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ORIGINAL ARTICLE

The histopathological evaluation based on the indocyanine green fluorescence imaging of regional lymph node metastasis of splenic flexural colon cancer by near-infrared observation

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Abstract

Purpose The purpose of this study was to investigate the relationship between the fluorescence on indocyanine green fluorescent imaging (ICG-FI) and the histopathological findings of regional lymph node (LN) metastasis of splenic flexural colon cancer.

Methods From July 2013 to December 2018, consecutive patients with splenic flexural colon cancer with a preoperative diagnosis of N0 who underwent laparoscopic surgery were enrolled. The distribution of cancer sites in metastatic LNs (completely/not completely occupied by metastatic foci) was evaluated with hematoxylin and eosin–stained preparations. We compared the relationship between the distribution of cancer site and fluorescence of paraffin block in metastatic LNs.

Results Seventy-two patients were enrolled, of whom 13 (18.1%) had metastatic LNs. A total of 25 metastatic LNs were evaluated. The median short axis of the occupied LNs was 4.5 mm, which was significantly larger than that of the non-occupied LNs (3.0 mm; $p = 0.036$). In the near-infrared observation of the paraffin block, the completely occupied LNs showed no fluorescence, regardless of the LN size, but 8 of 10 non-occupied LNs showed fluorescence ($p < 0.001$). Even the non-occupied LNs that showed fluorescence, the cancer site did not show fluorescence.

Conclusions The occupied LNs showed no fluorescence, but 80% of the non-occupied LNs showed fluorescence. Even in non-occupied LNs that showed fluorescence, the cancer site did not show fluorescence. This demonstrated LN dissection should not be omitted, even if no fluorescence is noted on intraoperative ICG-FI.

Keywords Colorectal cancer · Lymph node metastasis · Indocyanine green · Near infrared · Fluorescence

Introduction

In the last two decades, the usefulness of intraoperative navigation surgery has been reported in various surgical fields [1–7]. In particular, indocyanine green (ICG) fluorescence imaging (FI) has been used in clinical practice for colorectal cancer surgery to evaluate the lymph flow for optimal lymph node (LN) dissection and anastomotic perfusion in the field of colorectal cancer surgery [8–14].

We previously reported that the lymph flow evaluation for colon cancer located in the splenic flexure (SFx) using ICG-FI and intraoperative ICG-FI-facilitated complete mesocolic excision (CME) and central vascular ligation (CVL) [9]. However, when focusing on the dissected LNs, we often noted the presence of both fluorescent and non-fluorescent metastatic LNs. There have been no reports describing what kind of metastatic LNs show fluorescence on ICG-FI.

The present study investigated the relationship between fluorescence on ICG-FI and the histopathological findings of metastatic LNs.

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Materials and methods

Patients

This study protocol was approved by the Ethics Advisory Committee of Yokosuka Kyosai Hospital before initiation and was registered in the UMIN Clinical Trials Registry as UMIN-CTR000013047 (http://www.umin.ac.jp/ctr/index.htm). Written informed consent was obtained from all of the patients. From July 2013 to December 2018, consecutive patients with a preoperative histopathological diagnosis of primary colon cancer located in the SFx with a preoperative diagnosis of N0 who underwent laparoscopic surgery with LN dissection in Yokosuka Kyosai Hospital were enrolled in this study. The exclusion criteria were a history of colonic surgery, extended colorectal resection, allergic hypersensitivity to ICG, or allergic hypersensitivity to iodine.

We performed tumor marking with India ink under colonoscopic vision < 96 h before surgery. A stock solution of ICG (Diagnogreen; Dai-Ichi Pharmaceuticals, Tokyo, Japan) was prepared by dissolving 25 mg of powdered ICG in 10.0 ml of sterilized water; 1.0 ml of the suspension was used for each injection. The near-infrared camera system was provided by the Stryker Corporation (1588 AIM Platform; MI, USA) and Karl Storz (D-Light P; Tuttlingen, Germany). For the detection of the lymph flow, we laparoscopically injected ICG (2.5 mg/1.0 ml) into the subserosal-submucosal layer around the tumor at 2 points with a 23-gauge localized injection after trocar insertion, guided the tumor marking with India ink, and observed the lymph flow laparoscopically using the near-infrared camera system 30 min after ICG injection. LN dissection with CME and CVL was performed without regard to the fluorescence results.

