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REVIEW ARTICLE

# Guiding role of indocyanine green fluorescence lymphography compared to standard techniques in lymphadenectomy for gastric cancer during minimally invasive surgery: a systematic review and meta-analysis

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## Abstract

Real-time indocyanine-green (ICG) fluorescence lymphography in gastric cancer (GC) surgery is gaining traction for its potential to enhance lymphadenectomy during minimally-invasive procedures. This systematic review and meta-analysis evaluated efficacy and safety of ICG-guided lymphadenectomy versus standard techniques. Following PRISMA and Cochrane guidelines, this review (PROSPERO: CRD42024628572) included randomized controlled trials (RCTs) and non-randomized controlled studies (n-RCTs) comparing ICG-guided versus standard minimally-invasive lymphadenectomy in GC patients undergoing gastrectomy. Primary outcome was number of retrieved lymph-nodes (LNs). Secondary outcomes included ideal (≥30 LNs) and proper (≥16 LNs) lymphadenectomy rates, postoperative outcomes, recurrence, and mortality. Meta-analyses used a random-effects model; evidence quality was assessed via GRADE. 21 studies involving 8633 patients were included. ICG-guided surgery retrieved significantly more LNs (MD 6.91; 95%CI 5.47–8.35; $p<0.00001$; $I^2$ 68%). Subgroup analyses showed greater benefit in patients receiving neoadjuvant therapy (MD 9.3; 95%CI 6.73–11.88; $p<0.00001$; $I^2$ 0%) and in overweight/obese patients (MD 10.94; 95%CI 3.25–18.64; $p=0.005$; $I^2$ 79%). ICG significantly improved ideal lymphadenectomy rate (RR 1.29; 95%CI 1.15–1.45; $p<0.0001$; $I^2$ 74%), though proper lymphadenectomy rates were similar. ICG reduced operative time (MD − 6.56; 95%CI − 12.31 to − 0.81; $p=0.03$; $I^2$ 75%) and blood loss (MD − 10.13; 95%CI − 17.44 to − 2.82; $p=0.007$; $I^2$ 83%). No significant differences emerged for postoperative complication, recurrence, or mortality. ICG lymphography significantly improves nodal yield and ideal lymphadenectomy rates in minimally-invasive GC surgery, enhancing efficiency and reducing blood loss, without increasing complications. Broader implementation is supported, especially in challenging subgroups, like obese or neoadjuvantly treated patients.

**Keywords** Gastric cancer · Gastrectomy · Indocyanine green fluorescence · Fluorescence-guided surgery · Lymphadenectomy · Meta-Analysis

## Introduction

Over the past 15 years, research on fluorescence-guided surgery has grown significantly, yet some applications remain quantitatively and qualitatively underexplored.

One such area is gastric cancer (GC) surgery [1–4]. Real-time fluorescence lymphography may guide and help define the extent of lymphadenectomy for GCs, shaping the most sensitive "game-changer" factor for the prognosis of operable patients [5–9].

The scientific literature considers a "proper" lymphadenectomy one that ensures accurate and adequate staging [10, 11], and this appears to be achieved when at least 16

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lymph nodes (LNs) are retrieved [12, 13]. However, an "ideal" lymphadenectomy would further improve survival by optimising surgical sensitivity, meaning the removal of the tumour's potential for recurrence and/or progression [14, 15] within an acceptable morbidity threshold. For this latter definition, a cut-off of at least 30 LNs [16–20] is often proposed, while still following the Japanese guidelines for the selection of relevant nodal stations [21].

When injected subserosally or submucosally, Indocyanine Green (ICG), through near-infrared (NIR) light, allows for easy identification of lymphatic stations [22–24], with no obvious alterations of the surgical field as ICG is invisible as fast as the surgeon switches to white light [25, 26]. ICG is also known to be extremely safe [27, 28], with pseudo-allergic and anaphylactoid reactions being exceptionally rare.

The aim of this systematic review and meta-analysis is to analyse the impact of ICG fluorescence in guiding minimally invasive lymphadenectomy for GC, assessing clinical-pathological and therapeutic aspects, safety and feasibility, and prognosis.

## Methods

We conducted a systematic review and meta-analysis, following PRISMA guidelines [29–31], and referring to the Cochrane Handbook for Systematic Reviews of Interventions [32]. The study protocol was registered on PROSPERO (PROSPERO: International Prospective Register of Systematic Reviews) [33] under the identifier CRD42024628572.

### Objectives

The PICO(S) framework (Population, Intervention, Comparison, Outcomes, Studies) was applied to define the research question:

- *Population (P)*: patients diagnosed with GC submitted to minimally invasive gastrectomy.
- *Intervention (I)*: fluorescence-guided lymphadenectomy using real-time ICG lymphography.
- *Comparison (C)*: standard minimally invasive lymphadenectomy without tracer use.
- *Outcomes (O)*:
  - *Primary*: number of retrieved LNs, reported as mean and standard deviation (SD).
  - *Secondary*: "ideal" lymphadenectomy rate (proportion of patients with at least 30 LNs retrieved); "proper" lymphadenectomy rate (proportion of patients with at least 16 LNs retrieved); number of metastatic retrieved lymph nodes; surgery duration; estimated blood loss; time to first flatus; time to first liquid intake; length of postoperative hospitalization; incidence of major postoperative complications; recurrence; mortality.
- *Studies (S)*: randomized controlled trials (RCTs) and controlled observational studies (n-RCTs), conducted on the human species. For studies with duplicate data, only articles with the most complete and detailed datasets were included. Only full texts available in English were finally included.

### Search strategy

From inception until 15th of March 2025, MEDLINE, PubMed Central (PMC) and NCBI Bookshelf were searched via PubMed, whereas CENTRAL (Cochrane Central Register of Controlled Trials) was separately investigated. The full search strategy is available as supplementary material (Supplementary Material. Full search strategy). Reference lists from included studies and relevant reviews were also manually searched. No filters were applied (e.g. language or study type). Abstract and full-text screening was conducted via Rayyan [34].

### Assessment of the risk of bias

For included RCTs, the Cochrane Collaboration's Risk of Bias 2 tool (RoB 2) [35] was used to assess the risk of bias arising from five domains: randomisation, deviations from intended interventions, missing outcome data, outcome measurement, and selection of the reported result. The risk was classified as "low", "some concerns", or "high".

For n-RCTs, the latest Risk Of Bias In Non-randomized Studies—of Interventions (ROBINS-I V2) [36] was applied, covering seven domains: confounding, classification of interventions, selection of participants, deviations from intended interventions, missing data, outcome measurement, and selection of the reported result. Judgements on risk were "low", "moderate", "serious", or "critical".

