


Evaluation of lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time indocyanine green fluorescence imaging

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Abstract

Purpose The treatment of splenic flexural colon cancer is not standardized because the lymphatic drainage is variable. The aim of this study is to evaluate the lymph flow at the splenic flexure.

Methods From July 2013 to January 2016, consecutive patients of the splenic flexural colon cancer with a preoperative diagnosis of N0 who underwent laparoscopic surgery were enrolled. Primary outcome is frequency of the direction of lymph flow from splenic flexure. We injected indocyanine green (2.5 mg) into the submucosal layer around the tumor and observed lymph flow using the laparoscopic near-infrared camera system in 30 min after injection.

Results Thirty-one patients were enrolled in this study. The lymph flow was visualized in 31 patients (100 %) without any complications. No case exhibited lymph flow in both the left colic artery (LCA) and left branch of the middle colic artery (It-MCA) areas. There were 19 cases (61.3 %) with lymph flow directed to the area of the root of the inferior mesenteric vein (IMV), regardless of the presence of the left accessory aberrant colic artery. Lymph node metastases were observed in six cases (19.4 %), and all of the involved lymph nodes

existed in lymph flow areas determined by real-time indocyanine green fluorescence imaging.

Conclusions The findings of the lymph flow pattern of splenic flexure suggest that lymph node dissection at the root of the IMV area is important, and it may be not necessary to ligate both the It-MCA and LCA, at least in cases without widespread lymph node metastases.

Keywords Indocyanine green · Colorectal surgery · Near infrared · Lymph flow · Splenic flexure · Colon cancer

Introduction

Many clinical trials have demonstrated the benefits and safety of laparoscopic surgery for colon cancer compared with open surgery [1–4]. Moreover, the long-term outcomes after laparoscopic surgery for colon cancer are equal to that of open surgery [5–7]. Therefore, laparoscopic surgery for colon cancer has become an alternative to open surgery. Complete mesocolic excision (CME) and central vascular ligation (CVL) are current state-of-the-art colon cancer treatments that may provide improved oncologic outcomes [8, 9]. However, laparoscopic surgery of colon cancer located in the splenic flexure is not standardized. Patients with tumors in this location have been excluded from randomized controlled trials because of difficulty in deciding on the appropriate operative procedure, as well as technical difficulties with laparoscopic lymph node dissection. The lymphatic drainage at this site is variable, and the exact site of lymphatic dissection is uncertain [10–16].

It is considered that carcinoma at this site has several lymphatic drainage roots. The lymphatic drainage roots may be the left branch of the middle colic artery (It-MCA) and left colic artery (LCA) areas [12], in addition to the left accessory aberrant colic artery (LAACA) area when present [17].

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Moreover, aberrant metastatic pathways to the infrapancreatic lymph node region and the splenic hilum have been reported [11, 16]. However, to date, there have been no systematic data in the literature regarding the frequency of lymphatic drainage roots at this site.

In recent years, the usefulness of indocyanine green fluorescent imaging (ICG-FI) has been reported in various surgical oncology studies, such as the identification of sentinel lymph nodes in breast cancer [18]. In the colorectal cancer field, many reports have evaluated the blood flow, whereas reports on the lymph flow have been scarce [19–23].

The aim of this study was to evaluate the lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time ICG-FI.

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>]. The procedure used during this study was explained, and written informed consent was obtained from all of the patients. From July 2013 to January 2016, consecutive patients with a preoperative diagnosis of colon cancer at the splenic flexure with a preoperative diagnosis of N0 who underwent laparoscopic surgery with lymph node dissection in Yokosuka Kyosai Hospital were enrolled in this study. The splenic flexure was defined as the junction between the distal third of the transverse colon and the first part of the descending colon [14, 24]. The cancer site was diagnosed using colonoscopy and a barium enema. The clinical stage was diagnosed using contrast-enhanced computed tomography (CT) of the chest and abdomen. A lymph node with a minor axis measuring >10 mm on CT was defined as a metastatic lymph node in this study. The exclusion criteria were a past history of colonic surgery, extended colorectal resection, allergic hypersensitivity to indocyanine green (ICG), or allergic hypersensitivity to iodine. The primary outcome was the frequency of the direction of the lymph flow of splenic flexural colon cancer. Secondary outcomes were identification of the lymph flow (in percentage), number of fluorescent lymph nodes, and total number of dissected lymph nodes.

