



# Feasibility of quantitative analysis of colonic perfusion using indocyanine green to prevent anastomotic leak in colorectal surgery

Juan-Carlos Gomez-Rosado<sup>1</sup> · Javier Valdes-Hernandez<sup>1</sup> · Juan Cintas-Catena<sup>1</sup> · Auxiliadora Cano-Matias<sup>1</sup> · Asuncion Perez-Sanchez<sup>1</sup> · Francisco-Javier del Rio-Lafuente<sup>1</sup> · Cristina Torres-Arcos<sup>1</sup> · Yaiza Lara-Fernandez<sup>2</sup> · Luis-Cristobal Capitan-Morales<sup>1</sup> · Fernando Oliva-Mompean<sup>1</sup>

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## Abstract

**Background** The aim of this study was to quantify Fluorescence angiography with indocyanine green (ICG) in colorectal cancer anastomosis, determine influential factors in its temporary intensity and pattern, assessing the ability to predict the AL, and setting the cut-off levels to establish high- or low-risk groups.

**Methods** Retrospective analysis of prospectively managed database, including 70 patients who underwent elective surgery for colorectal cancer in which performing a primary anastomosis was in primary plan. In all of them, ICG fluorescence angiography was performed as usual clinical practice with VisionSense™ VS Iridium (Medtronic, Mansfield, MA, USA), in Elevision™ IR Platform (Medtronic, Mansfield, MA, USA). Parameters measured at real time or calculated were  $T_0$ ,  $T_{max}$ ,  $\Delta T$ ,  $F_{max}$ ,  $\%_{pos}$ ,  $F_{pos}$ , and Slope.

**Results** 70 patients were included, 69 anastomosis were performed and one end colostomy. Arterial hypertension demonstrated higher  $F_{max}$ , as well as the location of the anastomosis (the nearest to rectum, the most intensity detected). A statistical relationship was found between AL and the lower  $F_{pos}$  and Slope. The decision of changing the subjectively decided point of division did not demonstrate statistical difference on the further development of AL. All parameters were analyzed to detect the cut-off related with AL. Only in case of  $F_{pos}$  lower than 158.3 U and Slope lower than 13.1 U/s p-value were significant. The most valuable diagnostic parameter after risk stratification was the Negative Predictive Value.

**Conclusion** Quantitative analysis of ICG fluorescence in colorectal surgery is safe and feasible to stratify risk of AL. Hypertension and location of anastomosis influence the intensity of fluorescence at the point of section. A change of division place should be considered to avoid AL related to vascular reasons when intensities of fluorescence at the point of section is lower than 169 U or slopes lower than 14.4 U/s.

**Keywords** Colorectal cancer · Intestinal perfusion · Indocyanine green (ICG) · Quantitative fluorescence imaging · Anastomotic complications

Colorectal cancer surgery involves inherent risks, with high postoperative morbidity and mortality, of up to 33% of the procedures. One of the most feared ones is anastomotic leak (AL), not only because of the health implications for the patient but also because of the high impact of its costs [1, 2].

AL appears in 1–3% of ileocolic anastomoses, increasing as the anastomosis is performed more distally, up to 10–20% in low colorectal, depending on the series [3–5]. Its appearance has a significant negative impact on the quality of life of the patient in the short or long term, with the need for reoperations and temporary or permanent stomas up to 64% of the occasions [3]. Furthermore, it decreases survival and increases local recurrences [6] and the duration of postoperative hospital stay, which can raise up to 28 days on average [3, 7], with the consequent increase in hospitalization costs, which can increase from € 11,000 to € 22,000 [1, 3, 7–9].

For all the aforementioned, reducing the incidence of AL should be a main objective in this type of surgery and research has been focused to minimize the effect of the main

✉ Juan-Carlos Gomez-Rosado  
dr.gomez.rosado@gmail.com

<sup>1</sup> Colorectal Surgery Unit, General and Digestive Surgery, University Hospital Virgen Macarena, Dr Fedriani s/n, 41009 Seville, Spain

<sup>2</sup> General and Digestive Surgery, University Hospital Virgen Macarena, Seville, Spain

risk factors. It should be noted that, despite the multifactorial etiology, technical factors and insufficient blood perfusion in some of the ends to be anastomosed are one of the most frequent causes involved in AL [3, 10–12].

