의학석사 학위논문

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**2020년 2월**

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**A thesis of the** Master's **degree**

# Near-infrared fluorescence-guided surgery using indocyanine green facilitates secure infrapyloric lymph node dissection during laparoscopic distal gastrectomy for early gastric cancer

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Department of Medicine

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# ABSTRACT

# Near-infrared fluorescence-guided surgery using indocyanine green facilitate secure infrapyloric lymph node dissection during laparoscopic distal gastrectomy for early gastric cancer

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**Introduction:** Infrapyloric lymph node (LN) dissection is technically challenging procedure in laparoscopic distal gastrectomy. We aimed to investigate the usefulness of near-infrared indocyanine green (ICG)-enhanced fluorescence guidance for infrapyloric LN dissection during laparoscopic distal gastrectomy.

**Methods:** This prospective study enrolled patients with early gastric cancer (cT1NxM0) scheduled for laparoscopic distal gastrectomy between 2017 and 2018. After intraoperative submucosal injection of ICG (0.1 mg/mL), LN dissection was conducted under near-infrared ICG fluorescence guidance. The operation time, bleeding event, event of tearing of transverse mesocolon during infrapyloric LN dissection were analyzed. Cases of this protocol were retrospectively propensity-score matched to patients who underwent laparoscopic distal gastrectomy without ICG injection (non-ICG group), using 1:3 matching.

**Results**: Upon propensity-score analysis of clinicopathologic characteristics, the 20 patients in the ICG group were matched to 60 controls (non-ICG group). The mean time from midline omentectomy to exposure of the right gastroepiploic vein was significantly shorter in the ICG group than in the non-ICG group ($13.05 \pm 5.77$ vs $18.68 \pm 7.92$ min; $p = 0.001$), and the incidence of bleeding during infrapyloric LN dissection was lower in the ICG group (20% vs 68.3%, $p < 0.001$). Tearing of the transverse mesocolon did not occur in the ICG group but was detected in three patients from the non-ICG group (0% vs 5%; $p = 0.569$). The two groups did not differ significantly regarding the number of LNs retrieved from the infrapyloric area ($p = 0.434$).

**Conclusion:** Near-infrared ICG fluorescence guidance facilitates safe and fast infrapyloric LN dissection during laparoscopic distal gastrectomy.

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**Keywords**: Indocyanine green, Laparoscopic surgery, Lymph node dissection, Near-infrared imaging, Gastric cancer

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# INTRODUCTION

Lymphadenectomy in the infrapyloric area, which is categorized as lymph node (LN) station #6 according to the Japanese Gastric Cancer Association (1-4), is considered a technically challenging procedure when performed during laparoscopic distal gastrectomy. The anterior layer of the mesoduodenum is covered by membranous connective tissue of the greater omentum and transverse mesocolon. These structures lie in the immediate proximity of the pancreas and sometimes form fusion fascia (2-4). Moreover, the lymphatic and vascular drainage from the infrapyloric area exhibits numerous drainage patterns at the confluence of the right gastroepiploic vein (RGEV) and anterior superior pancreaticoduodenal vein (5-7). Thus, careless handling and manipulations in the infrapyloric area carry a high risk of tissue injury leading to intra- or postoperative complications such as bleeding, pancreatic fistula, and damage to the mesocolon vasculature (8-10). Moreover, if LN dissection is not performed correctly along the avascular plane, LN retrieval may be inadequate. Therefore, it is essential for infrapyloric LN dissection to be conducted safely and within a reasonably short span of time.

To overcome these difficulties of infrapyloric LN dissection, Shinohara et al. (2, 3) introduced a technique that helps to identify and trace the dissectable layers based on anatomical landmarks. Shibasaki et al. (4) recently reported a new dissection procedure for infrapyloric LN dissection, which employs a medial approach with dissection along the outermost layer. These previously described techniques are very successful but require adequate anatomical knowledge and a high level of surgical experience. Moreover, the success rate and applicability of such techniques in obese patients remain unclear.

In patients with tumors in the lower third of the stomach, the infrapyloric LNs drain the lymphatic flow mainly from the right gastroepiploic lymphovascular pedicle (1). With the recent advances in laparoscopic surgery providing a highly magnified view, it is now possible to clearly visualize the lymphatic channels and LNs using the near-infrared (NIR) indocyanine green (ICG)-enhanced fluorescence technique. For example, in our experience with sentinel LN navigation surgery, the mesogastric tissue of LN station #6 can easily be distinguished from the adjacent mesocolon using ICG-enhanced fluorescence under the NIR mode (11, 12). In contrast to previous studies which focused on the use of ICG fluorescence for assessment of bowel perfusion, vessel shape identification, and oncologic evaluation of metastatic nodes (13-19), we hypothesized that NIR imaging combined with ICG submucosal injection would help alleviate the technical difficulties of infrapyloric LN dissection during laparoscopic distal gastrectomy.

In the present study, we aimed to investigate the usefulness of NIR ICG-enhanced fluorescence guidance for reducing the duration and improving the safety of infrapyloric LN dissection during laparoscopic distal gastrectomy.

## Materials and Methods

### Patients

This was a prospective phase-II study of NIR-guided infrapyloric LN dissection during laparoscopic distal gastrectomy for early gastric cancer. Between July 2017 and June 2018, 20 ICG-enhanced fluorescence-guided surgeries were performed at Seoul National University Hospital using a real-time endoscopic NIR imaging system (PINPOINT; Novadaq Inc., Mississauga, ON, Canada).

