Mapping the mesentery using ICG

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Abstract (150 words)

Indocyanine green fluorescent (ICG) fluorescent imaging (ICG-FI) has been used in colorectal surgery to assess intra operative blood flow to the colon. Recently its use has expanded to allow imaging of the lymphatic drainage in cancer resections. This technique can been used for real time visualisation of lymph nodes, the detection of sentinel lymph nodes, lateral sidewall nodes, metastatic lymph nodes and peritoneal metastases. The technique is cheap and easy to use by the surgeon intra operatively. ICG lymphangiography has the potential to aid the surgeon to ensure complete lymphadenectomy is performed in cancer resections.

Indocyanine green fluorescent (ICG) fluorescent imaging (ICG-FI) has been established in colorectal surgery as a technique to perform fluorescence angiography. Using this technique the surgeon can assess intraoperative blood flow of the colonic conduit and so help guide decisions about resection margins, assessment of the anastomoses and the need for diverting stomas [1, 2].

The importance of lymph node harvest in reducing local recurrence in colorectal cancer is well established and part of most modern guidance on optimal oncological surgery is a minimum requirement of 12 lymph nodes in the resected specimen [3, 4]. It follows that colorectal surgeons have investigated the use of ICG-FI in lymphangiography in cancer resections.

In this review we describe its use in the real time visualisation of lymphatic drainage, detecting sentinel lymph nodes and for the detection of metastatic lymph nodes.

Indocyanine Green Fluorescence

ICG is a sterile, tricarboncyanine dye approved for clinical use, which fluoresces after excitation under near infra red light (806nm). It is detected with the use of specialized scope and camera [5]. Several camera models are available for open, laparoscopic and robotic surgery. The technique is inexpensive and the indications for its use continue to expand.

Intravascular injection has been used since the 1950s, side effects are rare [6]. The liver rapidly extracts ICG, appearing unconjugated in the bile about 5-8 min after intravenous injection, depending on liver vascularisation and function [7]. It is anionic and highly water-soluble, though relatively hydrophobic, and rapidly binds to plasma proteins, particularly lipoproteins. For lymphangiography ICG is injected interstitially. Due to the high protein content of lymph, it accumulates in the lymphatic pathways and loco regional lymph nodes, and is associated with longer periods of visualisation of ICG compared to intravenous administration.

Technique of ICG Intraoperative Administration for Lymphangiography

The injection can be placed into the peri tumoral region, either as an extra luminal/subserosal or intra luminal (endoscopic)/submucosa injection. Soares et.al have described a technique of laparoscopic injection with 10mg of ICG diluted into 4mls (+3mls chaser for the connector tubing) of saline injected using a butterfly needle at 4 points in the periphery of the colonic tumour. The wings of the butterfly needle are shortened with scissors to allow the needle to be passed via the laparoscopic port closest to the tumour. The needle is inserted subserosally and 1ml is injected at each of the 4 points, with care to avoid spillage, which would contaminate the field. Surgery is then continued, and the intra red mode camera is used 30-40 minutes after the injection to image the area, with the mesentery being examined on both sides [8].

ICG-FI for real time lymphangiography in colorectal cancer surgery

Intra operative real time laparoscopic observation of lymph flow in the mesentery may be useful to determine the extent of mesentery division, and so improve the accuracy of oncological resection. This is especially relevant for resection for tumours located at hepatic or splenic flexure, as lymphatic drainage at these sites can vary and surgical technique is not standardised. It has been reported that complete mesocolic excision (CME) with central vascular ligation may improve prognosis [(9),] however these is on going debate regarding the benefits of this technique. ICG may be used as an adjunct to perform more precise CME oncological resections by more clearly delineating lymph-containing mesocolon.

In 2016 Watanabe et al. [(10)] published a series of 20 consecutive patients where ICG-FI was used intra operatively to identify the lymph node flow before mesentery dissection. ICG (0.5mL) was injected using a 23G needle at two sites in the submucosal layer during intra operative colonoscopy in the peritumour region and lymph node flow was observed 30 minutes after injection. The authors reported the technique was feasible and helpful, and changed the extent of mesentery excision in 5 cases (25%). In 2017 Nishigori et al. published a similar study, reporting the use of ICG changed the surgical plan for lymphadenopathy in 23.5% [(11).]

