Application of Near-Infrared Fluorescence Imaging with Indocyanine Green in Totally Laparoscopic Distal Gastrectomy

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ABSTRACT

Purpose: Recently, totally laparoscopic gastrectomy has been gradually accepted by surgeons worldwide for gastric cancer treatment. Complete dissection of the lymph nodes and the establishment of the surgical margin are the most important considerations for curative gastric cancer surgery. Previous studies have demonstrated that indocyanine green (ICG)-traced laparoscopic gastrectomy significantly improves the completeness of lymph node dissection. However, it remains difficult to identify the tumor location intraoperatively for gastric cancers that are staged ≤T3. Here, we investigated the feasibility of ICG fluorescence for lymph node mapping and tumor localization during totally laparoscopic distal gastrectomy.

Materials and Methods: Preoperative and perioperative data from consecutive patients with gastric cancer who underwent a totally laparoscopic distal gastrectomy were collected and analyzed. The patients were categorized into the ICG (n=61) or the non-ICG (n=75) group based on whether preoperative endoscopic mucosal ICG injection was performed.

Results: The ICG group had a shorter operation time and less intraoperative blood loss. Moreover, significantly more lymph nodes were harvested in the ICG group than the non-ICG group. No pathologically positive margin was found and there was no significant difference in either the proximal or distal surgical margins between the 2 groups.

Conclusions: Near-infrared fluorescence imaging with ICG can be successfully used in totally laparoscopic distal gastrectomy, and it contributes to both the completeness of D2 lymph node dissection and confirmation of the gastric transection line. Well-designed prospective randomized studies are needed in the future to fully validate our findings.

Keywords: Gastric cancer; Gastrectomy; Indocyanine green; Tumor; Lymph node dissection

INTRODUCTION

Gastric cancer is one of the most common cancers worldwide, and surgery remains the only curable treatment method for malignant gastric tumors. A radical gastrectomy with adequate lymph node dissection is the standard surgical treatment [1]. In recent decades,
laparoscopic-assisted gastrectomy, a minimally invasive procedure, has been shown to have advantages in short-term follow-up studies [2-4] and to be similar to open surgery in terms of long-term survival [5]. As an advanced technique, the totally laparoscopic gastrectomy has been accepted by many surgeons, and preliminary results have shown it to be superior to the laparoscopy-assisted gastrectomy [6,7]. Despite these advantages, the totally laparoscopic gastrectomy also has a number of limitations. Indeed, one of the major issues is determining the proper transection line according to the tumor location in cases where the tumor cannot be located in the serosa of the stomach intraoperatively. Carbon nanoparticle suspension, a senior generation India ink, has previously been used in surgeries to determine tumor location and trace the lymph nodes [8,9]. However, one of the major disadvantages of this method is that it may cause intraperitoneal spraying and make locating the tumor, as well as dissecting the lymph nodes, more difficult [10]. Real time intraoperative endoscopic location of the tumor is another frequently used method; however, this approach increases the workload of endoscopists and leads to a prolonged operation time [11]. In addition, performing the surgery may become more difficult as a result of the limited space caused by gastrointestinal tract distention.

In recent years, near-infrared fluorescence imaging with indocyanine green (ICG) has been introduced for the laparoscopic gastrectomy. It was initially used in sentinel lymph node mapping for early stage gastric cancer [12-14], but more recently, it has been shown to have advantages in terms of the completeness of lymph node dissection [15-17]. Thus, near-infrared fluorescence imaging with ICG is considered to be an important technique in China specifically, given that the rate of advanced stage cancer with tumors located in the lower third of the stomach is considerably greater than that in Japan and Korea.

Another recent trend in the field of gastric cancer is that more aggressive cancers are being diagnosed at a relatively early stage, such as clinical stage II (cT2N0, cT2N1, cT3N0), due to the wide acceptance of screening endoscopy in this population. In these cases, standard D2 dissection of lymph nodes and precise gastric resection are considered to be essential for ensuring positive oncological outcomes and preserving the normal function of the remnant stomach to the maximum extent. Although the use of ICG in determining tumor location has been discussed in a previous retrospective study [18], to our knowledge, no studies have reported both standard D2 dissection of lymph nodes and precise stomach resection using this technique, especially for totally laparoscopic gastrectomy. The aim of this retrospective study was to assess the application of near-infrared fluorescence imaging using ICG for both the lymph node dissection and precise stomach resection.

