The Use of IndoCyanine Green Fluorescence in the Assessment of Bowel Perfusion in Emergency and Elective Colorectal Surgery

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Abstract

Background The use of Indocyanine Green (ICG) fluorescence is a well-established technique in colorectal surgery for the evaluation of bowel stump perfusion. However there is still no definitive acceptance, except intraoperative macroscopic evidence, with reference to the incidence of anastomotic leakage (AL). The objective of this study is to confirm the same efficacy and reliability of ICG in elective colorectal surgery, and emergency cases, which would be more exposed to complications related to inadequate vascularization.

Methods From January 2019 to June 2020, we used ICG to evaluate the perfusion of colonic

stumps before and after packaging the anastomosis in right and left hemicolectomy, rectal resection and Hartmann's reversals.

Results A total of 40 patients underwent surgery, 21 (52.50%) had benign pathology and 19 (47.50%) exhibited malignant neoplasia. 13 (32.50%) were emergency surgeries and 27 (67.50%) were planned elective surgeries. In almost all cases, the postoperative course was regular, in only 1 (2.5%) case of TaTME there was an AL. Other complications were not related to the anastomosis, but some validated the excellent perfusion despite episodes of prolonged acute ischemia due to postoperative hemorrhage. Data were finally compared to a control group of 39 patients where the ICG fluorescence was not used.

Conclusions The study confirms the validity of the use of ICG fluorescence as a method for intraoperative assessment of bowel perfusion even in emergency conditions and in acute postoperative hemorrhage, detecting an incidence of 2.5% (1 case out of 40) of AL. It's evident that to validate our results, further randomized studies on a larger data set are required. It would also be beneficial to evaluate quantitatively the fluorescence between the mucous and serous layer, to confirm the reduction of AL rate, the better evaluation of bowel perfusion and, especially in emergency surgeries, the potential reduction of further operations.

Introduction

Anastomotic leakage (AL) is the most feared complication in intestinal surgeries, particularly in colorectal surgeries. The incidence varies between 1.2% and 19.2% for all interventions [1–3], with a peak of 39% in rectal cancer with low or ultra-low localization [4–8],with a higher incidence and variability in emergency surgery cases [9].

Despite the important technological advances of the last number of years in terms of technique and surgical devices, AL complications has always remained significant and unchanged in terms of incidence, with inevitable prolongation of hospital stay and costs. In 95% of cases, the anastomotic leak required further surgery with a high mortality rate [4, 10, 11]. Furthermore, more than half of the patients requiring re-operation will have a permanent stoma [12]as a consequence of this further surgery. Although the etiopathogenesis involves several factors, the main and most determining factor is the adequate

evaluation of the vascular supply to the intestinal tissues [13–17]. To date the assessment of good vascular supply has been evaluated only using clinical criteria (color of the tissues, bleeding of the margins, arterial pulse). These clinical criteria are insufficient to make objective and quantifiable judgements during surgery.

The angiographic visualization with fluorescence, by means of a dedicated near infra-red (NIR) endoscopic video camera, allows the surgeon to view in NIR light the presence in the tissues of a fluorescence dye, Indocyanine Green (ICG). ICG is administered intravenously, which binds to plasma proteins and reaches all organs and regions of the body with excellent tolerability, few side effects and extremely low toxicity with the ability to absorb light in the NIR wavelength between 600 and 900 nm. The intensity of its visualization is proportional to its quantity in the tissues and, therefore, to the vascular perfusion of the same. The purpose of this study is to evaluate the impact of this intraoperative imaging technique on the perfusion of the intestinal stump in order to obtain an optimal anastomosis, reducing the AL and, in emergency cases, to reduce re-operations and, at the same time to increase the number of primary anastomosis.

Materials And Methods

From January 2019 to June 2020 at the Department of General and Emergency Surgery of San Filippo Neri Hospital (Rome, Italy) we used fluorescence with ICG to evaluate the perfusion of the colonic stumps before and after the packaging of the anastomosis in emergency and elective colorectal surgery.

A total of 79 patients underwent colorectal surgery in this period. Thirtynine patients were included in the control group being operated without using ICG to evaluate the perfusion of the colonic stump and of the anastomosis, due to several factors such as inexperience of the surgeon, inadequate setting or timing of surgery. Of these patients, 20 (51.28%) were operated for colonic malignancy and 19 (48.72%) for benign pathologies, 15 (38.46%) in an emergency setting and 24 (61.54%) in an election setting. Twentyone patients (53.85%) had associated cardiovascular, pulmonary or mand metabolic comorbidities and 7 patients (17.95%) had a BMI greater than 30 (Table2).

In parallel, a group of 40 patients, the ICG-group, were evaluated with ICG method; 21 (52.50%) were operated for benign pathology and 19 (47.50%) for malignant neoplasia; 13 (32.50%) in an emergency setting and 27 (67.50%) in an elective setting. 19 patients (47.5%) had associated cardiovascular, pulmonary and metabolic comorbidities and six (15%) had a BMI greater than 30 (Table 1).

Table 1
Patients characteristics

	ICG (40 pts)	Control (39 pts)
Age **(yr)**		
Mean (SD)	62.6 (10.5)	67.74 (13.4)
<50 *n* (%)	3 (7.5)	1 (2.5)
50-70 *n* (%)	30 (75)	25 (64.2)
71-90 *n* (%)	7 (17.5)	13 (33.3)
Gender n **(%)**		
Female	23 (57.5)	20 (51.3)
Male	17 (42.5)	19 (48.7)
BMI kg/m^2 n **(%)**		
<20	0 (0)	0 (0)
20-24	28 (70)	11 (28.2)
25-29	6 (15)	19 (48.7)
>30	6 (15)	9 (23.1)
Comorbidity n **(%)**		
Hypertension	15 (37.5)	11 (28.2)
Diabetes	7 (17.5)	3 (7.7)
Ischemic heart disease	3 (7.5)	3 (7.7)
Previous abdominal surgery	2 (5)	2 (5.1)
Psycosis	2 (5)	2 (5.1)
Smoking	2 (5)	3 (7.7)
Dyslipidemia	1 (2.5)	3 (7.7)
Colorectal disease n **(%)**		

Value are expressed as mean (SD = standard deviation) or *n (%)*

ICG Indocyanine green-guided colorectal surgery; BMI Body mass index

ERAS Enhanced Recovery After Surgery

pts Patients; yr years

	ICG (40 pts)	Control (39 pts)
Benign	21 (52.5)	19 (48.7)
Malignancy	19 (47.5)	20 (51.3)
Setting n **(%)**		
Urgency	13 (32.5)	15 (38.5)
Election	27 (67.5)	24 (61.5)
ERAS n **(%)**	26 (65)	4 (10.3)
Value are expressed as mean (SD = standard deviation) or *n (%)*		
ICG Indocyanine green-guided colorectal surgery; BMI Body mass index		
ERAS Enhanced Recovery After Surgery		
pts Patients; yr years		