A more recent study by Chand et al. of 10 consecutive patients showed lymphatic uptake in 100% of patients using the alternative technique of subserosal peritumoral injection of ICG (1ml). Optimal timing to demonstrate lymphatic drainage occurred in 10 minutes, after which no further lymphatics were demonstrated [(8)]. In 2 patients (20%), additional or aberrant lymph nodes were localised outside the proposed

resection margin. In these cases the resection margin was extended to include the fluorescent node, and histopathology of the node was positive in both cases.

Watanabe et al. published a further case series of 31 patients with splenic flexure colon cancer, reported mesentery lymph flow was seen in all patients [10]. The direction of lymph node flow was towards the root of the IMV in 19 (61.3%) cases. Interestingly no case exhibited flow to both the left colic artery and the left branch of the middle colic artery, leading authors to speculate that it is not necessary to ligated both the middle colic and left colic artery, in the absence of widespread lymph node spread. Authors concluded that for distal transverse colon tumours, CME of the left branch of the middle colic and root of the IMV should be performed, whereas for tumors in the proximal descending colon, CME with dissection of the left colic artery and the root of the IMV should be performed.

The relationship between ICG-FI visualised mesenteric lymphatic flow and clinicopathological factors in colon cancer was investigated by Ushijima et al [12]. The rate of visualised lymphatic flow was significantly higher in patients with a lower clinical stage. Patients with stage III and IV colon cancer had a higher incidence of non-visualized lymphatic flow, suggesting the technique is less effective in higher stage disease.

ICG-FI for the identification of sentinel lymph nodes in colorectal cancer surgery

Lymph node status is the most important prognostic factor in colon cancer, however the use of sentinel lymph node (SLN) detection techniques has been controversial in terms of clinical significance, accuracy and prognostic value. The SLN is the first to receive drainage from the primary tumour, and its positivity indicates likely further lymph node involvement, and so the need for further lymphadenectomy. SLN positivity also has implications for prognosis and adjuvant therapies.

Various techniques have been described, including direct visualisation after injection of patent blue dye or methylene blue dye, scintigraphy after injection of radio colloid around the tumour [(13),] and most recently detection using ICG-FI. The reported sensitivity of the all techniques varies widely, from 33-100% [(14),] with no standardized technique described.

ICG-FI detected SLN detection in colorectal cancer was first reported by Nagata et al in 2006 [(12).] Authors reported a detection rate 97.9% and overall false negative rate of 46.2%. All false negative results occurred in T3 tumours, suggesting technique may be affected due to occlusion of lymphatic channels. Van der Pas et al. also noted the technique was less effective in larger (>7cm) tumours, concluding this may be due to occlusion of lymph vessels by tumor ingrowth or metastatic disease [(15).] A recent meta-analysis published by Villegas-Tovar et al. included 11 studies, reporting a pooled detection rate of 91% (80-98%) [(16).]

ICG for the identification of pelvic sidewall lymph nodes in rectal cancer

It has been reported 7% of patients undergoing TME will have positive pelvic sidewall nodes. The improved oncological outcomes have been reported with the resection of metastatic pelvic side wall nodes. ICG-FI has been used as an adjunct to detect lateral pelvic sidewall lymph nodes in rectal cancer[26]. Kazanowski et al. demonstrated the feasibility of pelvic sidewall node mapping in 2015 [(17).] 5 patients with low rectal tumours were injected with submucosal ICG at the tumour site via proctoscopy at commencement of resection. In all five cases, pathology confirmed the presence of lymph node tissue; none had cancer cells evident on pathological processing .In 2019 Zhou et al. demonstrated this technique by injecting ICG in the submucosal layer of the rectum endoscopically in patients with low to middle rectal cancer [(18).] Authors reported use of ICG-FI was associated with a lower intra operative blood loss and greater number of pelvic lymph nodes harvested.

ICG-FI for the identification of metastatic lymph nodes in colorectal cancer

The primary aim of colorectal cancer surgery is to perform an excision to remove the tumour and associated involved lymph nodes. It follows that detection of tumour containing lymph nodes would be useful and theoretically would allow surgeons to tailor resections to the patient. In early stage or polyp cancers, if the nodes are detected to be absent of metastatic disease, in theory a minimal resection could be performed. Alternatively, if nodes are detected as having metastatic disease, a more extensive, or extra anatomical resection could be performed.

