BJS Open, 2022, zrac151
https://doi.org/10.1093/bjsopen/zrac151
Randomized Clinical Trial

Indocyanine green for radical lymph node dissection in patients with sigmoid and rectal cancer: randomized clinical trial

Jinliang Wan[1,2,3], Shijie Wang[1,2], Botao Yan[1,2], Yuting Tang[1,2], Jixiang Zheng[1,2], Hongli Ji[1,2], Yaowen Hu[1,2], Baoxiong Zhuang[1,2], Haijun Deng[1,2] and Jun Yan[1,2,*]

[1]Department of General Surgery, Nanfang Hospital, The First School of Clinical Medicine, Southern Medical University, Guangzhou City, PR China
[2]Guangdong Provincial Key Laboratory of Precision Medicine for Gastrointestinal Cancer, Nanfang Hospital, Southern Medical University, Guangzhou City, PR China
[3]Department of General Surgery, Affiliated Hospital of Jiujiang University, Jiujiang City, PR China

*Correspondence to: Jun Yan, Department of General Surgery, Nanfang Hospital, The First School of Clinical Medicine, Southern Medical University, Guangzhou, Guangdong, 510515, PR China (e-mail: yanjunfudan@163.com)

Abstract

Background: D3 lymph node dissection is recommended for patients with advanced sigmoid and rectal cancer in Japan. This trial aimed to investigate the feasibility of indocyanine green (ICG) as a tracer to increase the nodal harvest during D3 lymph node dissection in patients with sigmoid and rectal cancer.

Methods: This prospective randomized clinical trial was performed between May 2021 and April 2022. The inclusion criteria were patients with stage I–III sigmoid or rectal cancer eligible for laparoscopic resection. Patients were 1: 1 randomized to either the ICG group (endoscopic ICG injection at the tumour site and intraoperative imaging to guide dissection) or the control group (routine laparoscopic white-light imaging). All patients were treated with D3 dissection, and the primary outcome was the number of harvested lymph nodes at the D3 level.

Results: Out of 210 patients screened, a total of 66 patients were enrolled and randomized. Patients in the two groups presented similar ages and clinical stages (ICG group *versus* control group, median age of 58.0 *versus* 58.5 years; stage III 36.4 per cent *versus* 36.4 per cent, whereas the rate of rectal cancer was 27.3 per cent *versus* 48.5 per cent respectively). ICG imaging was helpful for completely dissecting D3 lymph nodes and could identify a median of more than 2 (range 1–6) D3 lymph nodes neglected by routine laparoscopic white-light imaging during surgery. The median number of D3 lymph nodes harvested in the ICG group was significantly higher than that in the control group (7.0 *versus* 5.0, $P = 0.003$); however, there was no significant difference in the median numbers of positive D1, D2, and D3 lymph nodes between the two groups.

Conclusion: ICG is safe and feasible to guide D3 lymph node dissection and can increase the number of harvested D3 lymph nodes in patients with sigmoid and rectal cancer.

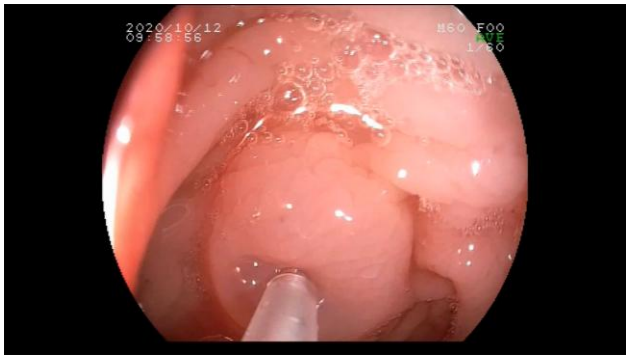
Registration number: NCT04848311 (http://www.clinicaltrials.gov).

Introduction

According to the report of Global Cancer Statistics 2020, colorectal cancer is the third most common malignancy and the second leading cause of cancer death[1]. Surgery is the treatment of choice for colorectal cancer, and adequate lymph node dissection is essential for colorectal surgery. The AJCC guidelines recommend that obtaining at least 12 lymph nodes is helpful for accurate colorectal cancer staging[2]. At present, harvesting more lymph nodes is considered more conducive to the prognosis of colorectal cancer surgery[3,4]. According to the Japanese classification of colorectal cancer, inferior mesenteric root nodes are defined as lymph nodes along the inferior mesenteric artery from the origin of the inferior mesenteric artery to that of the left colic artery, and these lymph nodes should be removed during D3 dissection for sigmoid and rectal cancer[5]; however, optimal lymphadenectomy for D3 lymph nodes remains controversial[6]. Dissection of D3 lymph nodes has advantages and disadvantages in laparoscopic surgery for patients with sigmoid and rectal cancer. On the one hand, D3 lymph node dissection has a longer long-term survival in D3-positive patients with sigmoid and rectal cancer[7,8]. On the other hand, the D3 lymph node metastasis rate is 0.3–8.6 per cent[9] and D3 lymph node dissection is time consuming, technically demanding, and sometimes damages the peripheral autonomic nerve[10,11].

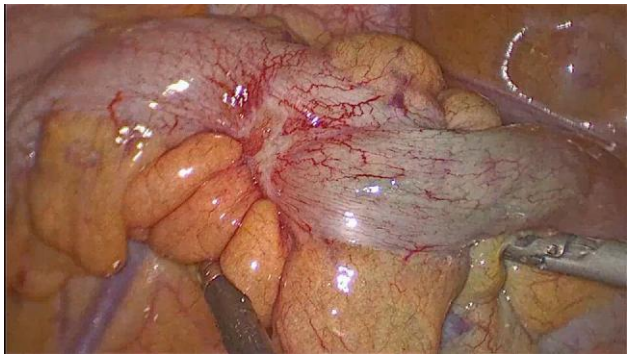
With the development of surgical tracing technology, indocyanine green (ICG) has been used to assist laparoscopic surgery[12]. ICG is a near-infrared (NIR) fluorescent dye with excellent biocompatibility. When ICG is injected into the submucosa of the gastrointestinal tract, it quickly enters the peripheral lymphatic system and is swallowed by macrophages in lymph nodes[13]. ICG NIR imaging can better penetrate tissue

Received: June 15, 2022. **Revised:** October 05, 2022. **Accepted:** October 19, 2022



Video 1 Endoscopic ICG injection

ICG was injected at two to four points around the tumour. The injection volume at each position was approximately 2–3 ml. Before ICG injection, 0.5 ml saline was used to raise the submucosal bleb in the wall of the colon to avoid puncturing the wall and drug leakage into the abdominal cavity. ICG, indocyanine green.