To verify correct perfusion, the surgeon's subjective perception was traditionally based on clinical findings, but several studies concluded that this subjectivity usually underestimates the risk of AL, regardless of the surgeon's experience [12–15]. Therefore, it was hypothesized that any reproducible and reliable technique, aimed to evaluate the micro-perfusion of the area to be anastomosed intraoperatively, would be very useful [16].

Indocyanine green (ICG) is a dye approved in 1959 by the United States Food and Drug Administration (FDA). It is made up of a sterile and soluble tricarboyanin molecule that, once injected intravenously, binds to plasma proteins [17]. The plasma half-life is 3–5 min, with hepatic elimination and biliary excretion. The recommended dose for circulatory diagnosis is 0.1–0.3 mg/kg of body weight maximum, in the form of an intravenous bolus. Its fluorescence imaging is based on the principle that plasma protein-bound ICG emits light with a wavelength peak of 830 nm when illuminated with NIR light of 760–780 nm [12].

Therefore, with the appropriate equipment, it is possible to demonstrate fluorescence, so fluorescence angiography (FA) with indocyanine green (ICG) has been increasingly recognized by the scientific community to provide an intraoperative assessment of the anastomotic vascular supply in real time [18].

However, subjective assessment of fluorescence intensity based on the surgeon's visual judgment remains being a major limitation [19]. Therefore, quantified, objective, and accurate measurements and patterns of fluorescence parameters are desirable to develop, in order to reduce the subjective factor of decision-making [12, 20, 21].

The aim of this study was to quantify fluorescence angiography with ICG in colorectal cancer anastomosis, determine if there were influential factors in its temporary intensity and pattern, as well as assessing the ability to predict the AL. In that case, to set the cut-off levels of those parameters in order to establish high- or low-risk groups.

## Materials and methods

### Patients

Study performed as a retrospective analysis of prospectively managed database, including patients who underwent elective surgery for colorectal cancer in which performing a primary anastomosis was in primary plan. In all of them, ICG fluorescence angiography was performed as usual clinical practice from July 2020 to February 2021 at a single

Colorectal Unit where more than 300 3D-laparoscopic colorectal resections are performed per year. Exclusion criteria are not considered, since the analysis was carried out retrospectively. One case, in which a terminal colostomy was performed, the quantitative analysis was included, but not related to AL for the anastomosis was not performed.

Written informed consent was obtained from all patients for the surgery as well as the use of their clinical data and the retrospective study was conducted after the approval of the Local Ethics Committee.

### Surgical procedure and ICG angiography

All procedures were performed by experienced surgeons, following a standardized technique. Nevertheless, ileocolic anastomoses were performed intracorporeally or extracorporeally depending on the surgeon's preference. Regarding the mobilization of the splenic flexure, it was carried out according to the anatomical or technical needs for the correct tension of the anastomosis and not systematically. Nutritional status was fully optimized before surgery in all cases, according to ERAS [22] criteria. Nutritional risk analysis was performed to all patients and in those in which the risk is moderate or high, we added nutritional supplements according to their needs. All patients took an arginine-rich immune-nutrition supplement for at least 10 days prior to surgery. Likewise, their hemoglobin figures are optimized, either with oral or intravenous ferrotherapy with ferric carboxymaltose, if necessary. Anterograde bowel preparation was performed in all cases.

Laparoscopic procedure was carried out with 3D-laparoscopic system Highlights Image 1S™ Platform (Dr.Kark-Stortz SE&Co KG, Germany), under general anesthesia.

