



# The Feasibility and Safety of Preoperative Fluorescence Marking with Indocyanine Green (ICG) in Laparoscopic Gastrectomy for Gastric Cancer

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## Abstract

**Background** Securing the surgical margin is the most essential and important task in curative surgery. However, it is difficult to accurately identify the tumor location during laparoscopic surgery for gastric cancer, and existing methods, such as preoperative endoscopic marking with tattooing and clipping, have multiple disadvantages.

**Aims** We investigated the feasibility and safety of indocyanine green (ICG) fluorescence marking for determining the tumor location during laparoscopic gastrectomy.

**Methods** We retrospectively analyzed preoperative and perioperative data from consecutive patients with gastric cancer undergoing planned laparoscopic distal gastrectomy. Data was maintained in a prospectively compiled surgical database, and patients were categorized into ICG ( $n = 84$ ) or non-ICG ( $n = 174$ ) groups based on whether they underwent preoperative endoscopic mucosal ICG injection. One-to-one propensity score matching (PSM) was performed to compare outcomes between the two groups.

**Results** We included 84 patient pairs after PSM, and there were no significant differences in preoperative patient characteristics. The ICG group had shorter procedure time ( $p < 0.001$ ), lower estimated blood loss ( $p = 0.005$ ), and significantly shorter post-operative hospital stay ( $p < 0.001$ ). Positive resection margins were confirmed in five cases (6.0%) in the non-ICG group, whereas there were none in the ICG group ( $p = 0.008$ ). Real-time confirmation was possible during laparoscopy, and the injected ICG did not affect the surgical procedure or result in adverse events.

**Conclusion** ICG fluorescence imaging is feasible and safe and can potentially be used as a tumor-marking agent for determining the surgical resection line.

**Keywords** Gastric cancer · Curative resection · Indocyanine green fluorescence imaging · Tumor localization · Total laparoscopic gastrectomy

## Introduction

Securing the correct surgical margin is the most essential and important task in curative surgery. However, in laparoscopic

surgery, determining the tumor location is difficult, and there is no standard protocol for the same. In Japan and other Asian countries with a high proportion of early gastric cancer patients, a preoperative endoscopic marking method, such as India ink tattooing and/or a clipping method, is usually performed to precisely identify the tumor location during surgery.<sup>1–7</sup> However, the use of India ink tattooing has resulted in reports of adverse events, such as peritonitis due to intraperitoneal spraying of the black ink and ileus obstruction due to adhesion, and the safety of this procedure remains unclear.<sup>8,9</sup> In addition, intraperitoneal spraying of India ink leads to poor recognition of the release layer and affects the procedure by making it impossible to recognize an appropriate

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The study has not been presented at any regional, national, or international meeting.

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resection line. On the other hand, the clipping method requires confirmation by palpation, which is not useful during laparoscopic surgery. Further, clipping may not be recognizable during a laparotomy because of excessive intestinal wall or fat thickness, making this an unreliable method. The reported confirmation method involving marking clips with intraoperative endoscopy<sup>7</sup> is cumbersome as the process is increased, and the preparation of the intraoperative endoscopy is necessary. Thus, these approaches require extraneous efforts, with increased operation time, the need for additional manpower, the possibility of additional frozen sections in suspicious cases of the gross margin.<sup>10</sup>

In recent years, indocyanine green (ICG) fluorescence imaging has received much attention in laparoscopic surgery. ICG binds to plasma proteins and emits fluorescence after stimulation using a laser beam<sup>11,12</sup> or near-infrared (NIR) light at a wavelength of approximately  $\geq 820$  nm.<sup>13,14</sup> The absorption maximum of ICG is approximately 807 nm while the emission maximum is approximately 822 nm.<sup>15</sup> The emitted ICG fluorescence is detected using specifically designated scopes and camera. Currently, ICG fluorescence imaging is being used for navigational laparoscopic gastrectomy, to detect the sentinel lymph nodes during gastric cancer and visualize blood flow,<sup>16–20</sup> and for determining the tumor location during laparoscopic colon tumor resection.<sup>21,22</sup> However, there are no reports on ICG fluorescence tumor marking for laparoscopic gastrectomy. Therefore, we assessed the usefulness and safety of a preoperative ICG marking method that used NIR fluorescence for activation and a laparoscopic imaging system during total laparoscopic gastrectomy.