Table 2
Surgical procedures

	ICG-CRS (40 pts)	Control (39 pts)
Type of surgery n **(%)**		
Right hemicolectomy	7 (17.5)	22 (56.4)
Left hemicolectomy	14 (35)	9 (23.1)
Trasverse colectomy	2 (5)	1 (2.6)
Rectal anterior resection	3 (7.5)	0 (0)
TaTME	4 (10)	0 (0)
Atypical resection	2 (5)	0 (0)
Hartmann's reversal	8 (20)	6 (15.4)
Subtotal Colectomy	0 (0)	1 (2.6)
Technique n **(%)**		
Laparoscopic	31 (77.5)	4 (10.3)
Open	9 (22.5)	35 (89.7)
Anastomosis ICG review n **(%)**		
Redo	0 (0)	0 (0)
Revision of strategy	3 (7.5)	0 (0)
Value are expressed as *n (%)*		
ICG Indocyanine green-guided colorectal surgery		
TaTME Transanal Total Mesorectal Excision; ICG Indocyanine green; Redo Reanastomosis		
pts Patients		

ICG powder (Verdye - 5 mL/25 mg, Diagnostic Green GmbH, Germany), is diluted in 10 mL of sterile pyrogen-free distilled water (for injections) and administered intravenously in the patient's peripheral vein, with the dose calculated per the patient's body weight.

ICG was developed for photography by Kodak as early as 1955 and approved for clinical use in 1959 by the US FDA. The molecule, when injected into the bloodstream, binds to plasma proteins (especially lipoproteins), it reaches arteries and veins in five to 50 seconds and it is rapidly excreted by the liver in the bile, where it appears after about eight minutes, depending on the vascularization and liver function. Fluorescence remains for several hours. The standard dose for clinical use (0.1-0.5 mg/ml/kg) is well

tolerated. The ICG gives off fluorescence when a laser or NIR light is excited at around 820 nm and is detected using dedicated cameras and optics (Fig. 1).

We used a dedicated laparoscopic system (KARL STORZ GmbH & Co. KG, Tuttlingen, Germany) with the image generated by a Full HD camera (IMAGE 1 SPIESTM, KARL STORZ) connected to a laparoscope at 30 degrees and 10 mm in diameter with a specific filter for NIR fluorescence. The light source (D-LIGHT P SCB, KARL STORZ) then allows, by the use of a foot pedal, to "switch" between NIR and standard light according to the surgeon's needs.

In the case of intestinal perfusion, in particular, the epiploic appendages and the mesentery are "colored" first, then it reaches the intestinal wall (the antimesenteric margin of the descending and transverse colon are paler due to the greater thickness) and, if well vascularized, the wall gradually turns a bright green.

The dose we used was 0.3-0.4 mg/ml/kg in a single administration before the resection of the proximal colonic stump or in administered twice (in a dose of 5 mL each) before the section of the stump and after the packaging of the anastomosis. Given the rapid appearance of the dye in the bloodstream, the timing of administration is practically instantaneous (about 50 seconds before the evaluation of perfusion). In case of anterior rectal resection, we also carried out endoluminal control of the anastomosis.

Furthermore, in the last 18 patients operated in order of time (seven left hemicolectomies, five right hemicolectomies, one TaTME, 3 anterior rectal resection, two Hartmann reversal) we carried out a double pre-anastomotic evaluation of the serous (Fig. 2) and mucous side (Fig. 3a-b) simultaneously (then also evaluated by post-anastomotic endoluminal view only the rectum), in consideration of the different sensitivity to ischemia of the two wall layers to of the mucous one, as evidenced by recent studies [18].

Due to the technological system in those cases operated in laparoscopy, in which the colic stump was prepared extracorporeally, and in all open operations, in order to clearly visualize the fluorescence and its intensity in the different colic segments, the chamber of operating theatre must be darkened with operating theatre lights reduced with the visual field reduced in diameter as necessary. This technological limit will be overcome with the imminent introduction of real-time unification of fluorescence images with infrared (IR) and simultaneous white light (real-time overlay).

All procedures performed in this study were in accordance with the Helsinki declaration and with the ethical standards of the hospital research committee that approved the study. All experimental protocols and all methods were carried out in accordance with relevant guidelines and regulations. All participants signed the informed consent prior to enrolling in this study.

Results

In general, surgical procedures guided by fluorescence with ICG have demonstrated advantages in the patient’s length of stay (LOS) in hospital, especially for colorectal surgery, with a reduction in anastomotic leakage rates and redo anastomosis, at the expense of a slight increase in operating time. This study as

well as multiple studies completed to date, confirms that this imaging technology is safe, effective and sustainable [19].

In the control group, a total of 39 patients (19 males and 20 females), with an average age of 67.7 ± 13.4, underwent colorectal surgery with primary anastomosis, nineteen for benign pathologies and twenty for malignancy. We performed nine left colectomies (two laparoscopic and seven open, one with ileus resection associated and one with splenectomy associated), twenty-two right colectomies (one laparoscopic, one with duodenal resection associated, one with ureteral resection associated, one with jejunal and bladder resection associated), one transverse resection, six Hartmann reversals (one laparoscopic) and one subtotal colectomy.

In the ICG-group, a total of 40 patients (17 males and 23 females), with an average age of 62.6 ± 10.5, underwent ICG-guided colorectal surgery,21 for benign pathology and 19 for malignant neoplasia. Of these, 13 operations were carried out in an emergency setting and 27 as elective procedures (Table 1).

In this group, we performed seven right hemicolectomies (two open and five laparoscopic), 14 laparoscopic left hemicolectomies, two laparoscopic transverse resections, three laparoscopic anterior rectal resection, four TaTME with protective ileostomy, eight Hartmann reversals (six open and two laparoscopic), one laparoscopic resection and anastomosis for colo-cutaneous stenosis and fistula after left hemicolectomy for neoplasia, one open resection and anastomosis for stenosis after left hemicolectomy for diverticulitis with ureteral reconstruction (Table 2).

In all the cases of malignant neoplasia, we have followed a classic medium-lateral approach with "high" vascular ligation. In cases of benign pathology and for Hartmann's reversal procedures we have opted, in most cases, for peripheral vascular ligatures, especially in the

case of elderly patients with significant associated comorbidities. The same strategy was used in emergency cases. The evaluation of the ICG perfusion was carried out before the resection of the proximal stump on the serous and mucous side (Fig. 2, 3a-b) and after the packaging of the anastomosis on the serous side (Fig. 4a-b), on the mucous one only in the case of rectal resection. In fact, in case of "low" rectal resection, the fluorescence evaluation of the distal stump perfusion was difficult to visualize on the serous side for anatomical reasons (distal part of the anorectal canal) and is almost exclusively entrusted to the evaluation of the mucous side by endoluminal view (Fig. 5). No adverse reactions were recorded during the administration of ICG. We did not perform a reanastomosis (redo) in the treated patients, but we did a revision of the section line (revision of decision) of the proximal ileal stump in a right hemicolectomy, in a Hartmann's reversal and in a left hemicolectomy (7.5%). In cases where we carried out a strategy review, we found no AL nor any other complication.

The postoperative course in the control group was characterized by a morbidity rate of 41.03%: there were five grade I complications according to the Clavien-Dindo classification, four grade II complications, two grade IIIa complications, two grade IIIb complications, five grade V complications.