### Measures and statistical methods

Statistical analyses were performed using Review Manager (RevMan 5.4.1 [37]). The effect measure was the mean difference (MD) with 95% confidence intervals (CIs) for continuous variables. For studies reporting median and interquartile ranges (IQRs), or median and range, data were properly converted using the Wan [38, 39] or the Hozo method [40], respectively. For dichotomous variables, the effect measure was the risk ratio (or relative risk, RR) with 95% CI.

All pooled analyses followed a random-effects model, anticipating expected heterogeneity among studies. Statistical heterogeneity was quantified by the $I^2$ value, with ≥ 50% considered "substantial", prompting sensitivity analyses. The $\chi^2$ test was used to evaluate the hypothesis of true heterogeneity.

In all statistical tests, $p < 0.05$ was considered indicative of statistical significance.

### Assessment of the quality of evidence

Evidence quality for each outcome was assessed using the GRADE approach (Grading of Recommendations, Assessment, Development and Evaluation [41]), through the GRADEpro GDT software [42], considering the overall risk of bias, inconsistency, indirectness, imprecision, and publication bias.

All stages of study identification, selection, quality assessment, risk of bias judgement and data extraction were carried out independently by two reviewers (L.D. and M.P.). Inconsistencies were resolved by discussion between the two reviewers until consensus was reached.

## Results

### Characteristics of studies and quality assessment

A total of 289 references were identified, with the last search conducted on 15th March 2025.

Of these, 15 were duplicates and 234 were deemed ineligible based on title and abstract evaluation. The remaining 40 articles were therefore considered for full-text review. Five studies with duplicate data sets [25, 26, 43–45], two studies whose design was unsuitable (ICG was used but did not guide lymphadenectomy) [46, 47], two studies with different outcomes [48, 49], two wrong publication types (one translation [50], one comment [51]), and two articles not available in English [52, 53] were excluded. Five additional articles were identified through reference evaluation, but none were eligible [54–58].

Figure 1 resumes the studies selection flow [31] that brought to the included 21 articles [59–79], whose general characteristics are summarised in Table 1.

One RCT and 20 n-RCTs were included. Studies were conducted in China (10 studies), South Korea (three), Italy (three), Spain (two), Vietnam (one), Japan (one), and Taiwan (one), and were published between 2017 and 2025. Data came from 8633 patients (3752 in the ICG-group and 4881 in the control group).

Pre-surgical staging characteristics were assessed, focusing on the distribution of cT ≥ 2 (advanced gastric cancer, AGC) and cN+: ten studies (7340 patients) provided cT

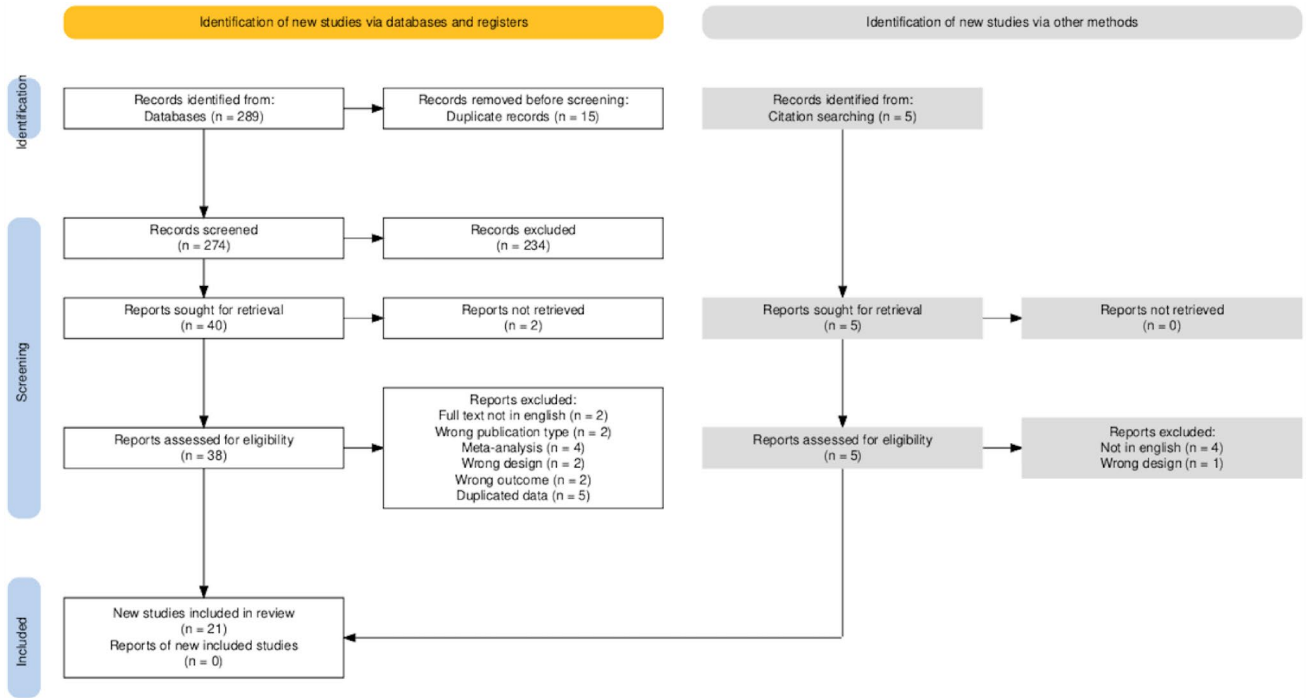



**Fig. 1** Study selection flow

**Table 1** General characteristics of the included studies

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Van Du N (2025) | Socialist Republic of Vietnam | N-RCT[o] | 2018 2023 | 160 (80 vs 80) | T1-3; N±; M0 | Excl[c] | LDG[f] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Ideal lymphadenectomy rate;* *Number of metastatic retrieved LNs[g];* *Surgery duration;* *Estimated blood loss;* *Time to first flatus* |
| Huang ZN (2024) | People's Republic of China | RCT[p] | 11-2020 05-2023 | 236 (118 vs 118) | T2-4a; N±; M0 | Only | LTG[j]; LDG[f] | D2; D2+ ICG[d] was *one* indicator for D2+ | Intraoperative laparoscopic subserosal injection | *Number of retrieved LNs[g];* *Ideal lymphadenectomy rate;* *Number of metastatic retrieved LNs[g];* *Surgery duration;* *Estimated blood loss;* *Incidence of major postoperative complications;* *Time to first flatus;* *Time to first liquid intake;* *Length of postoperative hospitalization* |
| Kim KY (2024) | Republic of Korea | N-RCT[o] | 01-2013 12-2021 | 5872 (2397 vs 3475) | T1-4a; N±; M0 | Excl[c] | LTG[j]; RTG[t]; LDG[f]; RDG[q]; LPG; RPG[r] | D1+; D2 D2+ ICG[d] beyond planned dissection wasn't considered, except for 14v | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Ideal lymphadenectomy rate;* *Proper lymphadenectomy rate;* *Surgery duration;* *Estimated blood loss;* *Incidence of major postoperative complications;* *Time to first flatus;* *Length of postoperative hospitalization* |

