Reply to the reviewers' comments

Dear Editor-in-Chief,

We thank you for your decision letter for our manuscript entitled "**Indocyanine green (ICG) fluorescence in gastrointestinal surgery: Appraisal of current evidence**" We have considered the reviewer's comments and provide below a point-by-point answer to each of them. Changes have been incorporated and highlighted in the revised manuscript. We are grateful to the reviewers for their comments which have helped us improve the manuscript. We hope that you will find this revised version suitable for publication in your esteemed journal.

With kind regards,
Authors

Response to comments

Reviewer 1

Nice manuscript with a lot of novelties within. One remark regarding the images: the authors should reveal the source if not from personal collection.

We thank the reviewer for the encouraging comments. All the images included in the review are from the author's personal collection and not taken from any other source.

Reviewer 2

Very well sectioned review of ICG in GI with ample bibliography and clear syntax making the review easy to read. Indocyanine green (ICG) has a long track of safety as it was used in medicine since the mid-1950s for a variety of applications in Ophthalmology to delineate the choroidal vessels, and subsequently to delineate cardiac and CNS vessels; however, its fluorescent properties have only recently been used in the GI literature and particularly in the

intraoperative evaluation (Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green: Historical Context, Current Applications, and Future Considerations. Surg Innov. 2016 Apr;23(2):166-75. doi: 10.1177/1553350615604053).

We thank the reviewer for the encouraging comments. As suggested by the reviewer we have added the reference "Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green: Historical Context, Current Applications, and Future Considerations. Surg Innov. 2016 Apr;23(2):166-75. doi: 10.1177/1553350615604053)" in the introduction section.

Reviewer 3

This manuscript presents a comprehensive review of ICG use in gastrointestinal surgery, showcasing evident writing skills. However, given the abundance of recently published reviews covering the same topic, I'm unable to identify a distinct element of novelty or innovation in this particular submission.

We thank the reviewer for the appreciation and the suggestion to improve the manuscript. The novel findings included in the manuscript are.

    i.   Use of ICG to identify bile duct in duodenum preserving pancreatic head resection.

    ii.   Use of ICG to assess the vascularity of the pancreatic cut surface prior to pancreaticoenteric anastomosis.

    iii.   Importance of quantitative evaluation of conduit vascularity

As suggested by the reviewer, we reviewed the literature again and added additional novel applications that are mentioned below.

    i.   Administering ICG in an aerosol form via nebulization to visualise the tracheobronchial tree during esophagectomy.

    ii.   Subcutaneous injection of ICG in the inguinal region to visualize the thoracic duct.

Editor's comment

I recommend the manuscript to be published in the World Journal of Gastrointestinal Surgery. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

As suggested by the editor, we reviewed the literature again and included two novel applications of ICG in the revised manuscript under future perspectives.

"Novel routes and formulations of ICG are being explored to widen its application. Thammineedi et al. reported the feasibility of administering ICG in an aerosol form via nebulization to visualise the tracheobronchial tree [72]. Tracheobronchial fluorescence could minimize the incidence of airway injury in patients undergoing minimally invasive salvage esophagectomy and those with extensive periesophageal fibrosis following neoadjuvant chemoradiotherapy. Tokumaru et al. reported that subcutaneous injection of ICG in the inguinal region is as effective as ultrasound-guided injection into inguinal nodes to visualize the thoracic duct [73]. The median time from the simple subcutaneous injection technique to the observation of ICG-induced fluorescence of the thoracic duct was 119 min. "

References

72. **Thammineedi SR**, Patnaik SC, Nusrath S, et al. Evaluation of indocyanine green tracheobronchial fluorescence (ICG-TBF) via nebulization during minimally invasive

esophagectomy [published online ahead of print, 2023 Sep 12]. Dis Esophagus. 2023;doad059. doi:10.1093/dote/doad059.

73. **Tokumaru S**, Kitazawa M, Nakamura S, Koyama M, Soejima Y. Intraoperative visualization of morphological patterns of the thoracic duct by subcutaneous inguinal injection of indocyanine green in esophagectomy for esophageal cancer. Ann Gastroenterol Surg. 2022;6(6):873-879. Published 2022 Jun 22. doi:10.1002/ags3.12594

Indocyanine green (ICG) fluorescence in gastrointestinal surgery: Appraisal of current evidence

Running title: Indocyanine green fluorescence in gastrointestinal surgery

Raja Kalayarasan, Chandrasekar Murugesan, Pothugunta Sai Krishna, Dasarathan Shanmugam

Department of Surgical Gastroenterology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry 605006, India

ORCID number: Raja Kalayarasan (0000-0003-4056-8672); Murugesan Chandrasekar (0000-0002-5006-3769); Pothugunta Sai Krishna (0000-0002-7009-5071); Dasarathan Shanmugam (0000-0001-5461-8218)

Corresponding author: Dr. Raja Kalayarasan, MS, MCh, FRCS (Edin) Additional Professor & Head, Department of Surgical Gastroenterology, Room no. 5442, 4th floor, Super specialty block, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry- 605006, India. Email: kalayarasanraja@yahoo.com Telephone**:** +91-9843015499

Indocyanine green (ICG) fluorescence in gastrointestinal surgery: Appraisal of current evidence

ABSTRACT

Applying Indocyanine green (ICG) fluorescence in surgery has created a new dimension of navigation surgery to advance in various disciplines. The research in this field is nascent and fragmented, necessitating academic efforts to gain a comprehensive understanding. The present review aims to integrate diverse perspectives and recent advances in its application in gastrointestinal surgery. The relevant articles were selected by using the appropriate keyword search in PubMed. The angiography and cholangiography property of ICG fluorescence is helpful in various hepatobiliary disorders. In gastroesophageal and colorectal surgery, the lymphangiography and angiography property of ICG is applied to evaluate bowel vascularity and guide lymphadenectomy. The lack of objective parameters to assess ICG fluorescence has been the primary limitation when ICG is used to evaluate bowel perfusion. The optimum dose and timing of ICG administration need to be standardized in some new application areas in gastrointestinal surgery. Binding tumor-specific ligands with fluorophores can potentially widen the fluorescence application to detect primary and metastatic gastrointestinal tumors. The narrative review outlines prior contributions, limitations, and research opportunities for future studies across gastrointestinal sub-specialty. The findings of the present review would be helpful for scholars and practitioners to explore and progress in this exciting domain of gastrointestinal surgery.

Core tip

Indocyanine green's unique absorption and emission spectrum allows its wide application in gastrointestinal surgery. The fluorescence cholangiography property of indocyanine green is widely used in hepatobiliary surgery to identify bile ducts. Its angiography property is used in liver surgery for tumor identification and to facilitate anatomic liver resection. Also, the angiography function is helpful in luminal surgeries to assess bowel perfusion. The limitations of indocyanine green fluorescence highlighted in the present review could guide future research on indocyanine green in gastrointestinal surgery.

INTRODUCTION

Fluorescence is the property of a substance to absorb light at one wavelength and emits at a higher wavelength, primarily within the visible spectrum, with a loss of photons. Since the late 19th century, the fluorescence property of many agents has been used in microscopic labelling for antigen or antibody detection [1]. Most commonly used fluorescence agents absorb and emit light within the visible spectrum. However, its use is limited in-vivo due to the absorption of wavelengths of visible light less than 650 nm and greater than 900 nm by blood hemoglobin and water, respectively. Hence, for in-vivo application, the fluorescence agent should be within the optical window of 650-900 nm [2]. Indocyanine green (ICG) absorbs light at 760 nm and emits at 820-830 nm, which is outside the visible human spectrum but can be identified with infrared camera devices [3]. The unique absorption and emission spectrum allow its wide application in gastrointestinal surgery.

ICG, approved for human use in 1956, is a non-toxic, anionic, hydrophilic tricarbocyanine with a wide safety margin. It is excreted unmetabolized by the liver with no enterohepatic circulation. ICG has a short half-life of 3-5 minutes. When administered parenterally, ICG binds to plasma proteins and remains within the intravascular compartment with insignificant interstitial space translocation. The initial application of ICG for cardiac output, renal blood flow, and hepatic function monitoring was based on its metabolic characteristics [4]. However, the fluorescence property of ICG expanded its application manifold, as documented by the phenomenal growth in publications and scholarly interest [Fig. 1].

In 1968, Kogure and Choromokos incidentally detected the fluorescence property of the ICG in an animal study [5]. The development of near infra-red video camera facilitated its use in real-time surgery as an angiography, cholangiography or lymphangiography agent. With extravascular administration, ICG is taken by draining lymph nodes through lymphatic channels. Utilizing this property, Kitai et al. used ICG for breast cancer sentinel lymph node mapping [6]. Subsequently, Miyashiro et al. demonstrated its usefulness in identifying lymphatic vessels and sentinel lymph nodes draining primary gastric tumors using intraoperative endoscopic peritumoral ICG injection [7]. Aoki et al. in 2008 reported the role of ICG

fluorescence in liver segmentation and cholangiography [8]. In 2011 Kawaguchi et al. demonstrated fusion imaging of ICG fluorescence and white light for real-time liver resection [9]. ICG-related publications peaked at 293 for 2022, making it the most productive year in this domain. Further, most ICG-related publications are from Japan (n=527), followed by the United States (n=451) and China (n=201) [Fig. 2].

SEARCH STRATEGY

All the authors did a PubMed search of relevant articles. The keywords and combinations included in the search were: "fluorescence"; "Indocyanine green"; "surgery"; "liver segmentation"; "hepatectomy"; "liver resection"; "cholangiography"; "transplantation"; "esophagectomy"; "gastrectomy"; "gastric conduit"; "colonic conduit"; "lymphadenectomy". Appropriate search syntaxes, such as parentheses and Boolean operators, were used as required for each topic. The search was limited to human studies and articles in English.

ICG FLUORESCENCE IN LIVER SURGERY

ICG fluorescence imaging facilitates intraoperative decision-making and improves patient safety through applications like liver segmentation, tumor detection, cholangiography and perfusion assessment.

Anatomical segmentation for liver resection

The surgical anatomy of the liver is complex, with the intersection of the portal pedicle and the hepatic vein in the liver parenchyma. It employs the imaginary plane of the portal pedicle and hepatic vein for liver segmentation. Surgical resection is the primary treatment for both primary and secondary liver tumors. Also, anatomical liver resection is preferred for less intraoperative bleeding and superior oncological outcomes in selected patients [10]. The surgeon utilizes preoperative computed tomography and intraoperative ultrasound images to create an imaginary visual plane of anatomical transection. ICG fluorescence with fusion image enables precise real-time visualization of this imaginary plane [11].

Kobayashi et al. demonstrated five techniques of staining portal vein territory for intraoperative liver segmentation. It includes single staining, multiple staining, counterstaining, paradoxical negative and negative staining. In single and multiple staining, ICG is directly injected into the portal pedicle of interest under ultrasound guidance, and the area of interest shows fluorescence. In counterstaining and paradoxical negative staining, the portal or hepatic vein to the region of interest is blocked by tumor thrombus, invasion, or embolism. Hence, direct injection of ICG to the portal vein results in positive staining of the liver parenchyma adjacent to the area of interest. In negative staining, the portal pedicle supplying the area of interest is clamped, and ICG is given intravenously to stain the normal area [Fig. 3]. The initial four techniques require direct puncture of portal vein branches. The ICG dose for positive and negative staining is 0.25 mg/ kg of body weight and 2.5 mg/ kg of body weight, respectively [12].