In all cases, the bowel was divided after adequate safe margin was secured, using a laparoscopic linear stapler Endo GIA™ Reloads with Tri-Staple™ Technology combined with Endo GIA™ Ultra Universal stapler or Signia™ Stapling System (Medtronic, Mansfield, MA, USA) or ECH-ELON FLEX™ Powered (Ethicon, Somerville, NJ, USA). Likewise, these devices were used for linear anastomoses. In case of transanal anastomosis, either Echelon Circular™ Powered Stapler (Ethicon, Somerville, NJ, USA) or EEA™ circular stapler with DST Series™ (Medtronic, Mansfield, MA, USA) was used.

For the ICG-FA, VisionSense™ VS Iridium (Medtronic, Mansfield, MA, USA), in Elevision™ IR Platform (Medtronic, Mansfield, MA, USA) was used, in “Colorectal profile” for Exo-camera vision, and “Standard profile” for laparoscopic vision. A 10-mm-diameter 30° forward oblique degrees optic is used. Camera is positioned 15 cm away from the bowel, despite the equipment is equipped with a distance sensor that allows to regulate the focus based on the distance of the device to always be able to see the blood perfusion in an optimal way, without

having to keep it at a fixed and unique distance. All external sources of light in operating room were turned off. The evaluation of fluorescence was performed in real time.

### Quantitative evaluation and definition of Fluorescence parameters

Before division of the distal end of bowel to be anastomosed, 7.5 mg of ICG (Verdye™, Diagnostic Green GmbH, Germany) was injected intravenously in 10-s bolus (reconstitution in 5 mg/ml of sterile injection water) and as soon as administration finished, time counter started and laser was activated.

Parameters considered to be measured at real time or calculated, according with other studies [12, 20, 21] were as follows:

$T_0$ : Time from end of infusion (start laser) to instant of minimal fluorescence detected, in seconds. The value recorded was just the time when fluorescence (described as *Autobase* in platform) begun to be detected: quantification was higher than zero, as well as green image was seen in monitor.

$T_{max}$ : Time from end of infusion (start laser) to instant of maximum fluorescence detected (described as *Autobase* in platform), in seconds. The value recorded for  $T_{max}$  was just the time when *Autobase* quantification finished to increase and reached the maximum level.

$\Delta T$ : Difference calculated ( $T_{max} - T_0$ ), in seconds.

$F_{max}$ : Maximum level of fluorescence detected (over 250 U possible).

$\%_{pos}$ : Percentage of Maximum fluorescence ( $F_{max}$ ) measured in the subjectively selected point of section.

$F_{pos}$ : Level of fluorescence calculated in the subjectively selected point of section.

Slope calculated from  $F_{max}/\Delta T$ , in U/s.

Once the  $F_{max}$  value is stabilized, we placed the equipment cursor at the place where we considered the transection line to be most suitable (previously marked with a clamp). Initially we started by measuring the intensity of fluorescence in the subjectively selected place, approximately at the midpoint of the line between the mesenteric and antimesenteric edges, but if any other one with a significantly higher percentage of fluorescence that forces us to modify the subjective decision was detected, the place selected to transect was changed and the  $F_{pos}$  and  $\%_{pos}$  at that point were recorded.

### Definition of anastomotic leak

AL was considered according with Rahbari [23] definition as a “communication between the intra- and extraluminal

compartments owing to a defect of the integrity of the intestinal wall at the anastomosis” and categorized in A–B–C severity grades. In every suspected leaks, C-Reactive Protein was requested, and computed tomography was performed in case of high levels. Therefore, all types of leak were included as AL.

### Statistical analysis

Continuous data were tested for normal distribution (Kolmogorov–Smirnov test). Normally distributed data are presented as mean and 95% CI, and categorical data as frequency and percentage values in descriptive tables.  $\chi^2$  or Levene test was used to analyze homogeneity between groups of comparison.

Differences between normal distributions was tested using Student’s unpaired t test for continuous data ( $p$  value),  $\chi^2$  and Fisher’s exact test for categorical data (reported as  $\chi^2$ ,  $p$  value). In case of not normally distributed variables, non-parametric test was used to compare hypothesis.