**Table 1** (continued)

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Senent-Boza A (2024) | Spain | N-RCT[o] | 02-2017 12-2022 | 84 (42 vs 42) | Tis-4; N±; M0 | Incl[e] | LTG[j]; LSTG[i] | D1+; D2; D2+ ICG[d] was the (*only?*) indicator for D2+ | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Cases with at least 15 LNs[g] retrieved; Number of metastatic retrieved LNs[g]; Incidence of major postoperative complications; Length of postoperative hospitalization; Recurrence; Mortality* |
| Jeon CH (2023) | Republic of Korea | N-RCT[o] | 01-2016 02-2022 | 393 (105 vs 288) ICG[d]-Laparoscopic: 61 ICG[d]-Robotic: 44 Control: 288 | *pS2; pS3 pT1-4; pN±; M0 Excluded: pS1; pS4* | Excl[e] | LTG[j]; LSTG[i]; RTG[t]; RSTG[s] | NA[k] *"All surgeries were performed adhering to the Korean Gastric Cancer guidelines, 2022"* | Intraoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Ideal lymphadenectomy rate; Proper lymphadenectomy rate* |
| Fujimoto D (2023) | Japan | N-RCT[o] | 03-2019 12-2022 | 94 (55 vs 39) | T1-4a; N±; M0 | NAC[l]: Excl[e]; NAR[m]:? (NA[k]) | RDG[q] | D1+; D2 | Intraoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Surgery duration; Estimated blood loss; Length of postoperative hospitalization* |
| Meng W (2023) | People's Republic of China | N-RCT[o] | 01-2017 10-2020 | 136 (68 vs 68) | *pT1-3; pN±; M? (NA[k])* | Excl[e] | LPG | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Number of metastatic retrieved LNs[g]; Surgery duration; Estimated blood loss; Recurrence; Mortality* |

**Table 1** (continued)

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Chen X (2022) | People's Republic of China | N-RCT[o] | 03-2019 12-2020 | 56 (18 vs 38) | T1-4a; N±; M0 | Excl[c] | LTG[j]; LDG[f]; LPG | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Number of metastatic retrieved LNs[g];* *Surgery duration;* *Estimated blood loss;* *Time to first flatus;* *Time to first liquid intake;* *Length of postoperative hospitalization* |
| Tian Y (2022) | People's Republic of China | N-RCT[o] | 11-2019 11-2020 | 59 (27 vs 32) | T1-4; N±; M0 | Excl[c] | RDG[q] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Surgery duration;* *Estimated blood loss;* *Time to first flatus;* *Length of postoperative hospitalization* |
| Puccetti F (2022) | Italy | N-RCT[o] | 04-2015 08-2021 *ICG[d] since 04-2018* | 102 (38 vs 64) | T≤3; N±; M0 | Incl[e] | LTG[j] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Number of metastatic retrieved LNs[g];* *Surgery duration* |
| Maruri I (2022) | Spain | N-RCT[o] | 02-2018 *End date = NA[k]* | 34 (17 vs 17) | T1-4; N±; M0 | Incl[e] | LTG[j]; LSTG[i]; LNTG[h] | D1.5 + *("all D2 stations except for 10 and 11 for TG, all D2 stations except for 12 for DG[a]")*; D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g];* *Number of metastatic retrieved LNs[g]* |

**Table 1** (continued)

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Wei M (2022) | People's Republic of China | N-RCT[o] | 01-2018 08-2019 | 195 (107 vs 88) | T1-4a; N±; M? (NA[k]) | Excl[c] | LTG[j]; LDG[f] | D2; D2+ ICG[d] was *the only* indicator for D2+ | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Number of metastatic retrieved LNs[g]; Surgery duration; Estimated blood loss; Incidence of major postoperative complications; Time to first flatus; Time to first liquid intake; Length of postoperative hospitalization; Recurrence; Mortality* |
| Zhong Q (2021) | People's Republic of China | N-RCT[o] | 11-2018 10-2020 | 514 (385 vs 129) | T1-4a; N±; M0 | Excl[c] | LTG[j]; LDG[f] | D2; D2+ ICG[d] was *one* indicator for D2+ | Preoperative endoscopic submucosal injection OR Intraoperative laparoscopic subserosal injection | *Number of retrieved LNs[g]; Number of metastatic retrieved LNs[g]* |
| Lu X (2021) | People's Republic of China | N-RCT[o] | 07-2015 10-2019 | 56 (28 vs 28) | T1-4; N±; M0 | NA[k] | LTG[j]; LDG[f]; LPG | D2 | Intraoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Surgery duration; Estimated blood loss; Incidence of major postoperative complications; Length of postoperative hospitalization* |
| Huang ZN (2021) | People's Republic of China | N-RCT[o] | 02-2010 10-2020 | 188 (94 vs 94) | T1–4a; N±; M0 | Only | LTG[j]; LDG[f] | D2 D2+ ICG was *one* indicator for D2+ | Intraoperative laparoscopic subserosal injection | *Number of retrieved LNs[g]; Surgery duration; Estimated blood loss; Time to first flatus; Time to first liquid intake; Length of postoperative hospitalization* |

**Table 1** (continued)

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Romanzi A (2021) | Italy | N-RCT[o] | 01-2017 08-2019 *ICG[d] since 09-2018* | 20 (10 vs 10) | *pT1-4b; pN±; pM0* | Incl[e] | RSTG[s] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Ideal lymphadenectomy rate; Proper lymphadenectomy rate; Number of metastatic retrieved LNs[g]; Surgery duration; Incidence of major postoperative complications; Time to first flatus; Length of postoperative hospitalization* |
| Ma S (2020) | People's Republic of China | N-RCT[o] | 12-2018 08-2019 | 65 (31 vs 34) | *pStages ≥ 2 BUT NOT T4b (Multiorgan) and NOT M + (Metastatic) (pT2-4a; N±; M0 except pT2N0M0)* | NA[k] | LDG[f] | D2 | Preoperative endoscopic injection | *Number of retrieved LNs[g]; Number of metastatic retrieved LNs[g]; Number of metastatic retrieved LNs[g]; Surgery duration; Estimated blood loss; Incidence of major postoperative complications; Time to first flatus; Length of postoperative hospitalization* |
| Park SH (2020) | Republic of Korea | N-RCT[o] | 07-2017 06-2018 | 80 (20 vs 60) | EGC[b] (T1; N±; M0) | NA[k] | LDG[f] | D1; D1 +; D2 | Intraoperative submucosal injection | *Number of retrieved LNs[g]; Surgery duration; Estimated blood loss; Incidence of major postoperative complications; Length of postoperative hospitalization* |