The negative staining technique is primarily used for major hepatectomy where the Glissonean pedicle of the hemi-liver or sector to be resected is ligated. For mono-segmentectomy and sub-segmentectomy, positive staining techniques are preferred [13]. However, the success of positive staining depends on the operator's ability to accurately puncture the segmental or sub-segmental portal vein under ultrasound guidance and inject the dye slowly without reflux to adjacent segments. Also, with the increasing use of the minimal access approach in liver surgery, positive staining by direct portal vein puncture using laparoscopic intraoperative ultrasound is challenging. To overcome that, Ueno et al. reported the use of interventional radiology-guided placement of microcatheter in the target segmental hepatic artery branch [14]. 2 mL of diluted ICG saline solution (0.125 mg/mL) is injected with the embolic agent (gelatin particles) to retain ICG in the area of interest [Table 1 and 2][14-16]. Qian et al. reported a high success rate of 80% in trans-arterial staining compared to 60% in portal venous staining method [16]. Li et al. reported a similar technique of super-selective arterial injection of ICG and noted a 100% success rate in identifying an appropriate intersegmental plane for hepatic resection [17]. However, this technique is contraindicated in patients with significant arteriovenous shunting in

the region of interest. In addition to failure to cannulate the target portal vein branch, reflux to the non-target segment, collateral to adjacent segment and uneven uptake due to tumor obstructing the pedicle are other causes of failure with a positive staining technique [18,19]. The main advantage of ICG fluorescence over other staining techniques is the ability to identify the intersegmental plane during deep parenchymal transection and the application of the pringle maneuver[20,21].

Hepatic Tumor Detection

ICG bound to lipoprotein is taken up by hepatocytes via organic anions-transporting polypeptides (OATP) and sodium taurocholate co-transporting polypeptides (NTCP) channels. Subsequently, ICG is secreted unmetabolized into the bile via multidrug resistance-associated proteins 2 (MDRP2). In well and moderately differentiated hepatocellular carcinoma (HCC) tumor cells, the ICG uptake mechanism is intact, but the excretion is impaired. Hence, HCC shows complete or partial fluorescence, depending upon the differentiation grade. In poorly differentiated HCC and metastatic liver tumors ICG uptake is also impaired, resulting in negative staining. However, some of these lesions may show rim fluorescence. It is due to immature hepatocytes in the peritumoral region that take up ICG but unable to secrete it into bile canaliculus [22]. Fluorescence imaging is primarily helpful for detecting subcapsular tumors within 10mm of the liver surface. However, it is not useful for deep parenchymal tumors except those on the cut surface of the liver parenchyma. Thus, ICG fluorescence and intraoperative ultrasound supplement each other [Table 3]. Despite the high sensitivity of fluorescence imaging to detect liver tumors, a false positive rate close to 40% due to regenerative nodules in cirrhosis patients and Von-Meyenburg complex underscores the need for further clinical assessment of fluorescent liver lesions [23]. Cai et al. reported the application of ICG for tumour margin identification in patients undergoing laparoscopic liver resection for HCC and colorectal liver metastases (CRLM) in 86 patients (Table 3). The authors reported a 96% positive rate of ICG staining for the hepatic malignant lesions [24].

Similarly, Piccolo et al. and Terasawa et al. reported a 100% and 85% high success rate in identifying malignant liver lesions [25, 26]. The dose and timing of ICG injection

for tumor imaging depend on the indication [27,28]. If ICG is primarily used for preoperative estimation of liver function with an added indication of tumor detection recommended dose is 0.5 mg/kg of body weight 7-14 days before surgery. If the primary indication is tumor detection 0.2 mg/kg of body weight is administered 1–2 days before surgery [29,30].

Liver transplantation

In living donor transplantation optimal site of bile duct division is critical to prevent biliary complications in both donor and recipient. Mizuno et al. documented its usefulness in delineating the right posterior sectoral bile duct following intrabiliary injection into the left hepatic duct in a donor undergoing left hepatectomy [31]. Hong et al. reported that intravenous ICG injection allowed real-time identification of the biliary tree around the hilar plate and facilitated optimal bile duct division in patients undergoing laparoscopic living donor hepatectomy [32]. The optimal route and timing of ICG administration in living donor transplantation are not standardized. However, intravenous ICG injection following inflow control of the ipsilateral hemi-liver facilitates the identification of the transection plane and bile duct division, as by the time the liver transection is completed, ICG would have been excreted into the bile duct.

ICG fluorescence imaging also allows the evaluation of graft perfusion and veno-occlusive regions. Hepatic venous outflow obstruction is a significant cause of graft dysfunction due to a reduction in functional liver volume. Kawaguchi et al. documented the usefulness of ICG fluorescence intensity in evaluating veno-occlusive regions [33]. They calculated the ICG concentration of the veno-occlusive and non-veno-occlusive regions from the plateau fluorescence intensity. The study showed that portal uptake is diminished to 40% in the veno-occlusive area compared to the non-veno-occlusive region. It allows real-time evaluation of functional liver volume and guides the need for venous reconstruction in veno-occlusive regions. Also, they documented that the reduction in portal uptake function in the veno-occlusive areas of S5 tends to be smaller compared to the similar regions in S8. The

difference is due to a more prominent communication vein to the right hepatic vein in segment 5 compared to segment 8. Hence, reconstruction is often needed for the segment 8 vein. Traditionally intraoperative ultrasound with Doppler is used for the evaluation of vascular anastomosis. However, intravenous ICG administration facilitates real-time evaluation of graft perfusion and complements Doppler evaluation for vascular thrombosis [33].

ICG FLUORESCENCE IN BILIARY SURGERY

The feasibility of fluorescence cholangiography-guided laparoscopic cholecystectomy was documented by Ishizawa et al. in 2008 [34]. Subsequently, multiple studies, including an RCT, have shown that fluorescence cholangiography is superior to conventional white-light imaging for identifying extrahepatic biliary anatomy before and after the dissection of the Calot triangle. While the first RCT comparing fluorescence cholangiography with conventional white light imaging was not adequately powered to identify differences in the rates of bile duct injuries between the two techniques, fluorescence cholangiography could reduce the risk of bile duct injuries related to anatomical variation or visual misperception of biliary anatomy [35]. Also, fluorescence cholangiography is helpful in delineating the biliary anatomy clearly in challenging cases such as acute cholecystitis and Mirizzi syndrome, thereby reducing conversion rate and bile duct injuries [36].

Compared to conventional intraoperative radiographic cholangiography, fluorescence cholangiography does not require ionizing radiation and provides images in real-time, even before the dissection of the Calot triangle. However, fluorescence cholangiography does not help visualize common bile duct stones and intrahepatic biliary anatomy. Also, fluorescence cholangiography is not helpful in obese patients with thick fat in the Calot triangle due to limited penetration of near infra-red light. While fluorescence cholangiography could reduce the need for radiographic cholangiography in the future, it cannot wholly replace the radiographic technique, and both will have a complementary role [37].

For fluorescence cholangiography, ICG can be injected intravenously or intrabiliary. Intravenous ICG can be injected from 30 minutes up to 24 hours before surgery at a

dose of 0.05 mg/kg body weight or a total dose of 2.5 mg. Until 24 hours, as the interval between intravenous injection and surgery increases, the bile duct to background liver contrast ratio improves, thereby facilitating the identification of the biliary tree [38]. As most laparoscopic cholecystectomy is done as a day-care surgical procedure, intravenous ICG is often administered at the time of induction of anaesthesia or 3-6 hours before surgery. For intrabiliary administration, like direct portal vein injection, dilute ICG (0.025 mg/mL) is administered through a trans cystic tube. While intrabiliary injection permits immediate visualization without background liver fluorescence, intravenous ICG injection is more convenient and avoids bile duct injuries associated with inserting a trans cystic tube. Also, spillage of ICG during an injection can obscure adequate visualization of the biliary tree due to background fluorescence. However, intrabiliary injection is preferred in patients with a preoperative percutaneous biliary drainage tube.

ICG FLUORESCENCE IN PANCREATIC SURGERY

In Pancreatic surgery, ICG is helpful for tumor localization, assessing pancreatic stump vascularity, and obtaining retroperitoneal margin in patients undergoing pancreaticoduodenectomy. Also, in patients undergoing duodenum preserving pancreas head resection, ICG helps localize the bile duct and assess duodenal vascularity. Pancreas is a highly vascular organ, and most pancreatic tumors, except neuroendocrine tumors, are poorly vascular [39]. In contrast to the fluorescence mechanism of liver tumors, pancreatic lesions were visualized based on the difference in vascularity between the lesions and the surrounding parenchyma. ICG fluorescence is more sensitive to detecting pancreatic neuroendocrine tumors and cystic neoplasms than pancreatic ductal adenocarcinoma. Pancreatic neuroendocrine tumors appear highly fluorescent, whereas cystic neoplasms appear as fluorescence defect lesions. Real-time visualization of pancreatic tumors permits parenchyma-preserving surgeries for low-grade tumors and improves the R0 resection rate with malignant tumors. Tumor visualization is potentially helpful in patients undergoing minimally invasive resection without tactile feedback. As fluorescence imaging fails to visualize minor differences in fluorescence intensity between tumor and

pancreatic parenchyma, pancreatic duct adenocarcinomas are not detected well with ICG. Also, fluorescence imaging cannot detect deeper parenchymal lesions. Hence, the current role of ICG-fluorescence imaging is to complement conventional intraoperative tumor detection techniques like visual inspection, palpation, and intra-operative ultrasound.

Strasberg et al. reported that the pancreatic neck region commonly used for pancreaticoenteric anastomosis following pancreaticoduodenectomy is the vascular watershed region of the pancreas. Ischemia of the pancreatic stump following pancreas mobilization was proposed as a contributing factor for pancreaticojejunostomy leak [40]. Rho et al. reported the usefulness of ICG in assessing the perfusion of the pancreatic stump. Pancreatic perfusion was observed within 30 seconds after an intravenous injection of 5 mg ICG. However, this technique's current limitations are the lack of objective quantification of perfusion, optimum ICG dosage, and time to assess perfusion [41]. As pancreatic hypo perfusion is not the only contributor to pancreatic leak, documenting the potential usefulness of ICG-fluorescence imaging in reducing pancreatic fistula is challenging.

A negative retroperitoneal margin is a significant predictor of long-term oncological outcomes in patients undergoing pancreaticoduodenectomy. In open pancreaticoduodenectomy, frequent palpation of the superior mesenteric artery guides the correct dissection plane. However, in a minimally invasive approach, due to the lack of tactile feedback differentiating pancreatic tissue of the uncinate process from the nerve plexus around the superior mesenteric artery could be a challenge. Rho et al. reported the usefulness of ICG-fluorescence-guided uncinate dissection in 10 patients undergoing laparoscopic pancreaticoduodenectomy. Following the intravenous injection of ICG, the initially detected fluorescence signal disappeared from the superior mesenteric artery. Whereas fluorescence signal gradually accumulated in the pancreas providing good contrast between the ICG-stained uncinate process of the pancreas and the superior mesenteric artery [42]. However,

their study could not demonstrate the oncological advantage of ICG-guided uncinate dissection due to the small sample size.

Duodenum preserving bile duct resection is indicated in patients with non-invasive tumors of the pancreas head and chronic pancreatitis patients with head mass. However, the reported incidence of bile leak due to injury to the intrapancreatic bile duct ranges from 11.8 to 16.7%. Lu et al. reported that intravenous administration of ICG 12-24 hours before surgery identified intrapancreatic bile duct in 93.3% of patients. Also, ICG injected 24 hours before surgery at a dose of 0.5 mg/kg had the highest signal-to-noise ratio [Fig. 4]. Also, in this group of patients, intraoperative administration of ICG permits the assessment of duodenal vascularity through the pancreaticoduodenal arcade along the medial wall of the duodenum [43].

ICG FLUORESCENCE IN ESOPHAGEAL SURGERY

In esophageal surgery, lymphangiography function of the ICG fluorescence is used for lymphatic mapping and visualization of the thoracic duct. The angiography function of the ICG is used to assess the vascularity of the conduit for esophageal reconstruction.

