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Blood Perfusion Assessment by Indocyanine Green Fluorescence Imaging for Minimally Invasive Rectal Cancer Surgery (EssentiAL trial)

A Randomized Clinical Trial

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Objective: The aim of the present randomized controlled trial was to evaluate the superiority of indocyanine green fluorescence imaging (ICG-FI) in reducing the rate of anastomotic leakage in minimally invasive rectal cancer surgery.

Background: The role of ICG-FI in anastomotic leakage in minimally invasive rectal cancer surgery is controversial according to the published literature.

Methods: This randomized, open-label, phase 3, trial was performed at 41 hospitals in Japan. Patients with clinically stage 0–III rectal carcinoma less than 12 cm from the anal verge, scheduled for minimally invasive sphincter-preserving surgery were preoperatively randomly assigned to receive a blood flow evaluation by ICG-FI (ICG+ group) or no blood flow evaluation by ICG-FI (ICG− group). The primary endpoint was the anastomotic leakage rate (grade A+B+C, expected reduction rate of 6%) analyzed in the modified intention-to-treat population.

Results: Between December 2018 and February 2021, a total of 850 patients were enrolled and randomized. After the exclusion of 11 patients, 839 were subject to the modified intention-to-treat population (422 in the ICG+ group and 417 in the ICG− group). The rate of anastomotic leakage (grade A+B+C) was significantly lower in the ICG+

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group (7.6%) than in the ICG− group (11.8%) (relative risk, 0.645; 95% confidence interval 0.422–0.987; $P = 0.041$). The rate of anastomotic leakage (grade B+C) was 4.7% in the ICG+ group and 8.2% in the ICG− group ($P = 0.044$), and the respective reoperation rates were 0.5% and 2.4% ($P = 0.021$).

Conclusions: Although the actual reduction rate of anastomotic leakage in the ICG+ group was lower than the expected reduction rate and ICG-FI was not superior to white light, ICG-FI significantly reduced the anastomotic leakage rate by 4.2%.

Keywords: anastomotic leak, fluorescence, indocyanine green, laparoscopic surgery, rectal cancer, randomized controlled trial

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Colorectal cancer is the second most common cancer worldwide, with nearly 2.2 million new cases and 1.1 million deaths in 2019.[1] Among these patients, approximately one-third of all colorectal cancers are localized in the rectum.[2] In rectal surgery, anastomotic leakage is one of the most critical complications, occurring in 11% to 15% of patients,[3–6] and it significantly worsens the short-term outcome by increasing the rate of reoperation and duration of hospitalization. Furthermore, long-term outcomes, such as the rate of local recurrence and concurrent cancer-specific survival, are also affected.[7,8]

Surgery-related factors for anastomotic leakage have been reported to be incomplete anastomosis,[9] anastomotic tension,[10] and anastomotic vascular perfusion.[11–15] In general, vascular perfusion to the anastomotic region is assessed by the surgeon intraoperatively by various parameters, such as active bleeding from the resection margin, palpable pulsation of the mesentery, and discoloration.[11] However, Karliczek et al[16] demonstrated these factors to be subjective and highly unreliable.

On the other hand, near-infrared fluorescence technology provides a rapid and reliable method of evaluating the anastomotic site perfusion in real time through angiography during minimally invasive surgery.[17] However, the role of indocyanine green (ICG) fluorescence imaging (ICG-FI) in anastomotic leakage in rectal surgery is controversial according to the published literature.[17–29] Although the IntAct and AVOID trials are currently underway as phase III randomized controlled trials (RCTs),[30,31] no RCTs with sufficient power in this field have yet been conducted.

The present RCT evaluated the superiority of ICG-FI in reducing the rate of anastomotic leakage in minimally invasive rectal cancer surgery.

METHODS

Trial Design and Participants

The EssentiAL study was an open-label, multicenter, phase III, RCT conducted at 41 hospitals within the framework of the Japan Society of Laparoscopic Colorectal Surgery. The original study protocol and statistical analysis plan were available online (Supplement 1, Supplemental Digital Content 1, http://links.lww.com/SLA/E611 and Supplement 2, Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The study protocol was approved by the Yokohama City University Certified Institutional Review Board, and the institutional review board of each participating hospital before the study was initiated. All the patients provided their written informed consent before enrolling in the study. This study was registered with the Japan Registry of Clinical Trials (jRCTs-CRB3180007) and the Japanese Clinical Trials Registry (UMIN-CTR000030240). The sponsor of the study, Stryker Japan K.K. (a subsidiary of Stryker), had no role in the study design, data gathering, analyses, or interpretation, or in the writing of the article or decision to submit the article for publication, other than funding the research.