Video 2 Intraoperative D3 lymph node dissection was performed in fluorescence mode in the ICG group

In the ICG group, after the exploration was completed at the beginning of the operation, tumour images and D3 lymph nodes images were obtained under white light, and then the light source mode was converted to collect the above images under fluorescence for comparison. Through fluorescence imaging, surgeons were able to determine the scope of dissection according to lymph node localization. For example, in the process of separating the root of the inferior mesenteric artery, D3 lymph node dissection was performed under ICG fluorescence mode, ICG imaging was helpful for completely dissecting D3 lymph nodes and could identify more D3 lymph nodes neglected by white-light mode during surgery. High ligation of the inferior mesenteric artery was routinely performed. During the dissection and ligation of the inferior mesenteric vein, the level of ligation was decided based on fluorescent lymph nodes eventually visualized. ICG, indocyanine green.

and more accurately identify lymph nodes in hypertrophic adipose tissue than other dyes, such as methylene blue and γ-ray-emitting radiotracers[14,15]. It can emit NIR light around a wavelength of 840 nm after excitation, and the tissue penetration depth of enhanced fluorescence ranges from 0.5 cm to 1.0 cm[16]. As a tracer for sentinel lymph nodes, ICG has a good detection rate in non-small cell lung cancer and breast cancer[17,18]. Recently, a clinical trial was conducted to explore the use of ICG tracer-guided lymph node dissection for gastric cancer and found that ICG is helpful for D2 lymph node dissection. This confirmed that ICG tracer-guided lymph node dissection can allow more lymph nodes to be harvested without increasing the operating time or rate of complications[19].

The research hypothesis in this trial was that it could be feasible to guide D3 lymph node dissection and increase the number of harvested D3 lymph nodes using ICG in patients with

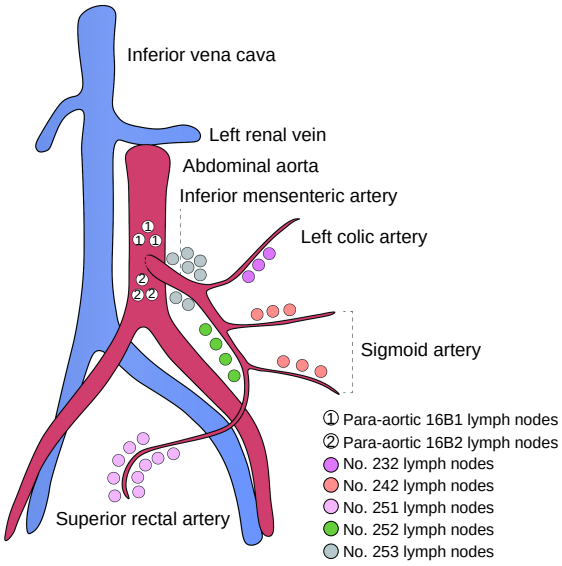


Fig. 1 Lymph node categorization

D1 lymph nodes are defined as lymph nodes along the marginal arteries, near the bowel wall, along the superior rectal artery and terminal sigmoid artery, which include no. 251 lymph nodes. D2 lymph nodes are defined as lymph nodes along the left colic and sigmoid arteries in sigmoid cancer or the inferior mesenteric artery between the origin of the left colic artery and the superior rectal artery in rectal cancer, which include no. 232, no. 242, and no. 252 lymph nodes. D3 lymph nodes are defined as lymph nodes along the inferior mesenteric artery from the origin of the inferior mesenteric artery to that of the left colic artery. Para-aortic 16B1 lymph nodes are defined as lymph nodes along the abdominal aorta between the level of the left renal vessels and the origin of the inferior mesenteric artery. Para-aortic 16B2 lymph nodes are defined as lymph nodes along the abdominal aorta between the origin of the inferior mesenteric artery and the bifurcation of the common iliac artery.

sigmoid colon and rectal cancer. Therefore, the primary aim was to compare the number of harvested lymph nodes using ICG and the control (laparoscopic white-light imaging). Secondary outcomes included operating time, estimated blood loss, postoperative hospital stay, and complications.

Methods

Study design

This is a prospective randomized clinical trial with a 1: 1 allocation ratio performed at Nanfang Hospital of Southern Medical University, which conducts 600–700 colorectal cancer surgeries per year. From May 2021 to April 2022, all consecutive patients eligible for laparoscopic resection of sigmoid and rectal cancer in Nanfang Hospital were recruited. The inclusion criteria were 18–80 years of age; ASA score 1–3; single sigmoid or rectal cancer confirmed by endoscopic biopsy; and planned laparoscopic radical resection. The exclusion criteria were previous abdominal tumour surgery; women who were pregnant or breastfeeding; emergency patients with obstruction or perforation; T4b cancer evaluated by CT, MRI, or endoscopic ultrasonography; pelvic or distant metastasis; T1 cancer planned for local excision; allergic constitution patients; preoperative neoadjuvant therapy; and low rectal cancer with lateral pelvic lymph node metastasis. This clinical trial was approved by the ethics committee of Nanfang Hospital. All patients signed informed consent before randomization.

Randomization and interventions

All the patients included in this study were randomly allocated to two groups (ICG group and control group) by sequentially