Among anastomotic complications, we registered seven cases of anastomotic leak (17.95%); two of them underwent relaparotomy but died despite resurgery because of a Multi Organ Failure (MOF), two others died without a relaparotomy. In addition to these patients, we registered 1 more decease due to a cerebrovascular accident, with a total mortality rate of 12.82%.

In the ICG group, the postoperative course was regular in most cases (Table 3). The overall morbidity rate was 20%. There have been three complications of grade IIIb according to the Clavien-Dindo classification, two of grade IIIa, one of grade II and finally two of grade I. Among the complications concerning more specifically the anastomosis, one case of anastomotic leak occurred (2.5%) in a patient operated on TaTME and affected by chronic ischemic heart disease. It was a small anastomotic leak ("delimited" according to the classification of Schein [20]), which contributed an intraoperative malfunction of the circular stapler with only partial suture of lumen circumference, which was treated and resolved with surgical drainage. There was also one case (2.5%) of stenosis of colorectal anastomosis after resection of the sigma for perforated diverticulitis. The total incidence of complications related to the packaging of the anastomosis in ICG-guided colorectal surgery was therefore 5%. Other complications (one death due to stroke after 30 days with a course complicated by massive haematemesis from duodenal stress ulcer, one reoperation for hemoperitoneum in post-operative day (pod) two, one reoperation for unrecognized small bowel lesion due to adhesiolysis in pod one, one enterocutaneous fistula from radiological drainage of abscess collection on perisplenic hematoma solved spontaneously, two superficial incisional wound infections SSI) did not concern the anastomosis, but rather validated excellent perfusion despite episodes of prolonged acute ischemia from postoperative hemorrhage (Table 3).

Table 3
Postoperative complications

	ICG-CRS (40 pts)	Control (39 pts)
Overall complications n (%)	8	22
Anastomotic leakage	1 (2.5)	7 (17.9)
Anastomotic stenosis (remote)	1 (2.5)	0 (0)
Abdominal pelvic abscess	1 (2.5)	2 (5.1)
Surgical reintervention	2 (5)	2 (5.1)
Surgical wound infection	2 (5)	5 (12.8)
Ureteral injury	0 (0)	1 (2.6)
Mortality	1 (2.5)	5 (12.8)
Clavien-Dindo classification scale n (%)		
No Complications *(Dindo grade 0)*	32 (80)	21 (53.8)
Complications *(Dindo grade I)*	2 (5)	5 (12.8)
Complications *(Dindo grade II)*	1 (2.5)	4 (10.3)
Complications *(Dindo grade IIIa)*	2 (5)	2 (5.1)
Complications *(Dindo grade IIIb)*	3 (7.5)	2 (5.1)
Complications *(Dindo grade IV)*	0 (0)	0 (0)
Mortality *(Dindo grade V)*	1 (2.5)	5 (12.8)
Value are expressed as *n (%)*		
ICG-CRS Indocyanine green-guided colorectal surgery		
pts Patients		

We investigated the difference in AL incidence between ICG and control groups with a simple chi-square test, obtaining a p-value of 0.022 (Table 4). Although further studies with larger samples are needed, our results allow us to assert that the use of the ICG has a statistically significant role in reducing the incidence of anastomotic leak.

Table 4

ICG-CSR	Non-ICG-CSR	AL ICG-CSR group (%)	AL non-ICG-CSR group (%)	p-Value
40	39	1 (2.5)	7 (17.9)	0.022

Discussion

In recent years we have witnessed important surgical technological advancements (advanced energy devices, electrical staplers with gradual compression and triple row of stitches, increasingly refined laparoscopic instruments, robotic surgery, high resolution imaging systems associated or not with 3D), but nevertheless the biggest and most dramatic problem of colorectal surgery and consequently also the greatest cause of concern for the

surgeon continues to be the packaging and consequently the tightness of the intestinal anastomosis, considering that the percentage of post-operative leakage have remained practically unchanged [21]. In fact, the AL rate varies from 1–19% depending on the cases and anatomical location: ileocolic (1% - 8%); colocolic (2% - 3%); ileorectal (3% -7%); colorectal or coloanal (5% -19%) anastomosis [22–24]. Several factors have been associated with an increased risk of AL: male gender, age, comorbidity, increased ASA score, malnutrition, obesity, cigarette smoking, immunosuppression and in particular cortisone therapy, alcohol abuse, neoadjuvant radio-chemotherapy, advanced staged tumors, diverticulitis, low rectal anastomosis, prolonged operating times, blood loss or perioperative transfusions and intraoperative sepsis conditions [22, 25, 26]. Above all, an adequate vascular perfusion of the anastomosis is essential for optimal healing and for the prevention of AL [15, 27, 28]. As a result, intraoperative evaluation of intestinal ischemia could significantly reduce its risk during surgery. In the evaluation of intraoperative intestinal perfusion, the use of fluorescence of ICG, a dye already used for other applicationssince the 1960's, seems to be a method that can potentially reduce the incidence of AL after colorectal surgery. Fluorescence Guided Surgery using ICG, allows the surgeon to view intestinal microperfusion in real time, is a fast and easy method to implement at the operating table, which can change the surgical resection strategy, as demonstrated by numerous recent studies [29–35].

In this retrospective study, the AL rate using guided NIR-ICG imaging is lower (3.6%) than the rate reported in the literature without using this method. If we also consider the case of remote stenosis of the colorectal anastomosis, which required endoscopic dilatative

treatment, our results show a relatively low and satisfactory level of complications (7.1%). We report the same percentage (7.1%) for changing surgical strategy (revision of strategy) due to insufficient perfusion as identified by fluorescence of the proximal intestinal stump which required further resection before the anastomosis. It is very likely that this is the key point of the effectiveness for ICG-based fluorescence, namely a better evaluation of perfusion compared to traditional macroscopic parameters (arterial pulse, bleeding, bowel color, peristalsis, etc.) [36].

Several studies have been published regarding the shifting of the resection line of the intestinal stump [21, 29, 32, 34, 35, 37–41]. Wada et al. reported that margin resection was changed in 16% of cases [32], while Jafari et al. reported in their Pillar II trial a rate of 6.5% of modification of margin resection [38]. Jun Watanabe et al. [39] in their guided ICG rectal resections, brought the section line more proximally towards a more adequate area of fluorescence in 12 cases (5.7%). Otero-Pineiro et al., during a comparative study on TaTME, reported a variation rate of the

proximal resection strategy of 28.7% with an incidence of AL of 2.5% as opposed to 11.3% of the non-ICG group [40]. Morales-Conde et al. [41], in a prospective study, hypothesized how the ICG could have a different utility depending on the type of surgery. They analyzed four group of patients: Group A, right hemicolectomy; Group B, segmental resection of the splenic flexure; Group C, left hemicolectomy; and Group D, anterior rectal resection. Fluorescence with ICG led to significant changes in the section line major in left hemicolectomy, followed by rectal resection and confirmed promise in reducing the AL rate, considering that it occurred in 5 patients (2.6%): 2 in group A (3%), 1 in C (1.2%), and 2 in D (5.7%).