Vascular assessment of oesophageal replacement conduit

Evaluation of conduit vascularity is the most studied application of ICG fluorescence in esophageal surgery. Anastomotic leak is a significant complication after esophageal replacement, and poor conduit perfusion is a considerable risk factor. After esophagectomy, ICG is used to assess the vascularity of the gastric or colon conduit before anastomosis. A meta-analysis of 17 studies reported a pooled anastomotic leak rate of 10.8%, comparable to the control group (without ICG), with a cervical leak rate of 12.3% and a thoracic anastomotic leak rate of 9.3%. However, when the analysis is restricted to six studies where specific intraoperative intervention was used to correct regions that showed poor perfusion, there was a 69% (5.7% vs. 22.9%) absolute risk reduction of the anastomotic leak. Interventions commonly used in patients with low conduit perfusion are changing the site of anastomosis and supercharging the conduit with microvascular anastomosis [44]. In contrast to cervical anastomosis, in patients undergoing intrathoracic anastomosis, a

recent meta-analysis reported that the use of ICG fluorescence for perfusion assessment did not reduce the anastomotic leak rate [45]. The discordant results regarding the usefulness of ICG fluorescence in reducing anastomotic leak rate could be explained by including many observational studies with small sample size and using subjective assessment tools like good and poor perfusion. Recent studies support using more objective parameters like time of appearance and disappearance of ICG fluorescence to assess arterial perfusion and venous congestion, the correlation of time of perfusion to the size of the area perfused, and software to quantitatively evaluate the ICG fluorescence [46]. Future studies should incorporate these objective parameters as primary endpoints.

Fluorescence guided lymphadenectomy

The lymphangiography property of ICG helps evaluate sentinel lymph nodes and visualization of nodal stations draining the primary tumor to improve the quality of lymphadenectomy (LND). Yuasa et al. studied the role of ICG-fluorescence in detecting sentinel nodes in esophageal cancer. Although ICG fluorescence had a sentinel node detection rate of 95%, a high false-negative rate of 25% is concerning. Hence, ICG fluorescence may not be helpful in avoiding lymphadenectomy in patients with negative sentinel node [47]. Hachey et al. first reported the feasibility of fluorescent lymphatic mapping in esophageal adenocarcinoma patients undergoing Ivor-Lewis esophagectomy. Four quadrant submucosal injection of diluted ICG mixture with human serum albumin or normal saline is given endoscopically before positioning the patient. Six out of 9 patients had an uptake of fluorescence in regional nodes aiding dissection. Further, the mixture of ICG to 25% human serum albumin resulted in better fluorescence retention in the lymphatics than normal saline [48]. As the dissection of recurrent laryngeal nodes is an important cause of lymphadenectomy-related morbidity, a prospective study evaluated the feasibility of mapping bilateral recurrent laryngeal nerve nodes. Authors reported that ICG-guided lymphatic mapping had 100% negative predictive value for recurrent laryngeal nodes, thereby guiding the optimum extent of lymphadenectomy [Table 4]. However, the study had only 29 patients with squamous cell carcinoma and more

studies with larger sample sizes are required to confirm the findings [49]. While ICG fluorescence could facilitate lymphadenectomy, the basic principles of lymphadenectomy in esophageal cancer should be followed to ensure good oncological outcomes.

Identification of Thoracic duct

Chyle leak is a potentially morbid complication of esophagectomy, and recent studies have shown the usefulness of ICG fluorescence in thoracic duct identification [Fig. 5]. Compared to subcutaneous injection of ICG in the dorsum of the foot between toes, injection into groin nodes under sonographic guidance has produced more consistent results. Vecchiato et al. demonstrated thoracic duct after a mean time of 52.7 minutes in 19 patients undergoing thoracoscopic esophagectomy by injecting 0.5 mg/Kg ICG in the bilateral superficial inguinal nodes with ultrasound guidance. However, the ICG dose is not standardized; while some studies have used 0.2 mg/kg, others have used 0.5 mg/kg. In addition to thoracic duct identification, ICG lymphangiography facilitates the identification of aberrant ducts, thereby preventing iatrogenic injury and postoperative chylothorax [50].

ICG FLUORESCENCE IN GASTRIC SURGERY

In gastric surgery, ICG fluorescence is used to detect sentinel lymph nodes in early gastric cancer patients and guide lymphadenectomy in patients with advanced gastric cancer. Also, ICG is utilized for tattooing tumor sites in early gastric cancer patients, facilitating intraoperative identification.

Sentinel node mapping for early gastric cancer

The aim of sentinel lymph node sampling in early gastric cancer patients is to avoid unnecessary lymphadenectomy. Traditionally radioisotopes are used for sentinel node detection. Compared to other techniques, low cost and easy availability are the main advantages of ICG lymphangiography. Both submucosal and subserosal ICG injections have been used to detect sentinel nodes. However, most studies used the endoscopic peritumoral submucosal injection of 2 mL ICG (5 mg/mL). Subserosal

injection requires tumor visualization or palpation, which may not be feasible in many patients with early gastric cancer. Also, the intraoperative subserosal injection might result in leakage of ICG into the surgical field. The timing of injection has been variable in different studies. However, preoperative injection a day before surgery has better accuracy and fewer false negative results than intraoperative injection before skin incision. Park et al. reported the utilization of ICG in the dissection of infrapyloric nodes in patients undergoing laparoscopic distal gastrectomy for gastric cancer. The mean time taken for the exposure of the right gastroepiploic vein was significantly shorter in the ICG group compared to non ICG group (p = 0.001) [51]. In a retrospective study, ICG guided laparoscopic lymphadenectomy resulted in a significantly increased number of retrieved lymph nodes (47.5 ± 1.7 vs 42.6 ± 1.7, P = 0.046) [52]. Similar results were reported by Cianchi et al. in robotic gastrectomy for gastric cancer [53]. A Korean multicentre randomized trial reported that a dual tracer that combines Te^{99-} labelled Human serum albumin and ICG improved the overall detection rate of sentinel nodes. Also, the Korean study showed that sentinel basin dissection has better lymph node yield than the pick-up method used in other studies [54]. A reliable intraoperative diagnostic technique to detect metastatic tumor cells is an essential component of sentinel node navigation surgery. However, as highlighted in the JCOG0302 trial frozen section investigation with only one plane has a high false negative rate of 46% [55]. Hence, all lymph nodes retrieved from the sentinel basins were evaluated in the SENORITA trial. Also, for nodes larger than 4 mm, serial sections at 2 mm intervals were taken for examination. Further, rapid molecular diagnostic techniques like one-step nucleic acid amplification assay might improve the sensitivity of intraoperative evaluation [Table 5]. Isozaki et al. reported an excellent gastric cancer-specific cumulative 5-year survival rate of 98.5% for ICG sentinel lymph node mapping in early gastric cancer [56].

Improving the performance of D2 lymphadenectomy

Studies comparing the ICG and non-ICG-guided lymphadenectomy have shown significantly better lymph node yield, less blood loss, and operative time in the ICG group. A meta-analysis of 13 studies with 1882 patients also showed significantly

better lymph node yield in the ICG group (40.33 vs. 33.40 nodes)[57]. In the randomized controlled trial, Chen et al. reported a significantly higher mean number of retrieved nodes (50.5 vs. 42, P < 0.001) and a lower noncompliance rate (31.8% vs. 57.4%, P < 0.001) with the ICG arm [58][Table 4]. Each lymph node station in gastric cancer has well-defined anatomical boundaries [Fig. 6]. ICG fluorescence complements proper surgical technique in achieving D2 lymphadenectomy. Improvement in compliance to D2 lymphadenectomy with ICG fluorescence is primarily seen in patients undergoing total gastrectomy. Also, patients in the ICG arm had better 3-year overall and progression-free survival [59]. Another potential utility of ICG-guided lymphangiography in patients with advanced gastric cancer is identifying metastatic lymph nodes beyond D2. However, the clinical utility of identifying and dissecting the nodes beyond D2 needs to be further studied.

ICG FLUORESCENCE IN COLORECTAL SURGERY

In large bowel surgery, ICG fluorescence is used to evaluate intraoperative bowel perfusion at the anastomotic site, identification of ureter, detection of metastases, lymphatic mapping, and tattooing of early colonic malignancy.

Assessment of bowel perfusion and anastomosis

The blood supply of the large bowel is variable in critical watershed areas due to diminished or absent collateral vessels. For satisfactory anastomosis healing, the bowel should be tension free and have sufficient vascular perfusion. Perfusion assessment is done before the bowel transection to identify the demarcation line and after anastomosis to decide for diversion in low rectal anastomosis [Fig. 7]. Multiple meta-analyses had reported lower anastomotic leak and complication rates with ICG fluorescence angiography. A recent meta-analysis reported a lower pooled anastomotic leak rate (4.3% vs. 9.43%, P<0.00001) with ICG use [60]. Emile et al. reported that ICG-guided vascular assessment resulted in the revision of the transection point in 9.6% of patients [61]. The main pitfall with using ICG for vascular assessment is the inability to assess mucosal perfusion. Sherwinter et al. first described the feasibility of assessing mucosal perfusion using a rigid sigmoidoscope

and 12 mm laparoscopic trocar to introduce a laparoscopic ICG camera to assess staple line [62]. Another limitation of the current technique is subjective perfusion assessment. Wada et al. reviewed the prospectively maintained video recordings of 112 patients undergoing left colonic resection with ICG fluorescence and used computer-based analysis to create fluorescent intensity to time curve and used parameters like Fmax, Tmax, and slope (F_{max}/T_{max}) to quantitatively assess the vascular perfusion [63]. Further studies to validate its application in surgery are awaited.

Ureter identification

Ureteral injury is reported in up to 0.6% of patients undergoing colorectal procedures. Preoperative ureteral stenting is done in high-risk patients with inflammatory disorders and advanced malignancy to aid ureteral identification and prevent injury. Meta-analysis of retrospective studies showed that stenting does not significantly reduce the chance of injury. However, it may help in the intraoperative identification of the damage [64]. Currently, many colorectal procedures are done using a minimally invasive approach. To identify the ureter, ICG is instilled along with ureteral stenting to aid in visual identification and preservation.

Lymphatic mapping and lymph nodal assessment

Colorectal malignancy spreads through the periarteriolar lymphatics to principal lymph nodes; hence, complete mesocolic excision and central vascular ligation is suggested. The Japanese also recommend D3 lymphadenectomy for clinical stage II and III colorectal malignancies based on a similar model. However, the regional lymph node zone varies and depends on the location of the tumor and its relationship to the feeding vessel. Nishigori et al. initially studied ICG fluorescence imaging in 21 patients undergoing colectomy with perioperative submucosal injection and observed lymph flow in 18 patients. Also, the principle lymph nodal basin was observed in 4 patients (23.5%), which changed the line of vascular ligation and extent of lymphadenectomy [65]. Similarly, Watanabe et al. did lymphatic mapping in 31 patients undergoing colectomy for splenic flexure malignancy. Splenic flexure, the watershed area between the superior and inferior mesenteric artery territory, is classically treated with complete mesocolic excision of the middle

and left colic regions. However, the study showed no bidirectional lymph flow. Hence the study hypothesized CME with middle colic ligation of distal third transverse colon cancer and CME with left colic ligation for proximal descending colon cancer [66]. The study, which evaluated the histopathological examination of the fluorescent and non-fluorescent metastatic lymph nodes, showed no uptake of ICG in lymph nodes with complete tumor infiltration. Also, it revealed no ICG uptake in cancerous areas within partially fluorescent nodes. Thus ICG fluorescence-guided lymphatic mapping is primarily suited for early malignancy with no bulky node. Meta-analysis on sentinel lymph nodal biopsy showed higher pooled accuracy in T_1 - T_2 (98%) compared to $T_3 - T_4$ (77%) [67].

Tattooing

Colorectal polyps are treated with colonoscopic polypectomy, and submucosal invasion on pathological examination mandates completion segmental colectomy. Conventionally India ink and radioactive colloids are used for tattooing these early lesions. However, both are associated with limitations like short levelling time, radiation exposure, and surgical plane diminution. Preoperative endoscopic ICG injection to the polypectomy region or early colonic malignancy helps identify the tumor site promptly with a near-infrared camera. The addition of albumin to the ICG injection increases the levelling time to up to 10 days [68].

FUTURE PERSPECTIVES

ICG and its fluorescence property have created a new era of navigation surgery with the potential for better postoperative outcomes. Currently, ICG fluorescence employs angiography and cholangiography features for tumor-to-background differentiation. Experimental studies of binding tumor-specific ligands with fluorophore have improved the detection of primary and metastatic tumors [69]. Boogerd et al. reported improved colorectal malignancy (primary, recurrent and metastatic) detection using fluorescent anti-CEA antibodies in 26 patients. This

enhanced detection led to a change in surgical management in about one-third of the patients [70].