The eligibility criteria were as follows: (1) histologically proven rectal cancer, (2) a lower margin of the tumor less than 12 cm from the anal verge, (3) clinically diagnosed as Union for International Cancer Control TNM classification (eighth edition)[32] stage 0-III, (4) scheduled for minimally invasive sphincter-preserving surgery with anastomosis [laparoscopic surgery, robotic surgery, or transanal total rectal resection (taTME) were eligible], (5) older than 20 years of age, and (6) Eastern Cooperative Oncology Group Performance status 0-2. The exclusion criteria were as follows: (1) patients with allergies to iodine or ICG, (2) patients planning to undergo surgery for other organ resection, and (3) patients with serious comorbidity. Complete inclusion and exclusion criteria are provided in Supplement 3 (Supplemental Digital Content 1, http://links.lww.com/SLA/E611).

Randomization and Masking

Randomization and data handling were performed using an electronic data capturing system [MARVIN Data Management System (XClinical, Munich, Germany)]. The patients were preoperatively subjected to 1:1 computerized randomization to groups either receiving a blood flow evaluation by ICG-FI (ICG + group) or not receiving such an evaluation (ICG− group). The sizes of the arms were balanced using the permuted block randomization method, according to the participating hospital, sex (male/female), and stage (0–I/II–III). Investigator surgeons were informed of treatment allocation via the internet and did the procedure. The patients and investigators were not masked to group assignments.

Procedure

In this trial, the surgeon's criteria were set according to the protocol (a total of 52 colorectal surgeons participated who had a qualified surgeon with endoscopic surgical skills endorsed by the Japan Society of Endoscopic Surgery[33] and had performed > 30 cases of laparoscopic rectal cancer surgery).

Total mesorectal excision with D2 or D3 dissection was performed according to the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma.[34] Central vessel ligation and colon or rectum mobilization (total mesorectal excision) were performed minimally invasive approach (laparoscopic, robotic, or transanal approach). The anastomosis method was not uniform in all study patients. There were no provisions regarding left colic artery preservation, splenic flexure mobilization, or diverting stoma.

Treatment Arm

The experience with ICG-FI was not specified. Therefore, an educational Digital Versatile Disc was used to educate all surgeons on how to evaluate ICG-FI to ensure quality control. Blood perfusion was evaluated by ICG-FI in addition to the conventional method. The protocol stipulated that the laparoscopic camera system used for near-infrared observation had to be the "endoscopic camera system 1588" (Stryker Corporation, MI) or the "endoscopic camera system 1688" (Stryker Corporation, MI) in all cases (even robotic surgery). The blood flow was evaluated immediately before performing anastomosis. Intravenously, 12.5 mg of ICG (5.0 mL of ICG solution, the

dilution as one 25 mg vial in 10 mL saline; Diagnogreen; Daiichi-Sankyo Pharma, Tokyo, Japan) was administered, and the infusion route was flushed with 20 mL of saline or extracellular fluids (Lactated Ringer's injection). The time point when the infusion route was flushed using 20 mL of saline or extracellular fluids (Lactated Ringer's injection) immediately after the administration of ICG was regarded as 0 seconds, and fluorescence observations were performed by switching to near-infrared light. The reference time from the administration of ICG to fluorescence observation of the proximal side of the anastomotic site of the intestinal wall was 60 seconds. If vascular perfusion via ICG fluorescence imaging was well visualized within 60 seconds at the planned proximal side of the anastomotic line, it was judged to be good perfusion, and anastomosis was performed with the planned anastomotic line. However, if fluorescence was observed after 60 seconds, it was judged to indicate poor perfusion. In cases of poor perfusion or no perfusion, after the identification of a good perfusion zone, a revised anastomotic line was set more than 1 cm from the edge of this good perfusion zone toward the proximal side. Subsequently, anastomosis was performed.

Control Arm

Blood perfusion was assessed without ICG-FI. Conventional methods, such as active bleeding from the resection margin, palpable pulsation in the mesentery, and a lack of discoloration were permitted for the assessment of blood perfusion. The use of these conventional methods was not dictated by the protocol and was entirely at the discretion of the surgeon.

