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REVIEW ARTICLE

Optimizing quantitative fluorescence angiography for visceral perfusion assessment

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Abstract

Background Compromised tissue perfusion is a significant risk factor for anastomotic leakage after intestinal resection, leading to prolonged hospitalization, risk of recurrence after oncologic resection, and reduced survival. Thus, a tool reducing the risk of leakage is highly warranted. Quantitative indocyanine green angiography (Q-ICG) is a new method that provides surgeons with an objective evaluation of tissue perfusion. In this systematic review, we aimed to determine the optimal methodology for performing Q-ICG.

Method A comprehensive search of the literature was performed following the PRISMA guidelines. The following databases were searched: PubMed, Embase, Scopus, and Cochrane. We included all clinical studies that performed Q-ICG to assess visceral perfusion during gastrointestinal surgery. Bias assessment was performed with the Newcastle Ottawa Scale.

Results A total of 1216 studies were screened, and finally, 13 studies were included. The studies found that *intensity* parameters (*maximum intensity and relative maximum intensity*) could not identify patients with anastomotic leakage. In contrast, the *inflow* parameters (*time-to-peak, slope,* and $t_{1/2}max$) were significantly associated with anastomotic leakage. Only two studies performed intraoperative Q-ICG while the rest performed Q-ICG retrospectively based on video recordings. Studies were heterogeneous in design, Q-ICG parameters, and patient populations. No randomized studies were found, and the level of evidence was generally found to be low to moderate.

Conclusion The results, while heterogenous, all seem to point in the same direction. Fluorescence *intensity* parameters are unstable and do not reflect clinical endpoints. Instead, *inflow* parameters are resilient in a clinical setting and superior at reflecting clinical endpoints.

Keywords Fluorescence angiography · Quantification · Indocyanine green · Anastomotic leakage · Optimization

One of the most feared complications following gastrointestinal surgery is anastomotic leakage (AL). AL is a complication with potentially dire consequences. Studies have shown an increased length of hospitalization, increased health expenses, increased risk of recurrence, reduced mobility, and reduced survival [1, 2]. AL is a multifactorial complication associated with several risk factors: Male gender, blood transfusion, steroids, nonsteroidal anti-inflammatory drugs, bacterial microbiome, and lack of surgical experience [3–6]. However, one of the most important reasons for AL is inadequate visceral perfusion [7–9]. Thus, securing good perfusion of the anastomosis is a vital piece of the puzzle towards reducing the rate of AL.

Traditionally perfusion assessment has been performed visually and manually by surgeons. They rely on the color of the tissue, peristalsis, bleeding from the resection line, and palpation of the mesenteric pulse to determine if the visceral perfusion is acceptable [10]. However, these methods are limited when performing minimally invasive surgery [10]. Furthermore, this traditional assessment is subjective to interpretation by the surgeon, which is demonstrated in

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studies showing that surgeons' sensitivity and specificity for predicting AL is low [11]. Hence, there is a need for a new method to evaluate visceral perfusion.

A strong contender for this method is fluorescence angiography (FA) with indocyanine green (ICG). FA can be divided into two methods: Visual fluorescence angiography (V-ICG), where the surgeon assesses the fluorescence signal, and quantitative fluorescence angiography (Q-ICG), where a computer algorithm interprets the fluorescence signal. In Q-ICG, the fluorescence signal is translated into a fluorescence–time curve. Subsequently, a computer algorithm can calculate different Q-ICG parameters (Fig. 1). The various Q-ICG parameters then reflect the perfusion of the examined tissue [12, 13].

Reviews have found that surgeries assisted by V-ICG tend towards a lower risk of AL in both esophageal and colorectal surgery [14–17]. However, the reviews considered the results biased, as they mainly included retrospective- and non-randomized studies. Two randomized-controlled studies exist, 1 found a significantly lower rate of AL in patients who underwent V-ICG compared with the controls (14.4 vs. 25.7%, $p = 0.04$) when performing low colorectal anastomoses [18]. On the contrary, the other found no significant difference between V-ICG and controls in the AL rate following colorectal resection [19]. Hence, several reviews of V-ICG have concluded that an objective (quantitative) methodology is needed to overcome the inherited observer bias of V-ICG [12, 14, 15, 17]. However, no gold standard regarding the optimal methodology and Q-ICG parameters have been established.

In this systematic review, we aimed to explore which Q-ICG parameters express the most robust association to clinical endpoints as re-resection or the occurrence of AL and search for the optimal methodology when performing Q-ICG.