Indocyanine green fluorescent imaging

We performed tumor marking with India ink under colonoscopic vision <96 h before surgery. A stock solution of ICG (Diagnogreen; Daiichi 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 laparoscopic NIR camera system was provided by 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 two 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.

Operative procedure

A single surgeon (JW) who was qualified by the endoscopic surgical skill qualification system of the Japan Society for Endoscopic Surgery (JSES) [25] performed CME with CVL. The operative procedure was performed with five ports: a 12-mm port in the umbilical region; 5-mm ports in the upper-right, left, and lower-left quadrants; and a 12-mm port in the lower-right quadrant. A 12-mm umbilical trocar was used as a camera port for a rigid scope. CVL and colon mobilization were performed laparoscopically. Lymph node dissection was performed without regard to the fluorescence results. The lymphatic drainage roots of the It-MCA and LCA and the root of the inferior mesenteric vein (IMV) areas were dissected by ligating the It-MCA, LCA, IMV (at the inferior border of pancreas), and the LAACA (when present). The specimen was extracted through the umbilical port, which extended to about 2–5 cm. To avoid contamination, a wound protector was used in every case. Functional end-to-end anastomosis (FEEA) was performed using the Endo GIA™ Universal or Echelon™60 stapling system extracorporeally. After the specimen was extracted through the umbilical incision, we excised the lymph nodes from the mesentery on the back table and observed the lymph nodes extracorporeally using the near-infrared camera system (HyperEye Medical System, Mizuho Corporation, Tokyo, Japan) to evaluate the number of fluorescent lymph nodes.

Statistical analysis

The data are presented as the mean and standard deviation or as the median and range. Statistical analyses were performed using the SPSS statistical software program (SPSS Inc., Chicago, IL, USA).

Results

A total of 31 consecutive patients were enrolled in this study. The clinicopathological characteristics and surgical outcomes of the patients are presented in Table 1. These patients included nine females and had a mean age of 67.5 years and a mean body mass index of 23.6 kg/m². The mean operative time was

Table 1 The clinicopathological characteristics and surgical outcomes

Variable	n = 31
Age, year, mean ± SD	67.5 ± 12.2
Gender, male/female	22/9
Body mass index (kg/m ²) mean ± SD	23.6 ± 3.24
ECOG performance status	
0	28
1	3
2	0
3	0
ASA physical status	
I	2
II	29
III	0
UICC stage	
0-I	13
II	18
III	0
Operative time, min, mean ± SD	206 ± 39.2
Blood loss, ml, mean ± SD	52.5 ± 106
Identification of the lymph flow, yes/no	31/0
Total number of dissected lymph nodes, mean ± SD	17.5 ± 7.60
Number of fluorescent lymph nodes, Mean ± SD	10.4 ± 4.73
Regional lymph node metastasis, yes/no	6/25
Postoperative complication, yes/no	3/28
Small bowel obstruction	2
Superficial surgical site infection	1
Anastomotic leakage	0
Length of hospital stay, day, median, range	5 (5–26)

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

UICC Union for international Cancer Control

208 min, and the mean blood loss was 52 ml. The lymph flow was visualized in 31 patients (100 %) intraoperatively (Fig. 1). All procedures were safely performed without any complications, and there were no issues with outshining lymph nodes near the tumor or spillage of ICG.