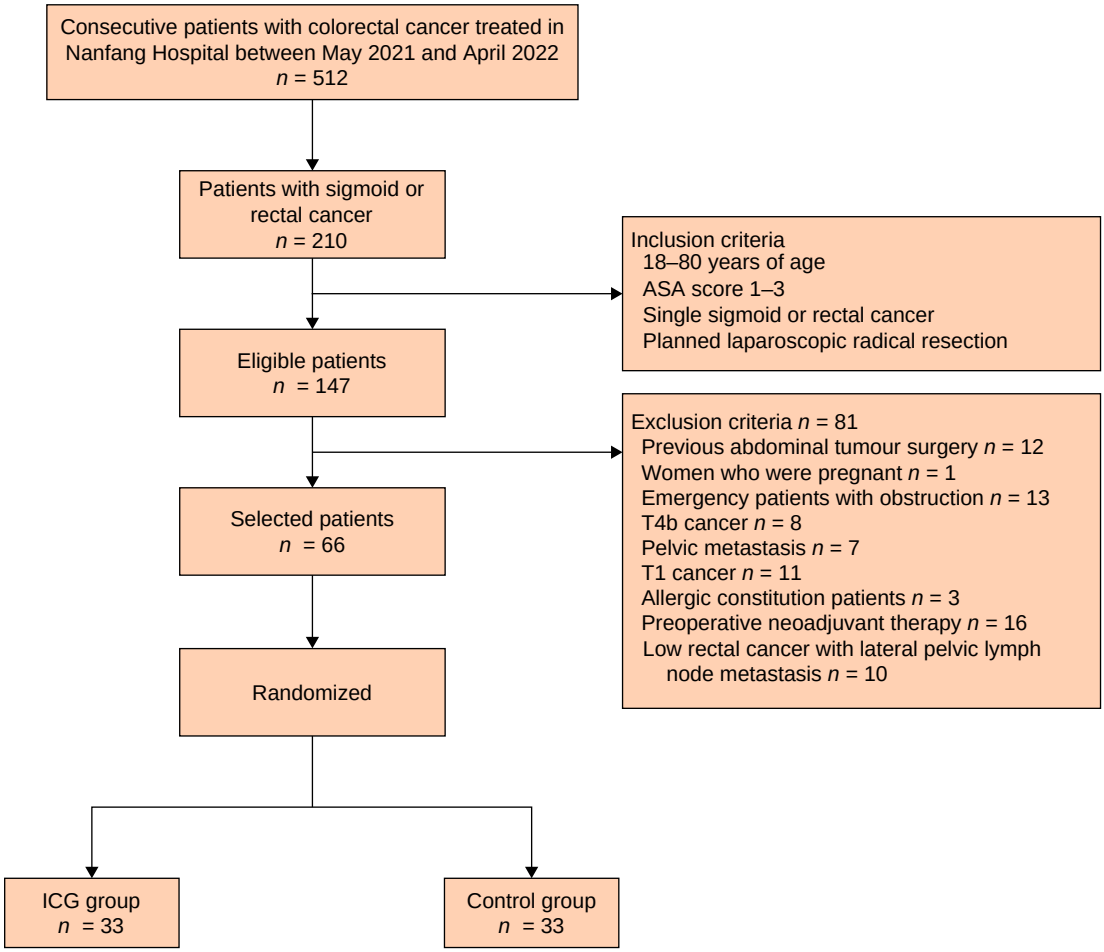


Fig. 2 Study flowchart

ICG, indocyanine green.

numbered, opaque, sealed envelopes. Randomization allocations were created by an independent statistician, and the clinician was responsible for recruiting and assigning participants. Patients in the ICG group received endoscopic submucosal injection of ICG 1 day before surgery. A total of 25 mg ICG powder (Dandong Yichuang Pharmaceutical Co.) was dissolved in 10 ml sterile water and then injected at two to four points around the tumour. The injection amount at each position was approximately 2–3 ml. Before ICG injection, 0.5 ml saline was used to raise the submucosal bleb in the wall of the colorectum to avoid puncturing the intestinal wall and drug leakage into the abdominal cavity (*Video* 1). During the operation, we used fluorescence imaging equipment (Storz, Germany) to obtain ICG images, and then D3 lymph node dissection was performed under fluorescence imaging. The control group did not receive tracer injection and D3 lymph node dissection was performed by routine laparoscopic white-light imaging.

Surgical procedure

All patients underwent surgery performed by an experienced senior attending doctor, following a detailed protocol. All details and outcomes were recorded in case report form. All surgeons participating in the study were personally instructed by the principal investigator. Intraoperative photo documentation was taken to maintain quality control. In the ICG group, after the exploration was completed at the beginning of the operation, tumour images, D1 lymph nodes images, D2 lymph nodes images, and D3 lymph nodes images were obtained under white light, and then the light source mode was converted to collect the above images under fluorescence for comparison. Through fluorescence imaging, surgeons can determine the scope of dissection according to lymph node localization. In the process of separating the root of the inferior mesenteric artery, D3 lymph node dissection was performed under ICG fluorescence mode (*Video* 2). High ligation of the inferior mesenteric artery was routinely performed. During disconnection of the inferior mesenteric vein, the location of disconnection depends on whether there are fluorescent lymph nodes. In the control group, the surgeon performed lymphadenectomy according to routine laparoscopic white-light imaging.

Nodal dissection and definitions

D1 lymph nodes are defined as lymph nodes along the marginal arteries, near the bowel wall, along the superior rectal artery, and terminal sigmoid artery. D2 lymph nodes are defined as lymph nodes along the left colic and sigmoid arteries in sigmoid cancer or the inferior mesenteric artery between the origin of the left colic artery and the superior rectal artery in rectal cancer. D3 lymph nodes are defined as lymph nodes along the inferior mesenteric artery from the origin of the inferior mesenteric artery to that of the left colic artery. Para-aortic 16B1 lymph nodes are defined as lymph nodes

Table 1 Baseline characteristics of the two groups of patients

Characteristics	ICG ($n = 33$)	Control ($n = 33$)	P
Age (years), median (range)	58.0 (39–79)	58.5 (44–79)	0.608
Sex (M/F)	21/12 (63.6%/36.4%)	24/9 (72.7%/27.3%)	0.428
BMI (kg/m^2), median (range)	23.4 (14.8–28.9)	23.4 (17.1–28.2)	0.908
ASA score			0.897
1	1 (3.0)	1(3.0)	
2	29 (87.9)	30 (90.9)	
3	3 (9.1)	2 (6.1)	
Tumour size (cm), median (range)	4 (2–10)	3 (2–8)	0.088
Tumour location			0.196
5–10 cm (middle rectum)	5 (15.2)	10 (30.3)	
10–15 cm (upper rectum)	4 (12.1)	6 (18.2)	
15–30 cm (sigmoid colon)	24 (72.7)	17 (51.5)	
cT category			0.597
cT2	2 (6.1)	4 (12.1)	
cT3	16 (48.5)	17 (51.5)	
cT4a	15 (45.4)	12 (36.4)	
cN category			0.796
cN0	11 (33.3)	12 (36.4)	
cN+	22 (66.7)	21 (63.6)	
pT category			0.425
pT2	6 (18.2)	7 (21.2)	
pT3	25 (75.7)	21 (63.6)	
pT4	2 (6.1)	5 (15.2)	
pN category			0.823
pN0	21 (63.6)	20 (60.6)	
pN1	8 (24.3)	10 (30.3)	
pN2	4 (12.1)	3 (9.1)	
AJCC stage			0.769
I	6 (18.2)	4 (12.1)	
II	15 (45.4)	17 (51.5)	
III	12 (36.4)	12 (36.4)	

Values are *n* (%) unless otherwise stated. ICG, indocyanine green.