Outcomes

The primary endpoint was the incidence rate of anastomotic leakage. Anastomotic leakage was classified into 3 categories, according to the degree of difference; grade A: radiological leakage (anastomotic leak confirmed solely by diagnostic imaging of the anastomosis site, despite the lack of clinical symptoms. These cases do not require any active therapeutic intervention); grade B: symptomatic leakage without re-laparotomy (anastomotic leak with clinical symptoms, which require therapeutic intervention by other means than surgery. It requires percutaneous or transanal drainage and antibiotic treatments); grade C: symptomatic leakage requiring re-laparotomy (anastomotic leak with clinical symptoms, which require surgical intervention).[35] In the present study, all grades of A, B, or C were defined as anastomotic leakage. Secondary endpoints included the incidence rate of anastomotic leakage (grade B+C), time to fluorescence at the resection margin on the proximal side, additional resection rate and length of the proximal resection margin, incidence rate of surgical adverse events, operative time, the 30-day incidence rate of complications, reoperation rate, and length of postoperative hospital stay.

Outcome measures were assessed during the hospital stay and at 1-month clinical follow-up visit at the outpatient clinic. The protocol stipulated that the following tests should be performed for the diagnosis of anastomotic leakage. Diverting stoma cases required enema contrast x-ray of the anastomotic site. No examination is required for cases with no diverting stoma and no clinical anastomotic leakage. Cases without a diverting stoma suspected of clinically anastomotic leakage required an enema contrast x-ray at the anastomotic site. Computed tomography (CT) was acceptable to perform if an enema contrast examination of the anastomosis site was not possible. Concerning the timing of contrast enema x-ray at the anastomotic site for diverting stoma cases, if a patient was discharged within 30 days after surgery, in principle, it would be performed before discharge, and it was also possible to perform it within 30 days after discharge. If a patient was not discharged within 30 days after surgery, the test would be performed within 30 days after surgery.

Statistical Analysis

Based on the published literature,[4,5,36,37] we considered the incidence rate of anastomotic leakage in the control arm to be 13%. We hypothesized that the study treatment would reduce the incidence rate of anastomotic leakage by 6% from the previous meta-analyses.[24,38] Thus, the expected incidence rate of anastomotic leakage in the ICG arm was 7%. Therefore, setting the 2-sided $\alpha = 0.05$ and $1-\beta = 0.8$, a total of 391 participants were deemed necessary in each arm based on the χ^2 test of independence. Considering dropouts, we set the total number of patients enrolled at 850. The main analysis set for the efficacy was the modified intention-to-treat (mITT) population. Patients were excluded from the analysis after randomization if intraoperative distant metastases were confirmed after the start of the study, cancer severely infiltrated other organs after the start of the study even if a combined resection of other organs could provide a curative resection, anastomosis was not performed due to surgical technique changes, surgery was not performed, or a severe violation of the protocol was committed.

Categorical variables were summarized as numbers and percentages, and differences between categorical variables were tested using the χ^2 test and Fisher exact test, which was presented as the relative risk (RR) with 95% confidence intervals (CIs) and risk differences with 95% CIs. Continuous variables were summarized as mean (SD) or as median (interquartile range: IQR), and differences between continuous variables were tested using the *t*-test and Mann-Whitney *U* test. All reported *P* values were 2-sided and unadjusted. Statistical analyses were performed using the statistical analysis software program, version 9.4 or later (SAS Institute Inc., Cary, NC, USA) and the R software program, version 3.5.0 or later (R Foundation for Statistical Computing, Vienna, Bundesländer, Austria), and conducted by an independent institution (Department of Biostatistics, Yokohama City University School of Medicine). To determine for which subjects a blood flow evaluation would be useful in an exploratory manner, a logistic regression analysis was conducted for each subgroup as a post hoc analysis, and forest plots of the odds ratio (OR) and their 95% CIs for the allocation arm were created.