**Table 1** (continued)

| Study (Year) | Country | Study design | Period of interventions | Sample Size (ICG[d] vs Control) | Clinical stages included | NAT[n] | Surgical method | LN[g] Dissection | ICG[d] injection modality | Outcomes of interest |
|---|---|---|---|---|---|---|---|---|---|---|
| Cianchi F (2020) | Italy | N-RCT[o] | 06-2014 06-2018 | 74 (37 vs 37) | *pT1-3; pN±; pM0* | NA[k] | RTG[t]; RDG[q] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Ideal lymphadenectomy rate; Proper lymphadenectomy rate;; Number of metastatic retrieved LNs[g]; Surgery duration; Length of postoperative hospitalization* |
| Liu M (2020) | People's Republic of China | N-RCT[o] | 08-2017 11-2019 | 136 (61 vs 75) | pT1-4; pN±; pM0 | Excl[c] | LDG[f] | D2 | Preoperative endoscopic submucosal injection | *Number of retrieved LNs[g]; Number of metastatic retrieved LNs[g]; Surgery duration; Estimated blood loss; Incidence of major postoperative complications; Time to first flatus; Time to first liquid intake; Length of postoperative hospitalization* |
| Lan YT (2017) | Republic of China (Taiwan) | N-RCT[o] | 01-2011 03-2016 | 79 (14 vs 65) | T1-3; N0-1; M0 | NA[k] | RTG[t]; RDG[q] | D1+; D2 | Intraoperative subserosal injection for the initial 9 patients Preoperative endoscopic submucosal injection for the other 5 patients | *Number of retrieved LNs[g]; Surgery duration; Estimated blood loss; Length of postoperative hospitalization* |

[a]*DG* Distal gastrectomy, [b]*EGC* Early gastric cancer, [c]*Excl* Excluded, [d]*ICG* Indocyanine green, [e]*Incl* Included, [f]*LDG* Laparoscopic distal gastrectomy, [g]*LN* Lymph node, [h]*LNTG* Laparoscopic near-total gastrectomy, [i]*LSTG* Laparoscopic subtotal gastrectomy, [j]*LTG* Laparoscopic total gastrectomy, [k]*NA* Not available, [l]*NAC* Neoadjuvant chemotherapy, [m]*NAR* Neoadjuvant radiotherapy, [n]*NAT* Neoadjuvant therapy, [o]*N-RCT* Non randomized controlled trial, [p]*RCT* Randomized controlled trial, [q]*RDG* Robotic distal gastrectomy, [r]*RPG* Robotic proximal gastrectomy, [s]*RSTG* Robotic subtotal gastrectomy, [t]*RTG* Robotic total gastrectomy

data, with *Zhong Q (2021)* showing a statistically significant excess of AGCs in the control group; nine studies (7260 patients) reported cN+data, with *Van Du N (2025)* exhibiting more clinical nodal metastatic cases in the ICG-group. *Park SH (2020)* only included early gastric cancers (EGCs).

All articles specified the type of gastrectomies performed. *Zhong Q (2021)* showed a significant imbalance, with more subtotal gastrectomies (STGs) in the ICG-group. *Puccetti F (2022)* solely focused on total gastrectomies (TGs), while *Van Du N (2025)*, *Fujimoto D (2023)*, *Meng W (2023)*, *Tian Y (2022)*, *Romanzi A (2021)*, *Ma S (2020)*, *Park SH (2020)* and *Liu M (2020)* only studied STGs.

We distinguished between D≥2 and D1+cases. Sixteen studies provided relevant data (7309 patients), with

*Senent-Boza A (2024)* showing a significant imbalance, due to an excess of D≥2 in the ICG-group.

Information on whether a neoadjuvant therapy (NAT) was administered was collected from 17 studies (8353 patients). Ten studies either excluded NAT-patients or had none in their datasets. Among the seven remaining studies: five were balanced, while *Puccetti F (2022)* showed a statistically significant excess of NAT-patients in the ICG-group, and *Maruri I (2022)* had more in the control group.

We performed adequate sensitivity and subgroup analyses to address baseline characteristics imbalances, particularly for the primary outcome (Supplementary Material Tables).

## Primary outcome assessment: Number of retrieved LNs

All included studies, involving 8633 patients (3752 in the ICG-group and 4881 in the control group) examined the number of retrieved LNs. ICG achieved a statistically significant higher harvest of LNs (MD 6.91; 95% CI 5.47–8.35; $p < 0.00001$, $I^2$ 68%) (Fig. 2).

Sensitivity analyses based on the risk of bias judgement confirmed a statistically significant advantage for nodal harvesting in the ICG-group. None of the single study exclusions significantly modified heterogeneity, which remained above 50%.

## Primary outcome and risk of bias

Apart from the only RCT included, which had an overall low risk of bias, all other studies showed at least a moderate risk. Seven studies had a serious risk, while two were classified as having a critical risk. The main concerns involved the selection of reported results (as no pre-determined analysis plan was available) and confounding factors (Fig. 3).

Two studies, *Senent-Boza A (2024)* and *Jeon CH (2023)*, had an overall critical judgement, for confounding factors. *Senent-Boza A (2024)* exhibited an imbalance in the distribution of lymphadenectomy types, with an excess of D≥2 cases in the ICG-group. Some other sources of confounding were noted in *Senent-Boza A (2024)* and *Jeon CH (2023)*, including the extent of gastrectomy. *Jeon CH (2023)* was problematic due to its three-group design (ICG-laparoscopic, ICG-robotic, control-laparoscopic), which also led to imbalances in mean age and surgical extent distribution. Additional concerns included the limitation to pT stages 2 and 3 (consequently controlling a post-intervention variable), and the absence of details on types of lymphadenectomies performed and their distribution.