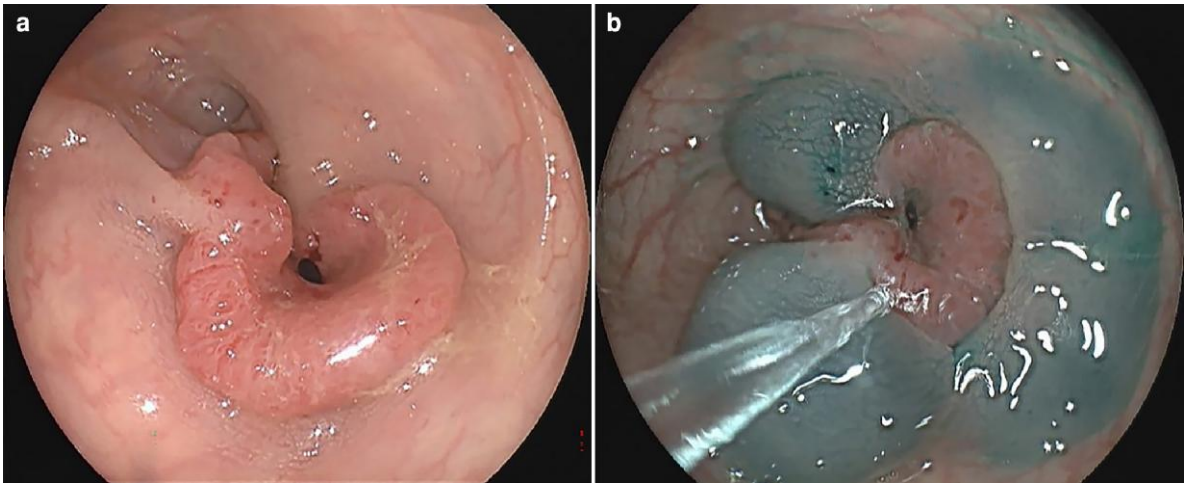


Fig. 3 Endoscopic ICG injection

a Endoscopic rectal cancer before ICG injection. **b** ICG was injected into the submucosa around the tumour. ICG, indocyanine green.

along the abdominal aorta between the level of the left renal vessels and the origin of the inferior mesenteric artery. Para-aortic 16B2 lymph nodes are defined as lymph nodes along the abdominal aorta between the origin of the inferior mesenteric artery and the bifurcation of the common iliac artery (*Fig. 1*).

Outcome of interest

The primary outcome was the number of harvested D3 lymph nodes. Secondary outcomes included the number of positive D3 lymph nodes, ratio of positive D3 lymph nodes (defined as the number of positive D3 lymph nodes divided by the total number of retrieved D3 lymph nodes), number of harvested D1 lymph nodes, number of positive D1 lymph nodes, ratio of positive D1 lymph nodes, number of harvested D2 lymph nodes, number of positive D2 lymph nodes, ratio of positive D2 lymph nodes, number of harvested total lymph nodes, number of total positive lymph nodes, ratio of total positive lymph nodes, operating time, estimated blood loss, postoperative hospital stay, time to first flatus, and complications. The lymph node data were obtained from the hospital pathological report within 1 week after the operation. The complications included

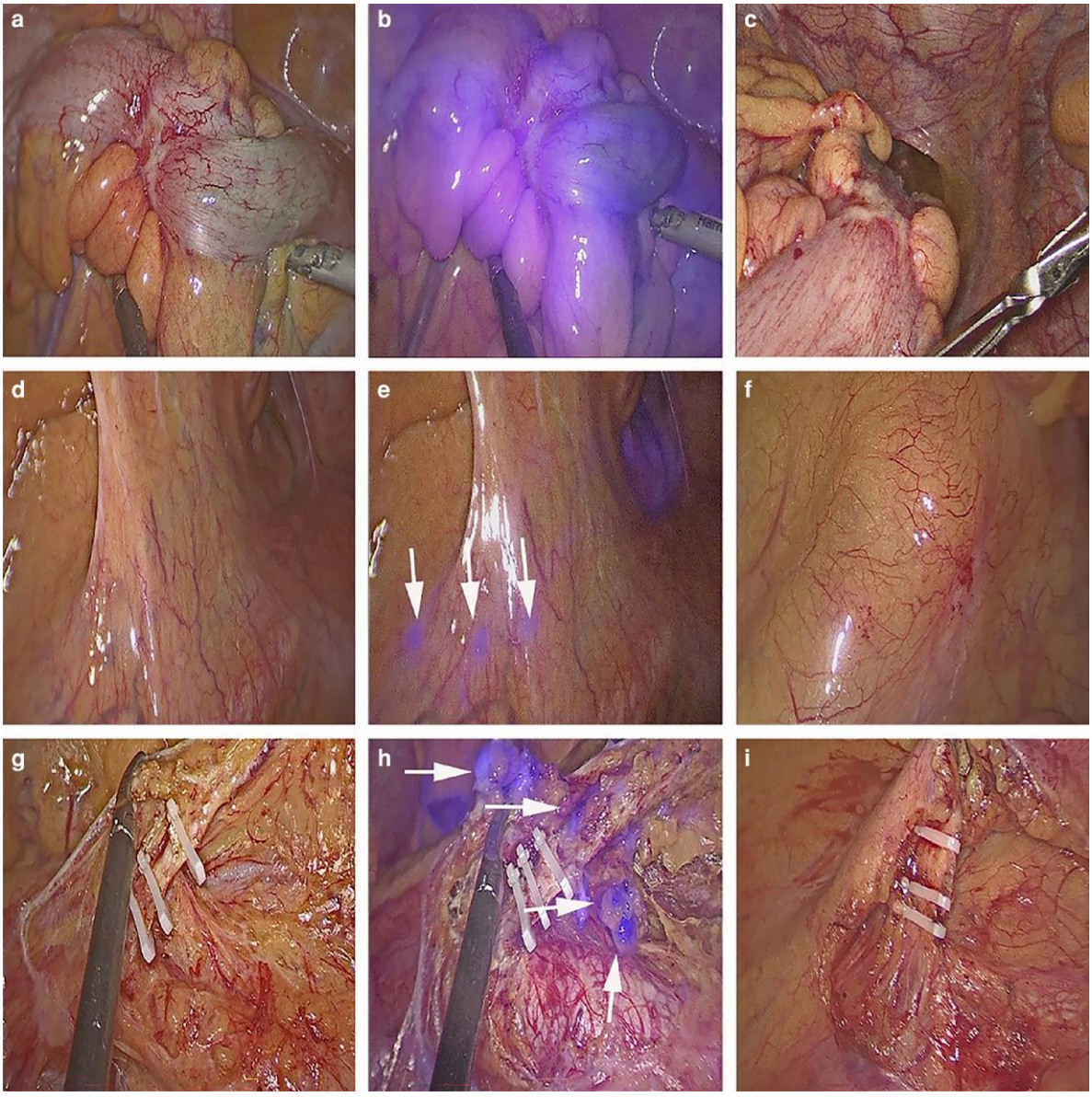


Fig. 4 Comparison of intraoperative tumours and D3 lymph nodes between the ICG group and the control group (pictures belong to the same patient before and after ICG)

a Tumour under white-light mode in the ICG group. **b** Tumour under ICG fluorescence mode in the ICG group. **c** Tumour under white-light mode in the control group. **d** Intraoperative D3 lymph node areas under white-light mode in the ICG group. **e** Intraoperative D3 lymph nodes were clearly identified under ICG fluorescence mode in the ICG group. The white arrow indicates D3 lymph nodes. **f** Intraoperative D3 lymph node areas under white-light mode in the control group. **g** The root of the inferior mesenteric artery was ligated under white-light mode in the ICG group. **h** The root of the inferior mesenteric artery was ligated under ICG fluorescence mode in the ICG group. The white arrow indicates D3 lymph nodes. **i** The root of the inferior mesenteric artery was ligated in the control group. ICG, indocyanine green.

intraoperative bleeding, anastomotic bleeding, anastomotic leakage, ileus, pneumonia, and incisional infection.