RESULTS

A total of 879 patients were screened and 850 patients were enrolled from 41 centers in Japan between December 2018 and February 2021. The number of registrations at each center is shown in Supplement 4 (Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The details are provided in the Consort diagram (Fig. 1). Eleven patients were excluded (Supplement 5, Supplemental Digital Content 1, http://links.lww.com/SLA/E611). Thus, 839 patients were subjected to the mITT analysis: 422 in the ICG+ group and 417 in the ICG− group. Details of the mITT population are presented in Supplement 6 (Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The patient backgrounds were well balanced between the groups, and the surgical outcomes were similar between the groups (Supplement 7, Supplemental Digital Content 1, http://links.lww.com/SLA/E611). Conversion to open surgery was

0.7% (3/422) in the ICG+ group and 0.7% (3/417) in the ICG − group.

Anastomotic leakage (grade A+B+C) occurred in 81 cases (9.7%). There were 56 patients diagnosed by contrast enema x-ray and 25 diagnosed by CT. The median time to the onset of anastomotic leakage was 15 (IQR 11–22) days postoperatively in grade A cases and 7 (IQR 5–13) days in grade B +C cases. The time to the onset of anastomotic leakage was significantly longer in grade A cases than in grade B+C cases ($P < 0.001$).

The rate of anastomotic leakage (grade A+B+C) was significantly lower in the ICG+ group (7.6%) than in the ICG− group (11.8%) (RR 0.645; 95% CI, 0.422-0.987; $P = 0.041$) (Supplement 8, Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The results for all randomized patients (ITT) showed that the incidence rate of anastomotic leakage in grade A +B+C was 7.5% (32/425) in the ICG+ group versus 11.8% (50-/425) in the ICG− group (RR 0.640; 95% CI, 0.419-0.977; $P = 0.037$).

The secondary endpoints were analyzed in the mITT populations (Supplement 8, Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The rate of anastomotic leakage (grade B+C) was 4.7% in the ICG+ group and 8.2% in the ICG− group ($P = 0.044$), and the time to fluorescence at the resection margin on the proximal side was 25 seconds (IQR 20–35 seconds). In the ICG+ group, there were 411 cases of good perfusion, 5 cases of poor perfusion, and 6 cases of no perfusion. The rate of anastomotic leakage was 7.3% (30/411) in good-perfusion cases, 20.0% (1/5) in poor-perfusion cases, and 16.7% (1/6) in no-perfusion cases. In one of the poor perfusion cases, additional resection was not performed due to intraoperative judgment by the surgeon. This patient had anastomotic leakage. The rate of change of the planned transection line based on ICG-FI was 2.4% (10/422) (95% CI, 1.1%-4.3%) (6 cases of no perfusion and 4 cases of poor perfusion). Among these 10 cases, 1 (10%) developed anastomotic leakage. The additional resection length at the proximal resection margin was 4.0 (IQR 1.5–10.0) cm. There was no perioperative mortality in either group. The details of the 30-day incidence rate of complications (the Clavien-Dindo classification) are presented in Supplement 9 (Supplemental Digital Content 1, http://links.lww.com/SLA/E611). The 30-day incidence rate of complications was 19.4% in the ICG+ group and 21.3% in the ICG− group ($P = 0.495$), and the respective incidence rate of intraoperative adverse events was 0.2% versus 0% ($P = 0.492$). There were no intraoperative adverse events related to ICG administration in the ICG+ group. The reoperation rate was significantly lower in the ICG+ group (0.5%) than in the ICG− group (2.4%) ($P = 0.021$). The reasons for reoperation in the ICG+ group were as follows: anastomotic leakage (n = 1); ileostomy changed to transverse colostomy due to high output (n = 1). The reasons for reoperation in the ICG− group were as follows: anastomotic leakage (n = 10). The length of postoperative hospital stay was 13 (IQR 9–16) days in the ICG+ group and 13 (IQR 10–17) days in the ICG− group ($P = 0.221$).

Subgroup analyses were performed for age (< 70 vs ≥ 70 yr old), sex (male vs female), body mass index (< 25 vs ≥ 25 kg/m^2), albumin (Alb; < 4 vs ≥ 4), clinical stage (0-I vs II vs III), tumor height from the anal verge (< 5 vs ≥ 5 cm), maximum tumor diameter (< 5 vs ≥ 5 cm), approach (laparoscopic surgery vs taTME vs robotic surgery), diverting stoma (absence vs presence), preservation of the left colon artery (no preservation vs preservation), and neoadjuvant chemoradiotherapy (no chemoradiotherapy vs chemoradiotherapy). The details of the subgroup analyses are presented in Figure 2.