The use of ICG angiography in liver segmentation and mapping improves precision and R0 resection rate. However, its impact on oncological outcomes has not been well studied. Gon et al. have published their study protocol to evaluate long-term outcomes like recurrence-free survival following ICG fluorescence-guided liver resection. The study has completed patient recruitment, and clinical results are awaited [71].

Novel routes and formulations of ICG are being explored to widen its application. Thammineedi et al. reported the feasibility of administering ICG in an aerosol form via nebulization to visualise the tracheobronchial tree [72]. Tracheobronchial fluorescence could minimize the incidence of airway injury in patients undergoing minimally invasive salvage esophagectomy and those with extensive periesophageal fibrosis following neoadjuvant chemoradiotherapy. Tokumaru et al. reported that subcutaneous injection of ICG in the inguinal region is as effective as ultrasound-guided injection into inguinal nodes to visualize the thoracic duct [73]. The median time from the simple subcutaneous injection technique to the observation of ICG-induced fluorescence of the thoracic duct was 119 min.

Experimental studies using ICG as a dual tracer that is detectable by radiography and fluorescent devices have increased its applicability as a marker for in-vivo mapping. The results of future clinical trials to establish the usage of ICG as a marker are anticipated [74]. He et al., in their clinical report regarding the fusion of lipiodol and Nano-ICG formulation, revealed its potential to enhance the photostability and fluorescence intensity of ICG [75]. The growing trend of navigation surgeries using ICG fluorescence implies the impending need for research studies to explore, validate and develop this arena. Future studies would expand the research scope, increase the application of ICG fluorescence and overcome limitations.

CONCLUSION

The use of ICG as a guiding tool for dissection in GI cancer surgery seems extremely promising. Further evidence from high-quality studies on larger sample sizes is needed to include ICG-guided surgery in standard practice. Our review has

examined the contribution of prior research in multiple disciplines of hepato-biliary-pancreatic and gastrointestinal surgery. This study provides a comprehensive and contemporary representation of the literature and enhances understanding of the current affairs of this domain. This may serve as a practical guide for researchers in developing their study to be highly relevant to both research and practice.

REFERENCES

1. **Renz M.** Fluorescence microscopy-a historical and technical perspective. Cytometry A. 2013 Sep;83(9):767-79. doi: 10.1002/cyto.a.22295. Epub 2013 Apr 12. PMID: 23585290.

2. **Kobayashi H**, Ogawa M, Alford R, Choyke PL, Urano Y. New strategies for fluorescent probe design in medical diagnostic imaging. Chem Rev. 2010 May 12;110(5):2620-40. doi: 10.1021/cr900263j. PMID: 20000749; PMCID: PMC3241938.

3. **Benson RC**, Kues HA. Fluorescence properties of indocyanine green as related to angiography. Phys Med Biol. 1978 Jan;23(1):159-63. doi: 10.1088/0031-9155/23/1/017. PMID: 635011.

4. **Reinhart MB**, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green: Historical Context, Current Applications, and Future Considerations. Surg Innov. 2016 Apr;23(2):166-75. doi: 10.1177/1553350615604053.

5. **Kogure K**, David NJ, Yamanouchi U, Choromokos E. Infrared absorption angiography of the fundus circulation. Arch Ophthalmol. 1970 Feb;83(2):209-14. doi: 10.1001/archopht.1970.00990030211015. PMID: 4983539.

6. **Kitai T**, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. Breast Cancer. 2005;12(3):211-5. doi: 10.2325/jbcs.12.211. PMID: 16110291.

7.   **Miyashiro I**, Miyoshi N, Hiratsuka M, Kishi K, Yamada T, Ohue M, Ohigashi H, Yano M, Ishikawa O, Imaoka S. Detection of sentinel node in gastric cancer surgery by indocyanine green fluorescence imaging: comparison with infrared imaging. Ann Surg Oncol. 2008 Jun;15(6):1640-3. doi: 10.1245/s10434-008-9872-7. Epub 2008 Apr 1. PMID: 18379850.

8.   **Aoki T**, Yasuda D, Shimizu Y, Odaira M, Niiya T, Kusano T, Mitamura K, Hayashi K, Murai N, Koizumi T, Kato H, Enami Y, Miwa M, Kusano M. Image-guided liver mapping using fluorescence navigation system with indocyanine green for anatomical hepatic resection. World J Surg. 2008 Aug;32(8):1763-7. doi: 10.1007/s00268-008-9620-y. PMID: 18543027.

9.   **Kawaguchi Y**, Ishizawa T, Masuda K, Sato S, Kaneko J, Aoki T, Beck Y, Sugawara Y, Hasegawa K, Kokudo N. Hepatobiliary surgery guided by a novel fluorescent imaging technique for visualizing hepatic arteries, bile ducts, and liver cancers on color images. J Am Coll Surg. 2011 Jun;212(6):e33-9. doi: 10.1016/j.jamcollsurg.2011.03.006. Epub 2011 Mar 29. PMID: 21450495.

10.   **Makuuchi M**, Hasegawa H, Yamazaki S, Takayasu K, Moriyama N. The use of operative ultrasound as an aid to liver resection in patients with hepatocellular carcinoma. World J Surg. 1987 Oct;11(5):615-21. doi: 10.1007/BF01655837. PMID: 2823489..

11.   **Aoki T**, Murakami M, Yasuda D, Shimizu Y, Kusano T, Matsuda K, Niiya T, Kato H, Murai N, Otsuka K, Kusano M, Kato T. Intraoperative fluorescent imaging using indocyanine green for liver mapping and cholangiography. J Hepatobiliary Pancreat Sci. 2010 Sep;17(5):590-4. doi: 10.1007/s00534-009-0197-0. Epub 2009 Oct 21. PMID: 19844652.

12.   **Kobayashi Y**, Kawaguchi Y, Kobayashi K, Mori K, Arita J, Sakamoto Y, Hasegawa K, Kokudo N. Portal vein territory identification using indocyanine green fluorescence imaging: Technical details and short-term outcomes. J Surg Oncol. 2017 Dec;116(7):921-931. doi: 10.1002/jso.24752. Epub 2017 Jul 10. PMID: 28695566.

13.   **Ueno M**, Hayami S, Sonomura T, Kawai M, Hirono S, Okada KI, Tanaka R, Yamaue H. Concomitant Use of Indocyanine Green Fluorescence Imaging and Interventional Radiology for Detection of Liver Segments During Laparoscopic

Anatomical Liver Resection: Pilot Feasibility Study. Surg Laparosc Endosc Percutan Tech. 2019 Aug;29(4):242-246. doi: 10.1097/SLE.0000000000000631. PMID: 30676538.

14. **Ueno M**, Hayami S, Sonomura T, Tanaka R, Kawai M, Hirono S, Okada KI, Yamaue H. Indocyanine green fluorescence imaging techniques and interventional radiology during laparoscopic anatomical liver resection (with video). Surg Endosc. 2018 Feb;32(2):1051-1055. doi: 10.1007/s00464-017-5997-8. Epub 2017 Dec 22. PMID: 29273869.

15. **Xu Y**, Chen M, Meng X, Lu P, Wang X, Zhang W, Luo Y, Duan W, Lu S, Wang H. Laparoscopic anatomical liver resection guided by real-time indocyanine green fluorescence imaging: experience and lessons learned from the initial series in a single center. Surg Endosc. 2020 Oct;34(10):4683-4691. doi: 10.1007/s00464-020-07691-5. Epub 2020 Jun 4. PMID: 32500459.

16. **Qian X**, Hu W, Gao L, Xu J, Wang B, Song J, Yang S, Lu Q, Zhang L, Yan J, Dong J. Trans-arterial positive ICG staining-guided laparoscopic liver watershed resection for hepatocellular carcinoma. Front Oncol. 2022 Jul 22;12:966626. doi: 10.3389/fonc.2022.966626. PMID: 35936704; PMCID: PMC9354495.

17. **Li WF**, Al-Taher M, Yu CY, Liu YW, Liu YY, Marescaux J, Cheng YF, Diana M, Wang CC. Super-Selective Intra-Arterial Indocyanine Green Administration for Near-Infrared Fluorescence-Based Positive Staining of Hepatic Segmentation: A Feasibility Study. Surg Innov. 2021 Dec;28(6):669-678. doi: 10.1177/1553350621996972. Epub 2021 Mar 31. PMID: 33787401.

18. **Lan X**, Tang Y, Wei W, Jiang K, Chen K, Du C, Hao X, Liu H. Indocyanine green fluorescence staining based on the "hepatic pedicle first" approach during laparoscopic anatomic liver resection. Surg Endosc. 2022 Nov;36(11):8121-8131. doi: 10.1007/s00464-022-09237-3. Epub 2022 Apr 25. PMID: 35469092.

19. **Uchiyama K**, Ueno M, Ozawa S, Kiriyama S, Shigekawa Y, Hirono S, Kawai M, Tani M, Yamaue H. Combined intraoperative use of contrast-enhanced ultrasonography imaging using a sonazoid and fluorescence navigation system with indocyanine green during anatomical hepatectomy. Langenbecks Arch Surg. 2011 Oct;396(7):1101-7. doi: 10.1007/s00423-011-0778-7. Epub 2011 Mar 29. PMID: 21918930.

20. **Berardi G**, Igarashi K, Li CJ, Ozaki T, Mishima K, Nakajima K, Honda M, Wakabayashi G. Parenchymal Sparing Anatomical Liver Resections With Full Laparoscopic Approach: Description of Technique and Short-term Results. Ann Surg. 2021 Apr 1;273(4):785-791. doi: 10.1097/SLA.0000000000003575. PMID: 31460879.

21. **Funamizu N**, Ozaki T, Mishima K, Igarashi K, Omura K, Takada Y, Wakabayashi G. Evaluation of accuracy of laparoscopic liver mono-segmentectomy using the Glissonian approach with indocyanine green fluorescence negative staining by comparing estimated and actual resection volumes: A single-center retrospective cohort study. J Hepatobiliary Pancreat Sci. 2021 Dec;28(12):1060-1068. doi: 10.1002/jhbp.924. Epub 2021 Mar 16. PMID: 33638899.

22. **Ishizawa T**, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, Hasegawa K, Beck Y, Fukayama M, Kokudo N. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer. 2009 Jun 1;115(11):2491-504. doi: 10.1002/cncr.24291. PMID: 19326450.

23. **Franz M**, Arend J, Wolff S, Perrakis A, Rahimli M, Negrini VR, Stockheim J, Lorenz E, Croner R. Tumor visualization and fluorescence angiography with indocyanine green (ICG) in laparoscopic and robotic hepatobiliary surgery - valuation of early adopters from Germany. Innov Surg Sci. 2021 Apr 22;6(2):59-66. doi: 10.1515/iss-2020-0019. PMID: 34589573; PMCID: PMC8435269.

24. **Cai X**, Hong H, Pan W, Chen J, Jiang L, Du Q, Li G, Lin S, Chen Y. Does Using Indocyanine Green Fluorescence Imaging for Tumors Help in Determining the Safe Surgical Margin in Real-Time Navigation of Laparoscopic Hepatectomy? A Retrospective Study. Ann Surg Oncol 2023; 30: 1981-1987 [PMID: 36484905]

25. **Piccolo G**, Barabino M, Diana M, Lo Menzo E, Epifani AG, Lecchi F, Santambrogio R, Opocher E. Application of Indocyanine Green Fluorescence as an Adjuvant to Laparoscopic Ultrasound in Minimally Invasive Liver Resection. J Laparoendosc Adv Surg Tech A. 2021 May;31(5):517-523. doi: 10.1089/lap.2020.0895. Epub 2021 Mar 2. PMID: 33651631.

26. **Terasawa M**, Ishizawa T, Mise Y, Inoue Y, Ito H, Takahashi Y, Saiura A. Applications of fusion-fluorescence imaging using indocyanine green in

laparoscopic hepatectomy. Surg Endosc. 2017 Dec;31(12):5111-5118. doi: 10.1007/s00464-017-5576-z. Epub 2017 Apr 28. PMID: 28455774.