Materials and methods

Search strategy

This study followed the PRISMA statement for systematic reviews, and the protocol was submitted to PROSPERO (reference number: CRD42020151477). This study

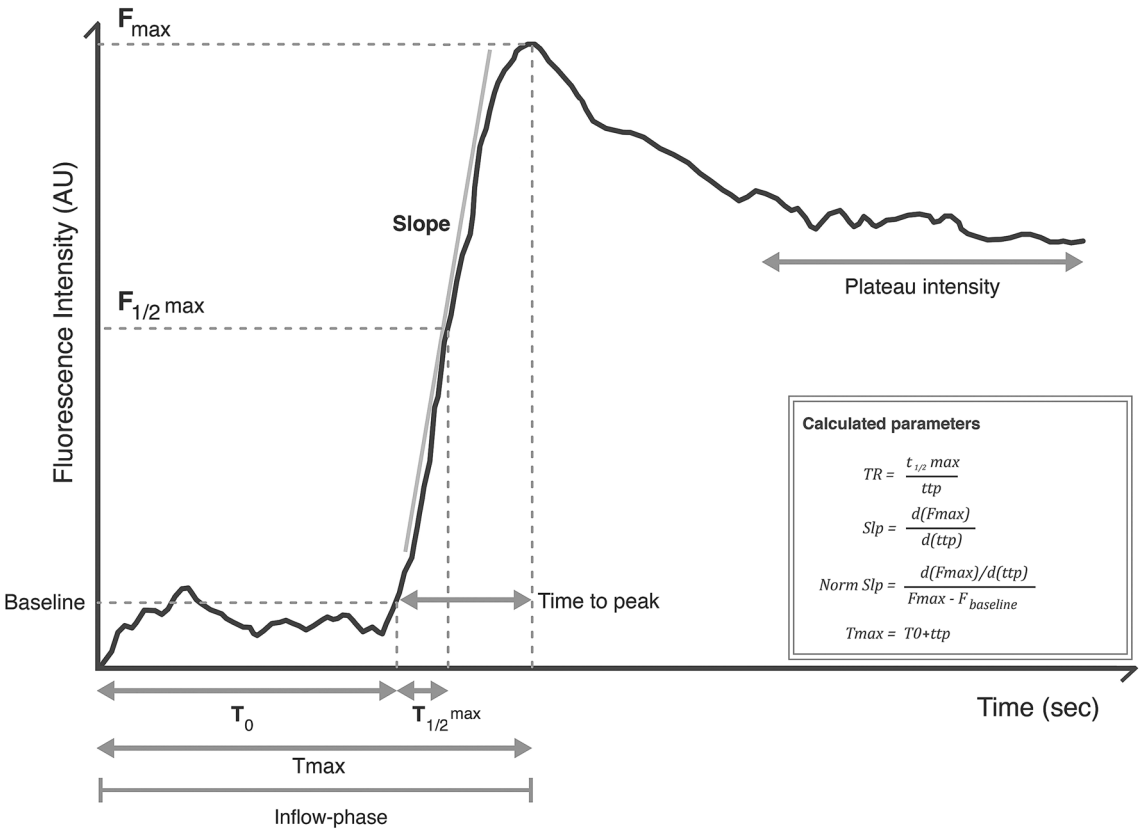

Fig. 1 An illustration of the different Q-ICG parameters and how they are calculated. The parameters are divided into two categories: (1) The *Intensity* category: *Fmax* Maximum fluorescence intensity, *R-Fmax* relative maximum fluorescence intensity, and plateau intensity. (2) *Inflow* category: *ttp* Time-to-peak, *slp* slope, *norm slp* normalized slope, $t_{1/2}max$ time to 50% of the maximum fluorescence intensity, *TR* time ratio, *Tmax* time from ICG injection to maximum fluorescence, and *T0* time to first fluorescence signal

required no approval from an institutional review board nor written consent. The search strategy was guided by the characteristics of our PICO-questions: Population: Clinical studies. Intervention: Q-ICG visceral perfusion assessment. Comparison: Different Q-ICG methodologies and parameters. Outcomes: Studies had to report a re-resection or the occurrence of AL.

We aimed for a very general search string as not to risk excluding relevant studies. The search string was constructed using the PubMed search builder. Subsequently, the following databases were searched: PubMed, Embase, Scopus, and Cochrane. The search was performed on the 16th of April 2020. The following search string was used:

> "(Fluorescence OR fluorescence angiography OR laser fluorescence videography OR fluorescence-Assisted Resection and Exploration OR near-infrared fluorescence angiography OR NIR fluorescence angiography OR angiography OR FLARE OR near-infrared imaging OR SPY OR near-infrared imaging OR video fluorescence OR Enhanced-Reality Video fluorescence) and (Indocyanine Green OR ICG OR Q-ICG) and (Quantitative Perfusion OR Perfusion angiography OR Perfusion monitoring OR Perfusion anastomotic OR Quantitative OR quantitative assessment OR Q-assessment OR Microcirculation)"

The selection process was assisted by an online tool (Rayyan®) [20]. Two investigators screened studies for inclusion and exclusion criteria. Any conflicts that arose during the evaluation were discussed in the author group to reach consensus. Finally, snowballing inclusion was performed through reference screening.