DISCUSSION

The EssentiAL trial was the first phase III RCT conducted to demonstrate the superiority of a blood flow assessment using ICG-FI compared with a conventional blood flow assessment in minimally invasive surgery for rectal cancer. We hypothesized that the study treatment would reduce the incidence rate of anastomotic leakage (grade A+B+C) by 6% according to previous meta-analyses.[24,38] However, the actual reduction rate was 4.2% (95% CI, 8.2%-0.2%; ICG− group 11.8%, ICG+ group 7.6%), which was less than the expected reduction rate. Although ICG-FI significantly reduced the rate of anastomotic leakage, the planned hypothetical reduction of 6% was not achieved.

To date, 3 RCTs have been reported.[27–29] The FLAG trial was the only report among the 3 RCTs in which ICG-FI significantly reduced anastomotic leakage.[29] However, the FLAG randomized trial (n = 377 patients) targeted elective patients with either malignant or benign sigmoid or rectal neoplasms, not just rectal cancer. The EssentiAL trial differs from the FLAG randomized trial in that it targeted rectal cancer located ≤ 12 cm from the anal verge. The report by De Nardi et al[28] (n = 240 patients) had a small sample size and therefore insufficient power. Furthermore, in addition to rectal cancer, these RCTs included patients with left-sided colonic cancer. The PILLAR III trial planned as a phase III trial for low anterior resection (planned sample size: n = 800 patients), discontinued enrollment in 347 cases and thus was also underpowered.[27] The present EssentiAL study included only cases of rectal cancer with tumor localization 12 cm or lesser from the anal verge and registered the largest number of patients thus far.

The causes of anastomotic leakage are multifactorial.[9,10,12,13] However, adequate perfusion is necessary to optimize anastomotic healing. In this study, the difference in anastomotic leakage between the ICG+ and the ICG− group was 4.2%. According to a systematic review of 27 studies that included 8786 patients, Emile et al[24] reported that changes to the surgical plan with regard to the level of transection and anastomosis based on the findings of ICG-FI were made in 331 (9.1%) of 3614 patients. The rates of such changes ranged from 0.6% to 28.7% across studies, and all changes were related to proximal transection. In the present study, the additional resection rate was 2.4% (95% CI, 1.1%-4.3%), which was lower than the preplanned rate. The reduction rate of anastomotic leakage (actually a 4.2% reduction) is within the 95% CI for the rate of additional bowel resection due to poor blood flow in the ICG+ group.

According to a systematic review in 2022, the dose of ICG varied and the majority of studies (10 studies) used a dose of 0.1 to 0.25 mg/kg of ICG, whereas some studies used a fixed dose (not based on body weight) of 5 to 25 mg.[24] Among the 27 trials, the ICG dose was 0.2 to 0.3 mg/kg (10–15 mg for 50 kg) in 8 trials and 10 to 25 mg/body in 5 trials.[24] In this study, the ICG dose was 12.5 mg (0.25 mg/kg for 50 kg), which is not a high dose. On the other hand, the time elapsed until fluorescence was 60 seconds in 13 studies, 30 seconds in 1 study, 90 seconds in 1 study, and 2 to 3 minutes in 1 study.[24] In most studies, the time elapsed before fluorescence occurred was 60 seconds. In addition, we evaluated the blood flow using 60 seconds as the evaluation standard.[18,39] Therefore, in the present study, the ICG dose was set at 12.5 mg/5 mL, and the reference value for the elapsed time until fluorescence was set at 60 seconds.