LN analyses

The harvested LNs were all submitted for a pathological diagnosis. After formalin fixation, the LNs were longitudinally cut in half and metastasis was evaluated using a cross section with hematoxylin and eosin (HE)–stained preparations. All metastatic LNs were targeted and evaluated. First, the distribution (occupied or non-occupied) of cancer site in metastatic LNs was evaluated with HE-stained preparations. Next, formalin-fixed paraffin-embedded tissues (paraffin blocks) were observed with the near-infrared camera system (PINPOINT Fluorescence Imaging System-SPY-PHI; Stryker Corporation) to evaluate the presence and distribution of the fluorescent signal. Finally, we compared the relationship between the distribution of cancer site and ICG fluorescence in the metastatic LNs. Occupied LN was defined as a lymph node with 100% complete loss of normal LN structure. Non-occupied LN was defined as LN with 1% or more of normal LN structure remaining. These assessments were performed by a single expert pathologist.

Statistical analyses

Categorical variables were expressed as the frequency and proportion (%), and numerical data were presented as the median and interquartile range (IQR). The SPSS Statistics 24 software program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Mann-Whitney *U* test and Fisher's exact test were performed for comparisons between independent groups when appropriate. All *p* values were 2-sided, and values < 0.05 were considered statistically significant.

Results

A total of 72 consecutive patients were enrolled in this study, of whom 13 (18.1%) had metastatic LNs. Table 1 shows the clinicopathological characteristics and surgical outcomes of the patients. These patients having metastatic LNs included 10 males and 3 females with a median age of 70 years old and a mean body mass index of 22.6 kg/m^2. The median operative time was 206 min, and the blood loss was 5 ml. Intraoperative lymph flow was observed in all 13 cases (100%) (Fig. 1). T factor was significantly more advanced in the lymph node metastasis group than in the no lymph node metastasis group. Other factors were not significantly different between the two groups.

A total of 229 LNs were harvested from these 13 patients, and 25 LNs (10.9%) were metastatic pathologically. All metastatic LNs were present in areas where lymph flow was observed on ICG-FI. The median number of harvested LNs was 17 (IQR, 14–24) and that of metastatic LNs was 2 (IQR, 1–3). Table 2 shows the details of the metastatic LNs. Of the 25 metastatic LNs, 15 were completely occupied by cancer. The median short axis of the occupied LNs was 4.5 mm (IQR, 3.8–7.0), which was significantly larger than that of the non-occupied LNs of 3.0 mm (IQR, 2.6–4.0) (p = 0.036). In the observation of the paraffin blocks using the near-infrared camera system, the occupied LNs showed no fluorescence, regardless of the LN size (Fig. 2), but 8 of 10 non-occupied LNs showed fluorescence (0% vs. 80%, p < 0.001). Even in the non-occupied LNs that showed fluorescence, the cancer site did not show fluorescence (Fig. 3). Two of 10 non-occupied LNs were non-fluorescent (Nos. 23 and 25), despite the remaining normal LN structure of 10% for No. 23 and 5% for No. 25.

Discussion

ICG-FI has been widely used to evaluate the blood flow in anastomosis [11–13, 15–17] and lymph flow for CME

Table 1 The clinicopathological characteristics and surgical outcomes

Variable	Lymph node metastasis (-), ***n* = 59**	Lymph node metastasis (+), ***n* = 13**	*p* value
Age, year*	72 (62.5–78)	70 (66–75)	0.786
Gender,			
Male	38	10	0.522
Female	21	3	
Body mass index, kg/m^2*	22.5 (20.9–24.9)	22.6 (18.6–23.5)	0.504
ECOG performance status			
0	54	12	1.000
1	5	1	
2	0	0	
3	0	0	
ASA physical status			
I	4	2	0.299
II	52	10	
III	3	1	
Tumor location			
Transverse colon	37	7	0.181
Transverse / descending colon	3	1	
Descending colon	18	5	
Surgical procedure			
Partial colectomy	55	10	0.106
Left hemi-colectomy	4	3	
Operative time, min*	190 (162–228)	206 (188–230)	0.200
Blood loss, ml*	7 (0–15)	5 (0–10)	0.212
Identification of the lymph flow			
Yes	58	13	1.000
No	1	0	
Number of dissected lymph nodes*	16 (12–24)	17 (14–24)	0.959
Number of metastatic lymph nodes*	-	2 (1–3)	-
Tumor diameter, mm*	25 (15–45)	35 (27–40)	0.223
Histological type			
pap	1	0	0.699
tub1	29	8	
tub2	28	5	
por	1	0	
Pathological T factor			
T1	25	0	< 0.001
T2	7	0	
T3	16	4	
T4	11	9	
Pathological N factor			
N1	-	12	-
N2		1	