Systematic reviews and meta-analysis also confirm that intraoperative use of fluorescence with ICG is a promising tool for reducing the risk of AL in colorectal surgery. However, the bias inherent in the fact that these are not RCT studies must be taken into consideration when interpreting these data sets. R. Blanco-Colino et al. [33] analyzed five articles on left colon surgery and report how the planned level of anastomosis was changed in 7.4% of cases (41 of 555 patients in the ICG-FI group), while showing an overall AL rate of 5.4%.

The meta-analysis by Renhui Shen et al. [42] reached the same result, even if conducted only on four non homogeneous non randomized studies. It is evident that the considerable heterogeneity and bias related to the mix of the cases operated in this study make difficult an accurate interpretation of the data. As a matter of fact, there are several studies that consider and compare different colorectal interventions. Impellizzeri G. et al. [43] analyzed left hemicolectomy, sigmoidectomy and rectal resection interventions, showing a change of strategy in 8.2% of cases with an AL rate of 3%. F. Ris et al. [37] in a multicenter phase II trial included benign and malignant pathologies operated with different colorectal procedures (right and left hemicolectomy, ileoanal and ileorectal pouches, rectal resections, Hartmann reversal) and they reported a revision rate of 5.8% with an incidence of AL of 2.4%.

In literature there are also two noteworthy multicenter RCTs (randomized controlled trials), one by De Nardi et al. [44], which evaluated 240 patients operated for left hemicolectomy and rectal resection, divided into two groups with and without ICG. They reported a revision rate of 11%, an AL rate of 5% (for the ICG group) vs 9% without ICG, but statistically not significant. Another key multicenter RCT which is still actively in progress, is the IntAct trial - [45] an ICG study of rectal surgery, which has the 90-day AL rate as its primary endpoint.

The evaluation of the ICG perfusion can be performed according to several steps: before and after the vascular preparation of the proximal intestinal stump, highlighting the fluorescence of the serous side; after the section of the stump on both sides, serous and mucous, and after the packaging of the anastomosis on the serous side.

In case of a "low" rectal resection (i.e. the distal part of the anorectal canal), the evaluation with ICG of the perfusion of the distal stump is always very difficult to visualize for anatomical reasons on the serous side and is almost exclusively entrusted to the evaluation of the mucous side by endoluminal view [46]. According to a recent experimental study, conducted by the group at the IHU Institute in Strasbourg [18], the perfusion of an ischemic segment of the pig colon was analyzed by fluorescence with ICG on the two

sides, serous and mucous, and simultaneously the lactate concentration was measured in different areas. The study showed that the average ischemic zone of the mucous side, measured in mm, is significantly wider than the serous one. From this, it can be seen that an evaluation of intestinal perfusion only on the serous side can underestimate the extent of the ischemia area. Knowing this, in our study we also

evaluated the mucous side with ICG during the preparation of the proximal stump before the introduction of the stapler and, for the rectum, the evaluation of the same layer by endoanal evaluation after the packaging of the anastomosis. In our study, we evaluated eight out of 28 patients (one RAR and five laparoscopic hemicolectomies, one laparoscopic right hemicolectomy and one open right hemicolectomy, four in emergency and four in elective surgery) and none of them experienced AL or other post-operative complications. Further studies will be required to predict the optimal resection margin and the anastomosis site.

Morever we believe that the use of fluorescence in surgery is justifiable in relation to costs, considering the significantly potential patient benefits. In fact, AL not only increas the risk of mortality and LOS [36], but it has been associated with a long-term reduction in tumor-specific survival [47, 48].

A similar discourse could be made in case of emergency surgery. Indeed, with this bowel perfusion assessment, it could be possible to reduce the number of intestinal derivative operations in favor of intestinal resections with primary anastomosis. As the most recent

data suggests [22] a net saving in costs is related to lower patient morbidity, reduced hospitalization and a reduced number of intestinal reconstructions. It is clear that the new technologies must be cost-effective in order for them to be used more widely. Regardless of the mortality and morbidity associated with AL, recent economic assessments suggest that,, hospital admission costs may increase between € 11,900 [49] and € 20,300 [50] directly associated with AL complications, and these costs do not include subsequent additional

costs related to recanalization, stoma care and to the loss of productivity related to the patient, post discharge. The cost for five years of these endoscopic systems is approximately € 110,000, while the cost of the ICG for three vials is approximately € 130. This shows that if NIR-ICG proved to be truly effective in reducing AL complications, the costs of the technology would be more than adequatelycovered. The quantitative definition of adequate or inadequate bowel perfusion remains poorly defined, because none of the laparoscopic ICG-FI systems currently available are able to quantify the fluorescent signal. Even if the operator reports a positive visual evaluation, a better standardization in the interpretation of the signal would allow for a more objective evaluation.

Indeed, the kinetic analysis of the maximum intensity signal could also reveal non-arterial perfusion effects (such as venous return). Automated analysis software programs for more precise and objective quantification have recently been developed [51, 52] and NIR-ICG systems with quantitative evaluation software are likely to soon enter the market.

Targeted fluorophores activated by specific cells or microenvironments (perhaps local tissue pH, hypoxia, lactate or other metabolites) are in development [53] and could allow a more precise identification of tissues [54] than ICG. Other experimental techniques have been described to evaluate the blood supply and the integrity of the anastomosis. These include intraoperative endoscopy, pulse oximetry, Doppler ultrasound and Doppler flowmetry, intramucous pH measurement, visible light oxygen spectroscopy and NIR oxygen spectroscopy. None of the techniques mentioned is regularly used, mainly due to its complexity and its high variability of measurements [55–57].

In this regard, Barberio M. et al. [58] analyzed quantitatively, in an animal model, intestinal perfusion with fluorescence-based enhanced reality (FLER) compared with Hyperspectral imaging (HSI).

HSI is another optical imaging technique that combines a spectroscope and a camera, enabling non-contrast, real-time, qualitative and quantitative tissue analysis based on tissue oxygen saturation (StO2). The limitation of HSI in the current phase of development is the lack of adequate video speed and the absence of a system for minimally invasive surgery. In the study, HSI produced more accurate results than fluorescence angiography so that in the future this technology, if made more functional and practical, could be a useful and contrast-free intraoperative tool to quantify intestinal ischemia..

In conclusion, it is evident that further randomized controlled studies on larger and more homogeneous data are necessary to validate our findings. In the future, in order to assess more precisely the bowel perfusion with NIR-ICG fluorescence, it also needs a quantitative analysis not only of the serous layer, but also of the mucous layer. However, as many studies and trials have already highlighted, the reduction of incidence of anastomotic leakage, the evidence of a better perfusion of the bowel and, especially in emergency, the possible reduction of derivative interventions in favor of primary anastomosis are clear.

Declarations

Ethics approval and consent to participate: All procedures performed in this study were in accordance with the Helsinki declaration and with the ethical standards of the hospital research committee that approved the study. All experimental protocols and all methods were carried out in accordance with relevant guidelines and regulations. All participants signed the informed consent prior to enrolling in this study.

Consent for publication: not applicable

Availability of data and material: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests: Drs. Biagio Picardi, Stefano Rossi, Simone Rossi Del Monte, Francesco Cortese, Edoardo Maria Muttillo, Gennaro Mazzarella and Irnerio Angelo Muttillo have no conflicts of interest or financial ties to disclose.

