.29% (50/108)
Tumour size	4.72 ± 1.92 (1.2–10) cm
Tumour pathology	Adenocarcinoma low grade 93.52% (101/108)
	Adenocarcinoma high grade 1.85% (2/108)
	Adenocarcinoma (grade N/A) 2.78% (3/108)
	Signet cell tumour 1.85% (2/108)
cT	T1 2.78% (3/108)
	T2 20.37% (22/108)
	T3 64.81% (70/108)
	T4a 12.04% (13/108)
cN	N0 25.00% (27/108)
	N1a 23.15% (25/108)
	N1b 28.70% (31/108)
	N1c 0% (0)
	N2a 15.74% (17/108)
	N2b 7.41% (8/108)
cM	M0 92.59% (100/108)
	M1a 7.41% (8/108)
	M1b 0% (0)

cT clinical tumour stage, *T1* tumour invasion into (but not through) submucosa, *T2* – tumour invasion into (but not through) muscular layer, *T3* tumour invasion through muscular layer into subserosa, or into non-peritonealised pericolic tissues, *T4a* tumour invasion of the visceral peritoneal layer, *cN* clinical nodal status, *N0* no evidence of nodal involvement, *N1a* involvement of one regional node, *N1b* involvement of 2–3 regional nodes, *N1c* deposits involving serosa or non-peritonealised pericolic/perirectal tissues without regional nodal metastasis, *N2a* involvement of 4–6 nodes, *N2b* involvement of ≥ 7 nodes, *cM* clinical evidence on distal metastases, *M0* no evidence of metastases, *M1a* distant metastases confined to one organ, *M1b* distant metastases to more than one organ, but without peritoneal metastases [15]

Regarding AFL along vascular tie, it was encountered in 99.01% (100 cases). Case with no recorded fluorescence along vascular pedicle were classified as pN0. During 87.13% (88) procedures fluorescence was observed along one vascular pedicle, and in 11.88% (12 cases) of cases two fluorescent pedicles were encountered. The latter group did not show any relationship between presence of fluorescence

Table 2 Surgical procedures

	ITT (107 patients*)	PP (101 patients)
Type of surgery		
Right hemicolectomy	21.10% (29)	25.74% (26)
Extended right hemicolectomy	7.47% (8)	6.93% (7)
Transverse colon resection	0.93% (1)	0.99% (1)
Extended left hemicolectomy	1.87% (2)	1.98% (2)
Splenic flexure resection	12.15% (13)	12.87% (13)
Left hemicolectomy	3.74% (4)	3.96% (4)
Sigmoid resection	35.51% (38)	36.63% (37)
High anterior resection	10.28% (11)	9.90% (10)
Proctocolectomy	0.93% (1)	0.99% (1)
Approach		
Laparoscopy	82.24% (88)	82.18% (83)
Laparotomy	1.87% (2)	1.98% (2)
Conversion: laparoscopy to open	1.87% (2)	1.98% (2)
Robot	14.02% (15)	13.86% (14)

*Data on 107 of 108 enrolled patients available for ITT analysis, because one of enrolled patients had atrial fibrillation on day of surgery and did not undergo surgical procedure, and therefore data on that case are not included into surgical procedures analysis

ITT intention-to-treat analysis, *PP* per-protocol analysis

and LN metastatic involvement: 41.67% (five cases) were pN0, 41.67% (five cases) were pN + without D2 involvement, 8.33% (one case) had lymphatic metastases along one pedicle and 8.33% (one case) had lymphatic metastases along both pedicles.

Specimen examination

Pathological examination included both investigation of conventional parameters and comparison of metastatic spread to ICG distribution. Data on pathological examination results are presented in Supplementary Table 4. According to pathological reports, 45.54% (46 cases) were found to be pN +. Data on the relationship between metastatic LNs, ICG distribution and RMs for these patients are presented in Table 4.