*Numerical data are indicated as medians. Values in brackets are IQR; first quartile–third quartile

ASA, American Society of Anesthesiologists; *ECOG*, Eastern Cooperative Oncology Group

[8–10, 18, 19] in the field of colorectal cancer surgery. Nishigori et al. reported that metastatic foci were observed in 10% of ICG-fluorescent LNs and in 5.3% of non-fluorescent LNs [19]. A sentinel LN biopsy for colorectal cancer has yet to be established because of the risk of false-negative results (low sensitivity) [20]. Therefore, we clarified the characteristics of non-fluorescent metastatic LNs by comparing the distribution of the tumor and ICG-

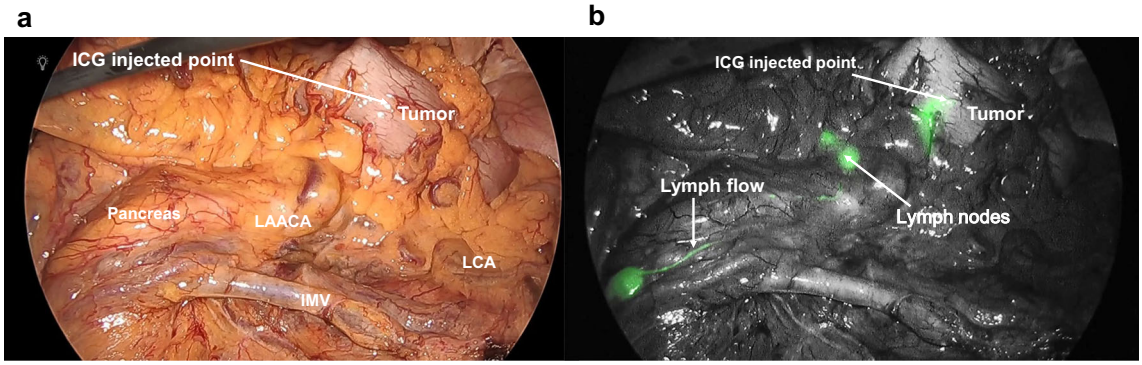

Fig. 1 **a** ICG (2.5 mg/1.0 ml) was injected into the submucosal layer around the tumor after trocar insertion, and the lymph flow was observed using the near-infrared camera system 30 minutes after injection. **b** The lymph flow and lymph node were visualized intraoperatively. ICG, indocyanine green; LAACA, left accessory aberrant colic artery; LCA, left colic artery; IMV, inferior mesenteric vein

FI in the LNs. This is the first report to reveal the relationship between metastatic LNs and ICG-FI.

In this study, we found that the non-cancerous region in the non-occupied LNs showed fluorescence on near-infrared observation; on the other hand, the cancer-occupied LNs (i.e., those completely replaced by cancer) did not fluoresce. This may be because ICG was unable to flow into the LNs due to lymphatic vessel occlusion. Due to the result of disruption of the lymph flow, the lymph flow may have been diverted to other areas, so caution must be practiced when performing

Table 2 Details of metastatic lymph nodes

Case	Number of metastatic LN	Location of metastatic LN	Long-axis (mm)	Short-axis (mm)	ICG fluorescence present	Occupation	% of occupation
1	1	Paracolic	7.0	4.0	No	Complete	100
1	2	Intermediate	9.0	8.0	No	Complete	100
2	3	Paracolic	4.5	4.0	No	Complete	100
3	4	Intermediate	5.0	5.0	No	Complete	100
3	5	Intermediate	9.0	6.0	No	Complete	100
3	6	Paracolic	6.0	4.0	Yes	Not complete	10
3	7	Paracolic	3.5	2.5	No	Complete	100
4	8	Paracolic	9.0	8.0	No	Complete	100
5	9	Paracolic	12.0	9.0	No	Complete	100
5	10	Paracolic	6.0	6.0	No	Complete	100
5	11	Paracolic	4.5	4.0	No	Complete	100
6	12	Paracolic	11.0	9.0	No	Complete	100
6	13	Paracolic	6.0	4.5	No	Complete	100
7	14	Paracolic	3.0	3.0	Yes	Not complete	30
8	15	Paracolic	2.0	1.5	yes	Not complete	40
9	16	Paracolic	5.0	3.5	No	Complete	100
9	17	Paracolic	3.5	2.5	Yes	Not complete	5
10	18	Paracolic	5.0	3.5	No	Complete	100
10	19	Paracolic	5.0	3.0	Yes	Not complete	5
10	20	Intermediate	3.0	2.0	No	Complete	100
11	21	Paracolic	5.0	4.5	Yes	Not complete	60
11	22	Paracolic	4.0	4.0	Yes	Not complete	20
11	23	Paracolic	4.5	3.0	No	Not complete	90
12	24	Paracolic	3.0	2.5	Yes	Not complete	40
13	25	Paracolic	8.0	6.0	No	Not complete	95

