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ORIGINAL ARTICLE

Fluorescence-guided lymphadenectomy in gastric cancer: a prospective western series

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ABSTRACT

Background Indocyanine green (ICG) has been recently introduced in clinical practice as a fluorescent tracer. Lymphadenectomy is particularly challenging in gastric cancer surgery, owing to the complex anatomical drainage.

Aim The primary outcomes of this study were the feasibility and usefulness of ICG-guided lymphadenectomy in gastric cancer surgery, considering both the success rate and improved understanding of the surgical anatomy of nodal basins. The secondary outcome was the diagnostic ability of ICG to predict the presence of nodal metastases.

Patients and methods We conducted a single-center prospective trial comprising 13 patients with gastric cancer. ICG was injected the afternoon prior to surgery or intraoperatively via the submucosal or subserosal route. Standard lymphadenectomy was performed in all patients, according to patient age and tumor stage, as usual, but after standard lymphadenectomy the residual ICG + nodes were harvested and analyzed. Each nodal station and each dissected node was recorded and classified as ICG + or ICG− (both in vivo and back table evaluation was utilized for classification). After pathological analysis, each nodal station and each dissected node was recorded as metastatic or nonmetastatic (E&E staining).

Results The feasibility rate was 84.6% (11/13). The mean number of dissected lymph nodes per patient was 37.9. Focusing on the 11 patients in whom ICG-guided nodal navigation was successfully performed, 81 lymph node stations were removed, for a total of 417 lymph nodes. Sixty-six stations (81.48%), comprising a total of 336 lymph nodes, exhibited fluorescence. No IC− node was metastatic; all 54 metastatic nodes were ICG + . A total of 282 ICG + nodes were nonmetastatic. In two cases, some nodes outside D2 areas were harvested, being ICG + (1 case of metastatic node).

Conclusions Fluorescence lymphography–guided lymphadenectomy is a promising new technique that combines a high feasibility rate with considerable ease of use. Regarding its diagnostic value, the key finding from this prospective series is that no metastatic nodes were found outside fluorescent lymph node stations. Further studies are needed to investigate whether this technique can help surgeons performing standard lymphadenectomy and selecting cases for D2 + lymphadenectomy.

Keywords Fluorescence-guided surgery · Indocyanine green · Gastric cancer · Lymphadenectomy · Navigation surgery

Introduction

Fluorescence-guided surgery is one of the most promising, recently developed, surgical techniques. The most common fluorophore used is indocyanine green (ICG). This molecule, developed during World War II as a photographic dye, was tested for human use by the Mayo Clinic in 1956 and received FDA approval in 1959. The clinical applications of ICG are wide-ranging and include liver function and cardiac output testing, as well as its use for retinal angiography during the 1970s [1–4]. More recently, new applications, including angiography, sentinel node-guided surgery and biliary tree visualization, are rapidly gaining widespread use [5–11].

Gastric cancer is one of the most common cancers worldwide and the third leading cause of cancer death [12]. Total or subtotal gastrectomy is usually associated with D1 + , D2 or D2 + lymphadenectomy. The feasibility of sentinel lymph node biopsy has been studied in T1 gastric cancer [10], but this technique is not applicable in advanced gastric cancer cases, which are more frequently diagnosed in Western

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countries. Thus, standard lymphadenectomy (D2) remains the gold standard [5, 13].

In this study, we prospectively evaluated the effectiveness of ICG fluorescence as a tracer during lymphadenectomy for gastric cancer. The primary goal was to detect the ability of ICG to provide accurate visualization of lymph node stations. The secondary goal was to assess the diagnostic value of ICG with respect to pathological analysis. This ability may prove particularly effective when additional fluorescence is visualized outside the standard dissection area.

Materials and methods

Ethics/study approval

Written informed consent was obtained from each patient included in this study. Experimental protocols conform to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008). IRB approval was obtained. The protocol also meets the Italian Guidelines for clinical research.

Patients

The subjects comprised 13 patients (four female and nine male) with gastric cancer who were candidates for radical surgery at Brescia University Hospital (ASST Spedali Civili) between January 2018 and March 2019. A total of 49 patients underwent gastrectomy in the same General Surgery department during the period. Inclusion criteria were as follows: a diagnosis of gastric cancer that made the patient eligible for radical surgery, the absence of contraindications to ICG injection (e.g., allergy to iodinated contrast medium, thyroid dysfunction), the same surgeon (GLB) acting as first operator during the gastrectomy, the availability of the camera system the day of the scheduled surgery, and a written informed consent from the patient. Exclusion criteria were pregnancy, palliative surgery, neoadjuvant chemotherapy, unfitness for standard lymphadenectomy (according to the international guidelines), and inability to sign the informed consent. All 13 consecutive patients meeting these inclusion criteria were included in this prospective study.

Administration of ICG

Twenty-five milligrams of ICG (Verdye, Diagnostic Green GmbH, Aschheim-Dornach, Germany) vials were utilized. Dosage and timing of ICG injection followed a standardized routine. Dilution was 2.5 mg/ml for injection the day before the intervention, and 0.25 mg/ml for intraoperative injection (both by submucosal and subserosal way). During endoscopic procedures, the four quadrants around the cancer were submucosally injected with a total volume varying from 1 to 3 ml. Alternatively, after abdominal exploration, ICG was injected by subserosal way in the four quadrants around the tumor, providing a total volume of 1–3 ml, followed by injection of air and clipping/stitching of the injection point.