The inclusion criteria were as follows: (i) pathologically proven primary gastric adenocarcinoma with a clinical stage of T1NxM0; (ii) patients who were scheduled for laparoscopic distal gastrectomy under the NIR mode; (iii) age 20−79 years. We excluded the patients with a history of hypersensitivity to iodide, severely impaired hepatic function, or previous major abdominal surgery.

Patient demographic data collected and analyzed in this study included age, sex, body mass index, operator, tumor location (along the longitudinal axis and along the circumference of the stomach), clinical stage, pathological stage (pT and pN category), and extent of lymphadenectomy. All postoperative outcomes and surgical complications were recorded and graded according to the Clavien-Dindo classification (20).

## ICG submucosal injection and NIR-guided laparoscopic distal gastrectomy

A high-concentration solution (2.5 mg/mL) of ICG (Daiichi Sankyo Pharmaceutical Co., Ltd., Osaka, Japan) was prepared and then diluted with 0.9% normal saline to a concentration of 0.1 mg/mL. Intraoperatively, a small volume of the diluted ICG solution (1 mL, 0.1 mg/mL) was injected into the submucosa at five location (on the lesser curvature side − low body and antrum; on the greater curvature side − mid body, low body and antrum) using a 23-gauge injection needle (Olympus Co., Ltd., Tokyo, Japan). Conventional laparoscopic distal gastrectomy was performed after confirming the initial uptake of ICG fluorescence under the NIR camera (PINPOINT; Novadaq Inc.) (Supplementary Video 1). The lymphatic channels and LNs could be distinguished from the surrounding blood vessels based on the ICG-enhanced fluorescence detected under NIR imaging, enabling the identification of dissectable layers between the mesogastric tissue and the mesocolon. (Fig. 1). The extent of LN dissection was determined according to the Japanese Gastric Cancer Treatment Guidelines [1].

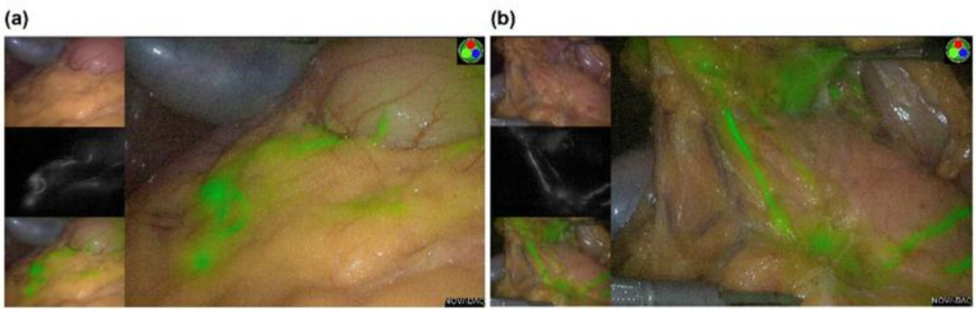


Figure 1. Indocyanine green-enhanced fluorescence uptake of lymphatic channels and lymph nodes in the infrapyloric area

## Procedure for selecting patients for the control group

To determine the potential benefits of the NIR ICG-enhanced fluorescence technique for infrapyloric lymphadenectomy, we conducted a comparative study of surgical outcomes and complications of laparoscopic distal gastrectomy with and without NIR ICG-enhanced fluorescence guidance (ICG group vs. non-ICG group). To identify patients for the non-ICG control group, we retrospectively surveyed our hospital's database to identify patients with early gastric cancer who, between July 2017 and June 2018, underwent laparoscopic distal gastrectomy without ICG injection (Fig. 2).

During the study period, 270 consecutive patients with primary gastric cancer underwent laparoscopic distal gastrectomy without ICG injection. Of these, 87 patients with tumors in the high or mid-body of the stomach, as well as 53 patients with clinically advanced gastric cancer were excluded. Of the remaining 130 patients with clinically early gastric cancer in the lower body of the stomach, 44 patients without laparoscopic video recording were excluded (Fig. 2). For the remaining 86 patients, a propensity score was estimated using logistic regression (SPSS version 25; IBM Inc., Chicago, IL, USA). Using nearest-neighbor matching within a caliper of 0.02 times the standard deviation of the propensity score, the patients in the ICG group (n = 20) were matched 1:3 to 60 controls treated during the same period (non-ICG group). The matching variables included age, sex, body mass index, operator, tumor location (along the longitudinal axis and along the circumference of the stomach), pathological stage (pT and pN category), and extent of lymphadenectomy.

This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 1602-009-738) and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. 20 patients enrolled in this prospective phase II study were provided with informed consent prior to their inclusion in the study. For the remaining patients in control group, the requirement for written consent was waived by IRB due to the nature of retrospective chart review. All patients underwent elective operation performed by three experienced surgeons who performed > 100 gastric surgeries per year.