## Methods

### Patients

This retrospective analysis used prospectively compiled surgical data from July 2015 to August 2017 at Department of Surgery, Osaka International Cancer Institute, Osaka, Japan. Data on consecutive patients who underwent scheduled laparoscopic distal gastrectomy (LDG) for gastric cancer were extracted from the database that included patient characteristics (age, sex, body mass index (BMI;  $\text{kg}/\text{m}^2$ )), preoperative data (clinical T and N stages and preoperative histology), surgical outcomes including intraoperative events (surgical approach, procedure time, and estimated blood loss), and postoperative course and outcome. We only used data from patients who fulfilled the inclusion criteria of gastric cancer without distant metastasis as determined by preoperative physical examination and imaging studies and who were physiologically normal, defined as the American Society of Anesthesiology physical status score of  $\leq 2$ . Patients with concurrent malignancies, those who had undergone preoperative

neoadjuvant chemotherapy (NAC), and those participating in other clinical trials were excluded from the study. We identified 258 patient records, and these were divided into two groups based on ICG use as patients with ICG marking (ICG group,  $n = 84$ ) and those without ICG marking (non-ICG group,  $n = 174$ ).

Gastric resection and anastomosis construction were performed intracorporeally in all cases and were a delta-shaped anastomosis or Roux-en-Y reconstruction, depending on the size of the remnant stomach after LDG. In addition, a D1+ or D2 lymphadenectomy was performed in all cases to comply with the treatment guidelines of the Japanese Gastric Cancer Association.<sup>23</sup>

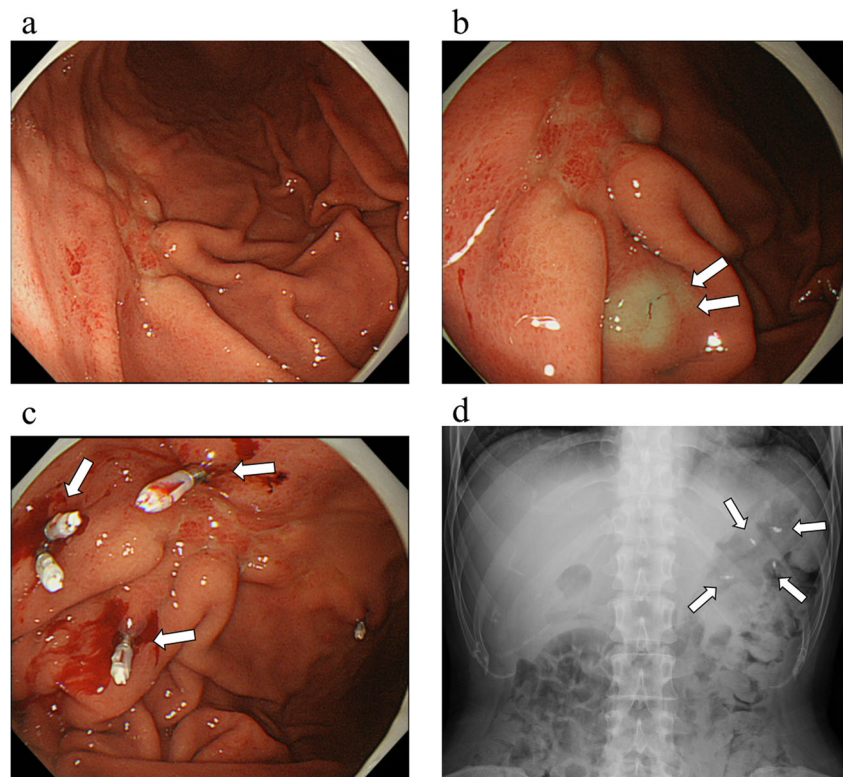
### Localization Procedure

ICG (Diagnogreen®) was purchased from Daiichi-Sankyo Co., Ltd. (Tokyo, Japan). All cases in the ICG group underwent GIF 1 day preoperatively, and ICG marking was performed around the tumor. The solution for ICG marking was prepared by dissolving Diagnogreen 1 V in 10 ml of distilled water; 0.2 ml of this solution was mixed with 9.8 ml of distilled water to obtain 10 ml of solution (0.05 mg/ml of Diagnogreen). The prepared solution (0.5 ml at a time) was submucosally injected under endoscopic guidance at four sites around the tumor (proximal side, distal side, and bilateral sides) (Fig. 1). To evaluate the spread of ICG from the injection site after the operation, clipping was performed at the injection point. In the non-ICG group, clipping was performed on the oral side of the tumor. ICG diffusion range from the clipping points was measured after tumor excision and recorded at four places.