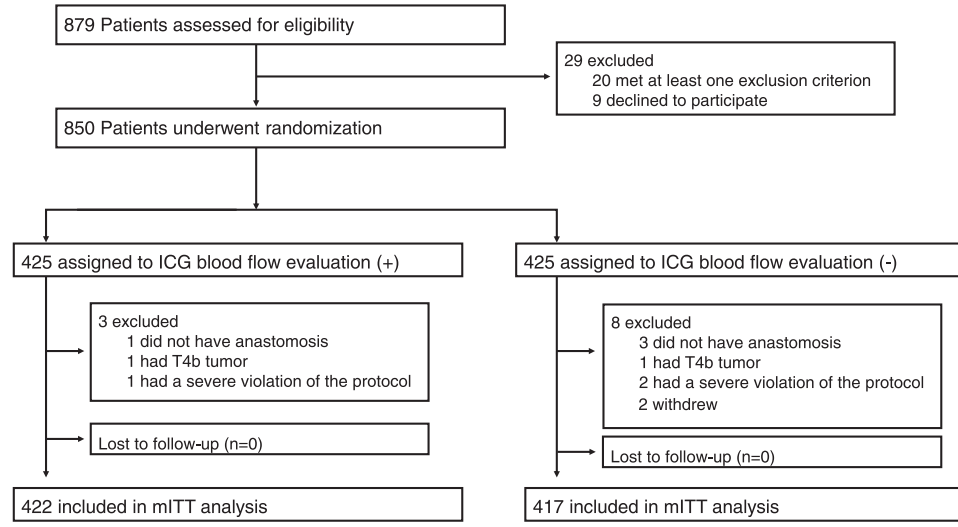

FIGURE 1. Trial profile. mITT indicates modified intention-to-treat.

In the subgroup analyses, the rate of anastomotic leakage in the ICG-FI group tended to be high in patients who had a tumor height of <5 cm from the anal verge (OR 1.500; 95% CI, 0.427-5.273) and received neoadjuvant chemoradiotherapy (OR 2.333; 95% CI, 0.354–15.36), which showed that these patients did not benefit from ICG-FI. The cause of anastomotic