27. **Marino MV**, Podda M, Fernandez CC, Ruiz MG, Fleitas MG. The application of indocyanine green-fluorescence imaging during robotic-assisted liver resection for malignant tumors: a single-arm feasibility cohort study. HPB (Oxford). 2020 Mar;22(3):422-431. doi: 10.1016/j.hpb.2019.07.013. Epub 2019 Aug 10. PMID: 31409539.

28. **Wakabayashi T**, Cacciaguerra AB, Abe Y, Bona ED, Nicolini D, Mocchegiani F, Kabeshima Y, Vivarelli M, Wakabayashi G, Kitagawa Y. Indocyanine Green Fluorescence Navigation in Liver Surgery: A Systematic Review on Dose and Timing of Administration. Ann Surg. 2022 Jun 1;275(6):1025-1034. doi: 10.1097/SLA.0000000000005406. Epub 2022 Feb 2. PMID: 35121701.

29. **Zhou Y**, Lin Y, Jin H, Hou B, Yu M, Yin Z, Jian Z. Real-Time Navigation Guidance Using Fusion Indocyanine Green Fluorescence Imaging in Laparoscopic Non-Anatomical Hepatectomy of Hepatocellular Carcinomas at Segments 6, 7, or 8 (with Videos). Med Sci Monit. 2019 Feb 26;25:1512-1517. doi: 10.12659/MSM.914070. PMID: 30806378; PMCID: PMC6400019.

30. **Kudo H**, Ishizawa T, Tani K, Harada N, Ichida A, Shimizu A, Kaneko J, Aoki T, Sakamoto Y, Sugawara Y, Hasegawa K, Kokudo N. Visualization of subcapsular hepatic malignancy by indocyanine-green fluorescence imaging during laparoscopic hepatectomy. Surg Endosc. 2014 Aug;28(8):2504-8. doi: 10.1007/s00464-014-3468-z. Epub 2014 Feb 25. PMID: 24566751.

31. **Mizuno S**, Isaji S. Indocyanine green (ICG) fluorescence imaging-guided cholangiography for donor hepatectomy in living donor liver transplantation. Am J Transplant. 2010 Dec;10(12):2725-6. doi: 10.1111/j.1600-6143.2010.03288.x. Epub 2010 Nov 9. PMID: 21062417.

32. **Hong SK**, Lee KW, Kim HS, Yoon KC, Ahn SW, Choi JY, Kim H, Yi NJ, Suh KS. Optimal bile duct division using real-time indocyanine green near-infrared fluorescence cholangiography during laparoscopic donor hepatectomy. Liver Transpl. 2017 Jun;23(6):847-852. doi: 10.1002/lt.24686. PMID: 27935193.

33. **Kawaguchi Y**, Akamatsu N, Ishizawa T, Kaneko J, Arita J, Sakamoto Y, Hasegawa K, Kokudo N. Evaluation of hepatic perfusion in the liver graft using fluorescence imaging with indocyanine green. Int J Surg Case Rep. 2015;14:149-51. doi: 10.1016/j.ijscr.2015.07.031. Epub 2015 Jul 31. PMID: 26275740; PMCID: PMC4573850.

34. **Ishizawa T**, Bandai Y, Kokudo N. Fluorescent cholangiography using indocyanine green for laparoscopic cholecystectomy: an initial experience. Arch Surg. 2009 Apr;144(4):381-2. doi: 10.1001/archsurg.2009.9. PMID: 19380655.

35. **Dip F**, LoMenzo E, Sarotto L, Phillips E, Todeschini H, Nahmod M, Alle L, Schneider S, Kaja L, Boni L, Ferraina P, Carus T, Kokudo N, Ishizawa T, Walsh M, Simpfendorfer C, Mayank R, White K, Rosenthal RJ. Randomized Trial of Near-infrared Incisionless Fluorescent Cholangiography. Ann Surg. 2019 Dec;270(6):992-999. doi: 10.1097/SLA.0000000000003178. PMID: 30614881.

36. **Roy M**, Dip F, Nguyen D, Simpfendorfer CH, Menzo EL, Szomstein S, Rosenthal RJ. Fluorescent incisionless cholangiography as a teaching tool for identification of Calot's triangle. Surg Endosc. 2017 Jun;31(6):2483-2490. doi: 10.1007/s00464-016-5250-x. Epub 2016 Oct 24. PMID: 27778170.

37. **Wang X**, Teh CSC, Ishizawa T, Aoki T, Cavallucci D, Lee SY, Panganiban KM, Perini MV, Shah SR, Wang H, Xu Y, Suh KS, Kokudo N. Consensus Guidelines for the Use of Fluorescence Imaging in Hepatobiliary Surgery. Ann Surg. 2021 Jul 1;274(1):97-106. doi: 10.1097/SLA.0000000000004718. PMID: 33351457.

38. **Pesce A**, Piccolo G, La Greca G, Puleo S. Utility of fluorescent cholangiography during laparoscopic cholecystectomy: A systematic review. World J Gastroenterol. 2015 Jul 7;21(25):7877-83. doi: 10.3748/wjg.v21.i25.7877. PMID: 26167088; PMCID: PMC4491975.

39. **Shirata C**, Kawaguchi Y, Kobayashi K, Kobayashi Y, Arita J, Akamatsu N, Kaneko J, Sakamoto Y, Kokudo N, Hasegawa K. Usefulness of indocyanine green-fluorescence imaging for real-time visualization of pancreas neuroendocrine tumor and cystic neoplasm. J Surg Oncol. 2018 Nov;118(6):1012-1020. doi: 10.1002/jso.25231. Epub 2018 Sep 27. PMID: 30261107.

40. **Strasberg SM,** Drebin JA, Mokadam NA, Green DW, Jones KL, Ehlers JP, Linehan D. Prospective trial of a blood supply-based technique of pancreaticojejunostomy: effect on anastomotic failure in the Whipple procedure. J Am Coll Surg. 2002 Jun;194(6):746-58; discussion 759-60. doi: 10.1016/s1072-7515(02)01202-4. PMID: 12081065.

41. **Rho SY**, Kim SH, Kang CM, Lee WJ. Is ICG-enhanced image able to help predicting pancreatic fistula in laparoscopic pancreaticoduodenectomy? Minim Invasive Ther Allied Technol. 2019 Feb;28(1):29-32. doi: 10.1080/13645706.2018.1479271. Epub 2018 Jun 5. PMID: 29869569.

42. **Rho SY**, Kim JS, Chong JU, Hwang HK, Yoon DS, Lee WJ, Kang CM. Indocyanine Green Perfusion Imaging-Guided Laparoscopic Pancreaticoduodenectomy: Potential Application in Retroperitoneal Margin Dissection. J Gastrointest Surg. 2018 Aug;22(8):1470-1474. doi: 10.1007/s11605-018-3760-7. Epub 2018 Apr 9. PMID: 29633118.

43. **Lu C**, Xu B, Mou Y, Zhou Y, Jin W, Xia T, Wang Y, Zhu Q, Fu Z. Laparoscopic duodenum-preserving pancreatic head resection with real-time indocyanine green guidance of different dosage and timing: enhanced safety with visualized biliary duct and its long-term metabolic morbidity. Langenbecks Arch Surg. 2022 Nov;407(7):2823-2832. doi: 10.1007/s00423-022-02570-0. Epub 2022 Jul 19. PMID: 35854048; PMCID: PMC9640461.

44. **Ladak F**, Dang JT, Switzer N, Mocanu V, Tian C, Birch D, Turner SR, Karmali S. Indocyanine green for the prevention of anastomotic leaks following esophagectomy: a meta-analysis. Surg Endosc. 2019 Feb;33(2):384-394. doi: 10.1007/s00464-018-6503-7. Epub 2018 Nov 1. PMID: 30386983.

45. **Casas MA**, Angeramo CA, Bras Harriott C, Dreifuss NH, Schlottmann F. Indocyanine green (ICG) fluorescence imaging for prevention of anastomotic leak in totally minimally invasive Ivor Lewis esophagectomy: a systematic review and meta-analysis. Dis Esophagus. 2022 Apr 19;35(4):doab056. doi: 10.1093/dote/doab056. PMID: 34378016.

46. **Talavera-Urquijo E**, Parise P, Palucci M, Olivari G, Turi S, Cossu A, Barbieri L, Elmore U, Rosati R. Perfusion speed of indocyanine green in the stomach before

tubulization is an objective and useful parameter to evaluate gastric microcirculation during Ivor-Lewis esophagectomy. Surg Endosc. 2020 Dec;34(12):5649-5659. doi: 10.1007/s00464-020-07924-7. Epub 2020 Aug 27. PMID: 32856151.

47. **Yuasa Y**, Seike J, Yoshida T, Takechi H, Yamai H, Yamamoto Y, Furukita Y, Goto M, Minato T, Nishino T, Inoue S, Fujiwara S, Tangoku A. Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative CT lymphography for superficial esophageal cancer. Ann Surg Oncol. 2012 Feb;19(2):486-93. doi: 10.1245/s10434-011-1922-x. Epub 2011 Jul 27. PMID: 21792510.

48. **Hachey KJ**, Gilmore DM, Armstrong KW, Harris SE, Hornick JL, Colson YL, Wee JO. Safety and feasibility of near-infrared image-guided lymphatic mapping of regional lymph nodes in esophageal cancer. J Thorac Cardiovasc Surg. 2016 Aug;152(2):546-54. doi: 10.1016/j.jtcvs.2016.04.025. Epub 2016 Apr 11. PMID: 27179838; PMCID: PMC4947564.

49. **Park SY**, Suh JW, Kim DJ, Park JC, Kim EH, Lee CY, Lee JG, Paik HC, Chung KY. Near-Infrared Lymphatic Mapping of the Recurrent Laryngeal Nerve Nodes in T1 Esophageal Cancer. Ann Thorac Surg. 2018 Jun;105(6):1613-1620. doi: 10.1016/j.athoracsur.2018.01.083. Epub 2018 Mar 5. PMID: 29518388.

50. **Vecchiato M**, Martino A, Sponza M, Uzzau A, Ziccarelli A, Marchesi F, Petri R. Thoracic duct identification with indocyanine green fluorescence during minimally invasive esophagectomy with patient in prone position. Dis Esophagus. 2020 Dec 7;33(12):doaa030. doi: 10.1093/dote/doaa030. PMID: 32448899; PMCID: PMC7720005.

51. **Park SH**, Berlth F, Choi JH, Park JH, Suh YS, Kong SH, Park DJ, Lee HJ, Yang HK. Near-infrared fluorescence-guided surgery using indocyanine green facilitates secure infrapyloric lymph node dissection during laparoscopic distal gastrectomy. Surg Today. 2020 Oct;50(10):1187-1196. doi: 10.1007/s00595-020-01993-w. Epub 2020 Apr 3. PMID: 32246228.

52. **Ushimaru Y**, Omori T, Fujiwara Y, Yanagimoto Y, Sugimura K, Yamamoto K, Moon JH, Miyata H, Ohue M, Yano M. The Feasibility and Safety of Preoperative Fluorescence Marking with Indocyanine Green (ICG) in Laparoscopic Gastrectomy

for Gastric Cancer. J Gastrointest Surg. 2019 Mar;23(3):468-476. doi: 10.1007/s11605-018-3900-0. Epub 2018 Aug 6. PMID: 30084063.

53. **Cianchi F**, Indennitate G, Paoli B, Ortolani M, Lami G, Manetti N, Tarantino O, Messeri S, Foppa C, Badii B, Novelli L, Skalamera I, Nelli T, Coratti F, Perigli G, Staderini F. The Clinical Value of Fluorescent Lymphography with Indocyanine Green During Robotic Surgery for Gastric Cancer: a Matched Cohort Study. J Gastrointest Surg. 2020 Oct;24(10):2197-2203. doi: 10.1007/s11605-019-04382-y. Epub 2019 Sep 4. PMID: 31485904.