Of all pN + cases, 95.65% (44) had D1 involvement. Invasion of LNs along vascular pedicle (s) was present in 47.83% (18 cases), and 4.35% (2 cases) had LN metastases in D2 ± D3 nodes skipping D1 level. Regarding lateral spread, LNs within 5 cm of the primary tumour were affected in 34.78% (16 cases), and in 4.35% (2 cases) this was encountered without metastatic involvement of nodes adjacent to the tumour. Lymphatic metastases within 5–10 cm from tumour were seen in 6.52% (three cases). There was one case (2.17%) with metastatic involvement of D1 LNs adjacent to the tumour and a single LN metastasis in

Table 3 ICG distribution in different tumour locations according to pathological report

	Proximal ICG spread (cm)	Distal ICG spread (cm)
Caecum (6 cases)	7.33+/-5.81 (3.00 – 19.00)	8.00+/-1.64 (6.00 – 10.00)
Ascending colon (15 cases)	4.70+/-2.29 (1.50 – 9.00)	6.93+/-3.16 (2.00 – 13.00)
Hepatic flexure (5 cases)	9.70+/-7.65 (4.50 – 23.00)	6.90+/-2.19 (4.00 – 10.00)
Proximal part of transverse colon (3 cases)	3.00+/-1.32 (2.00 – 4.50)	4.00+/-1.50 (2.50 – 5.50)
Middle part of transverse colon (2 cases)	8.50+/-0.71 (8.00 – 9.00)	4.50+/-0.71 (4.00 – 500)
Distal part of transverse colon (4 cases)	7.33+/-6.00 (4.00 – 16.30)	6.63+/-0.48 (6.00 – 7.00)
Splenic flexure (5 cases)	7.20+/-2.39 (5.00 – 11.00)	7.70+/-2.33 (5.00 – 9.00)
Descending colon (13 cases)	5.46+/-2.13 (2.50 – 10.00)	7.54+/-3.44 (3.00 – 15.00)
Sigmoid (48 cases)	5,74+/-2.08 (2.00 – 11.00)	4.97+/-1.86 (2.00 – 11.00)
All (101 cases)	5.87+/-3.20 (1.50 – 23.00)	5.89+/-2.54 (2.00 – 15.00)
pN+ (46 cases)	5.98+/-3.55 (1.5 – 23.00)	5.90+/-2.19 (2.0 – 13.00)
pN0 (55 cases)	5.78+/-2.91 (2.00 – 19.00)	5.89+/-2.82 (2.00 – 12.00)

Results of ICG lateral spread along pericolic lymph nodes for 101 patients who underwent ICG lympatic mappig procedure

Table 4 Relationship between ICG distribution and presence of metastases in lymphatic nodes