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References

1. Van Geldere D, Fa-Si-Oen P, Noach LA, Rietra PJ, Peterse JL, Boom RP. Complications after colorectal surgery without mechanical bowel preparation. *J Am Coll Surg*. 2002;194(1):40–47. doi:10.1016/s1072-7515(01)01131-0
2. Matthiessen P, Hallböök O, Rutegård J, Simert G, Sjödahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer: a randomized multicenter trial. *Ann Surg*. 2007;246(2):207–214. doi:10.1097/SLA.0b013e3180603024
3. Boccola MA, Lin J, Rozen WM, Ho YH. Reducing anastomotic leakage in oncologic colorectal surgery: an evidence-based review. *Anticancer Res*. 2010;30(2):601–607.
4. Law WL, Chu KW. Anterior resection for rectal cancer with mesorectal excision: a prospective evaluation of 622 patients. *Ann Surg*. 2004;240(2):260–268. doi:10.1097/01.sla.0000133185.23514.32
5. Montedori A, Cirocchi R, Farinella E, Sciannameo F, Abraha I. Covering ileo- or colostomy in anterior resection for rectal carcinoma. *Cochrane Database Syst Rev*. 2010;(5):CD006878. Published 2010 May 12. doi:10.1002/14651858.CD006878.pub2
6. Dehni N, Schlegel RD, Cunningham C, Guiguet M, Tiret E, Parc R. Influence of a defunctioning stoma on leakage rates after low colorectal anastomosis and colonic J pouch-anal anastomosis. *Br J Surg*. 1998;85(8):1114–1117. doi:10.1046/j.1365-2168.1998.00790.x
7. Enker WE, Merchant N, Cohen AM, et al. Safety and efficacy of low anterior resection for rectal cancer: 681 consecutive cases from a specialty service. *Ann Surg*. 1999;230(4):544–554. doi:10.1097/00000658-199910000-00010
8. Karliczek A, Harlaar NJ, Zeebregts CJ, Wiggers T, Baas PC, van Dam GM. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. *Int J Colorectal Dis*. 2009;24(5):569–576. doi:10.1007/s00384-009-0658-6
9. Meyer J, Naiken S, Christou N, et al. Reducing anastomotic leak in colorectal surgery: The old dogmas and the new challenges. *World J Gastroenterol*. 2019;25(34):5017–5025. doi:10.3748/wjg.v25.i34.5017

10. Choi HK, Law WL, Ho JW. Leakage after resection and intraperitoneal anastomosis for colorectal malignancy: analysis of risk factors. *Dis Colon Rectum*. 2006;49(11):1719–1725. doi:10.1007/s10350-006-0703-2
11. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. *Int J Colorectal Dis*. 2008;23(3):265–270. doi:10.1007/s00384-007-0399-3
12. Lindgren R, Hallböök O, Rutegård J, Sjödahl R, Matthiessen P. What is the risk for a permanent stoma after low anterior resection of the rectum for cancer? A six-year follow-up of a multicenter trial. *Dis Colon Rectum*. 2011;54(1):41–47. doi:10.1007/DCR.0b013e3181fd2948
13. Kingham TP, Pachter HL. Colonic anastomotic leak: risk factors, diagnosis, and treatment. *J Am Coll Surg*. 2009;208(2):269–278. doi:10.1016/j.jamcollsurg.2008.10.015
14. Boyle NH, Manifold D, Jordan MH, Mason RC. Intraoperative assessment of colonic perfusion using scanning laser Doppler flowmetry during colonic resection. *J Am Coll Surg*. 2000;191(5):504–510. doi:10.1016/s1072-7515(00)00709-2
15. Vignali A, Gianotti L, Braga M, Radaelli G, Malvezzi L, Di Carlo V. Altered microperfusion at the rectal stump is predictive for rectal anastomotic leak. *Dis Colon Rectum*. 2000;43(1):76–82. doi:10.1007/BF02237248 *27
16. Sheridan WG, Lowndes RH, Young HL. Tissue oxygen tension as a predictor of colonic anastomotic healing. *Dis Colon Rectum*. 1987;30(11):867–871. doi:10.1007/BF02555426
17. Kologlu M, Yorganci K, Renda N, Sayek I. Effect of local and remote ischemia-reperfusion injury on healing of colonic anastomoses. *Surgery*. 2000;128(1):99–104. doi:10.1067/msy.2000.107414
18. Seeliger B, Agnus V, Mascagni P, et al. Simultaneous computer-assisted assessment of mucosal and serosal perfusion in a model of segmental colonic ischemia [published online ahead of print, 2019 Nov 18]. *Surg Endosc*. 2019;10.1007/s00464-019-07258-z. doi:10.1007/s00464-019-07258-z
19. Vettoretto N, Foglia E, Ferrario L, et al. Could fluorescence-guided surgery be an efficient and sustainable option? A SICE (Italian Society of Endoscopic Surgery) health technology assessment summary. *Surg Endosc*. 2020;34(7):3270–3284. doi:10.1007/s00464-020-07542-3
20. Moshe Schein, Paul N. Rogers, Ari Leppäniemi, Danny Rosin, Jonathan E. Efron. Chapter 47 Anastomotic leaks and fistulas. Schein's Common Sense Emergency Abdominal Surgery, 4th Edition TFM Publishing, Shropshire, UK, 2016
21. Hellan M, Spinoglio G, Pigazzi A, Lagares-Garcia JA. The influence of fluorescence imaging on the location of bowel transection during robotic left-sided colorectal surgery. *Surg Endosc*. 2014;28(5):1695–1702. doi:10.1007/s00464-013-3377-6 *31 *43
22. Lee JM, Bai P Chang J, El Hechi M, et al. Hartmann's Procedure vs Primary Anastomosis with Diverting Loop Ileostomy for Acute Diverticulitis: Nationwide Analysis of 2,729 Emergency Surgery Patients. *J Am Coll Surg*. 2019;229(1):48–55. doi:10.1016/j.jamcollsurg.2019.03.007
23. Phitayakorn R, Delaney CP, Reynolds HL, et al. Standardized algorithms for management of anastomotic leaks and related abdominal and pelvic abscesses after colorectal surgery. *World J*

Surg. 2008;32(6):1147–1156. doi:10.1007/s00268-008-9468-1.