Correlation between quantitative variables was performed with Pearson correlation test (normal distribution) or Spearman correlation test (not normal distribution). In case of qualitative variables, ANOVA or Kruskal–Wallis test. They were applied to analyze the correlation between the existence of comorbidities (arterial hypertension, diabetes mellitus, cardiopathy, chronic renal failure, or current active smoker status), age, sex, ASA grade or BMI and fluorescence parameters.

After identifying statistical relationship between any fluorescence parameter and anastomotic leak a ROC curve was used to determine the cut-off values of perfusion factors with high sensitivity and specificity and two groups were created according to the risk of anastomotic leak detected. The cut-off value was the reference to consider low or high risk in each parameter, but statistical significance has been determinant to establish the parameter as predictor of risk. These groups were compared and diagnostic values were evaluated: Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV).

All statistical analyses were performed with 2-tailed hypothesis testing and a  $p$ -value of 0.05 was accepted as significant and was conducted with IBM SPSS Statistics for Macintosh Version 25.

### Results

70 patients were included, with a mean age of  $65.8 \pm 11.1$  years. Finally 69 anastomosis were performed, all of them stapled, and one end colostomy (not expected). Preoperative characteristics of patients and surgery are described in Table 1. The overall leak rate was 13% (7 patients presented type C leak and 2 patients Type A leak). It were 2 colocolic, 3 colorectal, and 4 ileocolic.

Among factors suspected to influence into the parameters of fluorescence, detailed in Table 2, statistical significance was only detected in  $F_{\text{pos}}$  (arterial hypertension as coexisting comorbidity demonstrated higher intensity than no hypertension), as well as the location of the anastomosis (the nearest to rectum, the most intensity detected). All other factors did not show statistical differences. Neither age nor BMI showed differences either.

Regarding the presence of anastomotic leak, a statistical relationship was found with the quantification of  $F_{\text{pos}}$  and Slope (both (lower in case of AL). Table 3.

The decision of changing the subjectively decided point of division did not demonstrate statistical difference on the further development of AL. Table 4.

All parameters were analyzed to detect the cut-off related with AL (Table 5). Only in case of  $F_{\text{pos}}$  lower than 158.3 U and Slope lower than 13.1 U/s  $p$  value were significant. Nevertheless, after stratification of high/low risk depending of cut-off point of all parameters analyzed (Table 6), and significance was obtained only in two parameters: Slope and  $\%_{\text{pos}}$ .

In Table 7 diagnostic values of perfusion parameters (risk-stratified) are shown. The most valuable is the Negative Predictive Value in all cases.

## Discussion

Although there are numerous factors that can influence the development of an anastomotic leak, probably those related to the correct perfusion of the ends to be anastomosed are the ones that have the greatest impact. In this sense, the appearance of fluorescence angiography techniques with ICG has constituted an advance in its prevention [3, 10–12, 24, 25]. However, ICG fluorescence has not been clearly demonstrated to reduce anastomotic complications at all, probably because there are still certain difficulties related to the subjectivity of their interpretation [20].

For this reason, scientific community is working on the development of fluorescence angiography techniques with quantitative evaluation, both of the intensity and its patterns in relation to time [12, 20, 21].

So far, authors have analyzed the fluorescence parameters with the intention of a real-time decision-making, although in reality, all of them have used in their methodology the ex post-evaluation of the recordings obtained [20]. In that case,  $T_{1/2\text{max}}$  (time to half maximal intensity of fluorescence) appears to be one of the most robust predictors of AL [20]. Relying on the measurements by viewing the recorded procedure, greatly limits the decision in real time from our point of view, hence, we have tried to analyze our results without this methodology, only with the information provided by the platform during the execution