ICG-based fluorescence imaging system

The fluorescence imaging system uses an intraoperative infrared camera that activates ICG with emitted light at a wavelength of 750 nm and filters out light with a wavelength below 810 nm. Three different systems were employed in this study: a NIR/ICG system based on the IMAGE1 Spies camera platform produced by Karl Storz SE & Co. KG (Tuttlingen, Germany), a 1588 Advanced Imaging Modalities [AIM] platform with ENV [Endoscope Near-Infrared Visualization]) modality, and the Novadaq PINPOINT camera system (both produced by Stryker (Kalamazoo, MI, USA). All gastric resections were video recorded in their entirety, and the videos were reanalyzed to reassess lymph node fluorescence.

Surgery

Gastrectomy was performed via either an open or a laparoscopic approach. A laparoscopic camera was utilized in all cases. In open surgery, during the staging phase of the intervention, the camera imaging head was positioned at a fixed distance of 15 cm from the surgical field to keep the intensity of the emitted signal constant, the surgical lights were turned off, and fluorescence images were displayed on a monitor in the operating room. The same procedure was repeated multiple times during the resection phases of the intervention, namely before each lymphatic basin harvestmen and after the gastrectomy completion.

In laparoscopic surgery, frequent switches before normal vision and fluorescent vision were performed using the Karl Storz system. Nodal dissection was performed under the fluorescence imaging vision with the Stryker system, keeping the backlight as high as possible, and switching to the normal vision in the case of bleeding occurring in the surgical field. Finally, with the Novadaq system the demolitive phase of the intervention was completely performed with the fluorescence imaging vision, which enables the superimposition of the green fluorescence upon the normally stained structures.

Examination of lymphatic basins and individual lymph nodes

Lymphadenectomy was performed according to the Italian guidelines for gastric cancer staging and treatment [14]. When residual lymph nodes exhibiting fluorescence

were identified outside the standard dissection area, these lymph nodes were also removed and classified. Dissection of lymphatic basins was performed during the gastric resection and completed on the back table, by separating and classifying the lymphatic stations according to the Japanese guidelines [15]. Both in vivo and ex vivo (back table) analyses were performed. Fluorescence activity was examined by the infrared camera. The presence (ICG+) or absence (ICG−) of fluorescence was recorded station by station. In addition, obvious single nodes in each station were evaluated during gastric resection or on the back table by the surgeon and singularly classified as ICG+ or ICG− nodes. Lymphatic stations were then separately sent to the pathologists; obvious single nodes further identified by the surgeon, with the indication of the nodal station to which they belonged, were also individually sent to the pathologists.

Figures 1, 2, 3 and 4 show examples of identification, removal, and classification of lymphatic stations and single lymph nodes with respect to ICG fluorescence (ICG+ versus ICG−) by in vivo and ex vivo (back table) analyses.

Pathological examination of the surgical specimens

All specimens were sent to the Pathology Department of our medical institution, where they were processed for both macroscopic and microscopic examinations. All surgical specimens were formalin-fixed, stained with hematoxylin/eosin and then examined by experienced pathologists. All nodes were microscopically