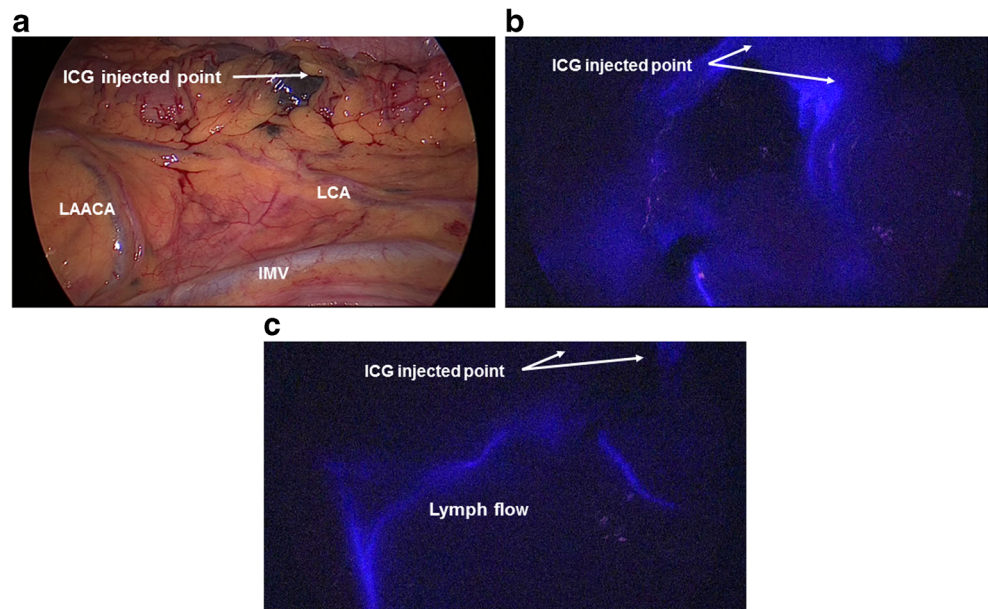
The presence of LAACA rate was 38.7 % (12 cases). In these cases, the prevalent direction of the lymph flow was the LAACA and LCA areas in five cases (16.1 %), the LAACA area in four cases (12.9 %), and the LAACA and It-MCA areas in three cases (9.7 %) (Fig. 2). Regarding cases in which the LAACA was absent, the prevalent direction of the lymph flow was the LCA area in eight cases (25.8 %), the It-MCA area in six cases (19.4 %), and the root of IMV which did not accompany the artery in five cases (16.1 %). Among eight cases in which the lymph flow ran along the LCA, we observed two cases (6.5 %) that had lymph flow running along the LCA at the beginning, followed by branching to the root of the LCA and to the root of the IMV (Fig. 3). Overall, no case

exhibited lymph flow in both the LCA and It-MCA areas. There were 19 cases (61.3 %) with lymph flow directed to the area of the root of IMV, regardless of the presence of the LAACA. The total number of retrieved lymph nodes was 17.5, and fluorescent lymph nodes were 10.4 (Fig. 4). Lymph node metastases were observed in six cases (19.4 %), and all of the involved lymph nodes existed in lymph flow areas determined by the fluorescence lymphatic flow evaluation.

Discussion

In CME for splenic flexural colon cancer, dissection of both the transverse and descending mesocolon must be considered [12]. In addition, due to blood vessel variation and complicated lymph flow, including aberrant lymph flow in the splenic flexure sites [10–16], lymph node dissection may be required depending on each individual case; however, it is quite difficult to determine the necessity during surgery. The blood supply to the splenic flexure has been shown to be variable; it is predominantly carried by the inferior mesenteric artery (IMA) via the LCA in 89 % of cases, while the remaining cases (11 %) are carried by the superior mesenteric artery (SMA) via the middle colic artery (MCA) [26]. Moreover, a LAACA can aberrantly originate from the SMA more proximal to the MCA, at the inferior border of the pancreas, which supplies the splenic flexure and the proximal part of the descending colon [17]. The frequency of appearance has been reported to be 4–49.2 % [17]. In this study, the frequency of the presence of LAACA was 38.7 % (12 cases). With respect to the selection of the operative procedure for CME for splenic flexural colon cancer, left hemicolectomy with CVL, ligating both the MCA at its origin, the ascending branch of the LCA, and, if it exists, the LAACA to remove the lymph nodes likely involved, has been advocated [10, 12]. However, there have been no systematic data in the literature on the choice of operative procedure at this site. Thus, understanding the lymph flow pattern of splenic flexural colon cancer would be useful. In this study, no case exhibited a lymph flow directed to the areas of both the LCA and It-MCA, and 19 cases (61.3 %) demonstrated a lymph flow directed to the area of the root of IMV, regardless of the presence of the LAACA. With regard to the distal third of the transverse colon cancer (six cases), the lymph flow ran along the It-MCA in all cases. With regard to the first part of the descending colon cancer (seven cases), the lymph flow ran along the LCA in all cases. Thus, in the case of the distal third of the transverse colon cancer, we should perform CME with lymph node dissection of the It-MCA and the root of the IMV areas; in the case of the first part of the descending colon cancer, we should perform CME with lymph node dissection of the LCA and the root of the IMV areas. In the case of just splenic flexural colon cancer, the lymph flow

Fig. 1 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 min after injection (a). The lymph flow was visualized intraoperatively (b, c)



could run in various directions. Therefore, the use of laparoscopic real-time ICG-FI may help to identify appropriate central vessels to be dissected, as well as determine the appropriate separation line of mesentery. We speculate that it is not necessary to ligate both the MCA and LCA, at least in cases without widespread lymph node metastasis, because no case with lymph flow directed to both the LCA and It-MCA was observed in this study.