	Proximal direction D1 spread				NT (tumour)	Distal direction D1 spread				D2-3 collector metastases
	Beyond 15 cm	N3O (10 - 15 cm)	N2O (5 – 10 cm)	N1O (0 – 5 cm)		N1A (0 – 5 cm)	N2A (5 – 10 cm)	N3A (10 – 15 cm)	Beyond 15 cm	
Caecum (case 1)					201 (6 /11)	211 (0/2)				202 (0/6) 203 (0/2)
Caecum (case 2)					201 (0/4)	201 (2/5)				202 (0/8)
Ascending colon (case 1)		201 (0/3)		201 (0/2)	211 (1/9)	221 (0/3)	211 (0/2)			202 (0/5)
Ascending colon (case 2)		201 (0/1)	201 (0/2)	201 (0/4)	201 (1/7)	211 (0/4)				202 (0/1) 203 (0/3) 212 (0/2)
Ascending colon (case 3)			201 (0/3)		201 (2/13)	201 (0/1)				202 (1/5)
Ascending colon (case 4)				201 (2/7)	201 (1/9)	211 (0/3)	211 (0/3)			202 (0/1) 212 (0/4)
Ascending colon (case 5)				201 (0/2)	201 (0/8)			211 (0/3)		202 (2/9) 203 (0/7)
Ascending colon (case 6)					201 (4/6)	201 (0/2)	201 (0/2)	211 (0/1)		202 (0/2)
Ascending colon (case 7)		201 (0/1)	201 (0/2)	201 (0/3)	211 (4/7)	211 (0/2)	211 (0/2)			202 (0/7) 203 (0/1) 223 (0/2)
Ascending colon (case 8)				211 (1/6)	201 (5/9)	211 (1/6)				202 (1/3) 203 (3/4)
Hepatic flexure (case 1)		201 (0/1)	201 (0/1)	221 (0/3)	221 (1/4)	221 (0/1)				202 (0/5) 203 (0/2)
Hepatic flexure (case 2)		201 (0/2)	211 (0/2)	211 (0/3)	211 (1/6)	221 (0/2)	221 (1/2)	221 (0/1)		202 (0/3) 203 (0/3) 222r (0/5) 223 (0/12)
Proximal transverse colon (case 1)		201 (0/5)	211 (0/1)	221 (0/4)	221 (4/7)	221 (2/3)	221 (0/1)			222r (2/3)
Proximal transverse colon (case 2)	201 (1/15)			211 (0/5)	211 (3/6)	221 (0/3)	221 (0/1)			221 (0/4)
Distal transverse colon (case 1)				221 (0/2)	221 (4/6)	221 (1/1)				222l (2/2) 232 (0/2)
Distal transverse colon (case 2)		201 (0/7)	211 (0/3)		221 (0/3)	221 (0/1)				222r (0/3) 222l (0/5) 223 (0/4)
Distal transverse colon (case 3)			221 (0/1)	221 (0/5)	221 (1/10)	221 (0/2)	231 (0/2)			222l (0/1) 223 (0/1)
Splenic flexure (case 1)			221 (0/3)	231 (1/2)	231 (4/4)	231 (0/5)				232 (0/3)
Splenic flexure (case 2)				221 (1/6)	231 (4/6)	231 (4/6)	231 (0/4)			222l (0/4) 232 (0/1)
Descending colon (case 1)				231 (0/1)	231 (5/7)		241 (0/3)			222l (0/1) 232 (2/4)
Descending colon (case 2)			221 (0/1)		231 (8/10)	241 (3/8)	241 (0/5)			232 (4/7) 242 (2/2) 252a (1/1) 253 (2/3)
Descending colon (case 3)			221 (0/2)	231 (0/3)	231 (1/7)	241 (0/2)				232 (0/1) 242 (0/3) 252b (0/2) 253 (0/2)
Descending colon (case 4)			231 (0/3)	231 (0/2)	231 (2/11)	231 (0/5)	231 (0/1)	231 (0/1)		222l (0/1) 232 (0/3)
Sigmoid (case 1)		241 (0/1)	241 (0/1)	241 (2/10)	241 (2/3)	241 (2/10)	251 (0/1)			242 (2/9) 252b (0/4)
Sigmoid (case 2)		241 (0/2)	241 (0/2)	241 (2/2)	241 (3/4)	241 (0/2)	251 (0/2)			242 (2/2) 252b (0/4)
Sigmoid (case 3)		241 (0/1)	241 (0/1)	241 (10/11)	241 (14/14)	241 (11/15)	241 (0/1)			242 (2/8) 252b (1/2) 252a (0/1)
Sigmoid (case 4)		241 (0/3)	241 (1/3)	241 (8/8)	241 (8/8)	241 (4/4)	241 (0/1)			242 (0/2) 252b (0/3) 252a (4/6) 253 (1/3)
Sigmoid (case 5)				241 (0/6)	241 (2/7)	241 (4/12)	241 (0/3)			242 (4/8) 252a (0/1)
Sigmoid (case 6)			241 (0/1)	241 (0/1)	241 (4/8)	241 (0/7)	241 (0/2)	251 (0/1)		242 (0/3) 252b (1/7) 252a (0/2)
Sigmoid (case 7)			241 (0/1)	241 (1/4)	241 (2/10)	241 (0/1)	241 (0/1)	251 (0/1)		242 (0/5) 252b (0/11)
Sigmoid (case 8)				241 (1/3)	241 (2/3)	241 (0/4)				242 (0/3) 252a (0/3)
Sigmoid (case 9)			241 (0/1)	241 (0/6)	241 (1/8)	241 (0/5)	241 (0/5)			242 (3/7)
Sigmoid (case 10)			241 (0/3)	241 (0/3)	241 (3/3)	241 (0/3)	241 (0/3)	251 (0/2)		242 (0/2) 252b (0/1) 252a (0/1)
Sigmoid (case 11)			241 (0/2)	241 (0/1)	241 (2/10)	251 (0/3)				242 (0/2) 252b (0/1) 252a (0/4)
Sigmoid (case 12)				241 (0/3)	241 (1/3)	241 (0/3)	241 (0/1)	251 (0/1)		252a (0/2)
Sigmoid (case 13)			241 (0/2)	241 (0/2)	241 (3/8)	251 (0/6)				252b (0/3)