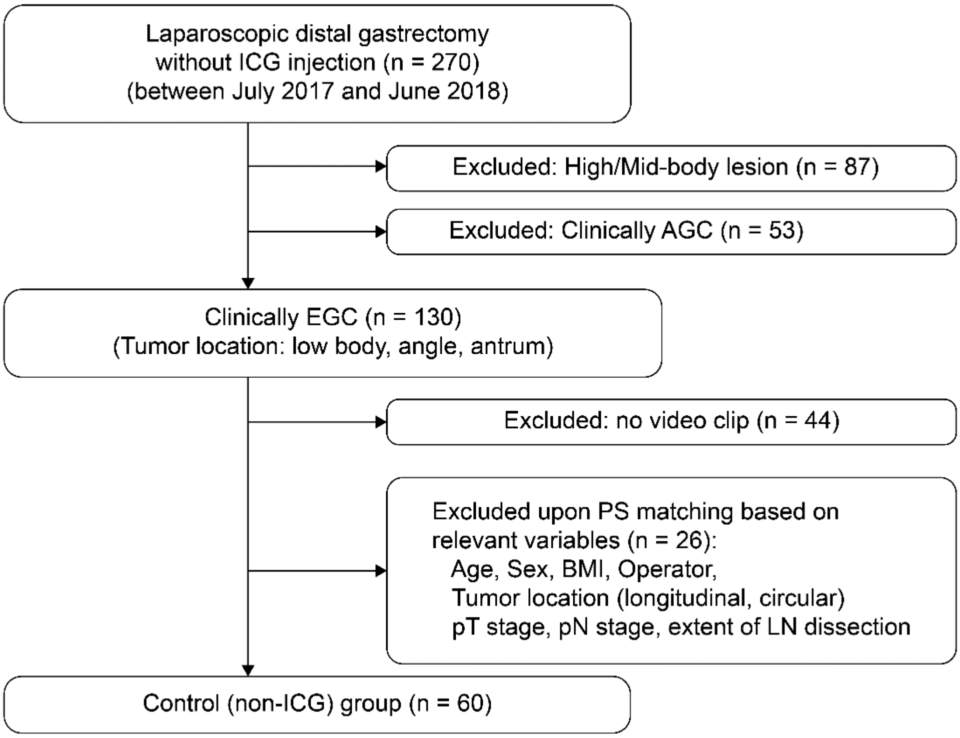



Figure 2. Flow chart of patient selection for the control group. Abbreviations: BMI = body mass index, GC = gastric cancer; ICG = indocyanine green; LN = lymph node; PS = propensity score

## Endpoints

The primary endpoints were operation time and the incidence of bleeding events during infrapyloric LN dissection. For the purpose of evaluating operation time, the infrapyloric LN dissection procedure was evaluated in each of two stages defined as follows: stage A, from midline omentectomy to RGEV exposure; stage B, from RGEV exposure to complete dissection of station #6. RGEV exposure was defined as follows: (a) The fat and lymphatics surrounding the RGEV have been peeled off, and both borders of the RGEV are visible with the vein-specific purple color; (b) If the contour of vein confluence is roughly shown by convergence of RGEV, (superior) right colic vein and anterior superior panceatico-duodenal vein.

The amount of intraoperative blood loss was measured separately for the two stages of infrapyloric LN dissection in the ICG group. As we did not have blood loss data pertaining specifically to the infrapyloric LN dissection procedure in the non-ICG group, we reviewed the video records in order to identify and define the bleeding events during infrapyloric LN dissection. And, the bleeding event count during infrayloric LN dissection assessed by video review was used as an alternative value for comparison in both groups. Bleeding events during infrapyloric lymphadenectomy were defined as follows (Fig. 3): (a) If the blood vessel is torn directly to bleed; (b) If blood is spurting directly from a vascular puncture site; and (c) If blood is not simply oozing, but continuously flowing out and bleeding control requires compression with gauze. Additionally, any event of tearing in transverse mesocolon was also reviewed and compared by recorded video in both groups (Fig. 4).

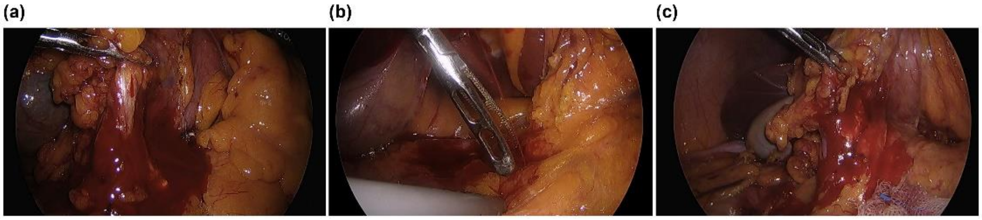



Figure 3. Definition of intraoperative bleeding events during infrapyloric lymphadenectomy

(a) If the blood vessel is torn directly to bleed

(b) If blood is spurting directly from a vascular puncture site

(c) If blood is not simply oozing, but continuously flowing out and bleeding control requires compression with gauze

Figure 4. Representative intraoperative picture showing tearing of the transverse mesocolon during infrapyloric lymphadenectomy

In the ICG group (n = 20), we also recorded the procedural time for ICG submucosal injection (from intubation to extubation of the gastroscope) and any other relevant intraoperative events such as ICG spillage and hypersensitivity reactions. The uptake of ICG fluorescence was recorded for each LN station.

## Statistical analysis

Data for each group (ICG and non-ICG) are presented as mean values with standard deviation. Independent continuous variables, including age, body mass index, tumor size, operation time, amount of blood loss, and hospital stay, were compared using the Student's t-test, whereas categorical variables, including sex, laparoscopic approach, reconstruction type, tumor location, cancer stage, and WHO classification, were compared using the chi-squared test, between the ICG and non-ICG groups. Repeated-measures ANOVA was used to compare the levels of serum inflammatory markers measured at baseline, postoperative day 2, and postoperative day 4 between the ICG and non-ICG groups. All tests were two-sided, and p-values of $<0.05$ were considered to indicate statistical significance. Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY).