54. **An JY**, Min JS, Hur H, Lee YJ, Cho GS, Park YK, Jung MR, Park JH, Hyung WJ, Jeong SH, Kim YW, Yoon HM, Eom BW, Kook MC, Han MR, Nam BH, Ryu KW; SEntinel Node ORIented Tailored Approach (SENORITA) Study Group. Laparoscopic sentinel node navigation surgery versus laparoscopic gastrectomy with lymph node dissection for early gastric cancer: short-term outcomes of a multicentre randomized controlled trial (SENORITA). Br J Surg. 2020 Oct;107(11):1429-1439. doi: 10.1002/bjs.11655. Epub 2020 Jun 3. PMID: 32492186.

55. **Miyashiro I**, Hiratsuka M, Sasako M, Sano T, Mizusawa J, Nakamura K, Nashimoto A, Tsuburaya A, Fukushima N; Gastric Cancer Surgical Study Group (GCSSG) in the Japan Clinical Oncology Group (JCOG). High false-negative proportion of intraoperative histological examination as a serious problem for clinical application of sentinel node biopsy for early gastric cancer: final results of the Japan Clinical Oncology Group multicenter trial JCOG0302. Gastric Cancer. 2014 Apr;17(2):316-23. doi: 10.1007/s10120-013-0285-3. Epub 2013 Aug 10. PMID: 23933782.

56. **Isozaki H**, Matsumoto S, Murakami S. Survival outcomes after sentinel node navigation surgery for early gastric cancer. Ann Gastroenterol Surg. 2019 Jul 25;3(5):552-560. doi: 10.1002/ags3.12280. PMID: 31549015; PMCID: PMC6749950.

57. **Pang HY**, Liang XW, Chen XL, Zhou Q, Zhao LY, Liu K, Zhang WH, Yang K, Chen XZ, Hu JK. Assessment of indocyanine green fluorescence lymphography on lymphadenectomy during minimally invasive gastric cancer surgery: a systematic review and meta-analysis. Surg Endosc. 2022 Mar;36(3):1726-1738. doi: 10.1007/s00464-021-08830-2. Epub 2022 Jan 25. PMID: 35079880.

58. **Chen QY**, Xie JW, Zhong Q, Wang JB, Lin JX, Lu J, Cao LL, Lin M, Tu RH, Huang ZN, Lin JL, Zheng HL, Li P, Zheng CH, Huang CM. Safety and Efficacy of Indocyanine Green Tracer-Guided Lymph Node Dissection During Laparoscopic Radical Gastrectomy in Patients With Gastric Cancer: A Randomized Clinical Trial. JAMA Surg. 2020 Apr 1;155(4):300-311. doi: 10.1001/jamasurg.2019.6033. PMID: 32101269.

59. **Zhong Q**, Zhong LY, Liu ZY, Zhuang LP, Chen QY, Huang C. 77P Long-term outcomes of indocyanine green fluorescence imaging-guided laparoscopic lymphadenectomy for gastric cancer: The FUGES-012 randomized clinical trial. Annals of Oncology. 2022;33:S1462. doi: 10.1016/j.annonc.2022.10.113.

60. **Tang G**, Du D, Tao J, Wei Z. Effect of Indocyanine Green Fluorescence Angiography on Anastomotic Leakage in Patients Undergoing Colorectal Surgery: A Meta-Analysis of Randomized Controlled Trials and Propensity-Score-Matched Studies. Front Surg. 2022 Mar 15;9:815753. doi: 10.3389/fsurg.2022.815753. PMID: 35372484; PMCID: PMC8964518.

61. **Emile SH**, Khan SM, Wexner SD. Impact of change in the surgical plan based on indocyanine green fluorescence angiography on the rates of colorectal anastomotic leak: a systematic review and meta-analysis. Surg Endosc. 2022 Apr;36(4):2245-2257. doi: 10.1007/s00464-021-08973-2. Epub 2022 Jan 13. PMID: 35024926.

62. **Sherwinter DA**. Transanal near-infrared imaging of colorectal anastomotic perfusion. Surg Laparosc Endosc Percutan Tech. 2012 Oct;22(5):433-6. doi: 10.1097/SLE.0b013e3182601eb8. PMID: 23047388.

63. **Wada T**, Kawada K, Takahashi R, Yoshitomi M, Hida K, Hasegawa S, Sakai Y. ICG fluorescence imaging for quantitative evaluation of colonic perfusion in laparoscopic colorectal surgery. Surg Endosc. 2017 Oct;31(10):4184-4193. doi: 10.1007/s00464-017-5475-3. Epub 2017 Mar 9. PMID: 28281123.

64. **Hird AE**, Nica A, Coburn NG, Kulkarni GS, Nam RK, Gien LT. Does prophylactic ureteric stenting at the time of colorectal surgery reduce the risk of ureteric injury? A systematic review and meta-analysis. Colorectal Dis. 2021 May;23(5):1060-1070. doi: 10.1111/codi.15498. Epub 2021 Jan 23. PMID: 33340227.

65. **Nishigori N**, Koyama F, Nakagawa T, Nakamura S, Ueda T, Inoue T, Kawasaki K, Obara S, Nakamoto T, Fujii H, Nakajima Y. Visualization of Lymph/Blood Flow in Laparoscopic Colorectal Cancer Surgery by ICG Fluorescence Imaging (Lap-IGFI). Ann Surg Oncol. 2016 Feb;23 Suppl 2:S266-74. doi: 10.1245/s10434-015-4509-0. Epub 2015 Mar 24. PMID: 25801355.

66. **Watanabe J**, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K. Evaluation of lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time indocyanine green fluorescence imaging. Int J Colorectal Dis. 2017 Feb;32(2):201-207. doi: 10.1007/s00384-016-2669-4. Epub 2016 Oct 1. PMID: 27695977.

67. **Burghgraef TA**, Zweep AL, Sikkenk DJ, van der Pas MHGM, Verheijen PM, Consten ECJ. In vivo sentinel lymph node identification using fluorescent tracer imaging in colon cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2021 Feb;158:103149. doi: 10.1016/j.critrevonc.2020.103149. Epub 2020 Nov 11. PMID: 33450679.

68. **Miyoshi N**, Ohue M, Noura S, Yano M, Sasaki Y, Kishi K, Yamada T, Miyashiro I, Ohigashi H, Iishi H, Ishikawa O, Imaoka S. Surgical usefulness of indocyanine green as an alternative to India ink for endoscopic marking. Surg Endosc. 2009 Feb;23(2):347-51. doi: 10.1007/s00464-008-9938-4. Epub 2008 Apr 29. PMID: 18443867.

69. **Kaushal S**, McElroy MK, Luiken GA, Talamini MA, Moossa AR, Hoffman RM, Bouvet M. Fluorophore-conjugated anti-CEA antibody for the intraoperative imaging of pancreatic and colorectal cancer. J Gastrointest Surg. 2008 Nov;12(11):1938-50. doi: 10.1007/s11605-008-0581-0. Epub 2008 Jul 30. PMID: 18665430; PMCID: PMC4396596.

70. **Boogerd LSF**, Hoogstins CES, Schaap DP, Kusters M, Handgraaf HJM, van der Valk MJM, Hilling DE, Holman FA, Peeters KCMJ, Mieog JSD, van de Velde CJH, Farina-Sarasqueta A, van Lijnschoten I, Framery B, Pèlegrin A, Gutowski M, Nienhuijs SW, de Hingh IHJT, Nieuwenhuijzen GAP, Rutten HJT, Cailler F, Burggraaf J, Vahrmeijer AL. Safety and effectiveness of SGM-101, a fluorescent antibody targeting carcinoembryonic antigen, for intraoperative detection of colorectal cancer: a dose-escalation pilot study. Lancet Gastroenterol Hepatol. 2018

Mar;3(3):181-191. doi: 10.1016/S2468-1253(17)30395-3. Epub 2018 Jan 30. PMID: 29361435.

71.   **Gon H**, Komatsu S, Murakami S, Kido M, Tanaka M, Kuramitsu K, Tsugawa D, Awazu M, Toyama H, Fukumoto T. Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study. BMJ Open. 2019 Aug 18;9(8):e030233. doi: 10.1136/bmjopen-2019-030233. PMID: 31427337; PMCID: PMC6701684.

72. **Thammineedi SR**, Patnaik SC, Nusrath S, et al. Evaluation of indocyanine green tracheobronchial fluorescence (ICG-TBF) via nebulization during minimally invasive esophagectomy [published online ahead of print, 2023 Sep 12]. Dis Esophagus. 2023;doad059. doi:10.1093/dote/doad059.

73. **Tokumaru S**, Kitazawa M, Nakamura S, Koyama M, Soejima Y. Intraoperative visualization of morphological patterns of the thoracic duct by subcutaneous inguinal injection of indocyanine green in esophagectomy for esophageal cancer. Ann Gastroenterol Surg. 2022;6(6):873-879. Published 2022 Jun 22. doi:10.1002/ags3.12594

74.   **Hayashi H**, Toyota T, Goto S, Ooishi A, Gao T, Ee LB, Hatayama H, Nomoto T, Fujinami M, Matsubara H. Development of a non-blurring, dual-imaging tissue marker for gastrointestinal tumor localization. Surg Endosc. 2015 Jun;29(6):1445-51. doi: 10.1007/s00464-014-3822-1. Epub 2014 Aug 30. PMID: 25171886.

75.   **He P**, Xiong Y, Ye J, Chen B, Cheng H, Liu H, Zheng Y, Chu C, Mao J, Chen A, Zhang Y, Li J, Tian J, Liu G. A clinical trial of super-stable homogeneous lipiodol-nanoICG formulation-guided precise fluorescent laparoscopic hepatocellular carcinoma resection. J Nanobiotechnology. 2022 Jun 3;20(1):250. doi: 10.1186/s12951-022-01467-w. PMID: 35658966; PMCID: PMC9164554.

Figure legends

Figure 1 - Annual publication trend of articles related to indocyanine green fluorescence.

Figure 2. Country-wise publication trend of articles related to indocyanine green fluorescence.

Figure 3. Negative indocyanine green staining technique used in a patient with hilar cholangiocarcinoma planned for right hepatectomy with caudate lobectomy. A) Portal and hepatic artery branches of the right hemiliver to be resected are ligated. B) line of transection for right hepatectomy identified with the help of ICG fluorescence.

Figure 4. Identification of bile duct during robotic duodenum preserving pancreas head resection. A) Pancreatic duct (arrow) divided at its junction with the bile duct. B) Indocyanine green fluorescence demonstrates bile duct.

Figure 5. Visualization of the thoracic duct during robotic esophagectomy in normal mode (A) is enhanced by indocyanine green fluorescence (B). Also, the branching pattern of the thoracic duct is well visualized in fluorescence mode (C).

Figure 6. Visualization of perigastric lymph node during robotic D2 gastrectomy in normal mode (A) and fluorescence mode (B).