LN, lymph node; *Long-axis*, long-axis diameter of lymph node; *Short-axis*, short-axis diameter of lymph node; *ICG*, indocyanine green; *Complete*, complete occupation of cancer; *Not complete*, not complete occupation of cancer

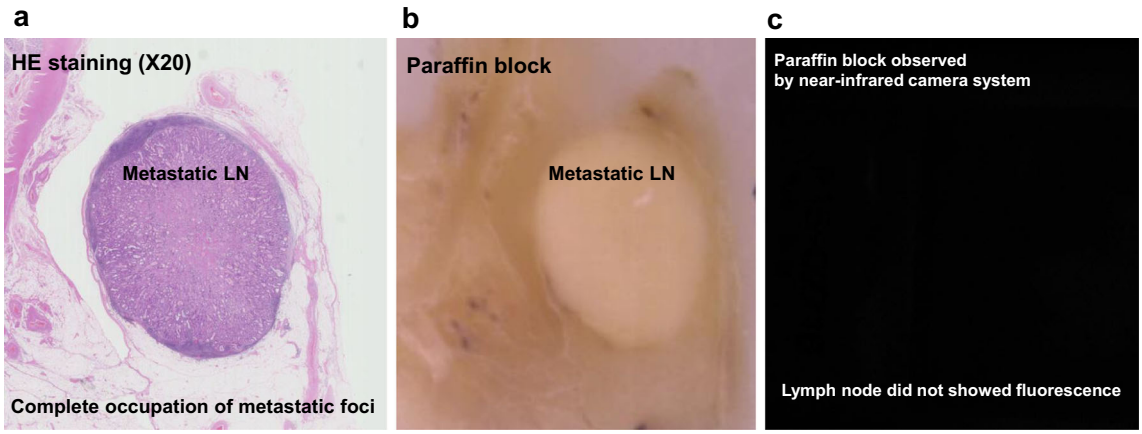

Fig. 2 Negative finding of ICG fluorescence in the LN completely occupied by metastatic foci (case 5, mLN No. 9). **a** HE stain (× 20). **b** Formalin-fixed paraffin-embedded tissue (paraffin block). **c** The paraffin block was observed using a near-infrared camera system. The lymph node did not show fluorescence

proper LN dissection [21]. For these reasons, this study enrolled patients with clinically no lymph node metastases on preoperative CT scans. In this study, according to the result of ICG application, we did not have any situation where we extended the resection or lymph nodes dissection because we performed lymph node dissection without regard to the fluorescence results. This procedure was pre-planned in advance in the study protocol in this study.

When detecting swollen LNs suspected of being metastatic on preoperative CT, we should dissect the area containing these LNs, even if it does not fluoresce in real-time during surgery. It should be noted that some lymph nodes, such as mLN No. 7 (3.5 × 2.5 mm) and 20 (3.0× 2.0 mm), were completely occupied by cancer even at small sizes. These lymph nodes cannot be pointed out as swollen lymph nodes by preoperative CT and do not fluoresce by ICG fluorescence. The presence of such LNs suggests that ICG-FI-guided lymph node dissection may be inadequate and that lymph node dissection in the fluorescent region only does not recommend.

On the other hand, there is a problem as to whether the fluorescent lymph nodes need to be dissected because ICG flows through the normal lymph node structure. In this study, 80% of non-occupied metastatic LNs fluoresce, so fluorescent lymph nodes should be dissected. In addition, if no metastatic foci are exposed on the cut surface of the LN, the metastatic LN is diagnosed as negative (false negative) in the permanent specimen. LNs in the fluorescent area might contain microcarcinomas and should therefore be dissected.