Eligibility criteria

The inclusion criteria were as follows: Studies had to use a numerical Q-ICG parameter from the time–fluorescence curve with relation to re-resection or AL. Studies had to assess the perfusion of gastrointestinal organs during a surgical procedure. Only English language studies published in peer-review journals were accepted. The exclusion criteria were: Wrong study design (case reports < 5 subjects, experimental animal studies, reviews, conference abstract, and editorials) and ex vivo studies.

Quality assessment

The quality assessment was performed by two authors independently. Studies were evaluated using the Newcastle Ottawa Scale (NOS) for retrospective cohort studies [21].

Statistics

Normally distributed data were given in mean ± SD and non-normal distributed data as median with range. *P* values < 0.05 were considered significant. An unpaired 2-sided *T* test was performed with IBM SPSS Statistics (v. 25, SPSS Inc, IBM, Chicago, IL, USA).

Results

Study characteristics

The initial search yielded a total of 1216 studies. Subsequently, 28 studies were included for a full-text screening, of which 13 were accepted for final inclusion [13, 22–33]. An overview of the process is depicted in a PRISMA flow diagram (Fig. 2).

The 13 studies included a total of 1918 patients. Q-ICG and V-ICG were performed in 1150 patients, while the remaining 768 patients were controls. The number of patients in the studies ranged from 9 to 657. The clinical studies were divided into the following fields: Two investigating esophageal surgery (n = 46) [27, 29], ten colorectal surgery (n = 1863) [13, 22–24, 26, 28, 30–33] and 1 gastrointestinal trauma surgery (n = 9) [25]. All studies were published during the period from 2015 to 2020. The surgical techniques varied among the studies; however, most of the studies used a laparoscopic technique (Table 1).

Bias evaluation

The results of the NOS-bias evaluation showed a low to moderate level of evidence. Only four studies had a high level of evidence [13, 23, 30, 32], while the remaining nine had a poor level of evidence. Studies mainly scored low due to a lack of multivariate analysis, inadequate blinding, and a description of follow-up. (Table 2).

Q-ICG methodology

Q-ICG was performed either intra-or postoperatively based on video recordings. Most studies used video recordings; however, two studies did perform intraoperative Q-ICG, both assisted by the SPY elite system [25, 31]. One study performed V-ICG transanally and examined the mucosal side of the anastomosis [22]. The remaining examined the serosa side of the anastomosis or tissue to be anastomosed.

The studies analyzed Q-ICG parameters of both the *intensity* category: Maximum fluorescence intensity (*Fmax*) and relative maximum fluorescence intensity (*R-Fmax*),) and the *inflow* category: Time-to-peak (*ttp*), slope (*slp*), normalized slope (*norm slp*), time to 50% of