**MATERIALS AND METHODS**

**Patient selection**
We retrospectively selected gastric cancer patients who successfully underwent totally laparoscopic distal gastrectomy and D2 lymph node dissection between August 2017 and November 2019, which were performed by a single group of surgeons at the Department of Gastrointestinal Surgery IV, Peking University Cancer Hospital & Institute, Beijing, China. The collected data included the following: baseline demographics (sex, age, and body mass index); American Society of Anesthesiology (ASA) physical status scores; intraoperative events (operation time, blood loss, and complications); postoperative events (complications, postoperative hospital stay, first passage of flatus); specimen measurements (proximal and...
distal margins); and pathological findings (pathological T and N stage, and lymph nodes retrieved). The exclusion criteria were as follows: treatment with neoadjuvant therapy before surgery; histological classification that was neither adenocarcinoma nor signet ring cell carcinoma; concurrent tumors detected during surgery; and ASA score >2. Patients who underwent near-infrared fluorescence imaging with ICG during surgery were classified as the ICG group and those who did not were classified as the non-ICG group. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Written informed consent was obtained from all the patients.

Preoperative ICG injection
For patients in the ICG group, ICG (Dandong Yichuang Pharmaceutical Co., Dandong, China) solution was injected into the submucosa layer 1 day (20–30 hours) before surgery by the endoscopist. Four points (proximal, distal, and bilateral to the tumor region) were selected and 0.5 mL of ICG, diluted to 0.625 mg/mL, was injected into each point (Fig. 1). One endoscopist, with extensive experience in the use of ICG, performed all the injections in the current study in order to ensure that the injection was performed correctly.

Surgical procedure
A regular 5-port trocar placement was used in all cases, and a small incision (3–5 cm) was made in the upper abdomen or around the umbilicus to remove the specimen. In the ICG group, the NOVADAQ fluorescence surgical system (Stryker Corp., Kalamazoo, MI, USA) was used. During laparoscopic lymph node dissection, different modes (spy, pinpoint, and color-segmented fluorescence) were used to improve the dissection (Fig. 2). In the tumor localization step, the color-segmented fluorescence mode was selected. We found that the core area (golden area) was a near-round shape, and the middle point of the longitudinal axis along the stomach was very close to the injection point. In our surgery, a 5 cm proximal margin was necessary. The determination of the proximal margin was as following. First, measuring the maximum length along the longitudinal axis of core area (defined as D); Second, from the proximal edge of the core area, measuring

![Fig. 1. Endoscopic findings of ICG injection. (A) The tumor lesion was located by preoperative endoscopy (white arrow); (B) ICG was injected into the submucosal layer of gastric cancer region at 4 points: proximal, distal, and 2 bilateral points. ICG = indocyanine green.](https://jgc-online.org)
5·D/2 cm, and marking as the transection line. (Fig. 3). For the non-ICG group, the high-definition laparoscopic surgical system (Karl Storz, Tuttlingen, Germany) was used, with
which the real time endoscopic location of the tumor could mark the proximal edge of the tumor before resection of the distal stomach.

For our totally laparoscopic distal gastrectomy, all the lymph node dissections and reconstructions of the gastrointestinal tract (uncut Roux-en-Y anastomosis) are performed intracorporeally. In both groups in this study, the specimens were checked immediately after the resection of the stomach, and if a positive margin was suspected, a frozen biopsy was used to confirm the result. Specimen management was performed by a surgeon from the surgical team immediately after the surgery (operator or 1st assistant). Lymph node-bearing soft tissue was separated from the specimens and divided into different stations. The resected stomach was completely unfolded, and photographs were taken with measurable tools.

**Statistical analysis**

All the statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The Student’s t-test was used to compare the continuous variables, and the $\chi^2$ test or Fisher’s exact test was used for categorical variables as appropriate. All the reported P-values were 2-sided, and a P-value <0.05 was considered statistically significant.

**RESULTS**

**Patient characteristics**

A total of 141 consecutive cases underwent totally laparoscopic distal gastrectomy during the study period. According to the exclusion criteria, 3 cases had received neoadjuvant treatments, concurrent colon cancer was found in one case, and squamous cell carcinoma was confirmed in another case in the final pathological report. Therefore, 136 cases were included in the final analysis. Among these, 61 received ICG injections and were allocated to the ICG group, while the other 75 did not receive ICG injections before operation and were allocated to the non-ICG group. No significant differences were observed in terms of the clinicopathological characteristics, as outlined in [Table 1](#).