**Table 1** Characteristics of patients ( $n=70$ )

Characteristic	Data
Age (years); mean $\pm$ SD	65.8 $\pm$ 11.1
Sex; $n$ (%)	
Male	44 (62.9)
Female	26 (37.1)
BMI (Kg/m <sup>2</sup> ); mean $\pm$ SD	27.4 $\pm$ 5.7
ASA grade; $n$ (%)	
I	3 (4.3)
II	28 (40.0)
III	39 (55.7)
Arterial hypertension; $n$ (%)	37 (52.9)
Diabetes mellitus; $n$ (%)	17 (24.3)
Cardiopathy; $n$ (%)	18 (25.7)
Chronic renal failure; $n$ (%)	3 (4.3)
Current active smoker; $n$ (%)	8 (11.4)
Radiotherapy <sup>b</sup> ; $n$ (%)	6 (31.6)
Splenic Flexure mobilization <sup>c</sup> ; $n$ (%)	22 (51.2)
Neoplasm site; $n$ (%)	
Caecum	8 (11.4)
Ascending colon	6 (8.6)
Hepatic flexure	1 (1.4)
Transverse colon	4 (5.7)
Splenic flexure	6 (8.6)
Descending colon	3 (4.3)
Sigmoid colon	21 (30.0)
Rectum	19 (27.1)
Total colon -adenomatous familiar polyposis-	2 (2.9)
Surgical approach; $n$ (%)	
3D laparoscopy	65 (92.9)
2D laparoscopy	1 (1.4)
Laparotomy	4 (5.7)
Conversion rate; $n$ (%)	2 (2.9)
Surgery duration (minutes); mean $\pm$ SD	165.7 $\pm$ 51.7
Colostomy -without anastomosis-; $n$ (%)	1 (1.4)
Stapled anastomosis <sup>a</sup> ; $n$ (%)	69 (100)
Anastomosis format <sup>a</sup> ; $n$ (%)	
Side to side	24 (34.8)
Side to end	35 (50.7)
End to end	10 (14.5)
Mode of anastomosis <sup>a</sup> ; $n$ (%)	
Linear open	3 (4.3)
Linear intracorporeal	7 (10.2)
Linear extracorporeal	14 (20.3)
Circular transanal	45 (65.2)
Overall anastomotic leak <sup>a</sup> ; $n$ (%)	9 (13.0)