24. 2015 European Society of Coloproctology collaborating group. The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit [published online ahead of print, 2017 Mar 6]. *Colorectal Dis*. 2017;10.1111/codi.13646. doi:10.1111/codi.13646
25. Kingham TP, Pachter HL. Colonic anastomotic leak: risk factors, diagnosis, and treatment. *J Am Coll Surg*. 2009;208(2):269–278. doi:10.1016/j.jamcollsurg.2008.10.015
26. Chadi SA, Fingerhut A, Berho M, et al. Emerging Trends in the Etiology, Prevention, and Treatment of Gastrointestinal Anastomotic Leakage. *J Gastrointest Surg*. 2016;20(12):2035–2051. doi:10.1007/s11605-016-3255-3
27. Rutegård M, Rutegård J. Anastomotic leakage in rectal cancer surgery: The role of blood perfusion. *World J Gastrointest Surg*. 2015;7(11):289–292. doi:10.4240/wjgs.v7.i11.289
28. Sparreboom CL, Wu ZQ, Ji JF, Lange JF. Integrated approach to colorectal anastomotic leakage: Communication, infection and healing disturbances. *World J Gastroenterol*. 2016;22(32):7226–7235. doi:10.3748/wjg.v22.i32.7226
29. Boni L, Fingerhut A, Marzorati A, Rausei S, Dionigi G, Cassinotti E. Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. *Surg Endosc*. 2017;31(4):1836–1840. doi:10.1007/s00464-016-5181-6
30. Jafari MD, Lee KH, Halabi WJ, et al. The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. *Surg Endosc*. 2013;27(8):3003–3008. doi:10.1007/s00464-013-2832-8 *44
31. Kawada K, Hasegawa S, Wada T, et al. Evaluation of intestinal perfusion by ICG fluorescence imaging in laparoscopic colorectal surgery with DST anastomosis. *Surg Endosc*. 2017;31(3):1061–1069. doi:10.1007/s00464-016-5064-x *45
32. Wada T, Kawada K, Takahashi R, et al. ICG fluorescence imaging for quantitative evaluation of colonic perfusion in laparoscopic colorectal surgery. *Surg Endosc*. 2017;31(10):4184–4193. doi:10.1007/s00464-017-5475-3
33. Blanco-Colino R, Espin-Basany E. Intraoperative use of ICG fluorescence imaging to reduce the risk of anastomotic leakage in colorectal surgery: a systematic review and meta-analysis. *Tech Coloproctol*. 2018;22(1):15–23. doi:10.1007/s10151-017-1731-8 *47
34. Guraieb-Trueba M, Frering T, Atallah S. Combined endoscopic and laparoscopic real-time intra-operative evaluation of bowel perfusion using fluorescence angiography. *Tech Coloproctol*. 2016;20(12):883–884. doi:10.1007/s10151-016-1547-y *48
35. Gröne J, Koch D, Kreis ME. Impact of intraoperative microperfusion assessment with Pinpoint Perfusion Imaging on surgical management of laparoscopic low rectal and anorectal anastomoses. *Colorectal Dis*. 2015;17 Suppl 3:22–28. doi:10.1111/codi.13031
36. McDermott FD, Heeney A, Kelly ME, Steele RJ, Carlson GL, Winter DC. Systematic review of preoperative, intraoperative and postoperative risk factors for colorectal anastomotic leaks. *Br J*

Surg. 2015;102(5):462–479. doi:10.1002/bjs.9697

37. Ris F, Liot E, Buchs NC, et al. Multicentre phase II trial of near-infrared imaging in elective colorectal surgery. *Br J Surg*. 2018;105(10):1359–1367. doi:10.1002/bjs.10844
38. Jafari MD, Wexner SD, Martz JE, et al. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. *J Am Coll Surg*. 2015;220(1):82-92.e1. doi:10.1016/j.jamcollsurg.2014.09.015
39. Watanabe J, Ishibe A, Suwa Y, et al. Indocyanine green fluorescence imaging to reduce the risk of anastomotic leakage in laparoscopic low anterior resection for rectal cancer: a propensity score-matched cohort study. *Surg Endosc*. 2020;34(1):202–208. doi:10.1007/s00464-019-06751-9
40. Otero-Piñeiro AM, de Lacy FB, Van Laarhoven JJ, et al. The impact of fluorescence angiography on anastomotic leak rate following transanal total mesorectal excision for rectal cancer: a comparative study [published online ahead of print, 2020 Feb 18]. *Surg Endosc*. 2020;10.1007/s00464-020-07442-6. doi:10.1007/s00464-020-07442-6
41. Morales-Conde S, Alarcón I, Yang T, et al. Fluorescence angiography with indocyanine green (ICG) to evaluate anastomosis in colorectal surgery: where does it have more value? [published online ahead of print, 2019 Oct 4]. *Surg Endosc*. 2019;10.1007/s00464-019-07159-1. doi:10.1007/s00464-019-07159-1
42. Shen R, Zhang Y, Wang T. Indocyanine Green Fluorescence Angiography and the Incidence of Anastomotic Leak After Colorectal Resection for Colorectal Cancer: A Meta-analysis. *Dis Colon Rectum*. 2018;61(10):1228–1234. doi:10.1097/DCR.0000000000001123
43. Impellizzeri HG, Pulvirenti A, Inama M, et al. Near-infrared fluorescence angiography for colorectal surgery is associated with a reduction of anastomotic leak rate [published online ahead of print, 2020 Apr 6]. *Updates Surg*. 2020;10.1007/s13304-020-00758-x. doi:10.1007/s13304-020-00758-x
44. De Nardi P, Elmore U, Maggi G, et al. Intraoperative angiography with indocyanine green to assess anastomosis perfusion in patients undergoing laparoscopic colorectal resection: results of a multicenter randomized controlled trial. *Surg Endosc*. 2020;34(1):53–60. doi:10.1007/s00464-019-06730-0
45. Armstrong G, Croft J, Corrigan N, et al. IntAct: intra-operative fluorescence angiography to prevent anastomotic leak in rectal cancer surgery: a randomized controlled trial. *Colorectal Dis*. 2018;20(8):O226-O234. doi:10.1111/codi.14257
46. Grieco M, Belia F, Persiani R. ICG fluorescence triple check during TaTME. Laparosc Surg 2020;4:12. doi: 10.21037/ls.2019.10.02
47. Katoh H, Yamashita K, Wang G, Sato T, Nakamura T, Watanabe M. Anastomotic leakage contributes to the risk for systemic recurrence in stage II colorectal cancer. *J Gastrointest Surg*. 2011;15(1):120–129. doi:10.1007/s11605-010-1379-4
48. Mirnezami A, Mirnezami R, Chandrakumaran K, Sasapu K, Sagar P, Finan P. Increased local recurrence and reduced survival from colorectal cancer following anastomotic leak: systematic review and meta-analysis. *Ann Surg*. 2011;253(5):890–899. doi:10.1097/SLA.0b013e3182128929