*Puccetti F (2022)*, *Maruri I (2022)*, *Zhong Q (2021)*, *Huang ZN (2021)*, *Park SH (2020)*, *Cianchi F (2020)* and *Lan YT (2017)* were classified as having a serious risk of bias, primarily due to unresolved imbalances in the groups. *Lan YT (2017)* was the only study rated serious in the "deviations from interventions" domain, as two of its 79 robotic cases experienced ICG leakage, which we considered an

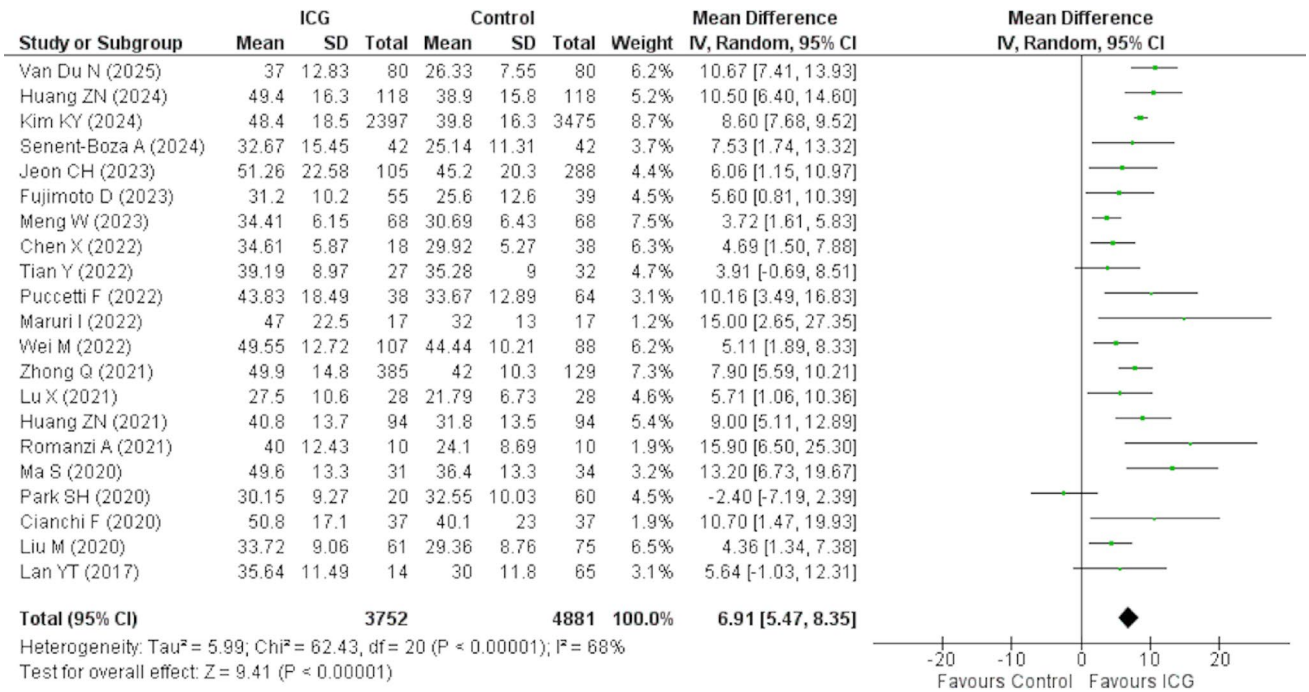
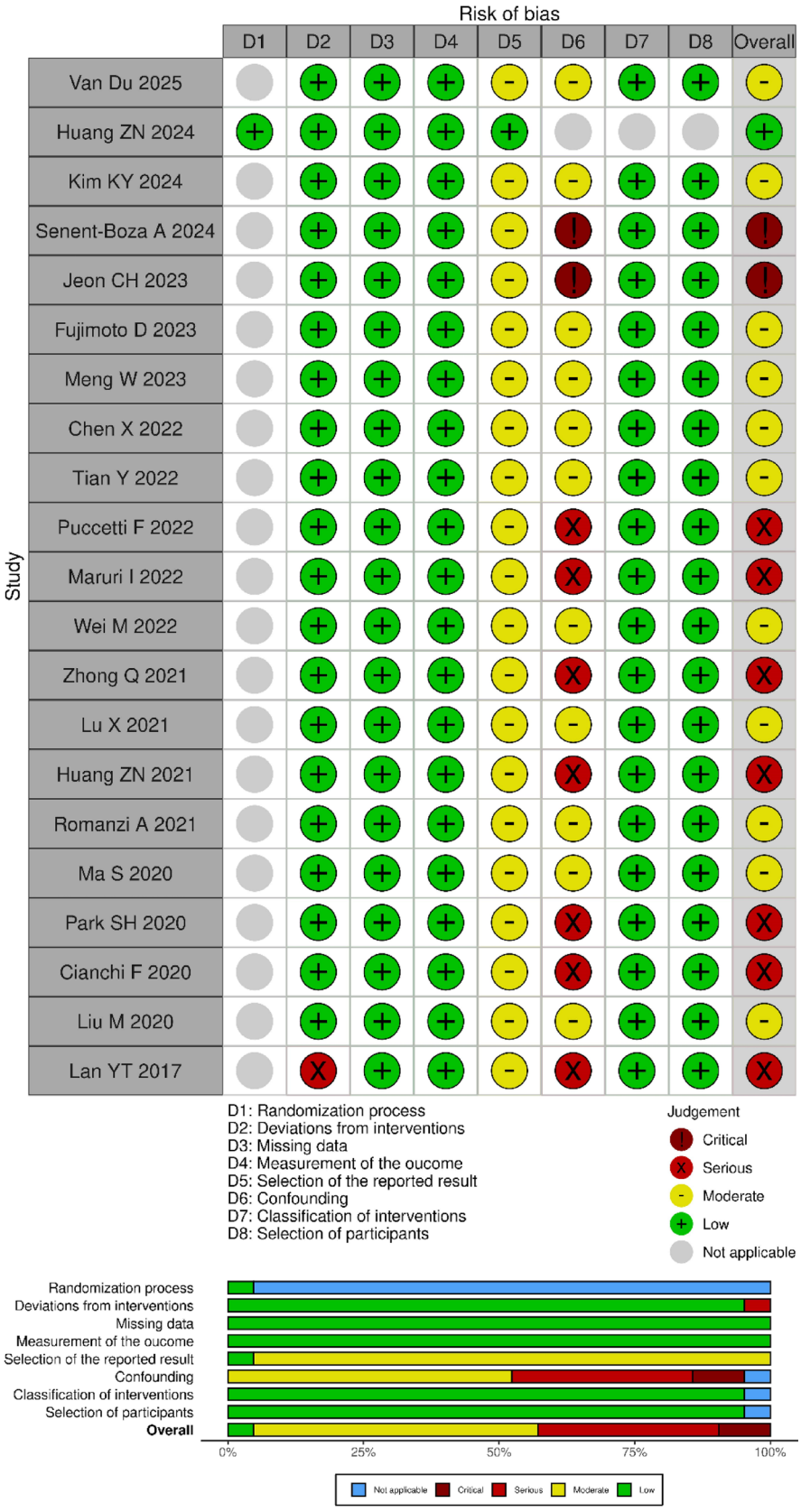



**Fig. 2** Primary outcome assessment

**Fig. 3** Primary outcome and risk of bias



unacceptable significant deviation that should have led to the exclusion of such cases.

## Primary outcome and subgroup analyses

Fifteen studies provided data specific to D2 stations (MD 7.96; 95% CI 5.95–9.97; $p<0.00001$; $I^2$ 73%; 3244 patients). Five studies provided data specific to D1 stations (1 to 7).