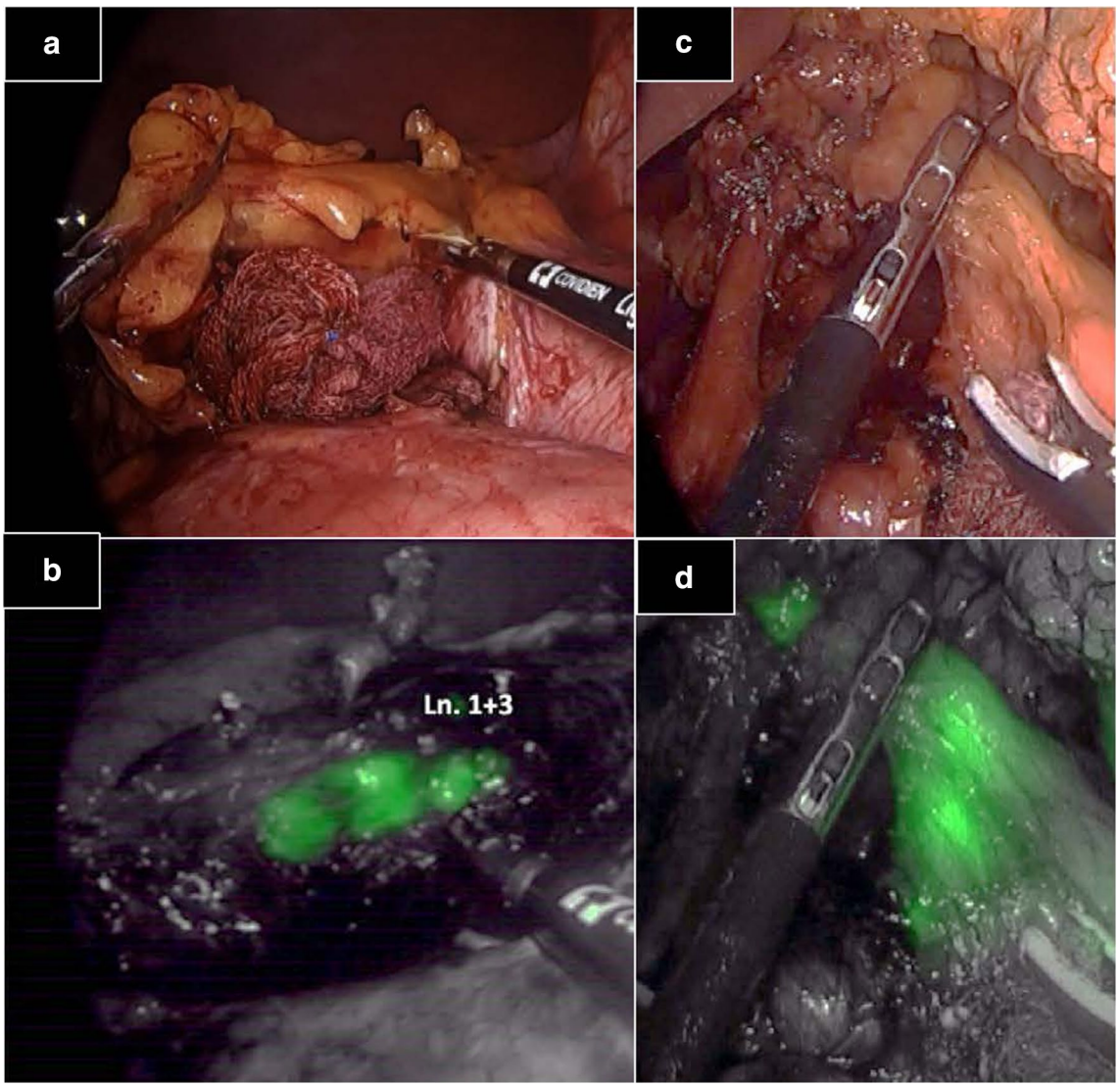

Fig. 1 Nodal stations: intraoperative detection, classification as ICG+ or ICG− and harvesting. In panels **a** and **b**: lymph nodal basins 1+3. In panels **c** and **d**: lymph nodal basin 11p.

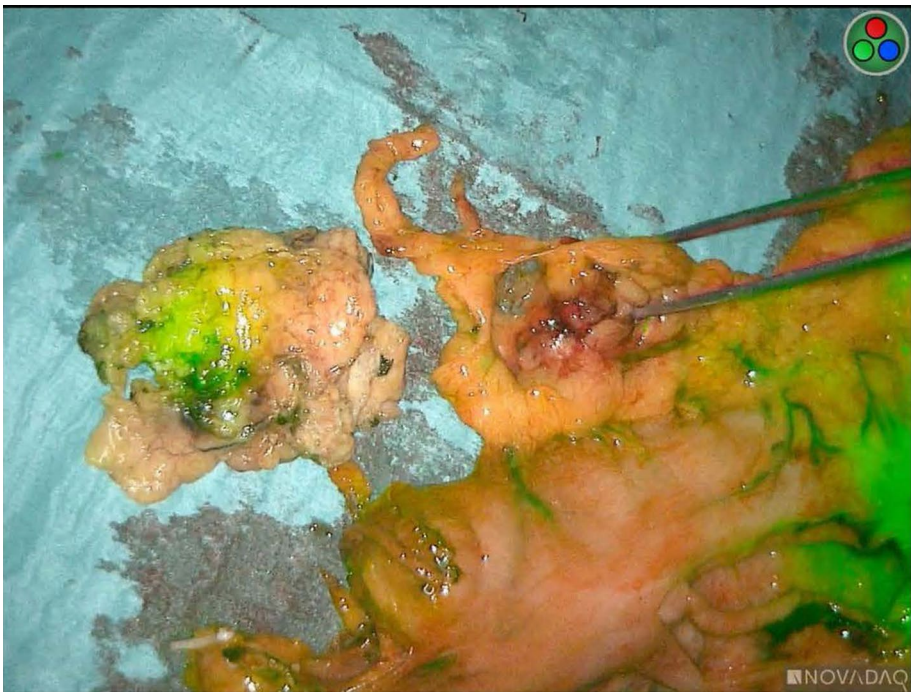

Fig. 2 Ex vivo (back table) detection, classification as ICG + or ICG− and harvesting of nodal station 7