### Laparoscopic Equipment

A laparoscopic laser-free FULL HD system was used for all procedures. Images were generated using a NIR/ICG telescope and camera head system (IMAGE1 S™ System, KARL STORZ, Tuttlingen, Germany) connected to a laparoscope (30" field of direction and 10 mm diameter) equipped with a specific filter for optimal detection of NIR fluorescence and white light without manual switching. The xenon light source (D-LIGHT P, KARL STORZ) provided both visible and NIR excitation light with enhanced background display. Switching between white light and NIR fluorescence mode was controlled by the surgeon using a footswitch. Visualization, both in standard and NIR light, was improved by an image enhancement system (IMAGE1 S™ System, KARL STORZ) that offered adjustable visualization modalities that could be selected according to the surgeon's preferences.

**Fig. 1** Preoperative endoscopic fluorescence tattooing and clipping. Endoscopic view. **a** Indocyanine green (ICG) was injected after locating the tumor lesion. **b** 0.5 mL was injected at four points, namely, proximal, distal, and bilateral, in the gastric cancer region (white arrow describes the ICG marking). **c** Marking clips were placed on the scar after injecting ICG (white arrows indicate marking clip positions at ICG injection points). **d** After ICG tattooing and clipping, we evaluated their position using X-rays (white arrow indicates the marking clip positions at the ICG injection points)

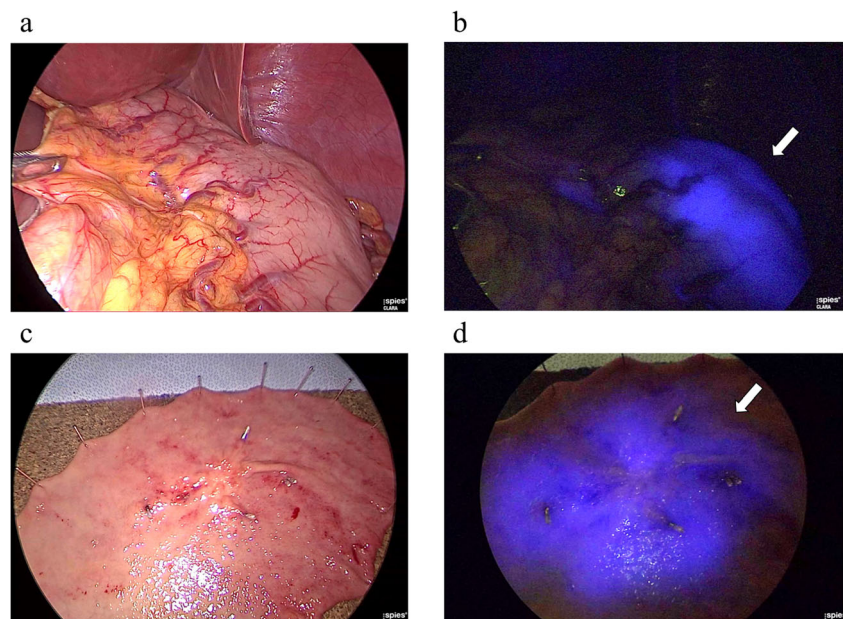


## Surgical Approach

The surgical procedure used for LDG has been described previously.<sup>24</sup> Briefly, the initial port was placed through a 2.5-cm infraumbilical incision made using the open method and a commercially available access port (EZ Access; Hakko, Nagano, Japan). During the procedure, a pneumoperitoneum was established using carbon dioxide insufflation at a pressure of

approximately 10–12 mmHg according to body type of the patient. LDG was performed using five ports (one 12-mm port in the umbilicus and 5- and 12-mm ports in the right and left lateral abdomen). We mainly used conventional straight graspers and an ultrasonic coagulation-cutting device (Harmonic scalpel; Ethicon Endo-Surgery, Cincinnati, OH, USA) to perform gastric mobilization and lymph node dissection. During laparoscopic gastrectomy, the stomach was first illuminated with white light (Fig. 2),