(MD 3.60; 95% CI 2.21–4.99; $p<0.00001$; $I^2$ 37%; 1018 patients). For D≥2-stations (8 onwards), data were provided by four studies (MD 2.80; 95% CI 0.81–4.78; $p = 0.006$; $I^2$ 85%; 504 patients).

Among TGs (three studies), the widest gap was reached (MD 11.03; 95% CI 8.59–13.47; $p<0.00001$; $I^2$ 0%; 966 patients). For DGs, data came from seven studies (MD 5.88; 95% CI 3.05–8.71; $p<0.0001$; $I^2$ 80%; 5360 patients). Only two studies specifically analysed PGs (MD 5.47; 95% CI 1.77–9.16; $p=0.004$; $I^2$ 76%; 547 patients).

Fifteen studies detailed laparoscopic cases (MD 6.79; 95% CI 4.98–8.61; $p<0.00001$; $I^2$ 68%; 2391 patients). Robotic technique application was detailed by five studies (MD 6.86; 95% CI 3.36–10.37; $p=0.0001$; $I^2$ 34%; 326 patients).

Four studies allowed specific investigation on NAT-patients (MD 9.3; 95% CI 6.73–11.88; $p<0.00001$; $I^2$ 0%; 514 patients). Overweight and obese patients were distinctly described by two studies (MD 10.94; 95% CI 3.25–18.64; $p=0.005$; $I^2$ 79%, 1866 patients).

Two different ICG injection techniques were described: intraoperative and preoperative. As for the intraoperative one, six studies reported specific data (MD 5.87; 95% CI 2.32–9.42; $p=0.001$; $I^2$ 73%; 1047 patients). The results were consistent when considering only studies based on preoperative injection (13 studies) (MD 7.33; 95% CI 5.45–9.22; $p<0.00001$; $I^2$ 71%; 6993 patients).

Restricting the analysis to the 16 Asian studies (MD 6.39; 95% CI 4.84–7.94; $p<0.00001$; $I^2$ 73%; 8319 patients) and the five European studies (MD 10.46; 95% CI 6.97–13.96; $p<0.00001$, $I^2$ 0%; 314 patients), the superiority of ICG was confirmed.

## Proper and ideal lymphadenectomy

The comparison on achieving a "proper" lymphadenectomy was based on five studies (Fig. 4A). No statistically significant difference was found (RR 1.01; 95% CI 1–1.03; $p=0.15$; $I^2$ 32%; 6443 patients). *Cianchi F (2020)* classified as proper a nodal harvest of at least 15. However, its exclusion did not alter statistical significance.

For "ideal" lymphadenectomy, ICG showed a statistically significant advantage (data from six studies) (RR 1.29; 95% CI 1.15–1.45; $p<0.0001$; $I^2$ 74%; 6755 patients) (Fig. 4B). *Cianchi F (2020)* based its results on a different threshold for "ideal" lymphadenectomy, that is more than 30 LNs. Excluding it resulted similarly.