# Results

## Clinicopathologic characteristics of the patients

After propensity score matching, the ICG group (n = 20) and non-ICG group (n = 60) were balanced in terms of baseline clinicopathological characteristics including age, sex, body mass index, operator, extent of lymphadenectomy, tumor location (along the longitudinal axis and along the circumference of the stomach), and cancer stage (Table 1).

**Table 1 Clinicopathological characteristics of patients who underwent laparoscopic distal gastrectomy for early gastric cancer**

| Variable | ICG group (n = 20) | Non-ICG group (n = 60) | *p*-value |
|---|---|---|---|
| Age, years | 60.10±11.09 | 61.67±11.47 | 0.598 |
| Sex | | | 1.000 |
| Male | 15 (75.0) | 45 (75.0) | |
| Female | 5 (25.0) | 15 (25.0) | |
| BMI, kg/m$^2$ | 24.89±3.22 | 24.09±2.80 | 0.331 |
| Laparoscopic approach | | | 1.000 |
| Laparoscopy-assisted | 9 (45.0) | 27 (45.0) | |
| Totally laparoscopic | 11 (55.0) | 33 (55.0) | |
| Reconstruction type | | | 0.896 |
| Billroth I | 11 (55.0) | 34 (56.7) | |
| Billroth II | 9 (45.0) | 26 (43.3) | |
| Operator | | | 0.964 |
| A | 10 (50.0) | 28 (46.7) | |
| B | 8 (40.0) | 26 (43.3) | |

| | | | |
|---|---|---|---|
| C | 2 (10.0) | 6 (10.0) | |
| **Lymphadenectomy** | | | 1.000 |
| ≤D1+ | 17 (85.0) | 51 (85.0) | |
| D2 | 3 (15.0) | 9 (15.0) | |
| **Tumor size, mm** | 3.03±1.25 | 2.82±1.60 | 0.556 |
| **Tumor location (longitudinal)** | | | 0.955 |
| Low body | 2 (10.0) | 5 (8.3) | |
| Angle | 4 (20.0) | 11 (18.3) | |
| Antrum | 14 (70.0) | 44 (73.3) | |
| **Tumor location (circumferencial)** | | | 0.195 |
| Lesser curvature | 6 (30.0) | 26 (43.3) | |
| Greater curvature | 7 (35.0) | 8 (13.3) | |
| Anterior wall | 4 (20.0) | 14 (23.3) | |
| Posterior wall | 3 (15.0) | 12 (20.0) | |
| Encircling involvement | 0 (0.0) | 0 (0.0) | |
| **Histologic classification** | | | 0.152 |

| | | | |
|---|---|---|---|
| Well differentiated | 4 (20.0) | 8 (13.3) | |
| Moderately differentiated | 5 (25.0) | 21 (35.0) | |
| Poorly differentiated | 0 (0.0) | 4 (6.7) | |
| Mucinous | 0 (0.0) | 0 (0) | |
| Signet-ring cell type | 4 (20.0) | 19 (31.7) | |
| Mixed | 7 (35.0) | 8 (13.3) | |
| Lauren classification | | | 0.632 |
| Intestinal type | 8 (40.0) | 31 (55.4) | |
| Diffuse type | 9 (42.9) | 23 (41.1) | |
| Mixed type | 3 (14.3) | 6 (10.0) | |
| pT category | | | 0.153 |
| ≤pT1 | 18 (90.0) | 59 (98.3) | |
| pT2–T4 | 2 (10.0) | 1 (1.7) | |
| pN category | | | 0.340 |
| pN0 | 17 (85.0) | 53 (83.3) | |
| pN1 | 1 (5.0) | 5 (8.3) | |
| pN2 | 2 (10.0) | 1 (1.7) | |
| pN3 | 0 (0.0) | 1 (1.7) | |

| | | | |
|---|---|---|---|
| **Cancer stage (7th AJCC)** | | | **0.097** |
| **≤I** | **17 (85.0)** | **58 (96.7)** | |
| **II–IV** | **3 (15.0)** | **2 (3.3)** | |

**The patients were stratified according to whether or not near-infrared fluorescence guidance with ICG was used during infrapyloric lymph node dissection. Data represent frequency (percentage) or mean ± standard deviation, as appropriate. The TNM stage was judged according to the 7th edition of American Joint Committee on Cancer (AJCC) TNM classification. Abbreviations: BMI = body mass index; ICG = indocyanine green; WHO = World Health Organization**

## Primary outcomes

The operative parameters and outcomes are summarized in Table 2. The mean operation time for stage A of infrapyloric LN dissection (from midline omentectomy to RGEV exposure) was significantly shorter in the ICG group than in the non-ICG group (13.05±5.77 vs. 18.68±7.92 min; $p = 0.001$), but no differences were noted regarding stage B (from RGEV exposure to complete station #6 dissection; 16.45±4.06 vs. 16.63±8.62 min; $p = 0.927$). In the ICG group, the mean time for ICG submucosal injection was 9.90±3.77 minutes, which accounted for the higher total operation time (by about 15 minutes) noted in this group relative to the non-ICG group. However, the total operation time did not differ significantly between the two groups (Table 2).