**Perioperative outcomes**

In the ICG group, ICG was successfully injected into the submucosa 1 day before surgery and all cases had a measurable proximal core area during surgery. There were no ICG related complications during injection or surgery. There were no significant differences in the proximal margins (ICG: 5.09±0.55 cm; non-ICG: 4.77±1.27 cm, P=0.064) or intraoperative complications (ICG: 1.6%; non-ICG: 1.3%, P=0.884) between the 2 groups. Compared to the non-ICG group, the ICG group had a shorter operation time (239.25±44.19 minutes vs. 207.21±33.63 minutes, respectively, P<0.001) and less blood loss (49.87±30.73 mL vs. 38.20±18.03 mL, respectively, P=0.010) ([Table 2](#)). There were no suspected positive margins; thus, no frozen biopsies were performed on the enrolled cases. The postoperative short term index was comparable between the 2 groups, including the postoperative complications (ICG: 9.8%; non-ICG: 8.0%, P=0.710), postoperative hospital stay (ICG: 7.95±1.99 days; non-ICG: 8.23±5.12 days, P=0.692), first flatus (ICG, 3.02±0.72 days; non-ICG, 3.07±0.74 days, P=0.691), and first oral intake (ICG, 7.15±3.00 days; non-ICG, 6.91±4.93 days, respectively, P=0.739). There were no complications ≥grade 3 according to the Clavien-Dindo classification.
The ICG group had a larger number of harvested lymph nodes than the non-ICG group (33.72±9.06 vs. 29.36±8.76, respectively, P=0.005), but there were no significant differences in the number of metastatic lymph nodes between the 2 groups (1.56±3.21 vs. 1.44±2.66, respectively, P=0.816). In the subgroup analysis, the number of D1 stations (No. 1, 3, 4sb, 4d, 5, 6, and 7) in the ICG group was not significantly different from that in the non-ICG group.

### Table 1. Clinicopathological characteristics of the ICG and non-ICG groups

| Characteristics       | ICG (n=61)        | Non-ICG (n=75)   | P-value |
|-----------------------|-------------------|-----------------|---------|
| Age (yr)              | 55.11±10.76       | 58.40±10.71     | 0.078   |
| Sex                   |                   |                 | 0.316   |
| Male                  | 33 (54.1)         | 47 (62.7)       |         |
| Female                | 28 (45.9)         | 28 (37.3)       |         |
| BMI (kg/m²)           | 23.75±3.49        | 23.51±2.51      | 0.636   |
| ASA score             |                   |                 | 0.362   |
| 1                     | 10 (16.4)         | 17 (22.7)       |         |
| 2                     | 51 (83.6)         | 58 (77.3)       |         |
| Tumor size (mm)       | 26 (12.9)         | 26.8 (12.1)     | 0.718   |
| Tumor location        |                   |                 | 0.713   |
| Middle third          | 23 (37.7)         | 26 (34.7)       |         |
| Lower third           | 38 (62.3)         | 49 (65.3)       |         |
| T stage               |                   |                 | 0.999   |
| T1                    | 30 (49.2)         | 38 (50.7)       |         |
| T2                    | 19 (31.1)         | 20 (26.7)       |         |
| T3                    | 7 (11.5)          | 12 (16.0)       |         |
| T4                    | 5 (8.2)           | 5 (6.7)         |         |
| N stage               |                   |                 | 0.989   |
| N0                    | 39 (63.9)         | 47 (62.7)       |         |
| N1                    | 10 (16.4)         | 13 (17.3)       |         |
| N2                    | 6 (9.8)           | 9 (12.0)        |         |
| N3                    | 6 (9.8)           | 6 (8.0)         |         |
| AJCC stage            |                   |                 | 0.579   |
| I                     | 37 (60.7)         | 44 (58.7)       |         |
| II                    | 18 (29.5)         | 20 (26.7)       |         |
| III                   | 6 (9.8)           | 11 (14.7)       |         |

Data are shown as mean±standard deviation or number (%).

TNM staging was based on the 7th edition staging manual of AJCC.

ICG = indocyanine green; BMI = body mass index; ASA = American Society of Anesthesiology; AJCC = American Joint Committee on Cancer; TNM = tumor, node, metastasis.