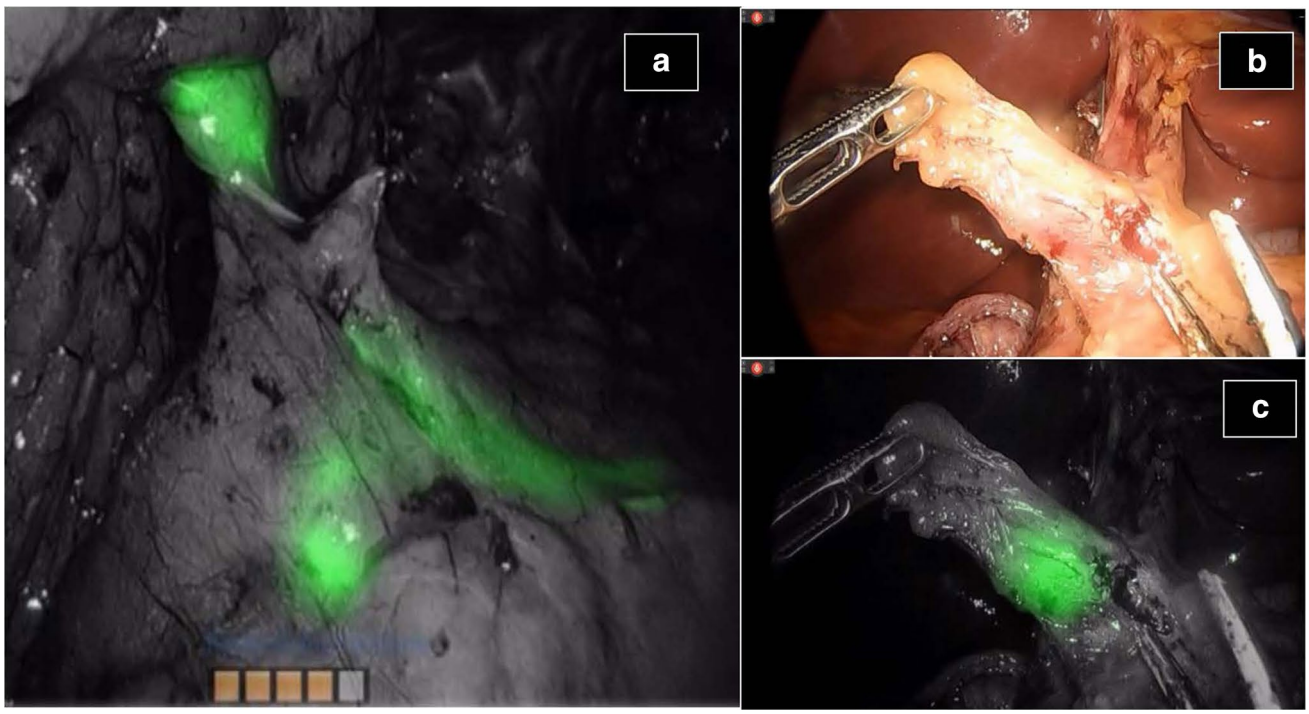

Fig. 3 Single lymph nodes: intraoperative detection, classification as ICG + or ICG− and harvesting. In panel **a**: lymph nodes from stations 7, 8a, 11p. In panels **b** and **c**: lymph node from station 8a

evaluated irrespectively of the presence or absence of ICG fluorescence (ICG + versus ICG−), and categorized

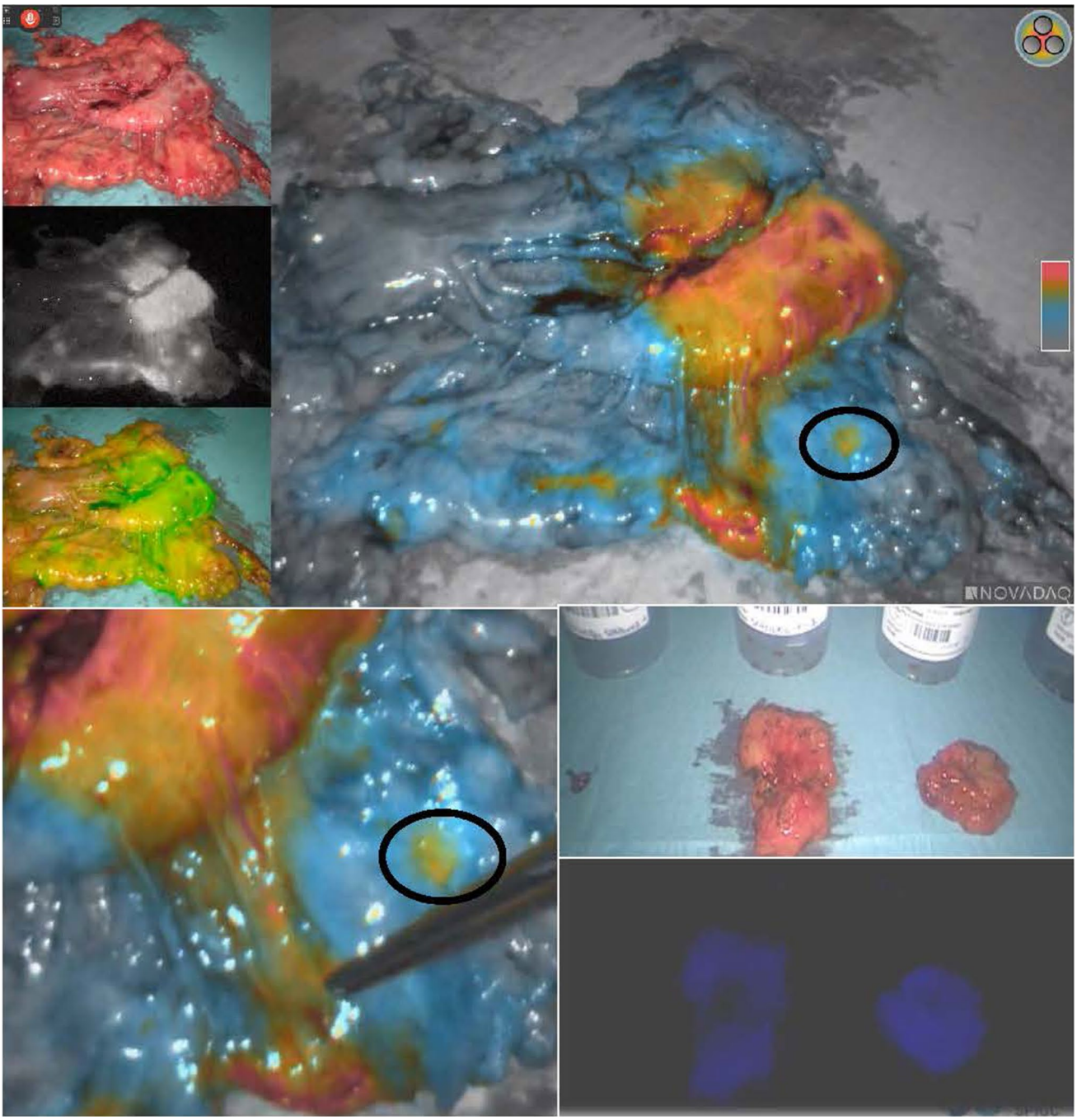

Fig. 4 Ex vivo (back table) detection, classification as ICG + or ICG− and harvesting of single lymph nodes

as either metastatic or nonmetastatic. Then, according to which lymphatic basin each single node belonged to, the basin itself was classified as either metastatic or nonmetastatic.

Data collection and recording

The following demographic, oncological, and surgical data were collected for each patient: age and sex, BMI, cancer

location, clinical and pathological staging according to the 8th AJCC-TNM classification, surgical procedure (total or subtotal gastrectomy), lymphadenectomy extent (D1/D2/D2+), surgical approach (open or laparoscopic), and duration of the intervention. In addition, the dose, timing, and route of the ICG injection were recorded. For each patient, each lymphatic station was recorded and classified as ICG+ or ICG−, and as metastatic or nonmetastatic. A similar taxonomy was applied to the nodes that were singularly dissected away from their nodal basin.