The rate of bleeding events was significantly lower in the ICG group than in the non-ICG group (20% vs 68.3%, $p < 0.001$). In the ICG group, the amount of blood loss was 2.18±4.22 and 4.61±8.74 mL, respectively, for stages A and B of infrapyloric LN dissection. Detailed blood loss data were not available for the non-ICG group. Tearing of the transverse mesocolon during infrapyloric lymphadenectomy did not occur in the ICG group but was noted in three patients from the non-ICG group; however, the difference between groups was not statistically significant (0% vs 5%; $p = 0.569$).

The total number of retrieved LNs was 30.15±9.27 for the ICG group and 32.55±10.03 for the non-ICG group ($p = 0.333$). No significant difference between the groups was observed regarding the number of LNs retrieved from station #6 (4.25±3.09 vs. 4.87±2.76; $p = 0.434$).

**Table 2** Operative outcomes and postoperative complications compared between ICG and Non-ICG group

| Variable | **ICG group (n = 20)** | **Non-ICG group (n = 60)** | *p* value |
|---|---|---|---|
| **Mean operation time, min** | | | |
| Total operation time | 228.85±33.17 | 213.87±38.48 | 0.102 |
| ICG injection time | 9.90±3.77 | - | - |
| Midline omentectomy to RGEV exposure | 13.05±5.77 | 18.68±7.92 | 0.001 |
| RGEV exposure to duodenal clearing | 16.45±4.06 | 16.63±8.62 | 0.927 |
| **Bleeding event** | 4 (20.0) | 41 (68.3) | <0.001 |
| **Amount of blood loss, mL** | | | |
| Midline omentectomy to RGEV exposure | 2.18±4.22 mL | - | - |
| RGEV exposure to duodenal clearing | 4.61±8.74 mL | - | - |
| **Tearing of the transverse mesocolon** | 0 (0) | 3 (5.0) | 0.569 |
| **Number of lymph nodes retrieved** | | | |
| From all stations | 30.15±9.27 | 32.55±10.03 | 0.333 |
| From station #6 | 4.25±3.09 | 4.87±2.76 | 0.434 |
| **ICG spillage** | 3 (15.0) | - | - |

| | | | |
|---|---|---|---|
| **Overall complications** | 1 (5.0) | 14 (23.3) | 0.099 |
| **Major complications** (Clavien-Dindo grade ≥IIIa) | 0 (0) | 7 (11.7) | 0.183 |
| **Comprehensive Complication Index** | 0.44±1.95 | 6.48±13.12 | 0.044 |
| **Surgical complication** | | | |
| Wound | 0 (0) | 1 (1.7) | 0.561 |
| Peri-pancreatic fluid collection | 0 (0) | 4 (6.7) | 0.567 |
| Intraabdominal bleeding | 0 (0) | 1 (1.7) | 0.561 |
| Stenosis | 0 (0) | 2 (3.3) | 0.408 |
| Motility disorder | 1 (5.0) | 3 (5.0) | 1.000 |
| Leakage | 0 (0) | 1 (1.7) | 0.561 |
| **Medical complication** | | | |
| Pulmonary | 0 (0) | 2 (3.3) | 0.408 |
| Renal | 0 (0) | 1 (1.7) | 0.561 |
| Urinary | 0 (0) | 1 (1.7) | 0.561 |
| **Duration of hospitalization, days** | 9.95±3.58 | 11.20±8.12 | 0.346 |

The patients were stratified according to whether or not near-infrared fluorescence guidance with ICG was used during infrapyloric lymph node dissection. Data represent frequency (percentage) or mean ± standard deviation, as appropriate. Abbreviations: ICG = indocyanine green; RGEV = right gastroepiploic vein

## Surgical complications

The overall rate of complications, as well as the rate of major complications (Clavien-Dindo grade ≥IIIa) tended to be lower in the ICG group than in the non-ICG group, but the difference was not statistically significant (5.0% vs. 23.3%, $p = 0.099$; 0% vs. 11.7%, $p = 0.183$; respectively). The Comprehensive Complication Index was significantly lower in the ICG group than in the non-ICG group (0.44±1.95 vs. 6.48±13.12; $p = 0.044$).

In the ICG group, no patient developed ICG-related adverse effects such as ICG toxicity or hypersensitivity reaction during or after the laparoscopic procedures. Leakage of ICG into the peritoneal space during submucosal injection occurred in three patients, but the spillage was minimal and did not interfere with the surgical field. Additionally, abnormally high serum amylase levels (>three times higher than normal; 585 U/L) were noted in one patient on the second postoperative day but recovered to normal levels without medication.

Peri-pancreatic fluid collection was not found in the ICG group but occurred in four patients from the non-ICG group; however, the difference between groups was not significant (0% and 6.7%, respectively; $p = 0.567$). Furthermore, there were no significant differences between the groups regarding the serum levels of inflammatory markers including white blood cell count (Fig. 5a) and C-reactive protein (Fig. 5b) or the length of hospitalization (Table 2).