**Fig. 2** Tumor localization using the intraoperative laparoscopic fluorescence imaging system. Laparoscopic view. Tumor location was visualized using near-infrared light-activated ICG fluorescence. **a, c** Under white light. **b, d** under near-infrared light-activated ICG. **a** White arrow indicates fluorescent outer wall of the stomach. **d** White arrow indicates fluorescent inner wall of the stomach



and ICG was subsequently activated with NIR light and viewed via a laparoscopic imaging system in patients injected with ICG to determine the tumor location. Afterward, the stomach was resected. After gross inspection and measurement of any serosal tumor involvement, the stomach was, in principle, opened along its greater curvature for examination from the mucosal side and measurement of the tumor size and length of the proximal and distal resection margins. If a positive resection margin was suspected, intraoperative histological examination of frozen sections was performed, and if indeed the resection margin was positive, the procedure was changed to laparoscopic total gastrectomy (LTG). Postoperatively, if the stump was also found to have positive margins, another LTG was planned as soon as possible, and the intracorporeal anastomosis technique was used for reconstruction.<sup>25,26</sup>

### Perioperative Surgical Outcomes

Data obtained from patient records included patient characteristics, procedure duration, intraoperative estimated blood loss, pathological findings (e.g., tumor depth, proximal and distal margins, number of lymph node dissected, and lymph node metastasis), postoperative morbidity rate, frequency of additional resection, and duration of postoperative hospital stay. Complications during surgery were assessed based on the Clavien–Dindo classification,<sup>27</sup> and TNM staging was based on the Japanese Classification of Gastric Carcinoma.<sup>28</sup> Outcomes specific to ICG usage were detection rate of ICG during gastrectomy, size of the visualized ICG, morbidity after preoperative upper endoscopy, and adverse effects after ICG injection.

### Statistical Analysis

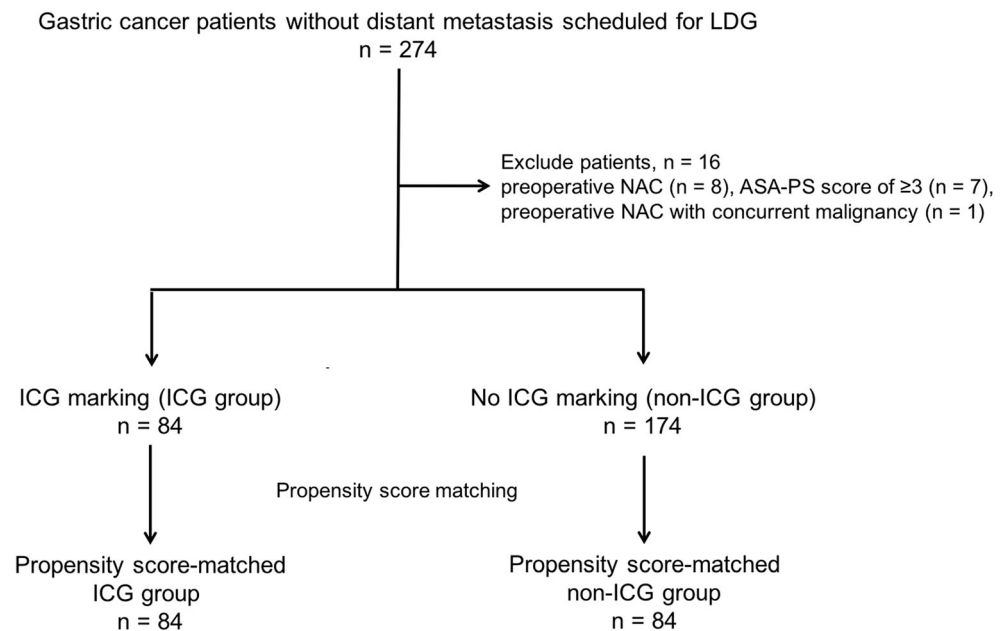
All statistical analyses were performed using the JMP® PRO software (JMP version 13.1.0, SAS Institute, Cary, NC). PSM analysis was conducted using a logistic regression model with the following covariates: age, sex, American Society of Anesthesiologists physical status classification (ASA-PS), BMI, histology, tumor location, clinical stage, and presence of preoperative ESD. We used a caliper width of 0.2 for the pooled standard deviation of the logit for calculating the propensity score for PSM. Demographic and clinicopathological characteristics were summarized and descriptively analyzed, and all qualitative values are presented as means and standard deviations, unless otherwise specified. The Student’s *t* test or the Mann–Whitney *U* test and Pearson’s  $\chi^2$  test were used to compare continuous and categorical variables, respectively. Categorical variables were also compared using the Fisher’s exact test. All values were two tailed, and *p* values < 0.05 were considered to be significant.