Table 4 (continued)

	Proximal direction D1 spread				NT (tumour)	Distal direction D1 spread				D2-3 collector metastases
	Beyond 15 cm	N3O (10 - 15 cm)	N2O (5 – 10 cm)	N1O (0 – 5 cm)		N1A (0 – 5 cm)	N2A (5 – 10 cm)	N3A (10 – 15 cm)	Beyond 15 cm	
Sigmoid (case 14)					241 (1/2)					242 (1/1) 252b (0/3) 252 (0/6)
Sigmoid (case 15)				241 (0/1)	241 (3/4)	251 (0/2)				252b (0/1) 252a (0/1)
Sigmoid (case 16)				241 (0/5)	241 (2/8)	241 (0/4)				242 (1/2) 252b (0/1) 252a (0/1)
Sigmoid (case 17)					241 (2/6)	241 (0/6)		241 (0/1)		242 (0/2) 252b (0/1)
Sigmoid (case 18)			241 (0/2)	241 (0/3)	241 (2/4)	241 (0/2)	251 (0/1)			242 (1/4) 252b (0/1) 252a (0/1)
Sigmoid (case 19)					241 (2/5)	241 (0/1)	241 (0/3)			242 (0/2) 252b (0/1) 252a (0/1)
Sigmoid (case 20)							251 (0/4)			242 (1/2) 252b (0/1) 252a (0/2)
Sigmoid (case 21)				241 (0/4)	241 (1/5)	241 (0/4)	241 (0/1)	241 (0/1)		242 (0/11) 252b (0/7)
Sigmoid (case 22)			241 (0/1)	241 (0/3)	241 (1/7)		251 (0/2)			242 (0/3) 252b (0/4) 252a (0/2)
Sigmoid (case 23)				241 (0/6)	241 (1/11)	241 (0/3)	241 (0/3)			242 (0/1) 252b (0/5)

Data on relation between ICG distribution and presence of metastases in lymphatic nodes for cases with lymph node metastases according to histopathology report. Grey color represents segments of pericolic (D1) collector removed during surgical procedure, Green color represents segments of D1 collector or vascular pedicle which where fluorescence was registered, Red color represents segments of D1 collector or vascular pedicle, where metastatic lymph noded were found during pathological examination, NT – pericolic lymph nodes adjacent to the tumour, N1O – pericolic lymp nodes located 0-5 cm cranially, N2O – pericolic lymph nodes located 5-10 cm cranially, N3O – pericolic lymp nodes located 10-15 cm cranially, N1A – pericolic lymp nodes located 0-5 cm caudally, N2O – pericolic lymph nodes located 5-10 cm caudally, N3O – pericolic lymp nodes located 10-15 cm caudally.

15 cm from primary tumour, with no lymphatic metastases between them.