Of note, there were two non-fluorescent LNs that were not occupied by metastases in the present study. In these two LNs (Nos. 23 and 25), 90% and 95% of the LN structure were occupied by metastatic foci. If most of the LN structure is destroyed, fluorescence might not appear due to lymphatic vessel destruction or obstruction, even though a small amount of normal structure of the LN remains.

In recent years, clinical applications of tumor-targeted fluorescence imaging have been reported [22]. Tumor-targeted fluorescence imaging may advance the current practice of

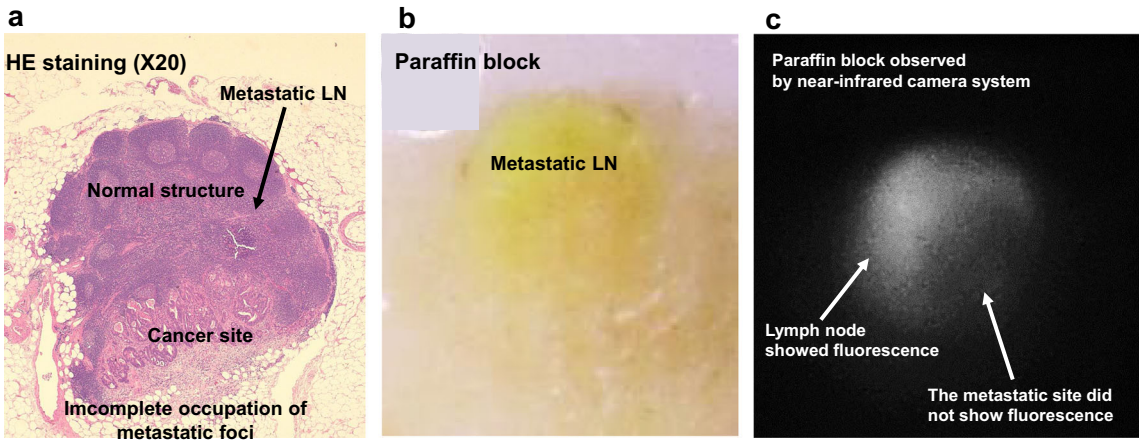

Fig. 3 Positive finding of ICG fluorescence in the LN unoccupied by metastatic foci (case 7, mLN No. 14). **a** HE stain (× 20). **b** Formalin-fixed paraffin-embedded tissue (paraffin block). **c** The paraffin block was observed using a near-infrared camera system. The lymph node showed fluorescence, but the metastatic site did not show fluorescence

tumor surgery by selectively highlighting malignant tissue during surgery. An open-label pilot study in the Netherlands evaluated the fluorescent anti-CEA monoclonal antibody SGM-101 in colorectal cancer patients with elevated serum CEA levels since diagnosis. Nineteen (43%) of 43 lesions were detected using fluorescence imaging and were not clinically suspected before fluorescent detection, which changed the treatment strategy in six (35%) of 17 patients. The development and clinical application of such tumor-targeted fluorescence imaging could complement non-fluorescence of cancer foci by ICG-FI. We believe that the combined use of ICG-FI with tumor-targeted fluorescence imaging will enable more accurate fluorescence-guided lymph node dissection.

Several limitations associated with the present study warrant mention. First, only a small number of patients and metastatic LNs were included. Second, it was unclear from this study how much displacement of normal structure to metastatic foci caused non-fluorescence on ICG-FI. Third, LNs were not evaluated by multi-section but a single cross section. Therefore, micrometastasis may have been overlooked. Further studies with a larger population are needed to confirm our findings.

In conclusion, the metastatic LNs that were completely occupied by cancer showed no fluorescence on ICG-FI, regardless of the LN size, because of lymph flow obstruction. In contrast, 8 of 10 metastatic LNs that were not completely occupied by cancer still showed fluorescence. Even in non-occupied LNs that show fluorescence, the cancer site may not show fluorescence, indicating that LN dissection should not be omitted even if the LNs do not fluoresce on intraoperative ICG-FI.

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Author contributions Jun Watanabe contributed to the study design. All of the authors contributed to the data collection, data analysis, and interpretation. Jun Watanabe and Manabu Kakizoe contributed to the statistical analyses. All of the authors contributed to the writing or review of the report and approved the final version.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval The study protocol was approved by the Ethical Advisory Committee of Yokosuka Kyosai Hospital.

Consent to participate All patients provided their written informed consent before enrolling in the study.

Consent for publication Not applicable.

Code availability Not applicable

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