The ICG staining was analyzed and assessed jointly by the lead surgeon (GLB) and a senior medical resident (SM) belonging to the surgical team. Both specialized surgeons independently examined the intraoperative images and the back table images of each lymphatic basin and each single node.

Statistical analysis

The results for both lymphatic stations and lymph nodes were analyzed on a dichotomous scale. Comparison between groups (preoperative vs intraoperative injection, submucosal versus subserosal injection) was performed by chi-square test; median and 95% CI was determined by Wilcoxon t test.

The number of false negative and false positive results of ICG-generated fluorescence was verified. The diagnostic accuracy of ICG-guided fluorescence for metastatic lymph nodes was determined using the standard epidemiological method of calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sensitivity was computed as the number of true positive lymph nodes/(number of true positive + false negative lymph nodes) × 100%. Specificity was computed as the number of true negative lymph nodes/(number of true negative + false positive lymph nodes) × 100%. Positive predictive value was computed as the number of true positive lymph nodes / (number of true positive + false positive lymph nodes) × 100%. Negative predictive value was computed as the number of true negative lymph nodes/(number of true negative + false negative lymph nodes) × 100%. Statistical analysis was performed with SPSS 21.0 software (IBM Inc., Chicago, IL). A P value of < 0.05 was considered statistically significant.

Results

Thirteen consecutive patients (four female and nine male) were included in this prospective study. The median age was 69 (range 33–85), the median BMI was 25.6. The clinical and pathological characteristics of the study participants, as well as information on the surgical procedures, are reported in Table 1.

Table 1 Patient cohort [$n = 13$] and surgical procedures

	N	%
Sex		
Female	4	30.8
Male	9	69.2
Median age [range]	69 [33–85]	
Median BMI [range]	25.6 [22.1–31.6]	
Tumor location		
Body	4	30.7
Antrum	7	53.8
Angulus	1	7.7
Pylorus	1	7.7
Clinical T stage		
T1-T2	6	46.1
T3	3	23.1
T4	4	30.7
Clinical N stage		
N0	7	53.8
N+	6	46.1
Clinical M stage		
M0	12	92.3
M+	1	7.7
Gastrectomy		
Subtotal	11	84.6
Total	2	15.4
Approach		
Laparoscopy	7	53.9
Open	6	46.1
Lymphadenectomy		
D1+	2	15.4
D2	9	69.2
D2+	1	7.7
Mean duration of surgery (minutes)	253.5	

Tumors were located mostly in either the antrum (53.8%) or the body (30.7%). Almost all procedures (84.6%) consisted of subtotal gastrectomies, whereas the percentages of open and laparoscopic procedures were almost the same. D2 dissection was the most frequently performed lymphadenectomy (in 69.2% of the cases), whereas D1+ and D2+ dissections were performed in selected cases: the former in early cancers in high-risk patients, and the latter in advanced cancers in surgically fit patients.

Table 2 shows the pTNM classification of the patients in this series, the dose, timing, and route of the ICG injection, and the number of harvested and fluorescent lymph nodes.

No patients experienced complications or adverse events after ICG injection. ICG was injected intraoperatively in ten patients: in five via the submucosal route and in five via the subserosal route. In the remaining three

Table 2 Patient pTNM Classification, Features of the ICG Injection, Number of Harvested and Fluorescent Lymph Nodes

Patient No	pTNM	ICG dose (mg)	ICG injection timing	ICG injection route	Harvested LN	Fluorescent LN
3	T1aN0M0	0.75	IO	SM	20	12
4	T4aN3aM0	0.25	IO	SS	56	52
5	T4aN3aM0	0.25	IO	SS	35	26
6	T2N0M0	0.75	IO	SM	42	42
7	T4aN3bM1	2.5	AbS	SM	44	44
8	T1bN0M0	0.25	IO	SM	35	12
9	T1aN0M0	0.25	IO	SM	14	13
10	T1bN1M0	0.25	IO	SS	30	13
11	T2N0M0	0.25	IO	SS	54	44
12	T2N2M0	2.5	AbS	SM	45	36
13	T1aN0M0	2.5	AbS	SM	42	42
Total	–-	–-	–-	–-	417	336

IO intraoperatively; *AbS* afternoon before surgery; *SM* submucosal; *SS* subserosal

In patients no. 1 and 2 the nodal navigation was unreliable (in patient n. 1 because of extraserosal injection with peritoneal spreading; in patient no. 2 because of a low injected volume of ICG, which prevented the detection of fluorescence in perigastric nodes)