Intra operative ICG FI has been used to detect metastatic lymph nodes in colon cancer. Clinically malignant lymph nodes appear more florescent than benign lymph nodes [19]. A signal to background ratio is used to determine which nodes are likely malignant, verses benign lymph nodes, which have the same fluoresce as surrounding tissue. A meta-analysis by Emile et al. in 2017 including 12 studies, reported a median sensitivity, specificity and accuracy of 73.3%, 100% and 75.7% [20]. In a more recent meta-analysis by Villegas-Tovar et al. in 2020 including 11 studies pooled sensitivity of 64.3% and specificity of 65% was reported suggesting a poorer performance of ICG for the detection of metastatic lymph nodes [16].

ICG for the identification of peritoneal carcinomatosis

The detection of peritoneal metastases (PM) is important to ensure accurate staging and treatment of colorectal cancer. Cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) is the standard treatment for patients with limited PM. Peritoneal metastases are often not apparent on pre operative staging imaging. The surgeon performing a visual assessment of the peritoneal cavity at the time of resection is the current method of detecting peritoneal metastases. ICG has been assessed as a method to improve detection of peritoneal metastases.

In 2016, Librale et al. reported the results of the first feasibility study of ICG for the detection of CRC PM [21]. Authors reported results of its use in 17 consecutive patients, reporting ICG-FG can detect PM in non mucinous cancer after intra

operative IV ICG injection with a sensitivity of 87.5%. ICG detected additional PM in 3 patients (21.4%), and the results of ICG led to a modification of the surgical plan in 4 patients (29%). In a similar proof of concept study published the same year, Barabino et al reported a sensitivity of 72.4% and specificity of 60%, with 29% change in proposed operation [22]. In 2018 Lieto et al. reported a sensitivity of 87.5% in non mucinous tumours, and specificity of 100% in non mucinous tumours, with 25% change in planned operation [23].

Limitations of the Technique

Cancer stage appears to impact on the sensitivity of the ICG-FG technique, with early stage cancers showing improved sensitivity. Theoretically the lymph node flow may be occluded in cases of extensive lymph node metastases or due to transmural spread from large tumours. Similarly the effect of neoadjuvant treatments on ICG uptake is unknown. It is likely the effect would be greatest in patient having neoadjuvant radiotherapy for rectal cancer. The body habitus of the patient may also impact on the efficacy of the technique. ICG-FI tissue penetration is limited to a few millimetres (approx. 15mm). In patients with thick mesenteries due to obesity this can mean reduced visibility. Further research is required to determine which patient groups would benefit most from the technique.

Future Directions

Recently reports of conjugated fluorophores have appeared in the literature. Fluorophores can be conjugated to radioisotopes or to cancer specific antibodies, such as anti-carcinoembryonic antigen (CEA). The use of radioisotopes such as Tc-99 has the advantage of increased visualation due to increased tissue penetration. This may be useful in obese patients with fatty mesenteries. The use of cancer specific antibodies has the advantage of accurately detecting the tumour alongside with metastases.

CEA is overexpressed in > 90% of colorectal cancer, making it an ideal target to conjugate with ICG. SGM-101 is a tumor-targeting agent consisting of a flurophore (BM-104) covalently bound a the monoclonal antibody that targets CEA [(24).] Boogerd et al. published the study in colorectal cancer patients, reporting successful detection of primary, recurrent and metastasised CRC during surgery, leading to an altered treatment plan in one-third of patients [(25).] A multiphase phase III study is in progress to assess the use of cancer specific fluorophores is underway. Given lymph node harvest is an independent prognostic factor for survival, the ability to perform real time accurate identification of lymph node metastases to guide the extent of resection is likely to have benefits to the surgeon, and a survival benefit for the patient.

Conclusion

The application of ICG-FI for lymphangiography has a lot of potential, and may allow surgeon to perform more precise, personalised cancer surgery. Early reports have

shown ICG-FI lymphangiography is a safe and easy technique with many potential applications. This review describes it applications, including real time lymph node mapping, detection of sentinel node lymph nodes, the detection of lateral sidewall nodes, and the detection of metastatic lymph nodes and peritoneal metastases. Further research is required to determine how to best apply these techniques into clinical practice.

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