Sample size and statistical analysis

The number of harvested D3 lymph nodes was the primary outcome. In a previous study from our group, the mean number of D3 lymph nodes retrieved from the control group was 3.15[20]. The present analysis was based on an α value of 0.05, and a power of 90 per cent, and the estimated difference in D3 lymph nodes between the two groups was two lymph nodes, revealing that a minimum of 29 patients should be included in each group. Given an expected dropout rate of 10 per cent, at least 33 patients were needed in each group, for a total of 66 participants.

Continuous variables are expressed as median (range) and categorical variables are expressed as frequency (percentage). The Mann–Whitney *U* test was used for the difference between the two groups of continuous variables, and the chi-square test or Fisher's exact test was used for the difference between the two groups of categorical variables. A *P* value less than 0.05 was considered a significant difference between the two groups. The statistical analysis was performed with SPSS® version 20.0 (IBM, Armonk, NY, USA).

Results

Figure 2 outlines the study population. Out of 210 patients screened, a total of 66 patients were randomized (median age

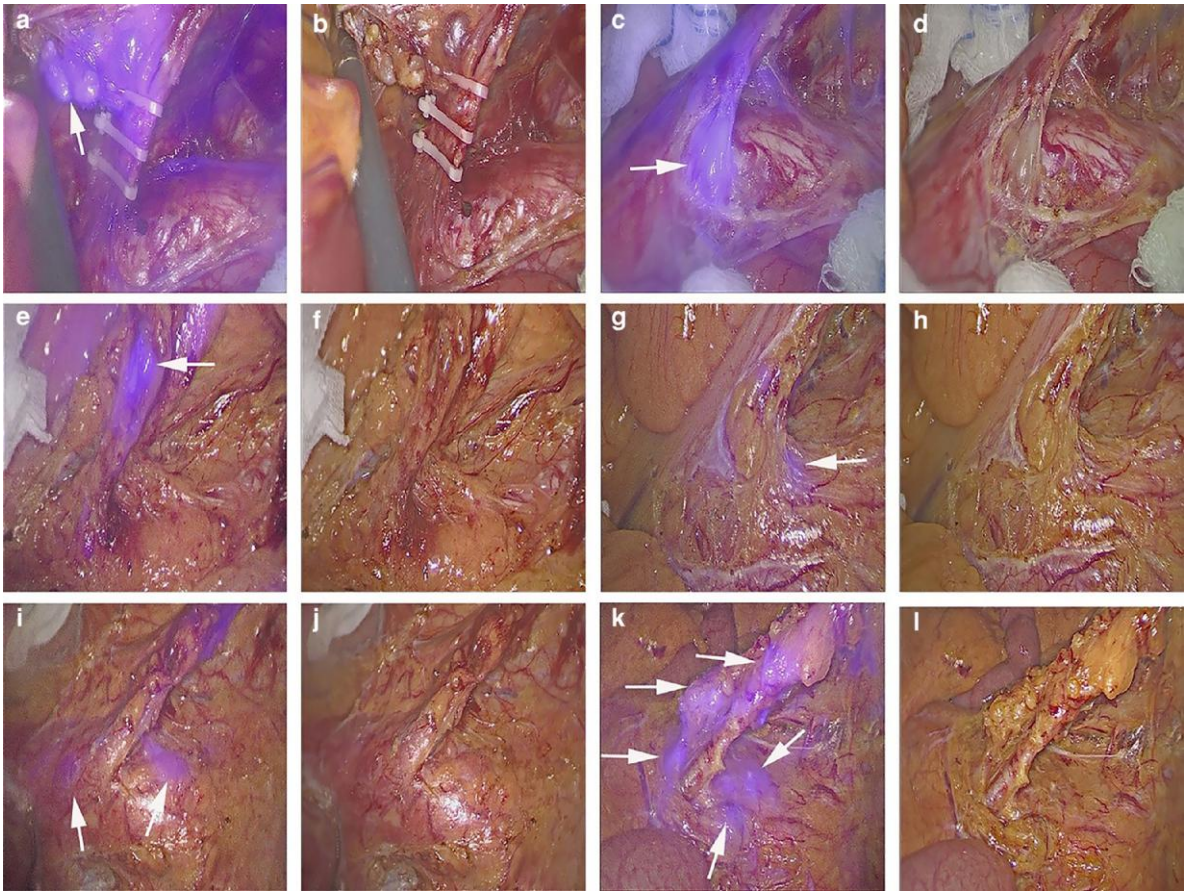


Fig. 5 The distribution of D3 lymph nodes in different patients was individualized via ICG imaging

a Intraoperative D3 lymph nodes were located on the left side of the inferior mesenteric artery under fluorescence mode in the ICG group. **b** Corresponding white-light imaging. **c** Intraoperative D3 lymph nodes were located on the right side of the inferior mesenteric artery under fluorescence mode in the ICG group. **d** Corresponding white-light imaging. **e** Intraoperative D3 lymph nodes were located in front of the inferior mesenteric artery under fluorescence mode in the ICG group. **f** Corresponding white-light imaging. **g** Intraoperative D3 lymph nodes were located behind the inferior mesenteric artery under fluorescence mode in the ICG group. **h** Corresponding white-light imaging. **i**. Intraoperative D3 lymph nodes were located in the anterior and posterior inferior mesenteric artery under fluorescence mode in the ICG group. **j** Corresponding white-light imaging. **k** Intraoperative D3 lymph nodes were located around the inferior mesenteric artery under fluorescence mode in the ICG group. **l** Corresponding white-light imaging. The white arrow indicates D3 lymph nodes. ICG, indocyanine green.

was 58.0 (range 39–79) years in the ICG group and median age was 58.5 (range 44–79) years in the control group). There were 33 patients in the ICG group and 33 patients in the control group. Baseline characteristics such as age, sex, BMI, ASA score, tumour size, tumour location, preoperative T and N category, and postoperative T, N, and AJCC category were recorded. The baseline characteristics of the two groups were comparable (*Table 1*). The trial ended when 66 eligible patients were enrolled and discharged after surgery.