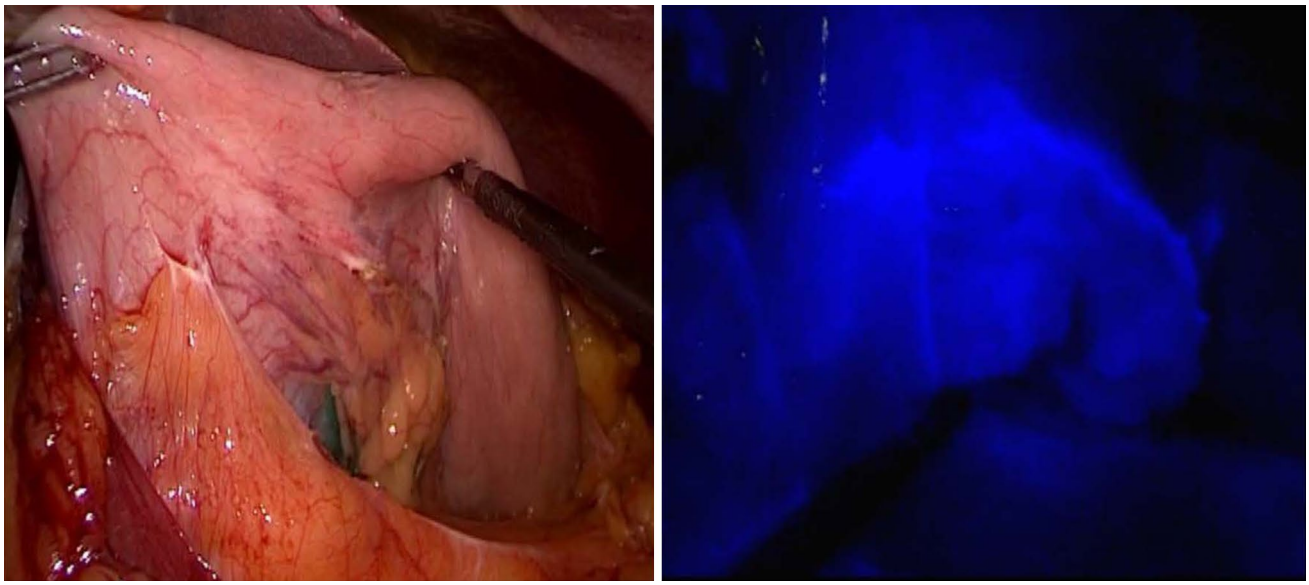

Fig. 5 In this patient, ICG injected via submucosal route spread out of the gastric wall; all the peritoneal surface surrounding the stomach was stained, preventing nodal navigation

patients, the injection was performed endoscopically via the submucosal route the afternoon prior to surgery. The dose of ICG (1 cc for 11 patients and 3 cc for the remaining two patients) was injected in the four quadrants around the cancer into normal tissue.

In 11 of the 13 patients (84.6%), we succeeded in detecting lymphatic drainage spreading from the ICG injection sites. The two problematic situations arose from the following events. In the first patient, ICG spilled out of the gastric wall, and all peritoneal surfaces were illuminated; thus, the identification of marked lymph nodes was not possible, as shown in Fig. 5.

In the second patient, ICG was ineffective because the intraoperative observation of fluorescence was restricted to a very limited number of lymph node stations (two fluorescent stations out of eight examined stations). The two patients in whom the approach was unsuccessful were excluded from the statistical analysis pertaining to the lymphadenectomy presented in the subsequent tables.

Table 3 Lymphadenectomy specimens according to fluorescence and metastatic status

	Lymph node stations basins		
	M+	M–	Total
ICG+	16	50	66
ICG–	0	15	15
Total	16	65	81
	Lymph nodes		
	M+	M–	Total
ICG+	54	282	336
ICG–	0	81	81
Total	54	363	417

In the 11 patients in whom fluorescence nodal navigation was deemed successful, no statistically significant difference was found between preoperative and intraoperative injection with regard to the total number of harvested lymphatic basins (median ± 95% CI 7 ± 2.11 versus 7 ± 1.98; $p = 0.529$) or the number of fluorescent lymphatic basins (median ± 95%CI 6 ± 1.55 versus 5.5 ± 1.73; $p = 0.386$). Similarly, no difference was found between submucosal and subserosal injection with regard to the abovementioned parameters (median ± 95% CI 7 ± 1.99 versus 7.5 ± 1.68; $p = 0.493$ for harvested lymphatic basins; median ± 95% CI 6 ± 0.95 versus 6.5 ± 1.13; $p = 0.786$ for harvested fluorescent lymphatic basins).

Table 3 reports that the number of examined lymph node stations of the 11 patients in whom ICG-guided nodal navigation was performed successfully was 81 (in some cases, stations 1–3 and stations 4sb-6 were dissected and sent to the pathologists together). In 66 stations almost one piece of positive ICG fluorescence was shown within the station (81.5%); 16 of 66 (24.2%) contained at least one metastatic node.

A total of 417 lymph nodes were removed (mean: 37.9 per patient), with 336 lymph nodes being ICG+. Among those positive nodes, 54 were metastatic. The key finding from this prospective series is that no metastatic nodes were ICG− or belonged to an ICG− lymph node station.

The sensitivity, specificity, positive predictive value and negative predictive value are reported in Table 4 with reference to lymphatic stations and to single nodes, separately.

For fluorescent nodes outside of the planned lymphadenectomy region, further tailored nodal dissection was performed. In one patient, the presence of a fluorescent lymph node in station 8p suggested an opportunity for removal. In other two patients, one undergoing total and one undergoing

Table 4 Diagnostic accuracy of ICG-guided fluorescence for metastatic lymph nodes

	Considering each lymph node station		
	Value	95% confidence lower boundary	95% confidence upper boundary
Pre-test likelihood	0.200197	0.110	0.2984
Sensitivity	1	1	1
Specificity	0.230	0.1328	0.333
Positive predictive value	0.242	0.1439	0.345
Negative predictive value	1	1	1
	Considering each lymph node		
	Value	95% confidence lower boundary	95% confidence upper boundary
Pre-test likelihood	0.1329	0.0897	0.161
Sensitivity	1	1	1
Specificity	0.223	0.180	0.265
Positive predictive value	0.160	0.121	0.20199
Negative predictive value	1	1	1