### Results

#### Baseline Demographics and Preoperative Assessment

The study flow chart is summarized in Fig. 3. We identified 274 consecutive cases that underwent LDG from July 2015 to August 2017. One hundred eighty-one cases in the previous term in continuous cases were in the non-ICG group and 93 cases in the later period were in the ICG group. We excluded 16 cases due to preoperative NAC (*n* = 8), ASA-PS score of  $\geq 3$  (*n* = 7), and preoperative NAC with concurrent malignancy

**Fig. 3** Study flow chart. The inclusion criteria are as follows: a normal physiologic condition defined as an ASA-PS score of  $\leq 2$ , the patients without concurrent malignancies, NAC, and those participating in other clinical trials. PSM was performed by logistic analysis using age, sex, ASA-PS, BMI, histology, tumor location, clinical stage, and presence of preoperative ESD as covariates. We used 0.2 times of the pooled standard deviation of the logit of the propensity score as caliper width for PSM



( $n = 1$ ). Eighty-four cases in the ICG group and 174 cases in the non-ICG group were adjusted by PSM and 84 pairs of cases were analyzed. The baseline characteristics of the ICG and non-ICG groups are summarized in Table 1. No significant differences were observed between the two groups with respect to preoperative characteristics.

### Perioperative Outcomes

All surgical outcomes analyzed are detailed in Table 2. All procedures were laparoscopic, and no case needed conversion to open gastrectomy. A comparison of surgical characteristics showed that the procedure time was significantly shorter, and estimated blood loss was significantly lower ( $p = 0.005$ ) in the ICG group than in the non-ICG group ( $p < 0.001$ ). Evaluation after surgical tumor resection used frozen sections of the resection line if the tumor border was unclear. In the non-ICG group, five patients had a R1 resection (positive resection margin), and four of these cases were identified by intraoperative frozen section assessment of the resection line; this resulted in the surgical procedure being converted to LTG. There was no significant difference in the rate of tumor retention after initial gastrectomy between the two groups ( $p = 0.238$ ). One case was diagnosed as a R1 resection margin on postoperative permanent specimens, and LTG was subsequently performed after 2 weeks postoperatively. Finally, R0 resection was achieved for all cases with gastric cancer.

There was no significant difference between the tumor size, proximal margin, distal margin, and histopathologic

classification between the two groups (Table 2). In the TNM classification, there was no difference between the two groups in the final stage or tumor depth, but N scores were higher in the ICG group ( $p = 0.002$ ). The number of lymph node dissections observed in the permanent specimens was significantly higher in the ICG group than in the non-ICG group ( $p = 0.046$ ). The rate of postoperative complications did not differ between the two groups, and there were no grade  $\geq 3$  complications, according to the Clavien–Dindo classification. There were also no cases of postoperative mortality and the postoperative hospital stay was significantly shorter in the ICG group than in the non-ICG group ( $p < 0.001$ ).

### Outcomes of ICG Injection

All ICG-specific outcome measures are described in Tables 2 and 3. ICG injection was confirmed intraoperatively in all cases, there were no complications associated with the upper gastrointestinal endoscopy performed for ICG injection, and no adverse effects were observed after ICG injection. During the surgery, tumor localization was identified and lymph flow was confirmed by injecting ICG. No adverse effects attributable to ICG use, such as intra-abdominal peritonitis or adhesion, were observed within the abdominal cavity during surgery. The visible range of ICG fluorescence was calculated to be 10–70 mm with a median of 25 mm. ICG diffusion in the tumor was assessed in four directions (proximal, distal, and bilateral), and there was no difference in ICG diffusion distance among these directions.