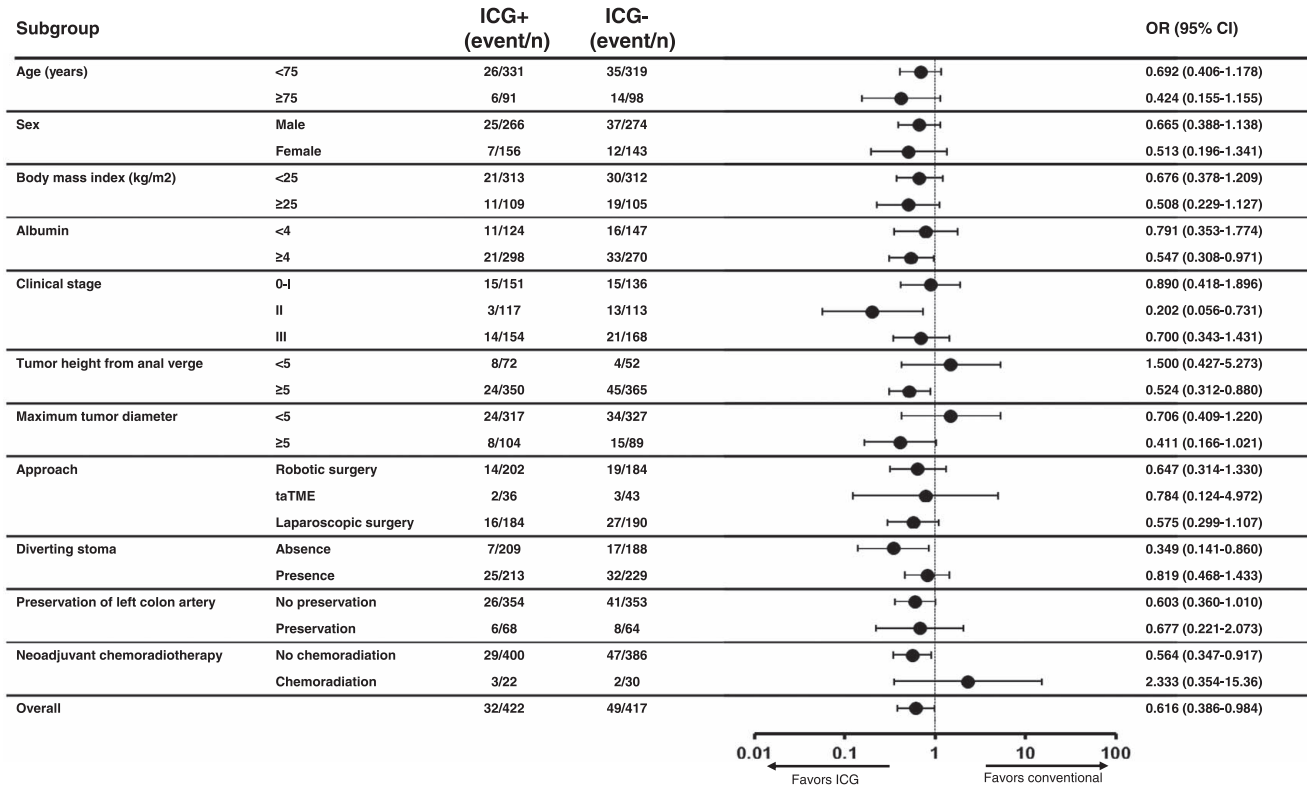

FIGURE 2. Subgroup analyses of the effect of ICG+ vs ICG– group on anastomotic leakage. To determine for which subjects a blood flow evaluation would be useful in an exploratory manner, a logistic regression analysis was conducted for each subgroup as a post hoc analysis, and forest plots of the odds ratio (OR) and their 95% confidence intervals (CIs) for the allocation arm were created. Subgroup analyses were performed for age (< 70 vs ≥ 70 yr old), sex (male vs female), body mass index (< 25 vs ≥ 25 kg/m^2), albumin (Alb; < 4 vs ≥ 4), clinical stage (0-I vs II vs III), tumor height from the anal verge (< 5 vs ≥ 5 cm), maximum tumor diameter (< 5 vs ≥ 5 cm), approach (laparoscopic surgery vs taTME vs robotic surgery), diverting stoma (absence vs presence), preservation of the left colon artery (no preservation vs preservation), and neoadjuvant chemoradiotherapy (no chemoradiotherapy vs chemoradiotherapy). ICG indicates indocyanine green; taTME, transanal total rectal resection.

leakage may be more strongly influenced by physical factors, such as ultra-low anastomosis and delayed wound healing after irradiation, than by insufficient blood flow. This was consistent with the PILLAR III trial, in which 64.6% (223/347) of patients received neoadjuvant chemoradiation and 83.0% (288/347) with lower/mid rectal cancer required low anastomosis.[27]

Several limitations associated with the present study warrant mention. First, there is a possibility of observational bias, as both the patients and surgeons were aware of the study group assignments. Second, the results are limited to the Japanese population. Third, in this study, the anastomosis method was not uniform in all study patients. There were no provisions regarding left colic artery preservation, splenic flexure mobilization, or diverting stoma. Although the proportions of these factors did not differ between the 2 groups, these variables may introduce bias, as leakage is not strictly due to suboptimal perfusion—it may occur due to a myriad of other factors, including tension. Fourth, a postoperative contrast enema assessment was not routinely performed. It would not be possible for investigators to detect a subclinical radiographic leak (grade A) if radiographic studies were not routinely performed. In the present study, the incidence of anastomotic leakage (grade A+B +C) may have been underestimated, as the study population included patients who did not undergo contrast enema. Fifth, in terms of patient characteristics of the mITT population, a 2×3 χ^2 statistic comparing ASA I versus ASA II versus ASA III between the ICG+ and ICG− groups did not show a significant difference ($P = 0.055$). In contrast, a χ^2 statistic comparing ASA I versus ASA II+III between the ICG+ and ICG− groups demonstrated a significant difference ($P = 0.026$). This may be a possible source of bias. However, the rate of anastomotic leakage in ASA PS class I/class II+III was 8.9% (32/358)/10.2% (49/481) with $P = 0.545$. There was no significant difference in the rate of anastomotic leakage between ASA PS class I and class II+III. The adjusted RR was 0.621 (95% CI, 0.389-0.993, $P = 0.047$) taking into account the ASA-PS (class I vs class II +III). This finding supported the consistency and robustness of the original analysis. Sixth, the fluorescence signal obtained using ICG-FI has not yet been quantified. The appearance of fluorescence differs depending on each fluorescent device. This can lead to different interpretations of blood flow assessments. To minimize this bias, we unified the fluorescent devices. Even with a unified laparoscopic system and settings, there are reportedly inter-user variations and a blood flow assessment by ICG-FI is not completely objective.[40] To address this issue, the objectivity of a blood flow evaluation by ICG-FI may be able to be improved by quantification of fluorescence signals[41,42] and automated real-time evaluations by trained artificial intelligence models in the future.[43]

In conclusion, although the actual reduction rate of anastomotic leakage in the ICG+ group was lower than the expected reduction rate of 6% and the addition of ICG-FI was not superior to white light (ICG− group), the addition of ICG-FI significantly reduced the anastomotic leakage rate by 4.2%.

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