In 43.6% (44 cases) lymphatic metastases were found only within borders of AFL appreciated intraoperatively. In two cases, lymphatic metastases were discovered outside AFL: one case of sigmoid tumour with subtotal involvement of adjacent pericolic LNs (sigmoid case 4 in Table 4) and one case of proximal transverse colon tumour with involvement of 3 D1 nodes adjacent to tumour and 1–15 cm proximally (proximal transverse colon case 2 in Table 4). In both cases non-fluorescent metastatic nodes were discovered proximally to the tumour. Given the low number of cases, it was difficult to conclude if it was coincidence.

Accuracy calculations

After completion of enrolment a 2×2 diagnostic accuracy table was created (Table 5). Accuracy calculations were performed using standard formulas. Sensitivity $= 44/(44+2) = 0.956$. Specificity $= 0/(0+55) = 0$. Positive predictive value (PPV) $= 44/(44+55) = 0.444$. Negative predictive value (NPV) $= 0/(0+2) = 0$. Accuracy $= (44+0)/(44+0+55+2) = 0.436$. Since sensitivity was found to be 0.956, which is beyond 0.960–0.990 interval, null hypothesis was not rejected.

Table 5 Accuracy calculations

	pN+	pN0
Fluorescence registered	Lymph nodes only within fluorescence margins (TP) – **44 cases**	pN0 in presence of fluorescence (FP) – **55 cases**
Fluorescence not registered	pN + with metastatic lymph nodes outside fluorescence zone/in the absence of fluorescence – false negative (FN) – **2 cases**	No fluorescence in pN0 (TN)- **0 cases**

TP true positive, *TN* true negative, *FN* false negative, *FP* false positive

Discussion

The primary aim of this study was to assess sensitivity of ICG LM for CC by comparing borders of fluorescence appreciated intraoperatively to results of pathological report. The calculated sensitivity turned out to be beyond confidence interval, so we could not conclude that it was sufficient. However, despite accepting null hypothesis, the calculated sensitivity of 0.956 was still very high and much higher than figures obtained in studies focusing on sentinel LN retrieval approach [1, 2]. It might mean that approach to ICG LM analysed in this study is more reliable than previously described ones; however, larger studies with more advanced pathological examination will be needed to prove that.

Regarding calculation of other accuracy parameters, PPV and NPV were as low as 0.444 and 0, with accuracy of 0.436. This situation is caused by initial choice of study hypothesis and primary end point calculation aiming at investigating sensitivity. A case of affected LNs only within AFL was regarded as a true positive case, no fluorescence in pN0 – true negative, pN + because of metastatic LNs outside AFL – false negative, pN0 despite presence of fluorescence – false positive. Even though this is an obvious limitation of the study, such approach allowed to focus on the most clinically relevant point of ICG LM for CC, the ability to highlight the area of potential/actual metastatic spread.

There were two cases when metastatic LNs were discovered beyond AFL. It might have been because they were located too deep within the mesentery to locate them with near-infrared light, absence of fluorescence because of metastatic replacement of tissue, or inefficient examination of mesentery. Pathological examination did not include methods allowing to check ICG uptake or proportion of LN metastatic involvement, so it was impossible to clarify the exact reasons behind failure to register fluorescence in those cases. This fact can be considered as a limitation to the present study, and suggests that more studies are needed to investigate reasons behind failure to identify metastatic LN during ICG LM procedure.

In current study presence of fluorescence was reassessed up until it appeared. This resulted in visualizing it in all cases and finding out that in 13.86% cases vascular tie fluorescence and in 31.25% cases D1 fluorescence took more than an hour to become detectable. Such result is different from figures obtained by other authors, who repeatedly reported lower detection rates [3, 18, 19]. However, the idea of extended observation helping to improve detection rates, although not currently being universally accepted, was previously mentioned. There are studies where 30–60 min after surgery was regarded as a timeframe when in majority of cases fluorescence starts to appear [10, 20–23]. So, even though biased by the aim to detect fluorescence in as many cases as possible, we obtained figures supporting concept of extended observation time and suggesting that lymphatics' fluorescence has a potential to appear in all cases. Currently there is no reliable data on factors that can predict time until detectable fluorescence, only theoretical considerations on the role ICG dosage and concentration, and patient's body mass index and tumour stage. Thus, a trial focusing specifically on influence of various factors on time until intelligible fluorescence is necessary for better understanding of time required for the fluorescence to appear, primarily to allow planning of fluorescence assessment during a surgical procedure.