<sup>a</sup>% calculated over total of 69 anastomosis

<sup>b</sup>% calculated over total of 19 rectum

<sup>c</sup>% calculated over total of 43 Left colon, sigmoid and rectum

**Table 2** Factors influencing parameters of fluorescence (correlation test)

Factor	$T_0$		$T_{max}$		$F_{max}$		$F_{pos}$		Slope	
	Data	<i>p</i> value	Data	<i>p</i> value	Data	<i>p</i> value	Data	<i>p</i> value	Data	<i>p</i> value
Sex	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Male	22.3 ± 13.7	0.10	40.9 ± 16.6	0.08	212.1 ± 12.0	0.50	167.9 ± 24.4	0.42	15.5 ± 7.8	0.27
Female	16.1 ± 10.7		32.1 ± 18.8		209.6 ± 18.0		162.9 ± 24.5		17.9 ± 7.1	
Age (years)	0.127 <sup>b</sup>	0.36	0.031 <sup>d</sup>	0.82	0.180 <sup>e</sup>	0.15	0.125 <sup>d</sup>	0.32	0.100 <sup>e</sup>	0.47
BMI (Kg/m <sup>2</sup> )	0.051 <sup>c</sup>	0.71	0.164 <sup>d</sup>	0.25	0.045 <sup>d</sup>	0.73	0.113 <sup>d</sup>	0.38	-0.122 <sup>d</sup>	0.39
Arterial hypertension	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Yes	23.2 ± 14.3	0.07	41.7 ± 19.9	0.15	213.8 ± 9.9	0.14	172.3 ± 24.7	0.02	16.4 ± 8.1	0.92
No	16.9 ± 10.7		33.9 ± 14.3		208.6 ± 17.5		159.9 ± 21.6		16.2 ± 7.2	
Diabetes mellitus	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Yes	20.4 ± 15.3	0.93	37.8 ± 16.2	0.70	214.8 ± 5.9	0.26	166.9 ± 26.3	0.81	16.0 ± 5.9	0.86
No	20.03 ± 12.1		37.9 ± 18.4		210.0 ± 16.1		165.8 ± 23.3		16.4 ± 8.2	
Cardiopathy	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Yes	24.9 ± 14.6	0.09	40.4 ± 17.8	0.40	214.5 ± 6.1	0.27	172.0 ± 27.6	0.17	17.4 ± 8.2	0.51
No	18.3 ± 12.0		36.9 ± 17.8		210 ± 16.2		164.0 ± 22.3		15.9 ± 7.5	
Chronic renal failure	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Yes	20.0 ± 8.7	0.99	43.3 ± 23.1	0.55	214.3 ± 9.6	0.70	148.3 ± 43.1	0.49	11.4 ± 5.4	0.25
No	20.1 ± 8.2		37.6 ± 17.5		211.0 ± 14.6		167.0 ± 22.8		16.6 ± 7.7	
Current smoker	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>	mean ± SD	<sup>b</sup>	mean ± SD	<sup>a</sup>
Yes	17.3 ± 4.7	0.23	32.1 ± 12.6	0.47	211.9 ± 5.0	0.89	161.8 ± 30.2	0.71	18.4 ± 9.2	0.44
No	20.5 ± 13.7		38.8 ± 18.2		211.1 ± 15.3		166.7 ± 23.1		16.0 ± 7.4	
ASA grade	mean ± SD	<sup>e</sup>	mean ± SD	<sup>f</sup>	mean ± SD	<sup>e</sup>	mean ± SD	<sup>f</sup>	mean ± SD	<sup>e</sup>
I	18.3 ± 7.2	0.82	33.7 ± 16.0	0.61	203.7 ± 21.2	0.65	149.6 ± 20.6	0.49	18.4 ± 10.8	0.86
II	18.9 ± 10.2		35.9 ± 18.4		211.8 ± 14.9		166.0 ± 22.7		15.8 ± 7.1	
III	21.1 ± 14.9		39.6 ± 17.6		211.4 ± 13.6		167.6 ± 25.0		16.4 ± 7.9	
Location of anastomosis	mean ± SD	<sup>e</sup>	mean ± SD	<sup>f</sup>	mean ± SD	<sup>e</sup>	mean ± SD	<sup>f</sup>	mean ± SD	<sup>e</sup>
Right sided	20.6 ± 11.1	0.43	38.8 ± 17.1	0.11	208.9 ± 10.8	0.65	146.5 ± 21.2	0.003	16.1 ± 7.1	0.11
Left sided	25.0 ± 19.1		51.1 ± 22.5		214.5 ± 7.3		172.0 ± 21.3		11.3 ± 4.3	
Rectum	18.6 ± 11.7		34.3 ± 15.4		211.1 ± 16.5		170.9 ± 22.4		17.6 ± 8.1	

<sup>a</sup>Unpaired t test<sup>b</sup>U Mann–Whitney test<sup>c</sup>Pearson correlation test (normal distribution)<sup>d</sup>Spearman correlation test (not normal distribution)<sup>e</sup>ANOVA<sup>f</sup>Kruskal–Wallis test

of the procedure. We are convinced that the ability to assess intraoperative perfusion accurately via an easy to use and accessible method is, therefore, of potential great importance [16].

The aim of this study was to quantify fluorescence angiography with ICG in colorectal cancer anastomosis, determine if there were influential factors in its temporary intensity and pattern, as well as assessing the ability to predict the AL.