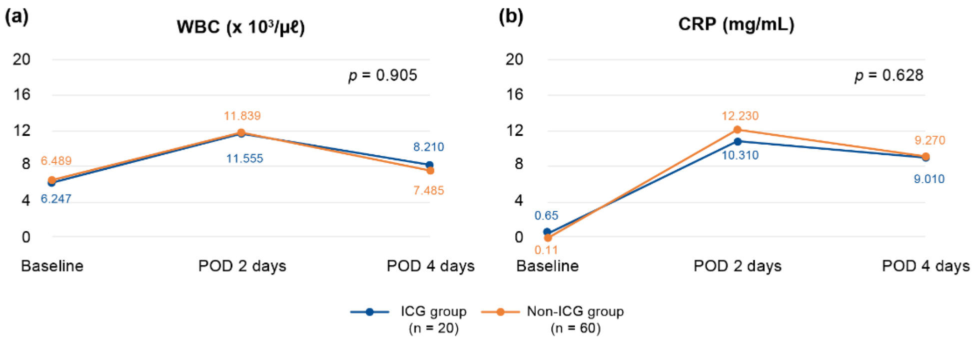



Figure 5. Serum levels of inflammatory markers (a) WBC (x $10^3/\mu\ell$) and (b) CRP (mg/mL) in patients who underwent laparoscopic distal gastrectomy for early gastric cancer. The patients were stratified according to whether or not near−infrared fluorescence guidance with ICG was used during infrapyloric lymph node dissection. Abbreviations: CRP = C−reactive protein; ICG = indocyanine green; POD = postoperative day; WBC = white blood cells

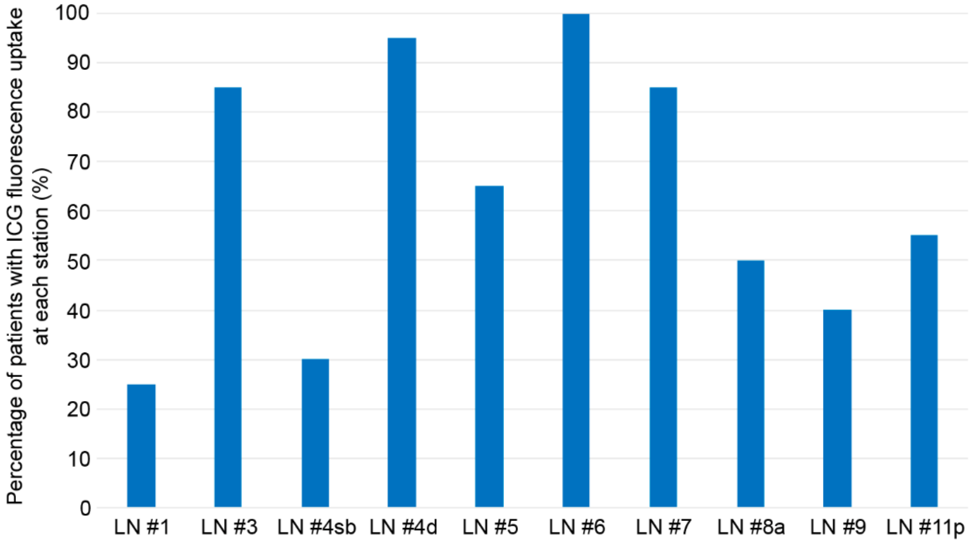
# Discussion

In this comparative study of laparoscopic distal gastrectomy with and without NIR ICG-enhanced fluorescence guidance, we found that NIR imaging guidance was associated with significantly reduced operation time for stage A of the infrapyloric LN dissection procedure (from midline omentectomy to RGEV exposure) ($p = 0.001$, Table 2), but did not provide any benefit regarding the operation time for stage B (from the RGEV exposure to complete station #6 dissection ($p = 0.927$, Table 2). The right gastroepiploic and infrapyloric vessels are covered with lymphatic tissue and fat, which makes it challenging to finely dissect and remove LNs from this area. The shorter time required from omentectomy to RGEV exposure in the ICG group is a result of adequate visualization under NIR fluorescence, which provided enhanced visual contrast between the mesocolon and the mesogastric tissue and thus facilitated the tracing of the dissectable layer. On the other hand, the use of NIR fluorescence did not help shorten the time from RGEV exposure to complete station #6 dissection, likely due to the fact that, by the time the RGEV became exposed, part of the lymphatic tissue in the infrapyloric area had already been peeled off, providing adequate view of the anatomical landmarks even in the absence of ICG fluorescence. Furthermore, the NIR fluorescence technique was associated with a significantly lower rate of bleeding events during infrapyloric LN dissection, likely due to the fact that it facilitated clear identification of the avascular plane and distinguishing the blood vessels from the surrounding lymphatic structures, which reduced the risk of injury to blood vessels.

Lan YT et al. (16) reported that a surgeon could harvest more infrapyloric LNs with the use of ICG fluorescence. However, in the current study, no statistically significant differences in the number of LNs retrieved in total or solely from station #6 were seen between the groups. In fact, if an experienced surgeon performed the operation, the number of retrieved LNs would not differ between the groups, regardless of the use of ICG. This can be supported by the fact that both groups in this study were operated by experienced three surgeons, while the control groups were established through propensity score matching with variables, including operator. We compared the prospectively collected outcomes of the ICG group with those of a control group from contemporary patients to eliminate the confounding factors, such as time difference. Therefore, we believe that ICG fluorescence contributed more to the stability of LNs dissection than to increasing the number of retrieved LNs. Further, NIR ICG fluorescence guidance enabled faster RGEV exposure and safer infrapyloric LN dissection without compromising the oncologic safety in terms of LN retrieval.