49. Ashraf SQ, Burns EM, Jani A, et al. The economic impact of anastomotic leakage after anterior resections in English NHS hospitals: are we adequately remunerating them?. *Colorectal Dis*. 2013;15(4):e190-e198. doi:10.1111/codi.12125
50. Hammond J, Lim S, Wan Y, Gao X, Patkar A. The burden of gastrointestinal anastomotic leaks: an evaluation of clinical and economic outcomes. *J Gastrointest Surg*. 2014;18(6):1176–1185. doi:10.1007/s11605-014-2506-4
51. Diana M, Agnus V, Halvax P, et al. Intraoperative fluorescence-based enhanced reality laparoscopic real-time imaging to assess bowel perfusion at the anastomotic site in an experimental model. *Br J Surg*. 2015;102(2):e169-e176. doi:10.1002/bjs.9725
52. Diana M, Noll E, Diemunsch P, et al. Enhanced-reality video fluorescence: a real-time assessment of intestinal viability. *Ann Surg*. 2014;259(4):700–707. doi:10.1097/SLA.0b013e31828d4ab3
53. Daly HC, Sampedro G, Bon C, et al. BF_2-azadipyrromethene NIR-emissive fluorophores with research and clinical potential. *Eur J Med Chem*. 2017;135:392–400. doi:10.1016/j.ejmech.2017.04.051
54. Nguyen QT, Tsien RY. Fluorescence-guided surgery with live molecular navigation–a new cutting edge. *Nat Rev Cancer*. 2013;13(9):653–662. doi:10.1038/nrc3566
55. Hirst NA, Tiernan JP, Millner PA, Jayne DG. Systematic review of methods to predict and detect anastomotic leakage in colorectal surgery. *Colorectal Dis*. 2014;16(2):95–109. doi:10.1111/codi.12411
56. Nachiappan S, Askari A, Currie A, Kennedy RH, Faiz O. Intraoperative assessment of colorectal anastomotic integrity: a systematic review. *Surg Endosc*. 2014;28(9):2513–2530. doi:10.1007/s00464-014-3520-z.
57. Karliczek A, Benaron DA, Baas PC, et al. Intraoperative assessment of microperfusion with visible light spectroscopy in esophageal and colorectal anastomoses. *Eur Surg Res*. 2008;41(3):303–311. doi:10.1159/000155880
58. Barberio M, Felli E, Seyller E, et al. Quantitative fluorescence angiography versus hyperspectral imaging to assess bowel ischemia: A comparative study in enhanced reality [published online ahead of print, 2020 Mar 26]. *Surgery*. 2020;S0039-6060(20)30074-X. doi:10.1016/j.surg.2020.02.008