**Table 1** Patient characteristics

	ICG group ( $n = 84$ )	Non-ICG group ( $n = 84$ )	$p$ value
Age*	66.2 $\pm$ 1.2	66.6 $\pm$ 1.2	0.811
Sex (M:F)	47 (56.0%):37 (44.1%)	46 (54.8%):38 (45.2%)	0.877
ASA-PS (1:2)	16 (19.0%):68 (81.0%)	15 (17.9%):69 (82.1%)	0.842
Height (cm)*	161.14 $\pm$ 0.99	161.28 $\pm$ 0.99	0.917
Weight (kg)*	59.80 $\pm$ 1.25	59.78 $\pm$ 1.25	0.986
BMI (kg/m <sup>2</sup> )*	22.92 $\pm$ 0.35	22.83 $\pm$ 0.35	0.860
Tumor location (M:L)	52 (61.9%):32 (38.1%)	55 (65.5%):29 (34.5%)	0.630
Tumor location (Less:Gre:Ant:Post:Circ)	37 (44.1%):13 (15.5%):15 (17.9%):17 (20.2%):2 (2.4%)	41 (48.8%):11 (13.1%):14 (16.7%):17 (20.2%):1 (1.2%)	0.946
cT (T1:T2:T3:T4)	55 (65.5%):15 (17.9%):9 (10.7%):5 (6.0%)	62 (73.8%):11 (13.1%):9 (10.7%):2 (2.4%)	0.500
cN (cN0:cN+)	74 (86.9%):11 (13.1%)	75 (89.3%):9 (10.7%)	0.634
cSt (I:II:III:IV)	67 (79.8%):11 (13.1%):6 (7.1%):0 (0%)	70 (83.3%):10 (11.9%):4 (4.8%):0 (0%)	0.773
Pathological classification (tub:por:sig:others)	40 (47.6%):12 (14.3%):32 (38.1%):0 (0%)	40 (47.6%):12 (14.3%):32 (38.1%):0 (0%)	1.000

Tumor location: upper third (U), middle third (M), lower third (L), lesser curvature (Less), greater curvature (Gre), anterior wall (Ant), and posterior wall (Post). TNM staging was based on the Japanese Classification of Gastric Carcinoma, 3rd English version

ASA-PS, American Society of Anesthesiologists Physical Status Classification; BMI, body mass index; ESD, endoscopic submucosal dissection; NAC, neoadjuvant chemotherapy; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; sig, signet-ring cell carcinoma

\* $p = 0.05$  was considered to be statistically significant; values are presented as mean  $\pm$  SE or number (%)

**Table 2** Perioperative outcomes

	ICG group (n = 84)	Non-ICG group (n = 84)	p value
Detection rate of ICG	84 (100%)	–	N/A
Procedure time (min)*	206.1 ± 5.0	237.0 ± 5.0	< 0.001
Operative blood loss (ml)*	10.1 ± 6.6	36.9 ± 6.6	0.005
Surgical procedure (DG: TG)	84 (100%):0 (0%)	80 (96.6%):4 (4.8%)	0.018
Surgical specimen resection margin <sup>a</sup> (positive:negative)	0 (0%):89 (100%)	5 (6.0%):79 (94.1%)	0.008
Residual tumor (R0:R1)	84 (100%):0 (0%)	83 (9.8%):1 (1.2%)	0.238
Proximal margin (mm)*	57.5 ± 3.2	54.0 ± 3.2	0.433
Distal margin (mm)*	76.7 ± 4.8	77.3 ± 4.8	0.935
Tumor size (mm)*	42.6 ± 2.3	38.7 ± 2.3	0.216
Pathological classification (tub:por:sig:others)	35 (41.7%):24 (28.6%):22 (26.2%):3 (3.6%)	38 (45.2%):25 (29.8%):20 (23.8%):1 (1.2%)	0.733
pT (T1:T2:T3:T4)	55 (65.5%):12 (14.3%):11 (13.1%):6 (7.1%)	59 (70.2%):5 (6.0%):15 (17.9%):5 (6.0%)	0.282
pN (N0:N1:N2:N3)	54 (64.3%):20 (23.8%):7 (8.3%):3 (3.6%)	64 (76.2%):4 (4.8%):8 (9.5%):8 (9.5%)	0.002
pStage (I:II:III:IV)	60 (71.4%):17 (20.2%):7 (8.3%):0 (0%)	60 (71.4%):12 (14.3%):12 (14.3%):0 (0%)	0.333
Number of retrieved LN (number)*	47.5 ± 1.7	42.6 ± 1.7	0.046
Overall surgical complications	2 (2.4%)	3 (3.6%)	0.649
Clavien–Dindo > III	0 (0%)	0 (0%)	N/A
Surgical site infection	1 (1.1%)	0 (0%)	
Liver enzyme evaluation <sup>b</sup>	1 (1.1%)	3 (3.6%)	
Morbidity of preoperative upper endoscopy	0 (0%)	–	N/A
Prevalence of adverse effects of ICG injection	0 (0%)	–	N/A
Postoperative hospital stay (days)*	7.5 ± 0.1	8.7 ± 0.1	< 0.001
Postoperative mortality	0	0	N/A