Several studies (2, 4, 21) examined the usefulness of new approaches for overcoming the technical difficulties in identifying and tracing the dissectable layer during infrapyloric LN dissection. One study [4] measured the operation time and the amount of bleeding during infrapyloric LN dissection conducted by expert (>100 laparoscopic distal gastrectomy procedures) vs. non-expert operators employing a medial approach with dissection along the outermost layer. The authors reported significantly shorter operation time (33 vs 60 min; $p < 0.001$) and relatively lower blood loss (15 vs 20 mL; $p = 0.195$) for procedures performed by expert operators than for those performed by non-expert operators, with no difference regarding the number of LNs dissected from station #6 (4 vs 4; p = 0.53) (4). These observations suggest that such techniques require adequate knowledge of the anatomical landmarks and thus might not be suitable for trainees or beginners. Even with advanced knowledge of the embryologic and anatomical background, the specific perception of color, spatial, and consistency features of the tissue differs according to the experience of each surgeon, suggesting that previously described techniques (2, 4, 21) may not provide a consistent ability to safely identify the dissectable layer. Therefore, enhanced visualization is desired.

In the present study, we were able to confirm the clinical feasibility and usefulness of an NIR ICG-enhanced fluorescence technique that helps reduce operation time and the incidence of bleeding during infrapyloric LN dissection. These findings support the potential role of this technique in guiding the surgeons through the infrapyloric lymphadenectomy procedure. Since the infrapyloric LNs station accommodates relatively wide-range lymphatics, we opted to perform the ICG injection at multiple sites rather than peritumorally, in an effort to stain all infrapyloric lymphatics. ICG fluorescence uptake in the infrapyloric area was successfully confirmed immediately after the ICG submucosal injection in all patients from the ICG group, which might explain the high success of the infrapyloric lymphadenectomy procedure in these patients (Supplementary Figure 1). Our present findings suggest that LN navigation using ICG fluorescence may be effective for visualization of the lymphatic pathway in stations #4d and #6, facilitating the adequate identification of lymphatics during infrapyloric LN dissection in patients with cancers located in the lower third of the stomach. (Supplementary Figure 1). Compared to previously described methods (2, 4, 21), the presently described technique of NIR ICG fluorescence may be more effective in guiding the surgeon by providing enhanced visualization and thus reducing the impact of less extensive surgical experience or very deep knowledge of the anatomical background.

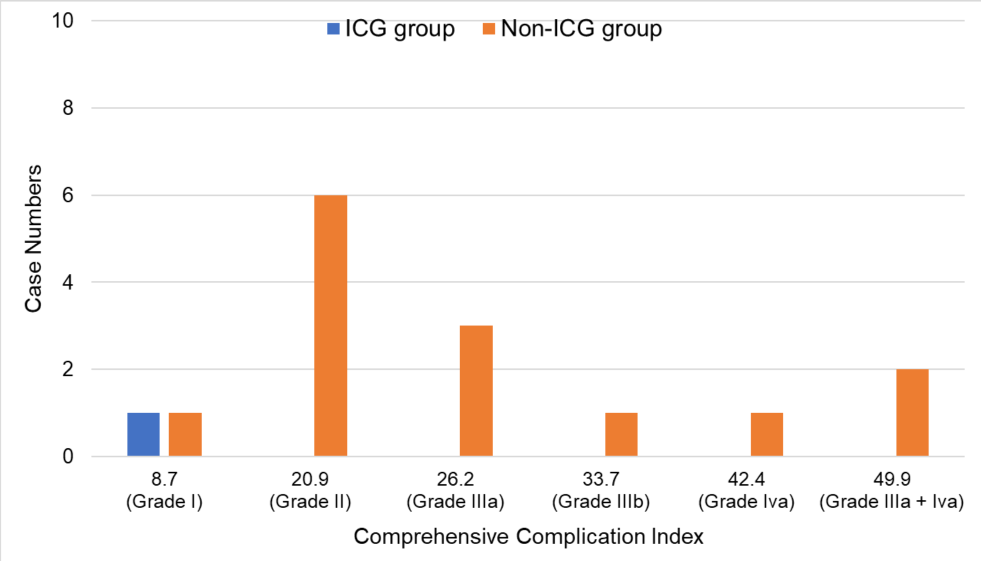



Supplementary Figure 1. Indocyanine green fluorescence uptake according to the LN station. Abbreviations: LN = lymph node

In clinical practice, high-risk cases are typically managed more carefully in order to avoid bleeding and reduce blood loss, which typically means that a longer time will be required for completing the infrapyloric LN dissection. On the other hand, if the operator's main aim during infrapyloric LN dissection is to minimize the operation time, there will be less concern about bleeding risk, which might result in reduced operation time but higher incidence of bleeding events or higher blood loss. In the present study, both the operation time and the rate of bleeding were considered primary endpoints. We found shorter operation time for exposing the RGEV, fewer bleeding events in the ICG group than in the non-ICG group.