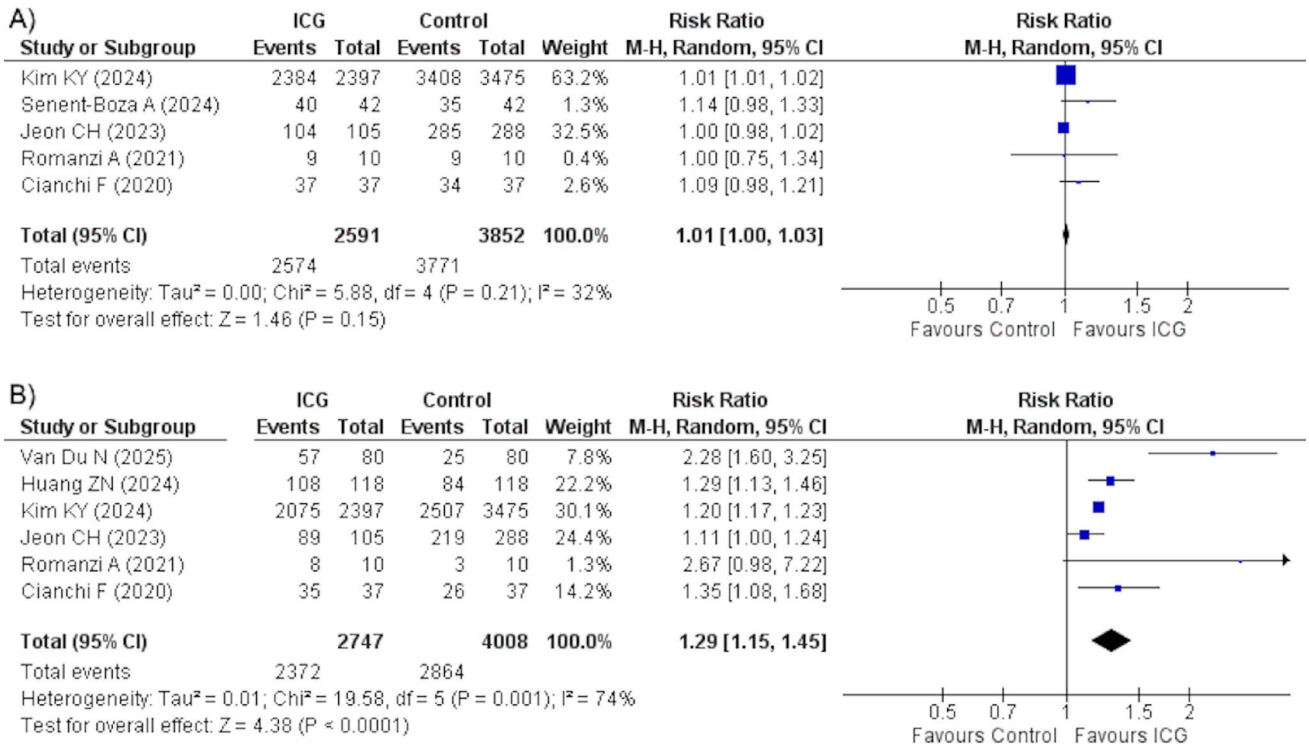



**Fig. 4** Lymphadenectomy Rates. **A** Proper lymphadenectomy assessment; **B** Ideal lymphadenectomy assessment

**Fig. 5** GRADE assessment

| Outcomes | Anticipated absolute effects* (95% CI) Risk with Control | Risk with ICG | Relative effect (95% CI) | № of participants (studies) | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| Number of Retrieved LNs | | MD **6.91 more** (5.47 more to 8.35 more) | - | 8633 (21 non-randomised studies) | ⨁⨁⨁◯ Moderate[a,b,c] | ICG likely results in a large increase in the Number of Retrieved LNs. |
| Proper Lymphadenectomy | 979 per 1.000 | **989 per 1.000** (979 to 1.000) | **RR 1.01** (1.00 to 1.03) | 6443 (5 non-randomised studies) | ⨁⨁◯◯ Low[b,d,e] | The evidence suggests that ICG results in little to no difference in obtaining a Proper Lymphadenectomy. |
| Ideal Lymphadenectomy | 715 per 1.000 | **922 per 1.000** (822 to 1.000) | **RR 1.29** (1.15 to 1.45) | 6755 (6 non-randomised studies) | ⨁⨁⨁◯ Moderate[b,f] | ICG increases the rate of achievement of an Ideal Lymphadenectomies. |
| Operation Time | | MD **6.56 lower** (12.31 lower to 0.81 lower) | - | 7608 (17 non-randomised studies) | ⨁⨁⨁◯ Moderate[b,h] | ICG likely results in a slight reduction in the Operation Time. |
| Postoperative Hospital Stay | | MD **0.36 lower** (0.55 lower to 0.18 lower) | - | 7294 (15 non-randomised studies) | ⨁⨁⨁⨁ High | ICG results in little to no difference in Postoperative Hospital Stay. |
| Incidence of Recurrence | 169 per 1.000 | **140 per 1.000** (91 to 213) | **RR 0.83** (0.54 to 1.26) | 505 (5 non-randomised studies) | ⨁⨁◯◯ Low[g,i] | ICG may result in little to no difference in the Incidence of Recurrence. |
| Mortality | 144 per 1.000 | **89 per 1.000** (55 to 148) | **RR 0.62** (0.38 to 1.03) | 505 (5 non-randomised studies) | ⨁⨁◯◯ Low[g,i] | ICG may result in little to no difference in Mortality. |

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

**CI:** confidence interval; **MD:** mean difference; **RR:** risk ratio

**GRADE Working Group grades of evidence**
**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.
**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

**Explanations**

a. The statistical heterogeneity is 68%. This represents the only real element indicating a need in reduction of the consistency. This should not be seen as a concrete limitation of the strength of the fluorescence advantage, but rather as an encouragement for higher quality and more standardized forms of research.
b. We decided to downgrade this domain because of the lack of standardization in the FGS. We observed excessive variability, especially in administration and dosage of ICG.
c. The funnel plot indicates the absence of small articles positioned to the left of the measured point effect.
d. The CI does not exclude the null hypothesis.
e. The funnel plot indicates the absence of articles positioned to the left of the measured point effect.
f. The funnel plot indicates the lack of articles positioned to the right of the measured point effect.
g. The sample size is too small, and the CI includes the null hypothesis.
h. The high statistical heterogeneity is likely due to different methodologies among the studies, such as the choice of reconstruction methods.
i. The funnel plot indicates the absence of small articles positioned to the right of the measured point effect.

The number of retrieved metastatic LNs showed no statistically significant difference, based on 13 studies (MD 0.05; 95% CI − 0.74–0.84; $p=0.90$; $I^2$ 52%; 1812 patients).

## Operative and short-term postoperative outcomes

The pooled analysis of 17 studies indicated a statistically significant advantage in using ICG in terms of reduction of operation time (MD − 6.56; 95% CI − 12.31 to − 0.81; $p=0.03$; $I^2$ 75%; 7608 patients). The high heterogeneity may stem from differences in reconstructive approaches or measurement methods (e.g. whether reconstruction time was included).

Similarly, ICG showed better performance in blood loss (MD − 10.13; 95% CI − 17.44 to − 2.82; $p=0.007$; $I^2$ 83%; 7412 patients).

Ten studies assessed time to first flatus, with no statistically significant pooled difference (MD 0.03; 95% CI − 0.08–0.13; $p=0.62$; $I^2$ 30%; 6987 patients).

No statistically significant difference was found in the pooled comparison of the time to first liquid intake (five studies) (MD 0.05; 95% CI − 0.16–0.27; $p=0.64$; $I^2$ 37%; 811 patients) (Supplementary Material Fig. 1).

Fifteen studies reported hospital stays (Fig. 5), with a pooled result of an advantage for ICG (MD − 0.36; 95% CI − 0.55 to − 0.18; $p=0.0001$; $I^2$ 0%; 7294 patients). *Kim KY (2024)*, *Senent-Boza A (2024)*, *Chen X (2022)*, *Ma S (2020)*, *Park SH (2020)* and *Cianchi F (2020)* did not specify of a post-operative stay. Their exclusion led to loss of statistical significance (MD − 0.28; 95% CI − 0.62–0.06; $p=0.11$; $I^2$ 0%; 1063 patients).

Ten studies assessed the incidence of Clavien-Dindo [80] ≥ 3 complications. The pooled result did not show statistically significant difference between ICG and controls (RR 0.78; 95% CI 0.59–1.02; $p=0.07$; $I^2$ 0%; 6932 patients) (Supplementary Material Fig. 2).

## Long-term outcomes

Five studies reported data on malignant recurrence, either as a 2-years estimate or absolute number out of the total

patients. No statistically significant difference was found (RR 0.83; 95% CI 0.54–1.26; $p=0.38$; $I^2$ 0%; 505 patients).

The same studies also assessed mortality, again showing no advantage of ICG in the pooled comparison (RR = 0.62; 95% CI 0.38–1.03; $p=0.07$; $I^2$ 0%; 505 patients) (Supplementary Material Fig. 3).

## Discussion

ICG significantly increases the total number of retrieved LNs in patients submitted to minimally invasive gastrectomy for GC. Using the GRADE approach, we synthesized that ICG likely results in a large increase in LN retrieval, with moderate certainty of evidence. We considered this a "large effect", not only because of the mere numerical value of the MD (nearly 7 additional LNs), but also due to our observation, with a moderate certainty of the evidence, of an increase in the success rate of an ideal lymphadenectomy: about 200 more harvests of at least 30 LNs, per 1000 procedures done. Conversely, evidence of lower quality suggests that ICG has minimal or no effect in improving the rates of achieving a proper lymphadenectomy. This is likely because standard techniques already suffice in obtaining at least 16 LNs in most cases. However, this outcome surely requires higher-quality studies, as the few available ones suffered from inconsistent definitions and imbalances in patient characteristics, factors we accounted for in sensitivity analyses.

To our knowledge, this is the first meta-analysis to evaluate such rates, going beyond the simple comparison on the harvest. We made sure to establish a link between the general value of nodal retrieval to the rate of a specific surgical benchmark (≥30 LNs) which is gaining evidence in literature of being a predictor of better prognosis [16–20], as it is incorporated for example by the latest NCCN guidelines [81].