The ICG group received endoscopic injection as described previously (*Video 1* and *Fig. 3*). Intraoperative ICG imaging was performed (*Video 2*) and compared with standard white light (*Fig. 4*). The dissection of D3 lymph nodes in different patients with sigmoid and rectal cancer was individualized via ICG imaging (*Fig. 5*). ICG imaging was helpful for completely dissecting D3 lymph nodes and could identify in median more than two (range 1–6) D3 lymph nodes neglected by routine laparoscopic white-light imaging (*Fig. 6*), In the ICG group, inferior mesenteric root nodes were marked by ICG along the inferior mesenteric artery from the origin of the inferior mesenteric artery to that of the left colic artery. As an example, para-aortic No.216 lymph nodes near the origin of the inferior mesenteric artery were also fluorescently observed, and these ICG marked lymph nodes were dissected in the ICG group (*Fig. 6* and *Video 2*).

Postoperative outcome measurements are shown in *Table 2*. The median number of harvested D3 lymph nodes in the ICG group was significantly higher than that in the control group (7.0 *versus* 5.0, $P = 0.003$). The median number of harvested D1 (14.0 *versus* 9.0, $P = 0.017$) and D2 (5.0 *versus* 3.0, $P = 0.019$) lymph nodes in the ICG group was also significantly higher than those in the control group. Of note, the lymph node grouping of tumour specimens in the ICG group and control group is based on *Fig. 1* according to the Japanese classification of colorectal cancer[5], and lymph nodes were harvested by pathologists. There was no significant difference in the median numbers of positive D1, D2, and D3 lymph nodes between the two groups. The median total number of lymph nodes harvested in the ICG group was also higher than that in the control group (28.0 *versus* 19.0, $P = 0.001$). There was no difference in the median number of total positive lymph nodes or in the ratio of total positive lymph nodes between the two groups. Regarding the other postoperative data, the estimated median volume of intraoperative blood loss (10 ml *versus* 20 ml, $P = 0.162$), and the median operating time (145 min *versus* 125 min, $P = 0.124$) was comparable between the ICG group and the control group. In terms of complications, one elderly patient in the ICG group experienced postoperative anastomotic leakage, and one patient in the control group had a postoperative anastomotic bleeding.

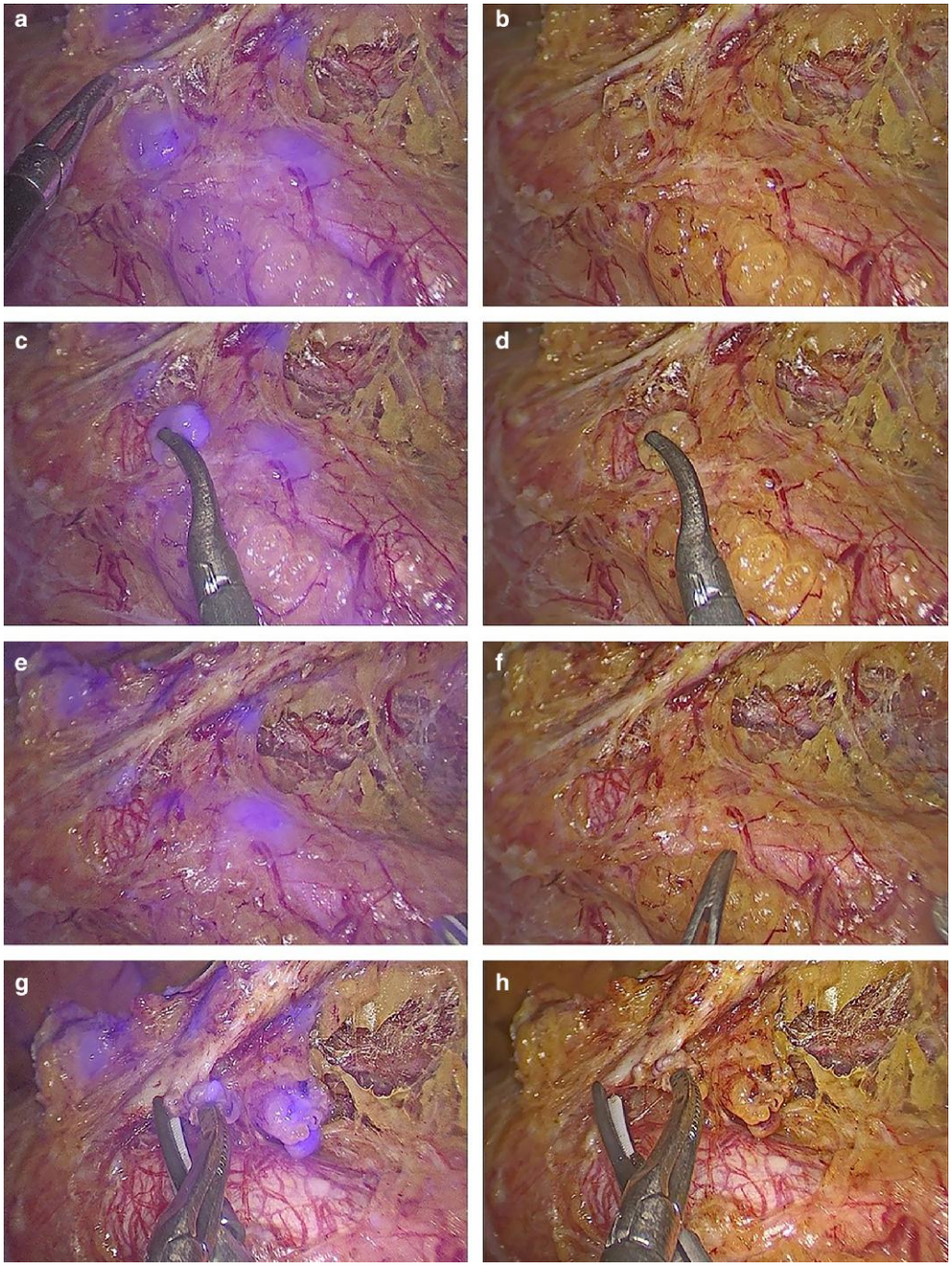


Fig. 6 ICG imaging for complete D3 lymph node dissection

a Intraoperative D3 lymph nodes could be easily visualized in fluorescence mode in the ICG group. **b** Corresponding white-light imaging. Intraoperative D3 lymph nodes were neglected under white-light mode in the ICG group. **c** Intraoperative D3 lymph nodes were identified under fluorescence mode and sent for frozen section biopsy in the ICG group. **d** Corresponding white-light imaging. **e** After the removal of one lymph node, other D3 lymph nodes were still easily found under fluorescence mode in the ICG group. **f** Corresponding white-light imaging. After the removal of one lymph node, other D3 lymph nodes could not be easily found under white-light mode in the ICG group. **g** Intraoperative D3 lymph nodes were easily found and dissected under fluorescence mode in the ICG group. **h** Corresponding white-light imaging. ICG, indocyanine green.