Figure 7. Objective evaluation of colonic perfusion using indocyanine green fluorescence during low anterior resection. Increasing fluorescence levels show the transition from blue (A) to red (B) and guide the selection of the line of bowel division. Fluorescence is displayed on a Grayscale (C). Fluorescence displayed in overlay mode (D).
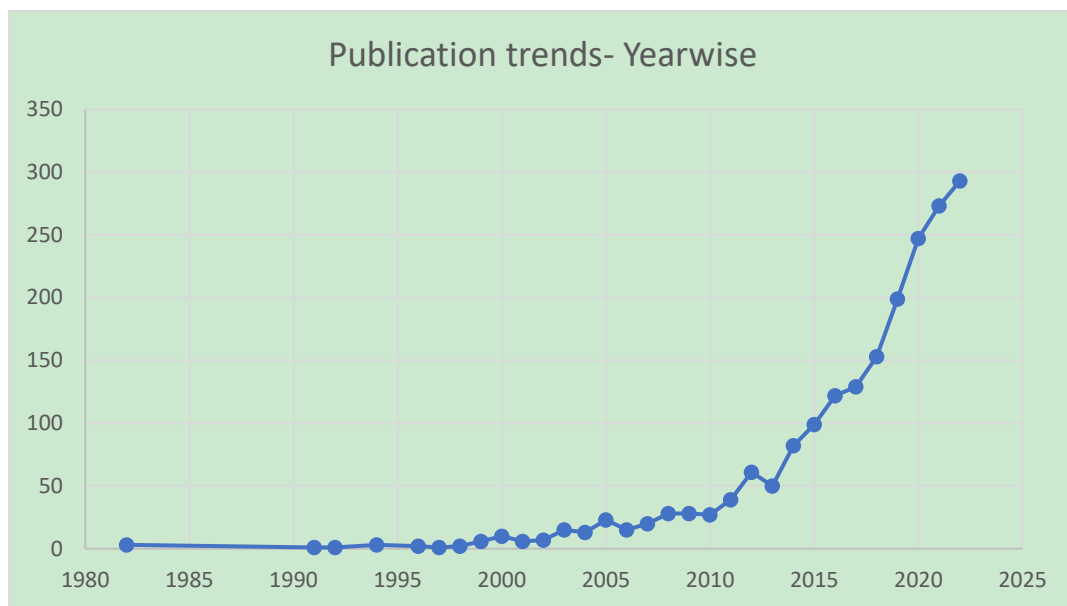
Figure 1 - Annual publication trend of articles related to indocyanine green fluorescence.
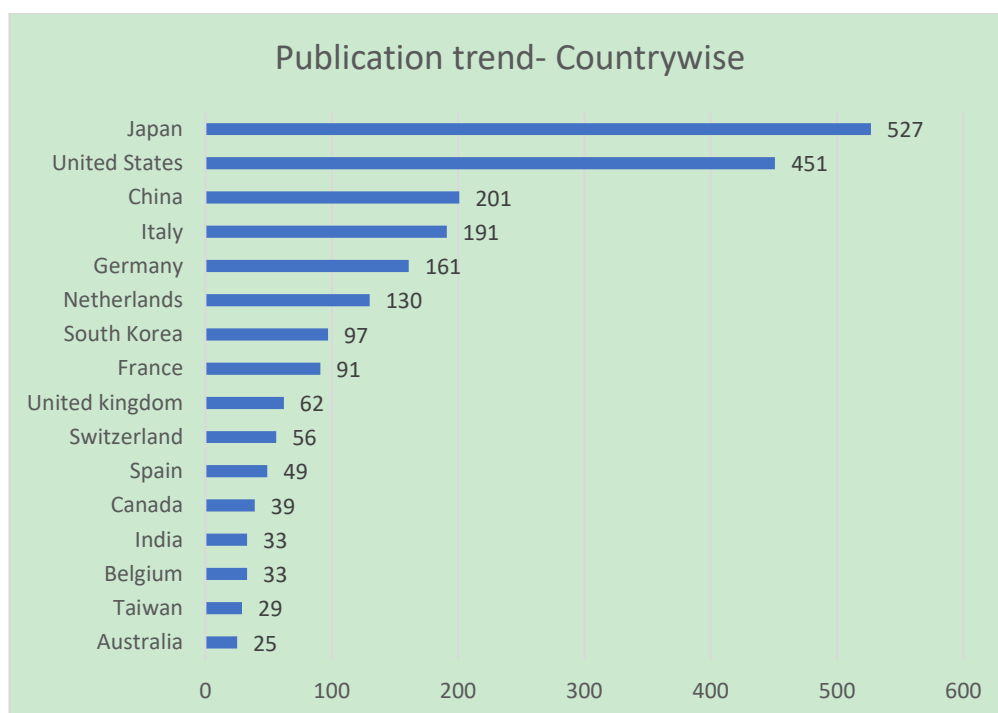
Figure 2 - Country wise publication trend of articles related to indocyanine green fluorescence.
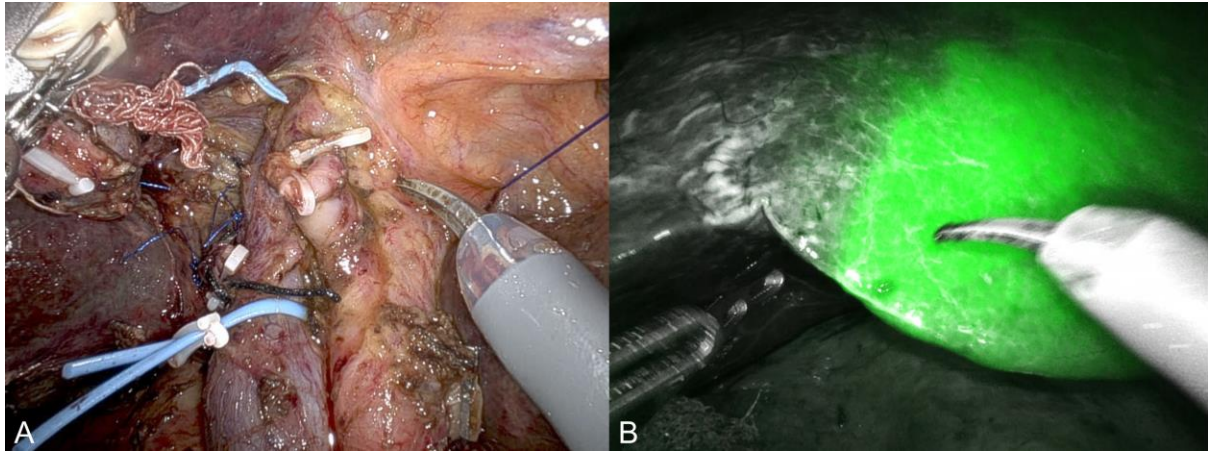
Figure 3. Negative **indocyanine green** staining technique used in a patient with hilar cholangiocarcinoma planned for right hepatectomy with caudate lobectomy. A) Portal and hepatic artery branches of the right hemiliver to be resected are ligated. B) line of transection for right hepatectomy identified with the help of ICG fluorescence.
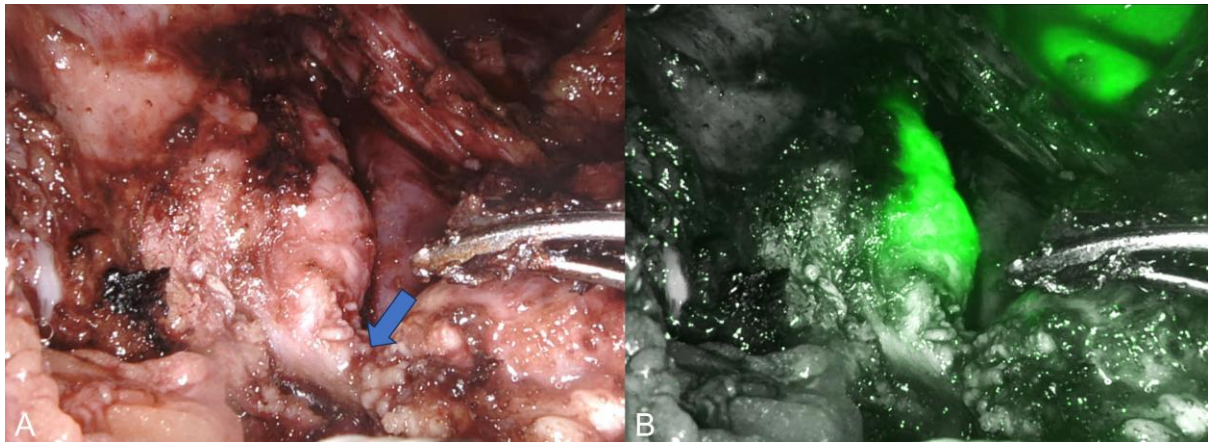
Figure 4. Identification of bile duct during robotic duodenum preserving pancreas head resection. A) Pancreatic duct (arrow) divided at its junction with the bile duct. B) Indocyanine green fluorescence demonstrates bile duct.
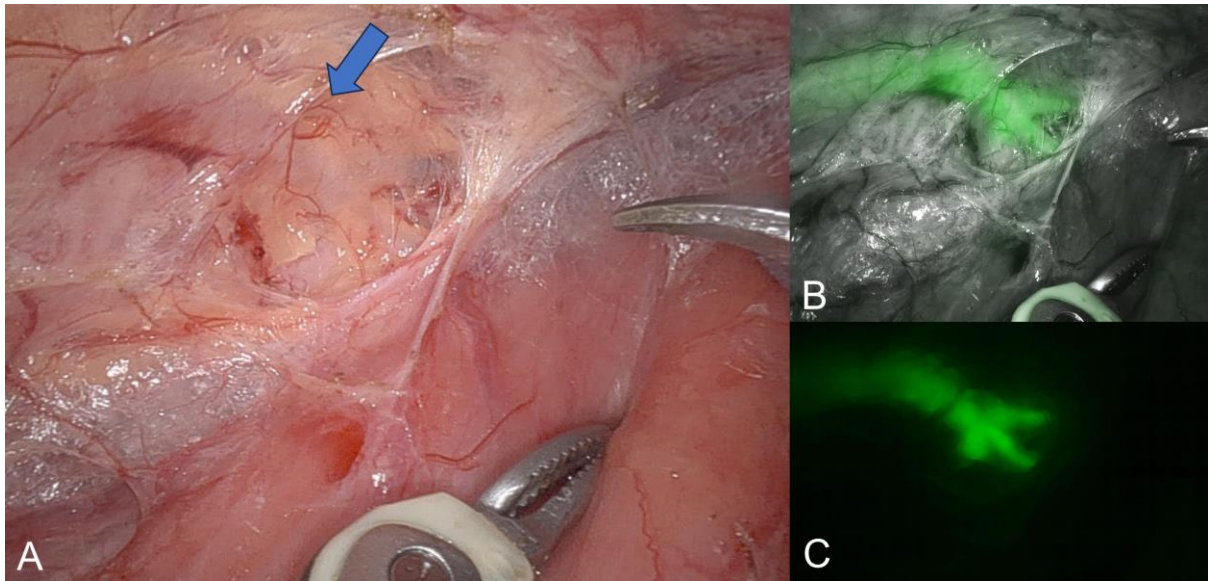
Figure 5. Visualization of the thoracic duct during robotic esophagectomy in normal mode (A) is enhanced by indocyanine green fluorescence (B). Also, the branching pattern of the thoracic duct is well visualized in fluorescence mode (C).

Figure 6. Visualization of perigastric lymph node during robotic D2 gastrectomy in normal mode (A) and fluorescence mode (B).
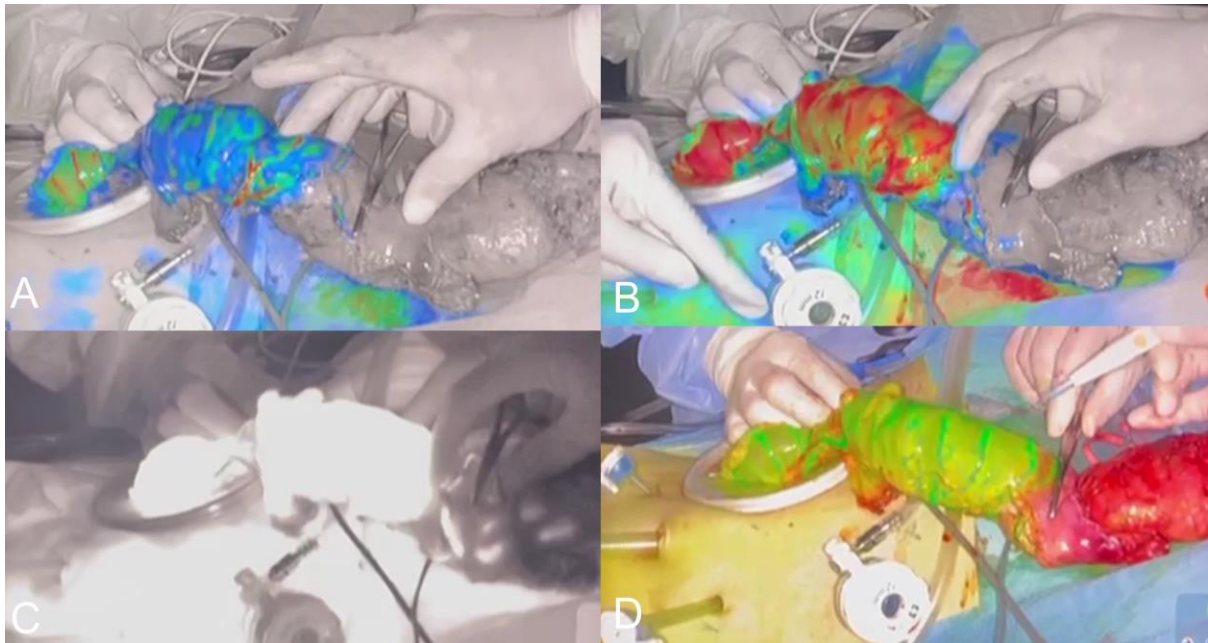
Figure 7. Objective evaluation of colonic perfusion using indocyanine green fluorescence during low anterior resection. Increasing fluorescence levels show the transition from blue (A) to red (B) and guide the selection of the line of bowel division. Fluorescence is displayed on a Grayscale (C). Fluorescence displayed in overlay mode (D).