Regarding the factors that influence the fluorescence parameters, we decided to include those that can affect microcirculation, such as hypertension, diabetes, heart disease, or smoking, as well as those that, in some way, can

affect the distribution or drug bioavailability, such as kidney failure, liver disease, or BMI, which, in addition to altering the pharmacodynamics of indocyanine, makes the technique difficult and prolongs the operative time. No liver disease was present that is the reason because we did not indicate it in our tables or have included it in the study. Only a statistical relationship was found between hypertension and  $F_{pos}$ , so that patients with hypertension presented more intensity of fluorescence at the section point, without greater intensity appearing at the point of maximum level ( $F_{max}$ ). It should be clarified that we refer to the existence of hypertension as comorbidity (yes or not) and not to the blood pressure at the moment of measurement of fluorescence parameters,

**Table 3** Quantification of Fluorescence parameters comparing anastomosis leak vs anastomotic integrity

Parameter	Anastomotic leak <sup>a</sup>		<i>p</i> value
	Yes ( <i>n</i> = 7) <sup>b</sup>	No ( <i>n</i> = 61) <sup>b</sup>	
$T_0$	19.9 ± 14.3	19.8 ± 11.8	0.98 <sup>c</sup>
$T_{max}$	47.6 ± 20.7	35.5 ± 16.6	0.10 <sup>d</sup>
$F_{max}$	207.9 ± 18.3	212.7 ± 12.0	0.35 <sup>c</sup>
% <sub>pos</sub>	72.1 ± 2.4	79.3 ± 9.4	0.05 <sup>d</sup>
$F_{pos}$	151.0 ± 13.1	169.0 ± 24.0	0.03 <sup>d</sup>
Slope	10.8 ± 6.2	17.4 ± 7.4	0.03 <sup>c</sup>

<sup>a</sup>Calculated over 69 anastomosis performed (1 colostomy not included)

<sup>b</sup>Excluded 1 patient (Failed quantification. Decision under subjective criteria)

<sup>c</sup>Unpaired t test

<sup>d</sup>U Mann–Whitney test

**Table 4** Relationship between decision of change subjectively selected point of section comparing anastomosis leak vs anastomotic integrity

Change point of section	Anastomotic leak <sup>a</sup>		<i>p</i> value
	Yes	No	
Yes	1 (6.3)	15 (93.8)	0.391
No	7 (13.5)	45 (86.5)	

<sup>a</sup>Calculated over 69 anastomosis performed (1 colostomy not included)

**Table 5** ROC curve analysis of fluorescence parameters related to anastomotic leak

Parameter	Cut-off value	AUC	<i>p</i> value	95% confidence interval
$T_0$	> 18.5	0.514	0.90	0.268–0.759
$T_{max}$	> 36.5	0.695	0.09	0.444–0.946
$F_{max}$	< 213.5	0.555	0.64	0.312–0.798
% <sub>pos</sub>	< 74.5	0.728	0.05	0.606–0.850
$F_{pos}$	< 158.3	0.741	0.04	0.612–0.870
Slope	< 13.1	0.737	0.04	0.546–0.927

**Table 6** Risk stratification of perfusion parameters for predicting anastomotic leak

	High risk	Low risk	<i>p</i> value
% <sub>pos</sub>	< 74.5	≥ 74.5	0.04
$F_{pos}$	< 158.3	≥ 158.3	0.08
Slope	< 13.1	≥ 13.1	0.04

**Table 7** Diagnostic values of perfusion factors for predicting anastomotic leak

	% <sub>pos</sub>	$F_{pos}$	Slope
Sensitivity	0.71	0.71	0.71
Specificity	0.72	0.68	0.71
PPV	0.23	0.22	0.28
NPV	0.95	0.95	0.94

PPV positive predictive value, NPV negative predictive value

which could be interesting to consider in subsequent studies. It may seem somewhat paradoxical, but despite the fact that hypertension is usually associated with alterations in the microcirculation, it can also be justified because the intraoperative blood pressure values of the patients could be increased and facilitate better irrigation at the time of measurement. Other authors demonstrated relation between  $T_{1/2}$  and AL [20] parameters that we did not analyzed.