The improved nodal harvest is likely attributable to enhanced tissue and surgical plane recognition, with effective differentiation of fat and vessels, and a clear highlighting of even smaller and deeply embedded LNs, despite probable cancerous and inflammatory alterations. A MD in favour of ICG was actually expected, as previous 2nd level studies had similar findings [82–85], but with smaller sample sizes, higher statistical heterogeneity, lower precision and poorer sensitivity analyses.

We found that, when restricting the data to laparoscopic cases, the advantage of ICG amounts to 6.79 LNs. A similar effect was observed in the robotic subgroup, when fluorescence is used. Although a huge prior meta-analysis [86] found robotic surgery to yield ~2,7 more LNs than laparoscopic surgery, only specifically designed comparative studies will be able to directly compare the two approaches when using ICG.

Our data suggest that ICG may be particularly beneficial in certain challenging patients, such as NAT-patients or those with a high BMI [87–91]. The advantage appears even more impactful in Western cohorts, where a higher prevalence of obese patients and more advanced stages at diagnosis are common.

We performed subgroup analyses based on different methodologies of ICG injection. Pre-operative injection yielded better results, as it did in past experiences [85], than intra-operative one. Cianchi et al. hypothesized that insufficient time for the tracer migration could explain the weaker performance. However, a direct head-to-head comparison of methods is needed to confirm any superiority. This could be particularly relevant, given prior concerns of Qi-Yue Chen et al. [92] on the potential to reduce the workload of endoscopists (and thus costs) as well as patient stress, while Meng Wei et al. [70] underlined that intra-operative injection prolongs surgical and pneumoperitoneum duration.

Certainty of evidence regarding the retrieval of metastatic LNs was low, and ICG appears to have little to no impact. Given the optimal information size criterion in GRADE approach, we believe that the sample size for this comparison was too small, so further studies are needed to confidently assess the impact of ICG on positive and negative count of LNs. Nevertheless, it is likely that, for metastases that can be identified through traditional histology with no immunohistochemical markers [93], surgery without fluorescence guidance is already sufficient, with the same logic of the assumption of its capability of ensure high rates of appropriate lymphadenectomies. A higher positive count is actually not necessarily beneficial, as studies clearly suggest that a lower ratio of positive LNs to the total harvested LNs is an independent prognostic factor [95–97]. At present, this is best achieved by increasing the number of harvested LNs [98, 99] including negative ones, reducing the risk of overlooked micrometastases.

Fluorescent intra-operative lymphography showed non-inferiority and, in several aspects, superiority over standard surgery in terms of feasibility and safety: it slightly reduced operation time, largely lowered, in proportion, blood loss, and minimally shortened hospital stay.

Unfortunately, we could not definitively confirm ICG's ability to reduce the incidence of recurrences and mortality, although the trends were promising. Limited sample sizes for these comparisons and lack of statistical significance resulted in low certainty of evidence. To our knowledge, this is the second meta-analysis of this kind attempting to reach conclusions regarding prognostic outcomes, and we are aware of a recent meta-analysis evaluating overall survival [100] which concluded that ICG-guidance does not

provide a survival advantage. Beyond the valuable contributions of these two meta-analyses, we believe that the most reliable evidence would ultimately come from specifically designed 1st level studies addressing these outcomes, with more direct, robust and intuitive methods, and less derivative in nature.

Compared to previously published meta-analysis in this research field [82–85] the present one is more up to date, more comprehensive (it includes more studies and patients); original for introducing four novel outcomes (including the two critical rates of "appropriate" and "ideal" lymphadenectomies), and richer and more robust in sensitivity and subgroup analyses.

One potential criticism concerns the absence of the so-called FUGES-012 study [26], included by Bo Dong et al. [82]. We excluded it, as *Zhong Q (2021)* explicitly incorporated those data, so we believe it was a mistake by Bo Dong and colleagues to include both studies, making a duplication.

The primary limitations of our work stem from variability among included studies, generating statistical, clinical, and methodological discrepancies, and from the quality of the included studies. This reflects the still pioneering nature of both the practical application of ICG to lymphadenectomy in GC (e.g. varying administration methods across studies) and the research itself, with even the most recent studies having small sample sizes. Furthermore, research on this field still lacks a significant amount of 1st level studies, especially RCTs, and it is still largely concentrated in Asia, with only a few promising attempts from Europe and a complete absence of American studies. All of this has consequences in terms of differing experiences in GC treatment, as well as differences in involved populations.

One of the sources for methodological heterogeneity derives from the different ICG administration protocols: both dose and timing of injection were inconsistent and often incompletely described. Considering the data we had, we believe that we partially addressed this issue through the subgroup analyses that compares pre-operative and intra-operative injection, respectively, to standard techniques. Results were consistent, showing a clear advantage for the ICG-guidance. Nevertheless, a direct comparison of the results for the two injections strategies would be strongly appreciated to definitively establish the superiority—and thus the opportunity—of one or the other modality. On the other hand, we could not evaluate the impact of the dosage, but it may be of secondary importance. Same applies to the exact timing of pre-operative injection, which was usually indicated as "the day before surgery", with minor variations, unlikely to have influenced the pooled results.

Unfortunately, studies included did not provide stratified data by stage, making unfeasible to conduct a separate subgroup analysis. We did, however, test the impact of excluding *Park SH (2020)*, which exclusively included EGCs, and the results remained consistent, likely suggesting that the inclusion of EGC cases may have underestimated rather than inflated the effect of ICG.

The pooled analysis of overall cases includes different types of lymphadenectomies. This could be seen as a limitation and a source of heterogeneity. We then performed subgroup analyses for specific stations (D1; D2; D≥2), that showed consistent results. Moreover, considering that D2 lymphadenectomies are the most common, we should underline the notably better result for D2 stations.

In addition, relevant variability in surgeon proficiency and institutional expertise across the included studies may have influenced the results, although this information was rarely reported in sufficient detail to allow stratification.

One last limitation is that none of the included studies reported on patients treated with immunotherapy, which represents a gap, considering its growing role in gastric cancer management.

Concluding, our systematic review and meta-analysis offers promising evidence regarding the implementation of ICG in GC lymphadenectomy, showing a significantly improved nodal retrieval, higher rates of "ideal" lymphadenectomies, potentially greater benefit in high-BMI patients, in NAT-patients and in limited case volume centres. Higher retrieval and ideal lymphadenectomies have previously been associated with better prognosis. However, high-quality RCTs are needed to confirm long-term benefits on overall survival, disease free survival, and years of life gained.

Future research must focus on standardizing ICG administration protocols (including dosage and timing), clarify the optimal patient subgroup, and expand studies outside of Asia.

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**Data availability** Not applicable.

## Declarations

**Competing interests** Luca Deidda, Adolfo Pisanu, Benedetto Ielpo and Mauro Podda have no conflicts of interest or financial ties to disclose.

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