The median duration of postoperative hospital stay (6 days *versus* 5 days, $P = 0.666$) and median time to first flatus (3 days *versus* 2 days, $P = 0.085$) was also comparable.

Discussion

In this study, ICG imaging was helpful for completely dissecting D3 lymph nodes and could identify more than two (median, range 1–6) D3 lymph nodes neglected by routine laparoscopic white-light imaging. Previously, the 5-year survival rate of patients with D3 lymph node metastases in rectal cancer and sigmoid colonic cancer was only 27.9 per cent[21]. In 2018, another study[22] confirmed, through a large sample retrospective study, that the number of metastatic lymph nodes was higher in patients with stage III colorectal cancer who underwent D3 dissection than in those who underwent D2 dissection (average,

Table 2 Comparison of postoperative data between the two groups

Variables	ICG ($n = 33$)	Control ($n = 33$)	P
Number of harvested D1 nodes, median (range)	14 (4–42)	9 (3–22)	0.017
Number of positive harvested D1 nodes, median (range)	0 (0–12)	0 (0–11)	0.921
Ratio of positive D1 nodes	37 of 542 (6.8)	32 of 372 (8.6)	0.318
Number of harvested D2 nodes, median (range)	5 (0–15)	3 (0–10)	0.019
Number of positive harvested D2 nodes, median (range)	0 (0–2)	0 (0–2)	0.964
Ratio of positive D2 nodes	5 of 177 (2.8)	6 of 122 (4.9)	0.527
Number of harvested D3 nodes, median (range)	7 (1–35)	5 (0–15)	0.003
Number of positive harvested D3 nodes, median (range)	0 (0–6)	0 (0–1)	0.299
Ratio of positive D3 nodes	8 of 274 (2.9)	1 of 153 (0.7)	0.226
Number of harvested total nodes, median (range)	28 (11–57)	19 (9–32)	0.001
Number of positive harvested total nodes, median (range)	0 (0–15)	0 (0–11)	0.976
Ratio of positive total nodes	50 of 993 (5.0)	39 of 647 (6.0)	0.386
Postoperative hospital stay (day), median (range)	6 (4–16)	5 (3–14)	0.666
Time to first flatus (day), median (range)	3 (1–7)	2 (1–5)	0.085
Estimated blood loss (ml), median (range)	10 (5–50)	20 (5–50)	0.162
Operating time (min), median (range)	145 (78–263)	125 (84–258)	0.124
Complications	1 of 33 (3.0)	1 of 33 (3.0)	1
intraoperative bleeding	0	0	
anastomotic haemorrhage	0	1	
anastomotic leakage	1	0	
ileus	0	0	
pneumonia	0	0	
incisional infection	0	0	

Values are *n* (%) unless otherwise stated.

3.1 *versus* 2.5; $P = 0.001$), and D3 lymph node status was found to be helpful for recurrence risk assessment in patients with stage III colorectal cancer. The 5-year survival rate of D3 lymph node-positive patients was lower than that of D3 lymph node-negative patients (stage IIIA, 72.5 per cent *versus* 94.9 per cent, $P < 0.001$; stage IIIB, 67.9 per cent *versus* 84.0 per cent, $P < 0.001$; stage IIIC, 42.4 per cent *versus* 70.6 per cent, $P < 0.001$). In 2019, a multicentre randomized clinical study comparing D2 and D3 lymph node dissection of colonic cancer reported that N-positive status was more common in the D3 group (46 per cent in D3 *versus* 26 per cent in D2, with a risk ratio of 1.81, $P = 0.044$)[23]. Therefore, obtaining more D3 lymph nodes would be beneficial to the prognosis of patients with sigmoid and rectal cancer because it allows for more accurate N categorization and can guide subsequent adjuvant therapy. In the present study, more D3 lymph nodes were found in the ICG group than in the control group, although the number of positive nodes was comparable.

ICG has been approved by the US Food and Drug Administration for clinical and research use in humans since 1956[24]. Applications for the use of ICG in neurosurgical research, cardiac vessel angiography, and surgical imaging were approved in 2003, 2005, and 2006 respectively[25]. ICG fluorescence has high contrast and sensitivity because the near-infrared light used to measure fluorescence makes tissues seem more translucent than at other wavelengths, allowing several millimetres of tissue to be probed [25]. ICG as a lymphatic tracer has been used in colorectal surgery, but previous studies were mostly conducted to detect sentinel lymph nodes in colorectal cancer[26,27]. Indeed, in the present study, peri-tumoural ICG injection-assisted laparoscopic D3 lymph node dissection was performed and regarded as feasible, without increasing the rate of surgical complications.

This study was limited by the single-centre experience, and the results need to be externally validated. Also, it lacks long-term follow-up to correlate the findings with patient prognosis, as previous studies in this field reported that resection of positive para-aortic lymph nodes had the potential to achieve R0 resection and longer survival[28,29], thus results should be evaluated further in the light of patients' long-term outcomes and possible recurrences.

Funding

This work was supported by the following: grant 82273360 from the National Natural Science Foundation of China; grant 2020B121201004 from the Guangdong Provincial Key Laboratory of Precision Medicine for Gastrointestinal Cancer; grant 202206010085 from the Science and Technology Planning Project of Guangzhou City; grant 320.2710.1851 from the Special Fund from the Clinical Research of Wu Jieping Medical Foundation; grant LC2016PY010 from the Clinical Research Project of Southern Medical University; grant 2019Z023 from the President Fund of Nanfang Hospital; grant 2014067 from the High-Level Research Matching Foundation of Nanfang Hospital; grant 201404280056 from the Scientific Research Foundation for High-Level Talents in Nanfang Hospital of Southern Medical University; grants pdjhb0100 and pdjh2017a0093 from the Special Funds for the Cultivation of Guangdong College Students' Scientific and Technological Innovation; grants 201912121008, 202012121091, 202212121011, S202212121092, and S202212121104 from the Training Programme for Undergraduate Innovation and Entrepreneurship; and grant 2018CR034 from the Clinical Research Project of Nanfang Hospital.