No significant differences in overall, major (grade≥IIIa), individual surgical and medical complications were seen between the two groups; however, only the mean value of CCI was significantly lower in the ICG group (0.44±1.95 vs. 6.48±13.12; p = 0.044, Table 2). The CCI integrates every postoperative complication of a patient and is based on the methods for operations risk index analysis. In the ICG group, most cases had the CCI score of 0, while only 1 patient had a value of 8.7. In the non-ICG group, only one patient had a CCI score of 8.7 and other cases with morbidity showed outlier pattern with the CCI score above or equal to 20.9 (Supplementary Figure 2). Significant difference in the number of cases with CCI score greater than or equal to 20.9 was seen between the ICG and non-ICG groups (0% vs 21.7%, p = 0.031). This statistical difference was equally found in the distribution of grade II or higher complication between the two groups. As most patients had a single complication except for 2 cases (CCI score = 49.9) with grade IIIa and IVa morbidity, the patients with CCI score of 20.9 or higher inevitably had at least grade II complication. Therefore, the skewed distribution of CCI scores above or equal to 20.9 caused a significant difference in the mean values of CCI;

however, it could not affect the difference in overall and major complications between the two groups.




Supplementary Figure 2. The distribution of complicated patients according to comprehensive complication index (CCI)

This study has some limitations. Since this was a phase-II study with retrospectively enrolled controls, we did not have data regarding blood loss during infrapyloric LN dissection in controls (non-ICG group). To facilitate comparison with the ICG group regarding bleeding risk, we examined the rate of bleeding events during infrapyloric LN dissection. Nevertheless, this study overlooked the fact that surgeons might not have paid much attention to minor bleeding of control groups, who were not enrolled in the clinical study. Second, although NIR fluorescence-guided surgery was associated with fewer bleeding events during infrapyloric LN dissection and shorter time required for exposing the RGEV, we could not clarify the exact clinical benefit in such patients. Future studies should aim to check for potential relationships between the operative parameters (e.g., operation time or rate of bleeding events during infrapyloric LN dissection) and the postoperative complications or serum levels of inflammatory markers (white blood cell count, C-reactive protein). Third, the ICG group spent 9.9 minutes on ICG injection time, but the total operation time tended to be longer than the non-ICG group by 15 minutes (p=0.102, Table 2). Since the ICG injection time is defined as the time from intubation to extubation of the gastroscope, the time for preparing the endoscopic equipment system and the ICG solution were not separately considered and reflected in results of the study. Forth, this was an exploratory phase II study that limited the number of subjects for ICG injection to 20 cases. Previous real-time enhancement studies using NIR-camera in the field of colorectal and gynecologic surgery have reported that 15 to 30 subjects were enough to confirm their feasibility (22-23). In the same time period of the study, 130 patients with early gastric cancer (cT1NxM0) located in low body to antrum received laparoscopic distal gastrectomy. Of these, the patients only who agreed to use the new technology were included in this prospective single arm study. Finally, this study did not evaluate the efficacy of infrapyloric LN dissection in patients with advanced gastric cancer who were scheduled to undergo laparoscopic distal gastrectomy. For these various reasons, based on the results of this study, randomized control trials or larger-scale prospective cohort studies are necessary in near future to validate the effectiveness of NIR-guided gastric cancer surgery and clarify its distinct advantages.

In conclusion, we demonstrated the usefulness of NIR ICG-enhanced fluorescence guidance, which enabled faster and safe infrapyloric LN dissection without compromising oncological safety related to LN retrieval. We hope these findings embolden further large-scale randomized studies on the potential benefits of adopting this technique in routine clinical practice.

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# 국문 초록

배경: 유문하 림프절 박리는 복강경 원위부 위 절제술에서 기술적으로도 어려운 과정이다.

목적 : 본 연구는, Indocyanine green (ICG)를 이용한 근적외선 형광발현이 복강경 원위부 위절제술에서의 유문하 림프절 박리에 도움이 되는지에 대해 조사하고자 한다.

방법: 이 전향적 연구는 2017 년부터 2018 년까지 복강경 원위부 위절제술이 예정된 조기 위암 (cT1NxM0) 환자를 등록하였다. 수술장내에서, 0.1 mg/mL 의 ICG 용액을 환자의 위 점막하에 주사한 뒤에, 근적외선 ICG 형광 발현하 림프절 절제가 시행되었다. 유문하 림프절 박리 동안의 수술시간, 출혈, 횡행 결장 장간막의 손상 등이 분석되었다. 이 환자군들은, ICG 주입을 하지 않은 복강경 원위부 위 절제술을 시행받은 환자들과 1:3 비율로 propensity-score matching 되었다.

결과: 임상 병리학적 특징에 기반한 propensity-score 분석을 통한 매칭 결과, 20 명의 실험군 환자는 ICG group, 60 명의 대조군 환자는 non-ICG group 으로 나누어졌다. The mean time from 중간단계의 대망 절제술에서 (midline omentectomy) 에서 우위 대망 정맥의 노출까지 걸린 평균 시간은 비 ICG 군 (18.68±7.92)보다 ICG 군 (13.05±5.77) 에서 유의하게 더 짧았다 ($p=0.001$). 또한, 유문하 림프절 박리 동안의 출혈 빈도는 ICG 군 (20%)에서 비 ICG 군(68.3%)보다 더 낮았다 ($p<0.001$). 횡행 결장 장간막의 손상은 ICG 군에서는 발생하지 않았지만 (0%),

비 ICG 군에서는 3 명 (5%) 이 발견되었다 (p=0.569). 두 군은, 유문하 림프절 박리 후에 얻어진 림프절의 수 측면에서 다르지 않았다 (p=0.434).

결론: ICG 를 이용한 근적외선 형광 유도하 수술은, 원위부 위 절제술 시행시 안전하고 빠른 유문하 림프절 절제를 가능하게 한다.

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주요어: Indocyanine green, 복강경 수술, 림프절 박리, 근적외선 이미지, 위암

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