DG, distal gastrectomy; TG, total gastrectomy

\*p = 0.05 was considered to be statistically significant; values are presented as mean ± SE or number (%)

<sup>a</sup> Intraoperative histological examination of frozen sections

<sup>b</sup> Liver enzyme evaluation: AST OR ALT > ULN-3.0xULN

## Discussion

During laparoscopic gastrectomy, the tumor location can be determined only based on images on the monitor and the sense of “touch” perceived through the forceps as it is impossible to palpate intra-abdominal organs. Further, in early gastric

cancer, the tumor cannot be visually identified, except in cases where the location can be recognized by resistance perceived through the forceps. Even in advanced gastric cancer, the accurate tumor location cannot be confirmed visually in laparoscopy, unless the tumor is exposed to serosa. Also, in both early and advanced cancers, the spread of cancer in the lumen cannot be recognized from outside the stomach wall. Previous reports have stated that every effort must be made to secure a negative proximal margin during the surgical treatment of gastric cancer because of the poor prognostic nature of a positive proximal margin.<sup>29,30</sup> Bissolati et al. have demonstrated that survival after gastrectomy is negatively affected by a positive resection margin.<sup>31</sup>

We have used ICG fluorescence to confirm tumor location by locally injecting it under endoscopic guidance into the stomach wall from the mucosal side prior to the gastrectomy. Switching between visible light and fluorescent images was possible using a footswitch, and as this could be performed while looking at the positional relationship between the tumor

**Table 3** Expansion degree of ICG marking

	Median	Range (min–max)	Mean	SE
PD (n = 72)	25.0	15.0–50.0	28.2	1.0
DD (n = 72)	25.0	10.0–70.0	27.0	1.0
RD (n = 72)	25.0	10.0–45.0	25.7	1.0
LD (n = 72)	25.0	10.0–50.0	25.8	1.0
Total (n = 288)	25.0	10.0–70.0	26.7	0.5

PD, proximal distance; DD, distal distance; RD, right-side distance (the right side of the long axis direction from the gastric cardia to the pylorus); LD, left-side distance (the left side of the long axis direction from the gastric cardia to the pylorus)

and the stomach wall, it was particularly useful for determining the resection line. Under these conditions, real-time laparoscopic surgery was possible under white light without the need for confirmation under night vision. This technology was also useful because there is no need to withdraw the stomach from the abdominal cavity during operation.

We performed clipping with ICG injection 1 day prior to the surgery; however, we did not perform intraoperative endoscopy. ICG was injected to surround the cancer, which enabled the determination of the exact localization of the gastric cancer. ICG injection was confirmed to have no complications and adverse events in all cases, including in those where ICG was injected a day prior to the surgery. Excision enabled easy R0 resection; as a result, no fluorescence from ICG was observed in the residual stomach. In addition, intraoperative endoscopy has been reported to be useful in localizing the tumor<sup>7,32,33</sup>; however, our method does not require confirmation of the tumor location by intraoperative endoscopy. In addition, confirmation of the marking clip localization by X-ray fluoroscopy is unnecessary. As a result, it is possible to reduce the operation time more efficiently and accordingly reduce the surgical invasion.

ICG also flows through the lymph duct, and occasionally, the regional lymph nodes may also be labeled by ICG, which may be useful for determining the lymphadenectomy range. This could account for the observation that the number of dissected lymph nodes in the ICG group was significantly larger than that in the non-ICG group. Nimura et al. have reported that infrared ray-based ICG fluorescence imaging make it easy to distinguish between lymphatic vessels and lymph nodes containing ICG and the surrounding tissues compared with naked eye observation.<sup>34</sup> Similarly, many studies have suggested that ICG fluorescence imaging during laparoscopic sentinel node biopsy for gastric cancer is feasible, useful, and safe.<sup>34–38</sup> We have previously reported on the usefulness of detecting sentinel lymph nodes in gastric cancer<sup>36,39–41</sup>; however, the results of a multicenter collaborative research showed an unacceptably high number of false negatives.<sup>42</sup> Therefore, the concept of sentinel lymph nodes in gastric cancer is still not popular, and it is premature to choose reduction surgery according to the results of sentinel lymph nodes. According to our research, LDG using ICG fluorescence enabled the dissection of more number of lymph nodes, and lymph flows such as lymph duct were clearly marked by ICG fluorescence. Thus, this procedure may be useful for intraoperative lymph flow mapping.