In the present study fluorescence was registered strictly within margins of initial resection plan in 98.02% (99 patients) of patients. This figure is in line with considerations of some authors [4, 24], but does not match other results [6, 7]. Possible explanation of this situation is presence of differences in understanding of "conventional RMs". Currently, there are two concepts of oncologically sufficient colonic resection. The "eastern," or "D3" or "segmental" colectomy approach focuses on feeding vessel ligation at the point of origin with relatively small proximal and distal colon resection clearance explained by available data on risks of D1 node groups involvement [25]. Complete mesocolic excision concept is contrary focusing on extended RMs and maximum LN harvest with emphasis on following embryonic plane between mesocolic and retroperitoneal fascia [26]. There are data on specimens obtained via segmental, "eastern" or "D3" approach being smaller than those resected as per CME rules [27]. This fact can explain why some investigators see ICG LM as a tool to decrease and tailor RMs to avoid established CME [4, 24], while others regard it as a way to extend plane of resection and retrieve more potentially metastatic LNs [6, 7, 11, 12, 28, 29]. In the present study an average of 25.36 ± 12.03 cm of colon were resected, because the centre, where it was conducted, follows principles of CME surgery when establishing resection plan [26, 30].

Regarding comparison of AFL and actual LN metastatic involvement, the current study confirmed that NIR picture reflects functional lymphatic pathways draining certain colon segment. Various relationships between D1 metastases and ICG spread were recorded (see Table 4) without any specific relation to tumour characteristics and N stage. The same type of variability was seen for fluorescence and metastatic involvement of vascular pedicles.

During analysis of AE, it was noted that the complication rate in PP group (30.69%) was significantly lower than among patients who could not undergo mapping procedure (71.43%). Even though, comparison including an extremely small sample (seven subjects) should not be performed owing to high risk of both type 1 and 2 errors, and no calculations were performed to compare samples, we noted a tendency of high interest that is worth being discussed. The main reason for cancelling mapping procedure was number of peritoneal adhesions rendering visualisation of tumour impossible prior to mobilization step. It made those operations more challenging and might have resulted in more eventful patient recovery.

Overall, the present trial design allowed us to obtain and describe relationships between AFL that were created during ICG LM and assess the actual spread of lymphatic metastases. It was shown that in majority of cases, the area of metastatic LN involvement is included into AFL, and the obtained sensitivity suggests that ICG LM might have a potential to guide RM in CC surgery.

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Author contributions L.P. prepared material and wrote the main manuscript; A.K. and A.P. participated in study design and and critical review of the data; A.M., T.G., M.K. and M.S. reviewed and edited the sections related to pathological examination of the specimen. All authors reviewed the manuscript.

Data availability Authors declare that data supporting this study are available within the paper and its Supplementary information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Trial protocol and informed consent form were approved by the Ethics Committee of NMRC of Oncology named after N.N. Petrov on 23 December 2023, protocol no. 8/328. No significant amendments were made to trial protocol after its launch.