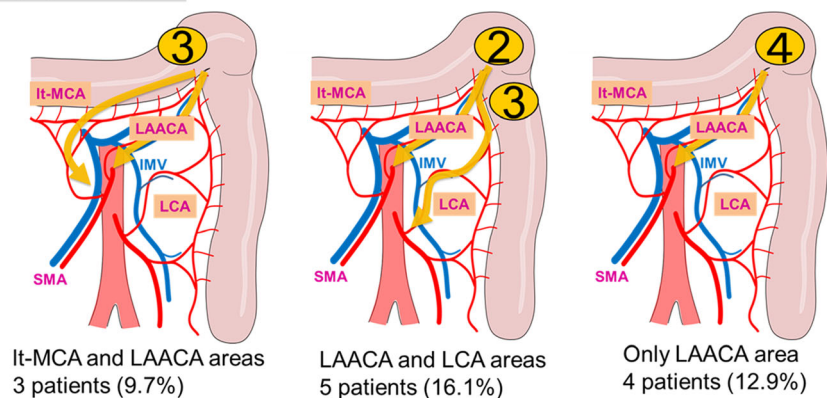
Direct lymphatic drainage to the splenic hilum and along the pancreatic tail has been demonstrated in anatomical studies of lymphatic drainage of the splenic flexure, and routine splenectomy with distal pancreatectomy has been advocated for splenic flexural colon cancer [24]. On the other hand, it has been reported that splenectomy and distal pancreatectomy for splenic flexural colon cancer did not improve the long-term survival [15]. In this study, no lymph flow directed to the pancreatic tail and the splenic hilum was observed. Therefore, we believe that routine distal pancreatectomy and

splenectomy are not necessary, at least in cases without widespread lymph node metastasis.

ICG-FI has been developed for the use of perioperative blood flow assessment and identification of sentinel lymph nodes in coronary artery bypass grafting and melanoma and breast cancer surgery [18, 27, 28]. In the field of colorectal cancer, it has been used for marking tumors [29], blood flow assessment for anastomotic sites [19–21, 23], and identification of sentinel lymph nodes [30]. In the colorectal cancer field, regarding false-negative results (low sensitivity), an optimum biopsy method for the sentinel lymph nodes has not yet been established, and further clinical trials are required for such a biopsy to be applied in actual clinical settings [31–33]. ICG-FI is capable of assessing not only lymph nodes but also the lymph flow in real time [22]. However, lymph flow assessments using ICG-FI have been scarcely reported [22]. In this study, the lymph flow was visualized in 31 patients (100 %) intraoperatively without any complications.

Fig. 2 The presence of LAACA rate was 38.7 % (12 cases). In these cases, the prevalent direction of the lymph flow was the LAACA and LCA areas in five cases (16.1 %), the LAACA area in four cases (12.9 %), and the LAACA and It-MCA areas in three cases (9.7 %)

LAACA was recognized at 12 patients (38.7%)
Lymph flow type



LAACL was not recognized at 19 patients (61.3%)

Lymph flow type

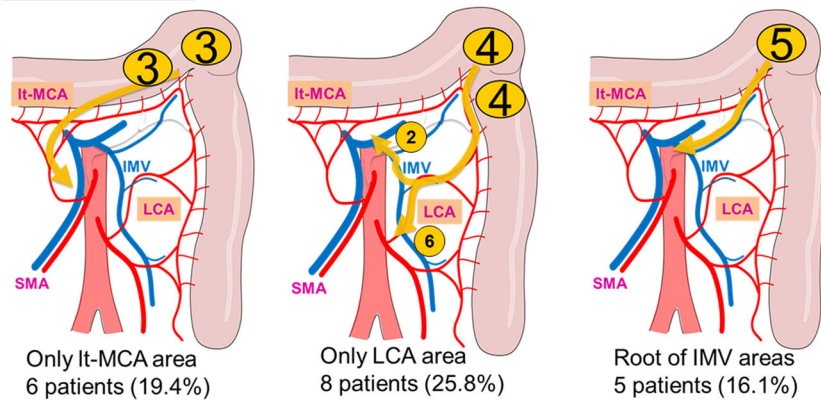


Fig. 3 Regarding cases in which the LAACA was absent, the prevalent direction of the lymph flow was the LCA area in eight cases (25.8 %), the It-MCA area in six cases (19.4 %), and the root of IMV which did not accompany the artery in five cases (16.1 %). Among eight cases in which

the lymph flow ran along the LCA, we observed two cases (6.5 %) that had lymph flow running along the LCA at the beginning, followed by branching to the root of the LCA and to the root of the IMV

This lymph flow evaluation differs from sentinel lymph node mapping in terms of the selective removal of the mesocolon to drain the tumor. Furthermore, it is possible to clinically apply this method in combination with the concept of CME without causing problems such as a false-negative result for the sentinel lymph nodes, helping to optimize and individualize CME. Indeed, in two patients histopathologically diagnosed with lymph node metastases, no metastases were detected in the fluorescent sentinel lymph nodes. These two patients were found to have skip metastases. However, all of the involved lymph nodes in these patients existed in the lymph flow areas observed on the fluorescence lymphatic flow evaluation.