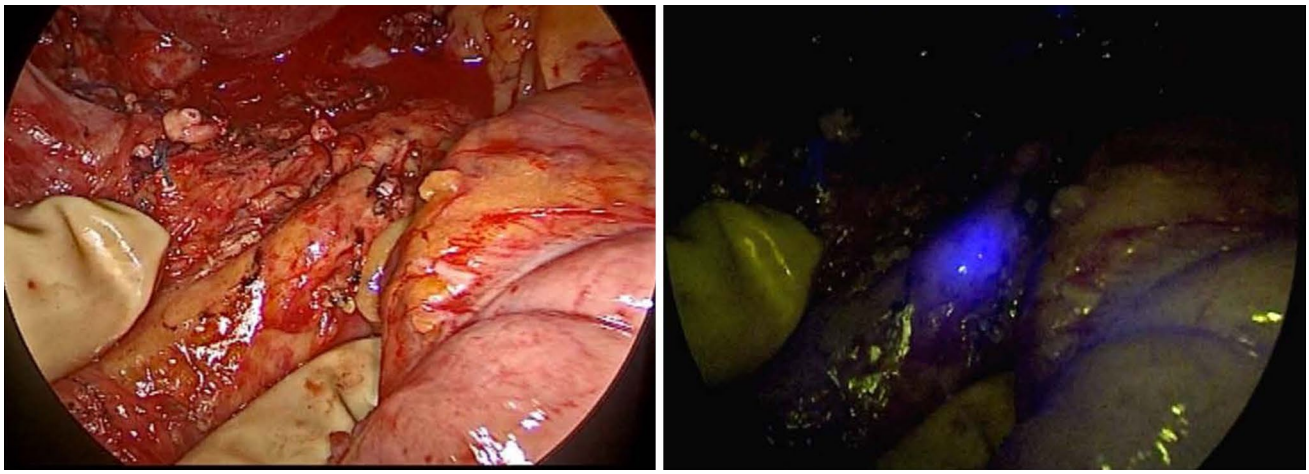

Fig. 6 In this patient undergoing a subtotal gastrectomy, the check of the surgical site using indocyanine green found an ICG + node in station 11d. The node was removed and the histological analysis confirmed that it was a metastatic node

subtotal gastrectomy, the evaluation of fluorescence after standard D2 lymphadenectomy highlighted residual illumination in station 11d, hence, we removed the lymph nodes. The node in station 8p and one node in station 11d were found to be negative upon pathological analysis. One metastatic lymph node was found—the second illuminated 11d node (Fig. 6).

In contrast, in other two patients, stations 8p and 11d did not exhibit intraoperative fluorescence, but the nodes were macroscopically suspect. The absence of fluorescence would have recommended to the surgeon the possibility of not removing these stations. Nevertheless, these stations were harvested for this study. No lymph node in those stations was metastatic.

Discussion

As it is well known, a complete lymphadenectomy is crucial to ensure a correct staging and to deliver more accurate oncological results in the surgical treatment of gastric cancer. This study analyzed the feasibility and safety of ICG as an intraoperative lymphographic tool to guide the surgeon in lymph node dissection. In our prospective study, effective ICG fluorescence was successfully attained in 11 of the 13 patients (84.6%), with more than 80% of lymph node stations exhibiting fluorescence. Errors during the ICG injection phase accounted for the two failure events: in one patient, ICG spilled outside the serosal layer into the peritoneum; in the other, a low injected volume of ICG resulted in an insufficient time interval between injection and lymphadenectomy.

In the analysis of the 11 successful cases, no dissimilarities were found in the accuracy of visualizing lymphatic stations and nodes between the different observation methods (open or laparoscopic) and different injection methods (subserosal or submucosal).

In a previous study, intraoperative subserosal injection resulted in lower accuracy than submucosal injection for the detection of sentinel lymph nodes [16]. Some studies support the injection of ICG the day before surgery even for intraoperative fluorescence-guided lymphadenectomy [17, 18]. Overall, preoperative submucosal injection seems to be the preferred method, even if additional gastroscopy is needed. Our study confirms the consensus in the literature concerning the absolute absence of serious side effects of ICG injection. The lethal dose is much greater than the commonly injected dose, and very few cases of possible—unconfirmed, but always minor—side effects have been reported.

Considering that ICG is a safe method based on existing studies, the next relevant question is whether ICG nodal navigation enables surgeons to perform more successful gastrectomies and lymphadenectomies.

Numerous previous studies reported the usefulness of ICG for detecting sentinel lymph nodes in early gastric cancer [5], demonstrating that ICG is more effective than other tracers. However, the use of fluorescence-guided radical lymphadenectomy for gastric cancer is still under evaluation. Some recent prospective studies have been published, in most of which gastrectomy was performed via robotic surgery. Herrera-Almario et al. first described fluorescence-guided lymphography during robotic gastrectomy [19]. The mean number of dissected lymph nodes per patient was 31 (range 17–61), and ICG was injected via the subserosal route 10 min before lymph node dissection. This study found that near-infrared fluorescence improved the visual reference of lymph node packages along the main gastric vessels. A pilot study by Lan et al. also using robotic surgery, reported a

number of retrieved lymph nodes (35.8 ± 11.8) similar to that in the non-ICG group [20]. However, the number of lymph nodes found in stations 4d and 6 was significantly higher in the ICG group than in the non-ICG group. All metastatic lymph nodes in both early and advanced gastric cancers were identified in the lymph node stations exhibiting ICG fluorescence. Kwon et al. compared groups treated with robotic gastrectomy with and without NIR [17] and concluded that fluorescence lymphography is a useful tool for performing complete lymphadenectomy and for assessing the adequacy of lymphadenectomy in real time. In the NIR group, these researchers found increased lymph node retrieval for pathological staging without any discernible disadvantages (48.9 versus 35.2 nodes; $p < 0.001$). Cianchi et al. confirmed the feasibility of this technique with robotic surgery, injecting ICG submucosally the day before surgery [18]. Additionally, in this series, the mean number of examined lymph nodes was higher in the ICG group than in the non-ICG group. An important paper recently appeared in this field. Chen and Co-workers published in 2020 a prospective randomized controlled trial performed in a single Chinese center with very high volume (> 800 gastrectomies per year) of gastric cancer cases. This RCT included 266 patients with cT1-4N0-1 gastric cancer cases, comparing ICG fluorescence-guided lymphadenectomy with standard surgery without fluorescence. In the experimental group a significantly higher number of nodes were retrieved, and a significantly lower rate of non-compliance with D2 lymphadenectomy was demonstrated [21].