References

1. Ankersmit M, Bonjer HJ, Hannink G, Schoonmade LJ, Van Der Pas MHGM, Meijerink WJHJ (2019) Near-infrared fluorescence imaging for sentinel lymph node identification in colon cancer: a prospective single-center study and systematic review with meta-analysis. Tech Coloproctol 23(12):1113–1126
2. Villegas-Tovar E, Jimenez-Lillo J, Jimenez-Valerio V, Diaz-Giron-Gidi A, Faes-Petersen R, Otero-Piñeiro A, De Lacy FB, Martinez-Portilla RJ, Lacy AM (2020) Performance of indocyanine green for sentinel lymph node mapping and lymph node metastasis in colorectal cancer: a diagnostic test accuracy meta-analysis. Surg Endosc 34(3):1035–1047
3. Currie AC, Brigic A, Thomas-Gibson S, Suzuki N, Moorghen M, Jenkins JT, Faiz OD, Kennedy RH (2017) A pilot study to assess near infrared laparoscopy with indocyanine green (ICG) for intraoperative sentinel lymph node mapping in early colon cancer. Eur J Surg Oncol 43(11):2044–2051
4. Chand M, Keller DS, Joshi HM, Devoto L, Rodriguez-Justo M, Cohen R (2018) Feasibility of fluorescence lymph node imaging in colon cancer: FLICC. Tech Coloproctol 22(4):271–277
5. Emile SH, Elfeki H, Shalaby M, Sakr A, Sileri P, Laurberg S, Wexner SD (2017) Sensitivity and specificity of indocyanine green near-infrared fluorescence imaging in detection of metastatic lymph nodes in colorectal cancer: systematic review and meta-analysis. J Surg Oncol 116(6):730–740
6. Cahill RA, Anderson M, Wang LM, Lindsey I, Cunningham C, Mortensen NJ (2012) Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. Surg Endosc 26(1):197–204
7. Nishigori N, Koyama F, Nakagawa T, Nakamura S, Ueda T, Inoue T, Kawasaki K, Obara S, Nakamoto T, Fujii H et al (2016) Visualization of lymph/blood flow in laparoscopic colorectal cancer surgery by ICG fluorescence imaging (Lap-IGFI). Ann Surg Oncol 23(Suppl 2):S266-274
8. Ushijima H, Kawamura J, Ueda K, Yane Y, Yoshioka Y, Daito K, Tokoro T, Hida J-I, Okuno K (2020) Visualization of lymphatic flow in laparoscopic colon cancer surgery using indocyanine green fluorescence imaging. Sci Rep. https://doi.org/10.1038/s41598-020-71215-3
9. Kakizoe M, Watanabe J, Suwa Y, Nakagawa K, Suwa H, Ozawa M, Ishibe A, Masui H, Nagahori K (2021) The histopathological evaluation based on the indocyanine green fluorescence imaging of regional lymph node metastasis of splenic flexural colon cancer by near-infrared observation. Int J Colorectal Dis 36(4):717–723
10. Kinoshita H, Kawada K, Itatani Y, Okamura R, Oshima N, Okada T, Hida K, Obama K (2023) Timing of real-time indocyanine green fluorescence visualization for lymph node dissection during laparoscopic colon cancer surgery. Langenbecks Arch Surg 408(1):38
11. Watanabe J, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K (2016) Real-time indocyanine green fluorescence imaging-guided complete mesocolic excision in laparoscopic flexural colon cancer surgery. Dis Colon Rectum 59(7):701–705
12. Watanabe J, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K (2017) Evaluation of lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time indocyanine green fluorescence imaging. Int J Colorectal Dis 32(2):201–207
13. Daibo S, Watanabe J, Suwa H, Sato S, Suwa Y, Ozawa M, Ishibe A, Endo I (2024) Short-term and mid-term outcomes of indocyanine green fluorescence imaging-guided laparoscopic right-sided colectomy: a propensity score-matched cohort study. Dis Colon Rectum 67(1):82–89