Figures

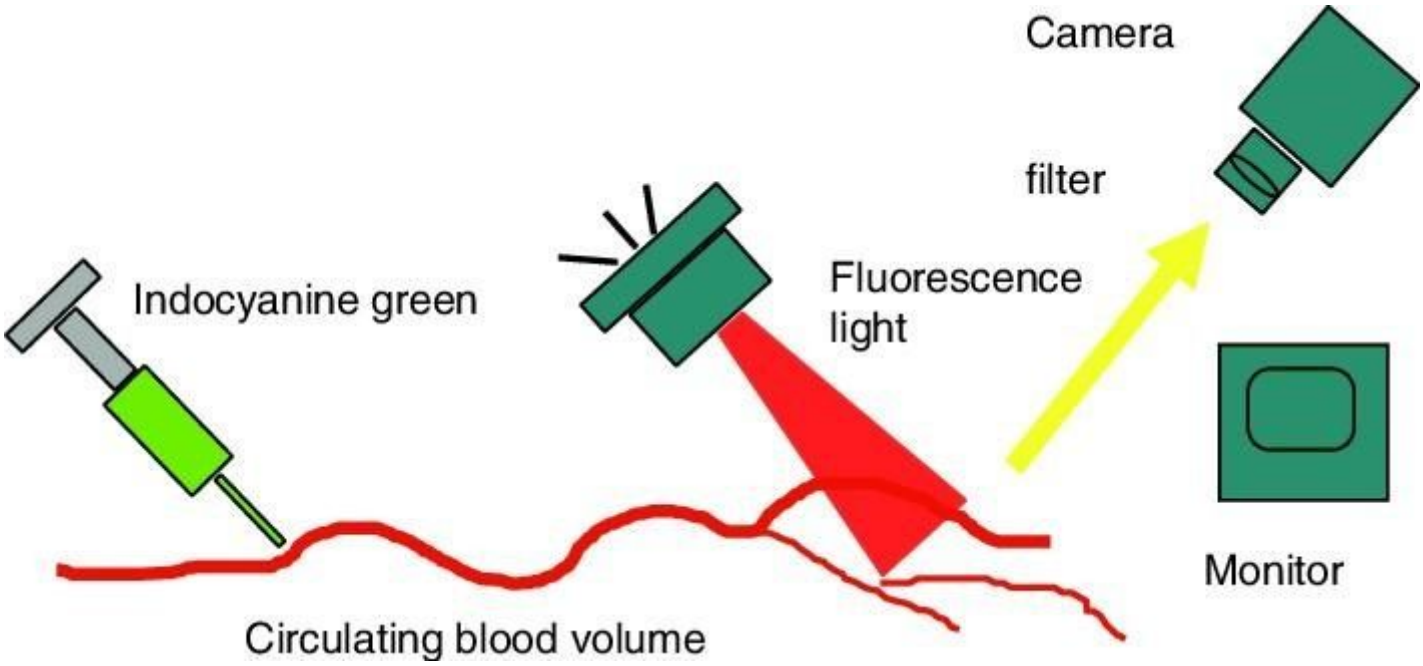

Figure 1

Mechanism of ICG fluorescence

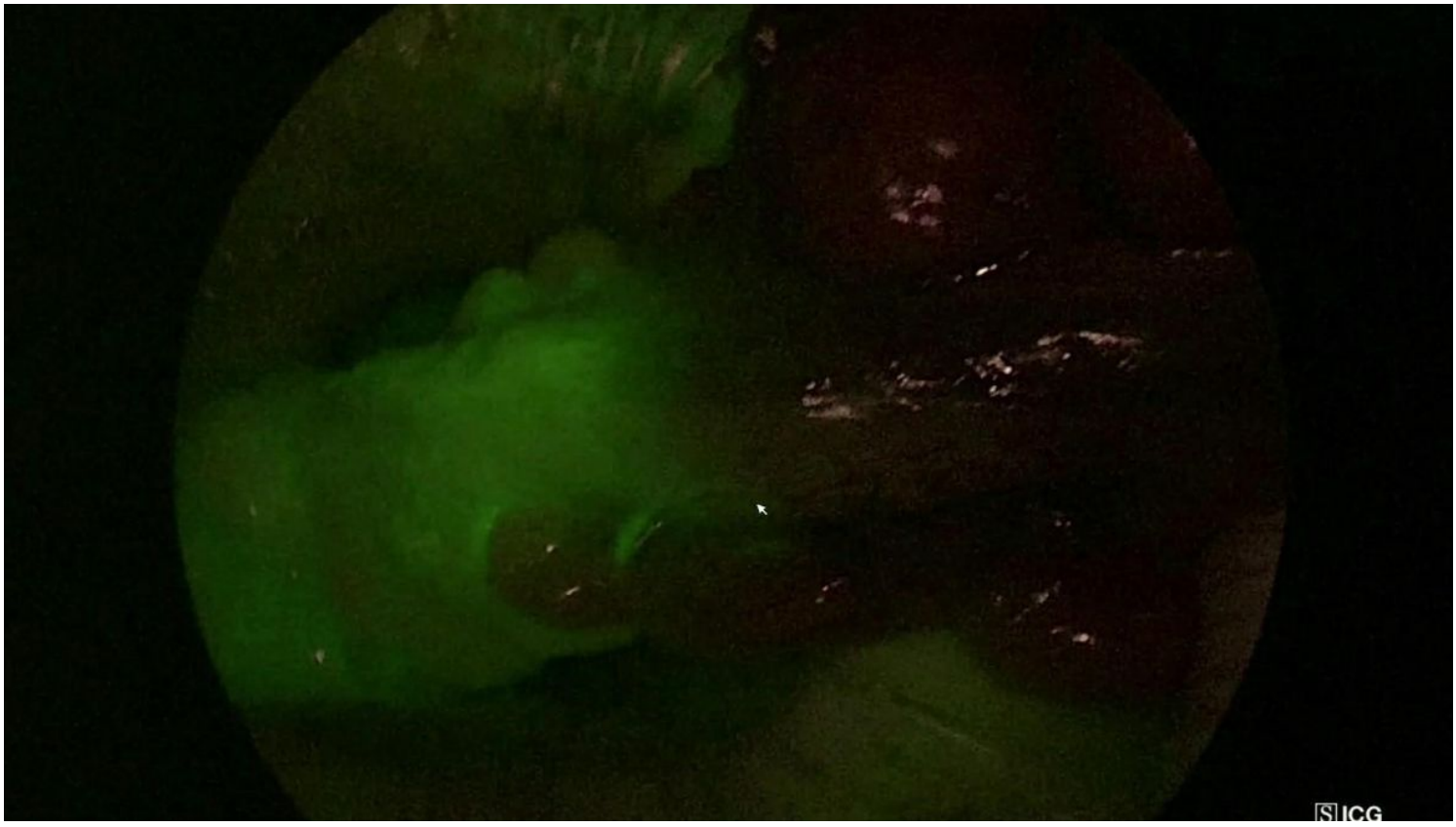

Figure 2

Pre-anastomotic check: serous side (ICG)

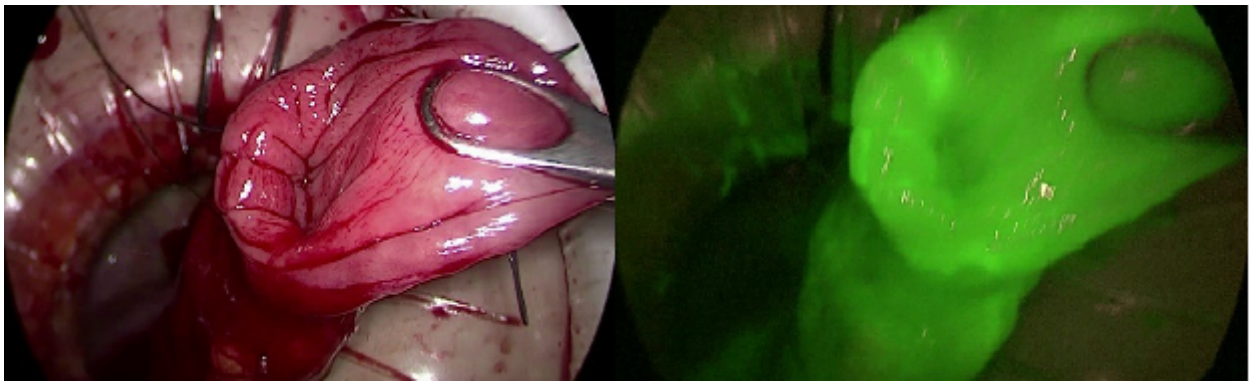

Figure 3

Pre-anastomotic check: mucous side Pre-anastomotic check: mucous side (ICG)

Figure 4

Post-anastomotic check: serous side Post-anastomotic check: serous side (ICG)

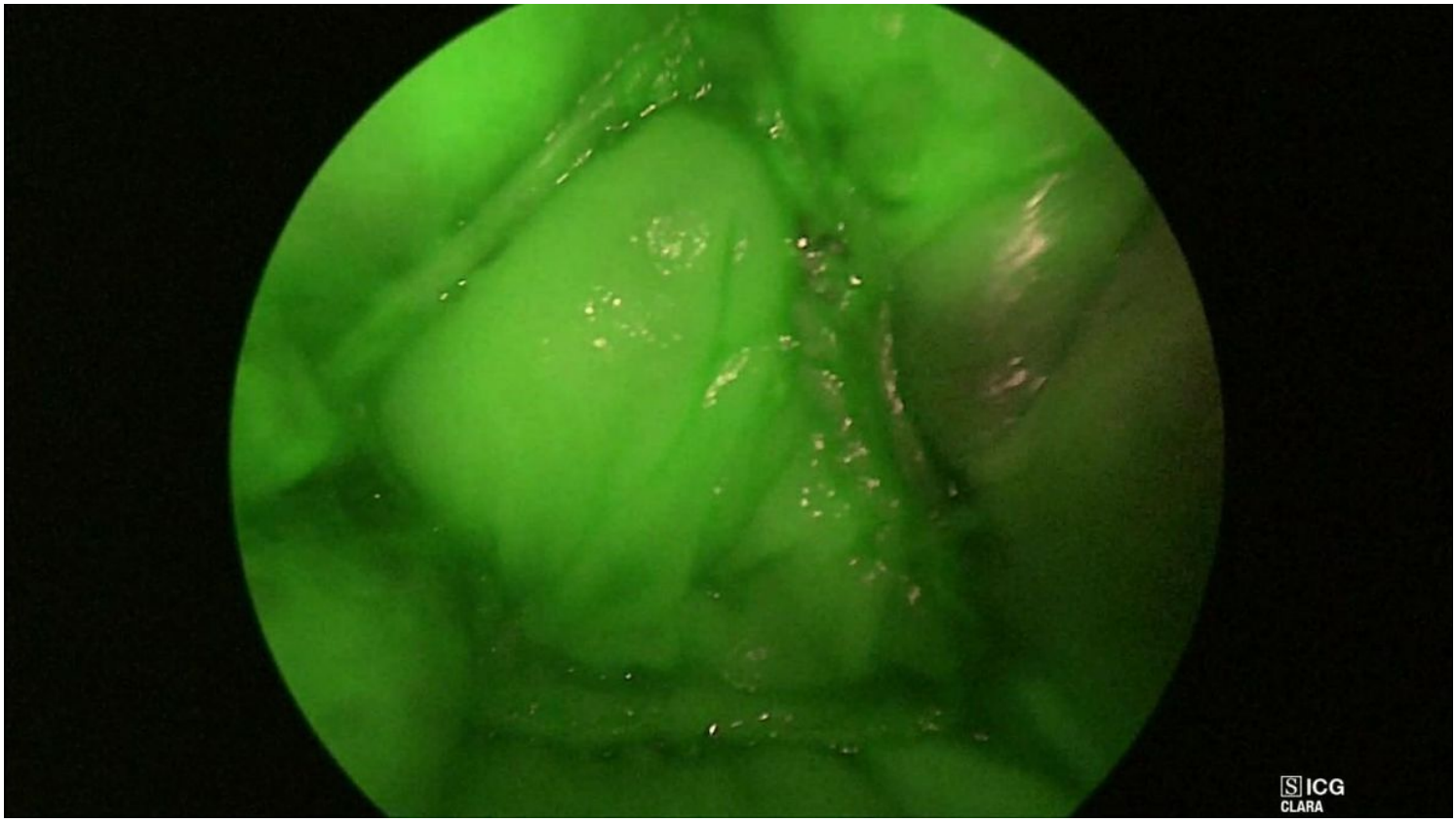

Figure 5

Endoluminal transanal post-anastomotic check (ICG)