### Table 2. Perioperative outcomes in the ICG and non-ICG groups

| Outcomes                      | ICG (n=61)        | Non-ICG (n=75)   | P-value |
|-------------------------------|-------------------|-----------------|---------|
| Operation time (min)          | 207.21±33.63      | 239.25±44.19    | <0.001  |
| Blood loss (mL)               | 38.20±18.03       | 49.87±30.73     | 0.010   |
| Intraoperative complications  | 1 (1.6)           | 1 (1.3)         | 0.884   |
| Proximal margin (mm)          | 50.9±5.5          | 47.7±12.7       | 0.064   |
| Distal margin (mm)            | 28.5±0.74         | 29.2±1.88       | 0.783   |
| Postoperative complications   | 6 (9.8)           | 6 (8.0)         | 0.701   |
| Emptying obstacles            | 3                 | 2               | -       |
| Lymphatic leak                | 0                 | 1               | -       |
| Abdominal infection           | 1                 | 0               | -       |
| Lung infection                | 1                 | 1               | -       |
| Anastomotic leakage           | 0                 | 1               | -       |
| Intraabdominal bleeding       | 1                 | 1               | -       |
| Postoperative hospital stay (days) | 7.95±1.99   | 8.23±5.12      | 0.692   |
| First flatus (days)           | 3.02±0.72         | 3.07±0.74       | 0.691   |
| First oral intake (days)      | 7.15±3.00         | 6.91±4.93       | 0.739   |

Data are shown as mean±standard deviation or number (%).

ICG = indocyanine green.

### Evaluation of lymph node dissection

The ICG group had a larger number of harvested lymph nodes than the non-ICG group (33.72±9.06 vs. 29.36±8.76, respectively, P=0.005), but there were no significant differences in the number of metastatic lymph nodes between the 2 groups (1.56±3.21 vs. 1.44±2.66, respectively, P=0.816). In the subgroup analysis, the number of D1 stations (No. 1, 3, 4sb, 4d, 5, 6, and 7) in the ICG group was not significantly different from that in the non-ICG group.
group, including both total retrieved (21.52±7.69 vs. 20.15±6.97, respectively, P=0.276) and the number of metastatic lymph nodes (1.31±2.59 vs. 1.17±2.15, respectively, P=0.735). In terms of D2 stations (No. 8, 9, 11p, and 12a), the ICG group had more harvested lymph nodes than the non-ICG group (12.16±3.84 vs. 9.27±4.87, respectively, P<0.001), but the number of metastatic lymph nodes were not significantly different between the 2 groups (0.26±0.73 vs. 0.27±0.81, respectively, P=0.974) (Table 3).

**DISCUSSION**

Radical surgery remains the primary potentially curable treatment for patients with resectable gastric cancer. A complete resection with a safety margin has been regarded as the standard goal, and subtotal gastrectomy is preferred for patients with distal gastric cancer, as this approach has similar outcomes with significantly fewer complications than a total gastrectomy.

For distal gastrectomy, it is crucially important to identify the location of the tumor and the appropriate resection line. Even though this may be relatively easy when operating on a gross tumor during open surgery since the tumor can be easily palpated, for tumors without serosa invasion it is important to locate the tumor boundary accurately, which can be particularly difficult during a traditional laparoscopic procedure. Currently, there are several methods for detecting tumor margins, such as the preoperative endoscopic injection of Indian ink, the use of metal clips, and intraoperative endoscopy [19-21]. Among these, the endoscopic injection of Indian ink is most common since it does not require any additional equipment and it has a lower cost. However, when using Indian ink, a large amount of ink needs to be injected to ensure that the serosa surface is visible, which often leads to blurring of the area of operation, affecting the safety of the operation. Preoperative endoscopic placement of a metal clip to mark the edge of the tumor is time-consuming because it requires intraoperative ultrasonography or X-ray examination. Additionally, if the metal clip falls off or cannot be detected for other reasons, it may lead to severe intraoperative complications [22,23]. Intraoperative endoscopy is the most reliable method and provides accurate information immediately before transection of the stomach. However, this is a time-consuming procedure since the relevant medical staff must be called into the operating room when the need arises, which prolongs the operation and may increase the incidence of perioperative complications. Moreover, the availability of endoscopists or endoscopic systems intraoperatively may be limited in some hospitals.