14. Panaiotti L, Karachun A, Muravtseva A, Petrov A: Protocol for a phase II interventional trial investigating indocyanine green (ICG) fluorescence guided lymph node mapping for determination of bowel resection margins in colon cancer (ISCAPE). *International Journal of Surgery Protocols* https://doi.org/10.1097/SP1099.0000000000000041.
15. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, de Santibañes E, Pekolj J, Slankamenac K, Bassi C et al (2009) The clavien-dindo classification of surgical complications: five-year experience. Ann Surg 250(2):187–196
16. Buderer NM (1996) Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Acad Emerg Med 3(9):895–900
17. Brierley JD GM, Wittekind C et al. (2017) TNM Classification of Malignant Tumours, 8th edition.;
18. Ahmed Z, Patil SM, Sekaran A, Rebala P, Rao GV (2023) Indocyanine green guided sentinel lymph node biopsy may have a high sensitivity for early (T1/T2) colon cancer: a prospective study in Indian patients. Turk J Surg 39(3):190–196
19. Burghgraef TA, Zweep AL, Sikkenk DJ, van der Pas M, Verheijen PM, Consten ECJ (2021) In vivo sentinel lymph node identification using fluorescent tracer imaging in colon cancer: a systematic review and meta-analysis. Crit Rev Oncol Hematol 158:103149
20. Khalafi S, Botero Fonnegra C, Reyes A, Hui VW (2024) Developments in the use of indocyanine green (ICG) fluorescence in colorectal surgery. J Clin Med. https://doi.org/10.3390/jcm13144003
21. Lucas K, Melling N, Giannou AD, Reeh M, Mann O, Hackert T, Izbicki JR, Perez D, Grass JK (2023) Lymphatic mapping in colon cancer depending on injection time and tracing agent: a systematic review and meta-analysis of prospective designed studies. Cancers (Basel). https://doi.org/10.3390/cancers15123196
22. Negrut RL, Cote A, Feder B, Bodog FD, Maghiar AM (2025) Enhanced lymph node detection in colon cancer using indocyanine green fluorescence: a systematic review of studies from 2020 onwards. J Pers Med. https://doi.org/10.3390/jpm15020054
23. Shevchenko I, Serban D, Dascalu AM, Tribus L, Alius C, Cristea BM, Suceveanu AI, Voiculescu D, Dumitrescu D, Bobirca F et al (2024) Factors affecting the efficiency of near-infrared indocyanine green (NIR/ICG) in lymphatic mapping for colorectal cancer: a systematic review. Cureus 16(2):e55290
24. Augestad KM, Merok MA, Ignatovic D (2017) Tailored treatment of colorectal cancer: surgical, molecular, and genetic considerations. Clin Med Insights Oncol 11:1179554917690766
25. Hashiguchi Y, Hase K, Ueno H, Mochizuki H, Shinto E, Yamamoto J (2011) Optimal margins and lymphadenectomy in colonic cancer surgery. Br J Surg 98(8):1171–1178
26. Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S (2009) Standardized surgery for colonic cancer: complete mesocolic excision and central ligation–technical notes and outcome. Colorectal Dis 11(4):354–364
27. West NP, Kobayashi H, Takahashi K, Perrakis A, Weber K, Hohenberger W, Sugihara K, Quirke P (2012) Understanding optimal colonic cancer surgery: comparison of Japanese D3 resection and European complete mesocolic excision with central vascular ligation. J Clin Oncol 30(15):1763–1769
28. Wan J, Wang S, Yan B, Tang Y, Zheng J, Ji H, Hu Y, Zhuang B, Deng H, Yan J (2022) Indocyanine green for radical lymph node dissection in patients with sigmoid and rectal cancer: randomized clinical trial. BJS Open. https://doi.org/10.1093/bjsopen/zrac151
29. Ribero D, Mento F, Sega V, Lo Conte D, Mellano A, Spinoglio G (2022) ICG-guided lymphadenectomy during surgery for colon and rectal cancer-interim analysis of the GREENLIGHT trial. Biomedicines. https://doi.org/10.3390/biomedicines10030541
30. West NP, Hohenberger W, Weber K, Perrakis A, Finan PJ, Quirke P (2010) Complete mesocolic excision with central vascular ligation produces an oncologically superior specimen compared with standard surgery for carcinoma of the colon. J Clin Oncol 28(2):272–278
31. Japanese Classification of Colorectal (2019) Appendiceal, and Anal Carcinoma: the 3d English Edition [Secondary Publication]. *J Anus Rectum Colon*, 3(4):175–195.