In our study, D1 + lymphadenectomy was performed in 15.4% of patients; D2, in 69.2%; and D2 +, in 7.7%. The mean number of lymph nodes removed per patient (37.9) in our series was lower than those reported in the above-mentioned series but compares favorably with that in our historical series and clearly respects the suggestions of the guidelines for radical lymphadenectomy. This increase may be attributed to the increased attention of surgeons to nodal dissection both in vivo and ex vivo on the back table because of the ICG fluorescence-guided lymphadenectomy approach or to more complete visualization of basins, which in turn enhances basin removal. A relationship between a greater number of examined nodes and improved patient survival has been suggested by studies on *N*-ratio staging. An optimal cutoff of 25 nodes was established for correct D2 lymphadenectomy, but no study has been published in which a case of ICG-guided lymphadenectomy included the analysis of less than 25 nodes.

The key finding in our prospective series is the absence of nodal metastases in ICG− nodes. Nodal metastasis was carefully evaluated by analyzing each of the 417 harvested nodes for both ICG fluorescence and metastatic cell infiltration. The search for single nodes was performed by the surgeon during gastric resection and on the back table after the intervention. In principle, this approach is a possible limitation of this study, because the pathologists were not present during nodal dissection. The total number of nodes harvested by the pathologists was clearly higher than the number of nodes harvested by the surgeons; however, nodes with evident fluorescence were easily dissected on the back table. This method of recording the fluorescence of single nodes appears simple and useful; it may be further improved by having the pathologists in the operating room during gastric resection or enabling the pathologists to search for nodes in fatty tissue via fluorescence technology. However, both options are difficult to implement in daily practice, mainly for organizational reasons, and should be restricted to cases that are included in clinical studies.

In two patients in our series, some ICG−nodes outside of the standard D2 lymphadenectomy area exhibited a suspicious appearance and were removed but were not affected by the tumor. These preliminary but encouraging findings were slightly different from other reports in the literature. Cianchi et al., for instance, found four patients with ICG− metastatic lymph nodes [18]. In one patient, histopathological analysis showed massive infiltration of the submucosa (linitis plastica) that might have occluded the lymphatic vessels and prevented ICG diffusion. In the other two patients, metastatic ICG− lymph nodes were located in lymphatic basins containing at least one fluorescent lymph node. Further studies, specifically focusing on ICG− metastatic nodes via deep histopathological and electron microscopy assessment, are needed to clarify the likelihood of lymphatic vessel thrombosis that would prevent ICG from reaching the nodes.

In addition, residual fluorescent tissue after standard lymphadenectomy is an even more interesting application for ICG-guided nodal dissection. Indeed, the presence of such tissue may be related to incomplete basin dissection (as in one patient in our series, in whom some nodes would have been left in station 11p) or to the spread of tumor cells outside the D2 area (as in two patients in our series in stations 8b and 11d). In some patients, residual tissue may be of utmost importance because incomplete lymphadenectomy may translate into local recurrence, with potentially fatal consequences. Notably, in one patient in our series, the residual node was metastatic; thus, ICG-guided dissection enabled more radical tumor clearance. Kim et al. studied residual fluorescence in the pyloric station after lymph node dissection as a first step and residual fluorescence after radical lymphadenectomy (D1 + or D2) [22]. A total of 15 fluorescent tissues were found in 14 of 50 patients (28%) after index node dissection. Two of the 15 fluorescent tissues (4%) were histologically confirmed as lymph nodes but showed no tumor involvement.

In conclusion, our study shows that intraoperative lymphatic mapping with ICG fluorescence during gastric cancer surgery is a feasible, safe and helpful technique. ICG

lymphography can assist the surgeon in guiding lymphadenectomy and, possibly, in decision making regarding extended lymphadenectomy. A larger number of enrolled patients are required in future studies to confirm the preliminary yet insightful findings of our pilot study. Advances in imaging systems will increase the use of fluorescence imaging as an intraoperative navigation tool that can enhance the safety and accuracy of open and laparoscopic lymphadenectomy in gastric cancer surgery. Considering the dire consequences of undetected metastatic nodes and the fact that ICG-based fluorescence imaging is a safe, easy, and relatively inexpensive intraoperative tool, further research to assess its clinical advantages is warranted.

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Author contributions GLB and FG developed the original idea and methodology of the project. BM wrote the first draft of the paper and incorporated the conceptual feedback sent by the authors. SM, LQ, and LA collected and analyzed the data. GA and SM checked as oncologist and pathologist the portions of the paper within their competence, respectively. MB made substantial scientific contributions to the project and data analysis. All authors critically revised and accepted the final version of the manuscript.

Compliance with ethical standards

Conflict of interest Prof. Baiocchi was the scientific organizer of two international workshops ["Intraoperative ICG Fluorescence Imaging in Hepatobiliary and Visceral Surgery: State of the Art and New Frontiers", (Brescia, Italy, October 21, 2017); and "ICG 2.0. Intraoperative ICG Fluorescence Imaging in Abdominal Surgery: Prevention of Complications and Oncological Perspectives", Milano, Italy, September 27–28, 2018) partly funded (travel expenses) by Karl Storz and Stryker companies. Prof. Baiocchi has a paid consultant relationship wit Stryker. Drs. B. Molteni, S. Molfino, G. Arcangeli, S. Manenti, L. Arru, F Gheza, Prof. M Botticini have no conflicts of interest or financial ties to disclose.

Research involving human participant and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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