Differences related to the location of the anastomosis were also found, with lower intensity of fluorescence at the section point ( $F_{pos}$ ) in right hemicolectomies than in left ones or in the rectum. The colonic arterial supply presents various critical points, as the splenic flexure and Sudeck's critical point at the sigmoid junction [26]. Consequently, it would be expected that the ligation of the inferior mesenteric artery during left colectomies and sigmoid resections would significantly affect the vascular supply of the rectal stump, so the angiographic measurement obtained at this level would be expected to be lower than that on the right side. Angiographic measures obtained in our study were actually opposite to this, as expected, and due to marginal vascular supply.

When fluorescence parameters are compared in patients with or without AL, lower intensity at the point of section ( $T_{pos}$ ) as well as lower slope are related with higher AL rate, as shown in other studies [12, 20]. This is completely understandable, since the better vascular supply, the greater the intensity of fluorescence, and the faster its increase over time, which, in turn, is related to a lower rate of dehiscence. In fact, when patterns are analyzed by artificial intelligence-based system higher slopes are related to lower anastomotic leak rates [27].

After risk was stratified according to cut-off points obtained in ROC curves of significant parameters, relationship between %<sub>pos</sub> and Slope were confirmed, in concordance with others, as well as positive and negative predictive values of those parameters [12, 20].

Regarding the absence of statistical relationship between the decision of change the subjective point of division and the anastomosis leak rate probably is due to the fact that that decision assumed was correct and based in a correct interpretation of quantitative values observed. As observed in our experience, in 16(23.2%) patients the decision to change the point of section was made, and although no significant differences were found, it is striking that in the cases in which

the section site was not modified, the AL rate is the double that in those in which it was decided to modify it. Despite statistical significance was not found, our results show that the incidence of AL when the point of section was changed was 1/16 (6.3%) even lower than actual AL of the group where the point of section was not needed to be changed, so we would expect a higher AL rate if the point of section had not been changed. This highlights the importance of a correct interpretation of fluorescence intensity, even more precise thanks to quantification.

We believe this is an added value to this study, for if this point of section was indeed not well perfused, changing the point of section would be related to a decrease in the AL rate. Understanding that in order to prove this fact, it would be necessary to perform a study comparing the AL rates when the anastomosis was made in this insufficient perfused spot or changed to a new well-perfused point in order to find the correlation with the AL. However, we believe that this study approach would be ethically debatable.

Our study has some limitations, mainly related to the retrospective nature of the analysis, the small number of patients and the real-time evaluation of the values, unlike Park's study [27], which uses real-time analysis based on artificial intelligence of the pattern of fluorescence. It will probably be the next step to get more consistent and accurate conclusions. Further investigation in this field would be desirable.

In conclusion, quantitative analysis of ICG fluorescence in colorectal surgery is a safe and feasible technique. It can help to identify patients with high or low risk of AL and it could be helpful in order to diminish the rate of postoperative complications and AL rate. Hypertension appears to be a factor influencing the intensity of fluorescence at the point of section, as well as the location of anastomosis (the nearest to rectum, the most intensity detected). For the moment and awaiting further studies, we can establish a relationship between anastomotic leak and intensities of fluorescence at the point of section lower than 169 U, as well as slopes lower than 14.4 U/s, so according with our results, when values under this cut-off point are found, a change of division place should be considered, in order to avoid anastomotic leak related to vascular reasons.

Anyway, some improvements in the technical procedure may be helpful, such as the use of real-time artificial intelligence-based systems that may help to analyze automatized measures, diminishing the subjective human factor.

Explanatory note:

At the moment of sending this manuscript, PILLAR-III was not yet published (published in August, 2021), so we were not able to consider as a valid reference. According to their conclusions, we could indeed consider that fluorescence with ICG does not provide any value for the prevention of AL. However, despite its good design and methodological rigor, it is not a quantitative analysis of fluorescence parameters, but a qualitative one. That is the great limitation

that we try to avoid with quantitative analysis: the subjectivity of the observer. Therefore, although, with the data in our hands, we could validate the technique only for colon cancer and not rectal cancer, our group thinks that quantification will permit to obtain greater precision in the measurement and those results could be questioned. Nevertheless, it is not the reason for our study.

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