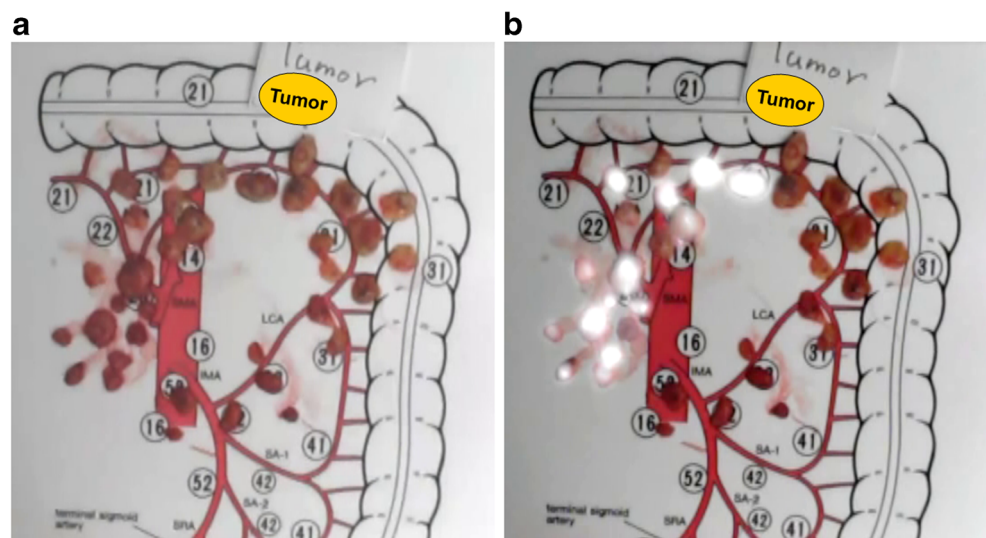
Sentinel lymph node mapping of patients with colorectal cancer is a difficult technique, and a learning curve of 20–30 cases has been described. However, this lymph flow evaluation is better suited for clinical application than sentinel lymph node biopsy and has less of a learning curve. Before

conducting the present study, we evaluated the intraoperative lymphatic flow using ICG in ten patients. Spillage was observed during injection of ICG in 1 of the 10 patients. In the present study, no ICG spillage occurred.

In this study, two patients had a body mass index >30 kg/m² (31.63 and 31.14 kg/m²). The lymph flow was visualized in both patients intraoperatively. We therefore believe that ICG fluorescence imaging is acceptable for patients with high BMI.

In cases with widespread lymph node metastasis as the lymph flow may be bypassed to other areas as a result of lymphatic vessel occlusion, this method may possibly misinterpret the dissection range [34]. For these reasons, the present study included cases without any obvious lymph node metastasis observed in the preoperative CT scan. However, a preoperative CT scan is not standard worldwide. The applicability of these findings is therefore limited to institutions where a preoperative CT scan is routinely performed.

Fig. 4 After the specimen was extracted through the umbilical incision, we excised the lymph nodes from the mesentery on the back table and observed the lymph nodes extracorporeally with white light (a) and using the near-infrared camera system to evaluate the number of fluorescent lymph nodes (b)



There are some limitations associated with this study. Only a small number of patients were included, and the follow-up period was short. Further studies with a larger population and a long-term follow-up are needed to confirm our findings.

In conclusion, the findings on the analysis of the lymph flow pattern using ICG-FI during laparoscopic splenic flexural colon cancer surgery suggest that lymph node dissection of the root of the IMV area is important, and it may not be necessary to ligate both the It-MCA and LCA, at least in cases without widespread lymph node metastases. The comprehension of this lymph flow pattern may be helpful for identifying appropriate central vessels to be dissected.

Compliance with ethical standards 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>]. The procedure used during this study was explained, and written informed consent was obtained from all of the patients.

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

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