The clinical application of ICG was approved by the US Food and Drug Administration in 1959. In recent years, the ICG-labeled fluorescent laparoscopic system has been used in clinical practice, and more recently, ICG markers have been used for laparoscopic gastrectomy. According to our clinical observation, the ICG solution spreads to form a

**Table 3.** Number of retrieved lymph nodes in the ICG and non-ICG groups

| Variables                     | ICG (n=61) | Non-ICG (n=75) | P-value |
|-------------------------------|------------|----------------|---------|
| Metastatic lymph nodes of D1 station | 1.31±2.59 | 1.17±2.15 | 0.735   |
| Number of D1 station lymph nodes | 21.52±7.69 | 20.15±6.97 | 0.276   |
| Metastatic lymph nodes of D2 station | 0.26±0.73 | 0.27±0.81 | 0.974   |
| Number of D2 station lymph nodes | 12.16±3.84 | 9.27±4.87 | <0.001  |
| Total metastatic lymph nodes  | 1.56±3.21  | 1.44±2.66  | 0.816   |
| Total lymph nodes harvested   | 33.72±9.06 | 29.36±8.76 | 0.005   |

Data are shown as mean±standard deviation. ICG = indocyanine green.
nearly round shape divided into 2 areas (golden and blue areas) under the color-segmented fluorescence mode. In addition, the middle point of the longitudinal axis in the golden area along the stomach is very close to the injection point, and a 5 cm safety margin can be easily measured during laparoscopy. Our results also confirm that this method allows for a relatively precise transection line compared to the endoscopic procedure, newly demonstrating its advantages in terms of the quality and safety of the surgery.

ICG was injected into the submucosa layer 1 day before surgery in our study, while some other studies have suggested intraoperative injection by the operation surgeons [24]. We believe that, compared to the intraoperative injection of ICG, a preoperative submucosa injection of ICG under endoscopic guidance is easier to control and is more time efficient. Furthermore, intraoperative injection is likely to cause intraoperative leakage of ICG into the surgical field or interference with the surgery by straying into the blood vessels.

D2 lymph node dissection is the standard curative surgical procedure for advanced gastric cancer in Asia [1,25]. In recent years, some studies have shown that D1 lymph node dissections are similar to the D2 procedure in terms of treatment for early stage advanced gastric cancer [26], resulting in a lower incidence of perioperative complications and faster recovery without affecting the oncological outcomes. However, in China, where there are far more advanced cases than there are in Japan and Korea, D2 dissection is still the mainstream procedure for gastrectomies. The first application of ICG for lymph node dissection was in sentinel lymph node mapping [27], but its advancement has not been significant until now. In the past 2 years, fluorescence-guided lymph node dissection has gained the attention of gastrointestinal surgeons, and preliminary studies have shown its advantages in lymph nodes dissections [15]. Our study demonstrated that more lymph nodes were harvested in the ICG group than in the non-ICG group, but what is more interesting is that our study also found a significant difference in D2 stations between the 2 groups. The dissection of D2 stations is a relatively difficult procedure to perform during laparoscopic gastrectomy, and some previous studies have suggested that the incomplete dissection of D2 stations could be a major reason for local recurrence after surgery [28,29]. Moreover, surgery under fluorescence mode can assist with finding small lymph nodes easily, which would otherwise be omitted during the traditional mode, which may also explain the likelihood of greater lymph node retrieval.

Our study also showed that the ICG group had less blood loss and a shorter operation time. This was considered to be partly due to the fact that fluorescence-guided laparoscopic surgery can provide a clear lymphatic drainage pathway during lymph node dissection. Consequently, this helps in identifying small spaces between lymph nodes and important structures, such as the pancreas and blood vessels, making it possible to avoid damaging lymph node capsules and adjacent organs and allowing for the operation to progress smoothly.

In a recently-published prospective trial [16], fluorescent lymph nodes were all removed, even outside the planned dissection areas, and some positive lymph nodes were found during this step. We did not perform this procedure because we had no evidence to suggest a relationship between fluorescent stained lymph nodes and metastasis until now. However, this novel finding will be used to guide future research to reduce local recurrence and improve oncological outcomes.

In conclusion, our retrospective analysis is the first to preliminarily illustrate the advantages of near-infrared fluorescence imaging with ICG, both in terms of achieving a
precise transection line and the completeness of D2 lymph node dissection during totally laparoscopic distal gastrectomy. This technique was associated with a shorter operation time, less blood loss, and comparable perioperative complications compared to the laparoscopic gastrectomy following intraoperative endoscopy for tumor localization. Although this was a retrospective study, we believe that the application of ICG has broad potential for the total laparoscopic distal gastrectomy.

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