Acknowledgements

J.W., S.W., and B.Y. contributed equally to this work.

Disclosure

The authors declare no conflict of interest.

Data availability

The data sets generated and analysed during the present study are available from the corresponding author on reasonable request. To gain access, data requestors will need to sign a data access agreement.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A *et al.* Global cancer statistics 2020: gLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;**71**:209–249
2. Weiser MR. AJCC 8th edition: colorectal cancer. *Ann Surg Oncol* 2018;**25**:1454–1455
3. Ahmadi O, Stringer MD, Black MA, McCall JL. Clinico-pathological factors influencing lymph node yield in colorectal cancer and impact on survival: analysis of New Zealand cancer registry data. *J Surg Oncol* 2015;**111**:451–458
4. Johnson PM, Porter GA, Ricciardi R, Baxter NN. Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer. *J Clin Oncol* 2006;**24**:3570–3575
5. Japanese Society for Cancer of the Colon and Rectum. Japanese classification of colorectal, appendiceal, and anal carcinoma: the 3d English edition [secondary publication]. *J Anus Rectum Colon*. 2019; **3**:175–195
6. Allaix ME, Fichera A. Extended lymphadenectomy in rectal cancer is debatable. *World J Surg* 2013;**37**:1814–1820
7. Kanemitsu Y, Hirai T, Komori K, Kato T. Survival benefit of high ligation of the inferior mesenteric artery in sigmoid colon or rectal cancer surgery. *Br J Surg* 2006;**93**:609–615
8. Ubukata M, Itabashi M, Kameoka S. Japanese D3 lymph node dissection in low rectal cancer with inferior mesenteric lymph node metastases. *World J Surg* 2014;**38**:2724–2729
9. Lange MM, Buunen M, van de Velde CJH, Lange JF. Level of arterial ligation in rectal cancer surgery: low tie preferred over high tie. A review. *Dis Colon Rectum* 2008;**51**:1139–1145
10. Kobayashi M, Okamoto K, Namikawa T, Okabayashi T, Araki K. Laparoscopic lymph node dissection around the inferior mesenteric artery for cancer in the lower sigmoid colon and rectum. *Surg Endosc* 2006;**20**:563–569
11. Liang J, Huang K, Lai H, Lee P, Sun C. Feasibility of laparoscopic D3 lymphadenectomy for male rectosigmoid cancer with clinically positive lymph nodes. *Surg Endosc* 2008;**22**:2514–2517
12. van Manen L, Handgraaf HJM, Diana M, Dijkstra J, Ishizawa T, Vahrmeijer AL *et al.* A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *J Surg Oncol* 2018;**118**:283–300
13. Ietto G, Amico F, Soldini G, Chiappa C, Franchin M, Iovino D *et al.* Real-time intraoperative fluorescent lymphography: a new technique for lymphatic sparing surgery. *Transpl P* 2016;**48**: 3073–3078
14. Gioux S, Choi HS, Frangioni JV. Image-guided surgery using invisible near-infrared light: fundamentals of clinical translation. *Mol Imaging* 2010;**9**:237–255
15. Schaafsma BE, Mieog JSD, Hutteman M, van der Vorst JR, Kuppen PJK, Löwik CWGM *et al.* The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J Surg Oncol* 2011; **104**:323–332
16. Chance B. Near-infrared images using continuous, phase-modulated, and pulsed light with quantitation of blood and blood oxygenation. *Ann N Y Acad Sci* 1998;**838**:29–45
17. Yamashita S, Tokuishi K, Anami K, Miyawaki M, Moroga T, Kamei M *et al.* Video-assisted thoracoscopic indocyanine green fluorescence imaging system shows sentinel lymph nodes in non-small-cell lung cancer. *J Thorac Cardiovasc Surg* 2011;**141**: 141–144
18. Valente SA, Al-Hilli Z, Radford DM, Yanda C, Tu C, Grobmyer SR. Near infrared fluorescent lymph node mapping with indocyanine green in breast cancer patients: a prospective trial. *J Am Coll Surgeons* 2019;**228**:672–678
19. Chen Q, Xie J, Zhong Q, Wang J, Lin J, Lu J *et al.* Safety and efficacy of indocyanine green tracer-guided lymph node dissection during laparoscopic radical gastrectomy in patients with gastric cancer. *JAMA Surg* 2020;**155**:300
20. Li K, Chen D, Chen W, Liu Z, Jiang W, Liu X *et al.* A case–control study of using carbon nanoparticles to trace decision-making lymph nodes around inferior mesenteric artery in rectal cancer. *Surg Endosc* 2019;**33**:904–910
21. Chin C, Yeh C, Tang R, Changchien C, Huang W, Wang J. The oncologic benefit of high ligation of the inferior mesenteric artery in the surgical treatment of rectal or sigmoid colon cancer. *Int J Colorectal Dis* 2008;**23**:783–788
22. Shinto E, Hida J, Kobayashi H, Hashiguchi Y, Hase K, Ueno H *et al.* Prominent information of jN3 positive in stage III colorectal cancer removed by D3 dissection: retrospective analysis of 6866 patients from a multi-institutional database in Japan. *Dis Colon Rectum* 2018;**61**:447–453
23. Karachun A, Panaiotti L, Chernikovskiy I, Achkasov S, Gevorkyan Y, Savanovich N *et al.* Short-term outcomes of a multicentre randomized clinical trial comparing D2 versus D3 lymph node dissection for colonic cancer (COLD trial). *Br J Surg* 2020;**107**:499–508
24. Feindel W, Yamamoto YL, Hodge P. The human cerebral microcirculation studied by intra-arterial radio-active tracers, Coomassie blue and fluorescein dyes. *Bibl Anat* 1967;**9**:220–224
25. Alander JT, Kaartinen I, Laakso A, Pätilä T, Spillmann T, Tuchin VV *et al.* A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging* 2012;**2012**:1–26
26. Hirche C, Mohr Z, Kneif S, Doniga S, Murawa D, Strik M *et al.* Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. *Int J Colorectal Dis* 2012;**27**:319–324
27. Cahill RA, Anderson M, Wang LM, Lindsey I, Cunningham C, Mortensen NJ. Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. *Surg Endosc* 2012;**26**:197–204
28. Lee J, Park H, Lee SY, Kim CH, Kim HR. Prognostic significance of enlarged paraaortic lymph nodes detected during left-sided colorectal cancer surgery: a single-centre retrospective cohort study. *World J Surg Oncol* 2021;**19**:9
29. Sakamoto J, Ozawa H, Nakanishi H, Fujita S. Oncologic outcomes after resection of para-aortic lymph node metastasis in left-sided colon and rectal cancer. *PLoS ONE* 2020;**15**:e241815