Table 1 Studies on liver segmentation and mapping using direct staining technique.

Author, Year	Country	Study Type	Patient	Camera device	ICG dose and time	Guide/Approach	Target liver	Success Rate	Procedure time
Qian et al., 2022[16]	China	Prospective Cohort	Grp A- 10 Grp C - 20	-	0.0125 mg/ml upto 10 ml	DSA- Hepatic artery USG - Portal vein	Grp A - seg, sub-seg Grp c - seg	Grp A - 80 % Grp C - 60 %	Grp A - 305.3 ± 23.2 min Grp C - 268.4 ± 34.7 min
Li et al., 2021[17]	Taiwan	Prospective Cohort	8	Karl storz, Pinpoint Stryker	conc -0.125 mg/mL,rate of 1 mL/min	DSA - Hepatic artery	segment Bisegment	100%	DSA - 32.7 +/5.3 min, Operative time - 242 +/118 min
Lan et al., 2022[18]	China	Retrospective cohort	24	Pinpoint Stryker	5 ml of 0.025 mg/ml	Glissonian pedicle approach	Hemiliver- 10 Left lateral - 7 Segment - 7	79.40%	Staining time - 25.92 ± 14.64 Operative time - 334.17 ± 98.65
Xu et al., 2020[15]	China	Retrospective cohort	9	Pinpoint Stryker	0.025 mg/ml 5-10 ml	Intraoperative ultrasound Glissonian pedicle approach	Segment, Section	56%	Median operative time- 260 minutes(Range 150-360 min).
Ueno et al., 2019[13]	Japan	Prospective Cohort	10	Pinpoint Stryker	0.125 mg/ml 2 ml ICG 2 ml Indigo carmine 1 ml Sonazoid embolic agent-Gelatin particles	DSA	Segment	100%	IVR procedures- 41 (30-102) minutes Median operating time with IVR time- 432 minutes
Ueno et al., 2018[14]	Japan	Prospective Cohort	5	Pinpoint Stryker	0.125 mg/ml 2 ml ICG 2 ml Indigo carmine 1 ml Sonazoid embolic agent-Gelatin particles	DSA	Segment	100%	Operative time - 432 (293–572) min
Aoki et al., 2010[11]	Japan	Prospective cohort	81	PDE-2	5 mg/ml	IOUS	Segment Sub-segment	73/81 (90.1%)	NA

IOUS – Intraoperative ultrasound, DSA – Digital subtraction angiography, USG – Trans abdominal ultrasound, ICG – Indocyanine green

Table 2 - Studies on liver segmentation and mapping using Counterstaining technique.

Author, Year	Country	Study Type	Patients	Camera device	ICG dose and time	Approach	Target liver	Success Rate	Median (range) operative time, in minutes
Funamizu et al., 2021[21]	Japan	Retrospective cohort	74	Viscera Elite II, Olympus Pinpoint, Stryker Hopkins II, Karl storz	0.5 mg	Glissonian pedicle approach	Monosegmectomy	100%	351
Xu et al., 2020[15]	China	Retrospective cohort	27	Pinpoint, Stryker	2.5 mg/ml	Glissonian pedicle approach	Hemiliver Section Segment	52%	260 (150-360)
Uchiyama et al., 2011[19]	Japan	Prospective cohort	22	PDE; Hamamatsu Photonic	0.5 mg/kg	CE-IOUS Glissonian pedicle approach	Hemiliver- 8 Section - 8 Segment - 6	100%	280 (140 - 380)
Berardi et al., 2019[20]	Japan	Retrospective cohort	86 patients HCC-55 CRLM-31	Viscera Elite II, Olympus Pinpoint, Stryker Hopkins II, Karl storz	0.5 mg	Glissonian approach	Section -14 Segment - 56 Sub-segment - 16	98.80%	328 (270–437)

HCC – Hepatocellular carcinoma, CRLM – Colorectal liver metastasis, CE-IOUS – contrast enhanced intraoperative ultrasound.

Table 3 - Studies on hepatic tumor detection with indocyanine green fluorescence.

Author, Year	Country	Study type	Patients	Device	ICG dose and time	Tumor detection rate	New lesions	Correlation with histology	Micro metastases Detection
Cai et al., 2022[24]	China	Retrospective cohort	Total- 86 HCC-52 CRLM-34	Pinpoint, Stryker	Dose - 0.5 mg/kg ICG <7 - 5 days prior ICG >7 - 7 days prior	96%	8	Positive(P-0.001)	False positive-3/8 (37.5 %)
Franz et al., 2021[23]	Germany	Prospective cohort	Total - 18 HCC - 9 CRLM - 4 IHCC - 2	Firefly, DaVinci NIR-ICG, Karl storz	Dose - 0.5 mg/kg 2-14 days prior	100%	27.8	-	False positive-39%
Piccolo et al., 2021[25]	Italy	Prospective cohort	Total lesions - 29 HCC - 14 CRLM - 13 IHCC - 1 Non-CRLM - 1	NIR-ICG, Karl storz	0.5 mg/kg HCC/IHCC - 7 days prior CRLM - 5 days prior	ICG-100 % LUS-72.4 %	Nil	R0- 100%	-
Marino et al., 2020[27]	Spain	Retrospective cohort	Total lesions- 59 HCC- 23 CRLM-27 IHCC-6 Hemangioma-2 Steatosis-1	Davinci-Firefly	0.5 mg/kg 5 days prior	52/59 (88.1%)	6 (11.5%)	R0-100%	False positive- 3/52
Zhou et al., 2019[29]	China	Retrospective cohort	ICG-21 IOUS-21	Pinpoint, Stryker	0.25 mg/kg 3–5 days prior	100%	-	-	NA
Terasawa et al., 2017[26]	Japan	Prospective cohort	Total lesions-59 CRLM-46 HCC-7 Others - 6	Pinpoint, Stryker	0.5 mg/kg body within 3 days	45/53 (85%)	22/45	R0-100%	True negative - 100 %
Kudo et al., 2014[30]	Japan	Prospective cohort	Total lesions- 32 HCC- 16 CRLM - 16	Olympus	0.5 mg/kg within 14 days	23/32 (71.8%)	-	-	-

HCC – Hepatocellular carcinoma, CRLM – Colorectal liver metastasis, IHCC – Intrahepatic cholangiocarcinoma, CE-IOUS – contrast enhanced intraoperative ultrasound, ICG – Indocyanine green, LUS – Laparoscopic ultrasound.

Table 4 - Studies on lymphatic mapping in esophageal and gastric surgery.

Author, Year	Country	Study type	Patient	Device	ICG dose and time	Surgery	Results	Long term outcome
Esophageal								
Hachey et al., 2016[48]	USA	Prospective PFS	9 - EAC	NOVADA Q, Stryker	2.5mg/ml, 1 cc, 4 sites, Endoscopic injection Diluted with sterile water /HSA 25%	Ivor Lewis esophagectomy	6/9 patients showed 2-6 regional NIR +	NA
S. Y. Park et al., 2018[49]	Korea	Prospective PFS	29 -ESCC, cT1	Firefly, Da Vinci	0.5 mg/ml 0.5 ml each quadrant 1 day prior	Robotic Mckeown Esophagectomy	High NPV	NA
Stomach								
Chen et al., 2020[58]	China	RCT	ICG - 129 Non-ICG - 129	NOVADA Q, Stryker	Submucosal Endoscopic 1 day prior 2 ml, O.5 ml/quadrant 0.625 mg/ml	Laparoscopic distal and total gastrectomy	Lymph node yield Mean (SD) ICG - 50.5 [15.9] Non-ICG - 42.0 [10.3]	3-yr DFS - 81.4% vs 68.2% OS - 86.0% vs 73.6%
Park et al., 2020[51]	Korea	Prospective feasibility study PSM	ICG - 20 Non-ICG - 60	NOVADA Q, Stryker	Endoscopic Sub-mucosal 0.1 mg/ml, 1 ml 5 sites	Laparoscopic distal gastrectomy Station 6 dissection	ICG vs Non-CIG Lymph node yield 30.15 vs 32.55 Bleeding events 4 (20.0) vs 41 (68.3)	NA
Cianchi et al., 2019[53]	Italy	Retrospective PSM	ICG - 37 Non-ICG - 37	Firefly, Da Vinci	Endoscopic Sub-mucosal 0.1 mg/ml, 0.5 ml, 4 quatrants	Robotic distal or total gastrectomy	Operative time mean(SD) - 293.1 ± 6 vs 321.2 ± 77.8 harvested lymph nodes- vs 50.8 ± 17 vs	NA
Ushimaru et al., 2019[52]	Japan	Retrospective PSM	ICG - 84 Non-ICG - 84	NIR, Karl storz	Endoscopic submucosal 0.05 mg/ml, 10 ml, 4 sites	Laparoscopic distal gastrectomy	Operative time mean(SD) - 206.1(5.0) vs 237.0(5.0) harvested lymph nodes- 47.5 vs 42.6	NA

ICG – Indocyanine green, PSM – Propensity score matching, SD – Standard deviation, NIR – Near infra-red, DFS – Disease free survival, OS – Overall survival.

Table 5 - Studies on sentinel nodal navigation in early gastric cancer.

Author, Year	Country	Study type	Patient	ICG dose	Surgery	Mean SN yield	Results	Long term Outcome
Isozaki et al., 2019[56]	Japan	Multicenter Retrospective cohort	100	Intraop Endoscopic submucosal 1 ml 2.5% ICG 4 sites wait 15 mins Sentinel node biopsy followed by Frozen	Open SNNS/DiG WDG - 3 1/2 DG - 18 PPG - 19 SG - 31 LR - 29	3.8	Sensitivity - 85.7% Specificity - 98.9% Accuracy - 98% SBD SB nodes - 59.7% Hot nodes - 23.4% Hot and green - 12% Green nodes - 4.9%	5-yr OS - 89.6% Gastric Cancer specific survival - 98.5%
An et al., 2020[54] (SENORITA)	Korea	Multicenter RCT	580 patients LSG - 292 LSNNS - 245	Dual Tracer ICG 2·5 mg/ml, 2 ml, and 99mTc-HSA, 2 ml	LSG group Open - 3 LSG- 266 LSNNS group LSNNS - 210	9	Operative time LSG - 180 mins LSNNS- 192.5 mins Upstaging - 7.4%	NA
Miyashiro et al., 2014[55] (JCOG0302)	Japan	Multicenter RCT	440 patients Suspended	Serosal injection 4–5 ml 25 mg/5 ml ICG	Sentinel node biopsy followed by Gastrectomy with lymphadenectomy	4	False negative- 46.4%	NA

RCT – Randomized controlled trial, ICG – Indocyanine green, SNSS – Sentinel node navigation surgery, WDG - wide-extent distal gastrectomy, LR – Local resection of stomach, SG – Segmental gastrectomy, PPG – Pylorus preserving gastrectomy, OS – Overall survival, DiG – Diminished gastrectomy, SBD – Sentinel basin dissection, HN – Hot nodes, SB – Sentinel basin, LSG - laparoscopic standard gastrectomy, LSNNS – Laparoscopic sentinel node navigation surgery.