In gastric cancer, it is difficult to locate the tumor from outside the wall; therefore, it is currently common to locally inject India ink in the vicinity of the tumor under endoscopic guidance preoperatively.<sup>5,6</sup> However, it is often difficult to confirm the preoperative tattooing with dye injection depending on the tumor location, the fat volume, and the thickness of stomach wall. In addition, it has been reported that the ink

spreads widely, resulting in uncertain localization.<sup>4</sup> Matsuda et al. have described a method that combines intraoperative laparoscopic ultrasonography with preoperative clipping and tattooing, but a disadvantage of this method is that clips may be applied erroneously in the anterior wall when the tumor is located at the posterior wall.<sup>4</sup>

In addition, peritonitis due to intraperitoneal/intestinal mucosal scattering through the intestinal puncture, intra-abdominal adhesion, and poor visual field are also disadvantages, and the effects of India ink under such conditions remain unknown.<sup>8,9,43</sup> In contrast, our method with ICG, a bio-absorbable drug whose safety is guaranteed, is very useful for marking tumors because its visibility is independent on local factors, such as the tumor location, fat volume, and thickness of the stomach wall. Even if puncture occurs outside the alimentary tract, unlike the India ink tattooing, there is no color change after its spread to the peritoneal cavity/mesentery, and it does not hamper the surgical procedure.

However, this study has several limitations. First, the additional equipment required for ICG detection is more expensive than that required for India ink Tattooing. Second, the amount of time required for gastrointestinal endoscopy to inject ICG is much higher than that required for tattooing. Third, in this study, we selected the cases who received ICG marking on the day before surgery to investigate the efficacy of preoperative endoscopy. Therefore, further investigation is needed for evaluating the appropriate timing of ICG injection (e.g., immediately before surgery, during intraoperative endoscopy, etc.). Finally, the number of cases in this series was small, and data was obtained from a single institute. Further, ICG cannot be used in patients prescribed drugs or those allergic to the contrast agent. Therefore, it is necessary to acquire data from larger multi-centric studies to evaluate the safety and usefulness of using ICG fluorescence for determining the tumor location.

## Conclusion

This is the first report that statistically proved that ICG fluorescence is useful in laparoscopic gastric cancer surgery. Determining the surgical resection line using ICG fluorescence is both useful and feasible as real-time confirmation is possible during the laparoscopic procedure itself. ICG use resulted in negative resection margins in all cases, shorter procedure time, lower estimated blood loss, and significantly shorter postoperative hospital stay and did not affect the procedure or result in adverse events. We think that ICG fluorescence is an invaluable marking method that can greatly facilitate next-generation surgery for gastric cancer.

**Author's Contribution** Ushimaru designed the study and wrote the initial draft of the manuscript. Omori contributed to the interpretation of the data and to the critical revision of the manuscript for important intellectual

content. All the other authors (Fujiwara, Yanagimoto, Sugimura, Yamamoto, Moon, Miyata, Ohue, and Yano) contributed to data collection and interpretation and critical review of the manuscript. All the authors have read and approved the final version of the manuscript and have agreed to the accountability of all aspects of the study, ensuring that any queries related to the accuracy or integrity of any part of the work are answerable.

## Compliance with Ethical Standards

This study was approved by the Institutional Review Board of the Osaka International Cancer Institute. All patients signed a written informed consent. Data collection and analysis were performed in compliance with the Helsinki Declaration of 1975.

**Disclosures** Drs. Yuki Ushimaru, Takeshi Omori, Yoshiyuki Fujiwara, Yoshitomo Yanagimoto, Keijiro Sugimura, Kazuyoshi Yamamoto, Jeong-Ho Moon, Hiroshi Miyata, Masayuki Ohue, and Masahiko Yano have no conflicts of interest or financial ties to declare.

**Conflict of Interest** There are no funding sources for this study or any associated financial conflicts.

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