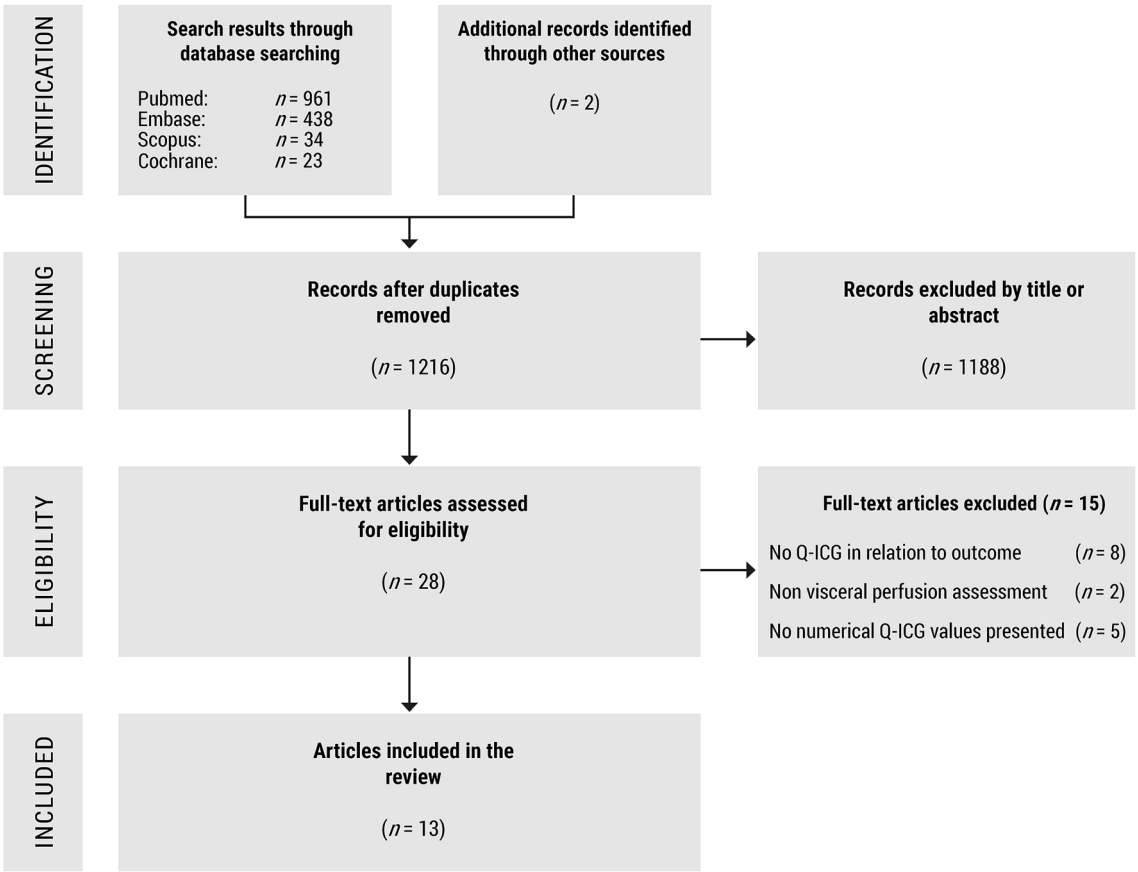

Fig. 2 PRISMA flowchart depicting the literature search

the maximum fluorescence intensity ($t_{1/2}max$), time ratio (*TR*), time from ICG injection to maximum fluorescence (*Tmax),* and time to first fluorescence signal (*T0*) (Fig. 1) [12, 13, 22, 30]. The relative Q-ICG parameters were calculated by selecting two or more region(s) of interest (ROIs). Many different FA systems were used, and the most popular was the Spy-Elite system, utilized in four [23–25, 31] of 13 studies. The most popular Q-ICG parameters were the intensity-based (*Fmax* or *R-Fmax*), and 6 studies only explored 1 of these 2 parameters [23–25, 30–32]. A detailed description of the applied Q-ICG parameters is listed in Table 1.

The dosing of ICG was heterogeneous as 9 of 13 studies used different dosing regiments. Most studies used a fixed dose ranging from 1.25 to 10 mg, while only two studies used a dosage depended on body weight [13, 22]. Finally, the timing of fluorescence measurement was only reported in seven studies [13, 24, 26–28, 31, 33]. Five studies had a recording period of approximately five minutes following the administration of ICG [13, 26–28, 33]. The remaining two studies only performed Q-ICG once at a predetermined interval. These studies sampled a *Fmax* value once at 60 s post-ICG injection [24, 31].

Clinical endpoint–Re-resection

In all but two studies, the decision to perform a re-resection was based on V-ICG and not Q-ICG [25, 31]. In one study investigating laparoscopic colorectal resection with intraoperative Q-ICG using *Fmax* with no fixed cut-off, four patients (5.2%) had re-resection followed by an uncomplicated postoperative course [31]. In the other study, the repair of nine military trauma-related bowel injuries was assisted with perioperative Q-ICG. Arbitrary cut-off values of *R-Fmax* > 25% or Fmax > 6AU were used, resulting in re-resection in 3 patients (33.3%) [25].

In four studies, Q-ICG was performed post-surgery to determine if patients who underwent re-resection could be distinguished from the patients without re-resection by a Q-ICG system. In one study, no significant difference in *Fmax* was observed at the re-resection site in colorectal patients (169 vs. 192AU, $p = 0.11$) [23]. Another study reported a lower *Fmax* in colorectal patients with re-resection but did not include a statistical analysis [24]. A study analyzed *Fmax* and *ttp* in the gastric phase of esophagectomy but did not find a significant difference in re-resection patients [27]. Finally, a single study found a significantly

Table 1 Descriptive characteristics of clinical studies

Studies	Demographics			Q-ICG description				Re-resection following Q-ICG
	Study design	*n*	Procedure	Imaging system	Software	Q-ICG parameters	ICG dose	Frequency
Green et al. [25]	Pros	9	Trauma: Open	SPY-Elite system[a]	SPY-Q software[a]	*Fmax and R-Fmax*	7.5 mg	33.3%
Protyniak et al. [31]	Pros	77	Colorectal: Laparoscopic	SPY-Elite system[a]	SPY-Q software[a]	*Fmax*	N/A	5.2%
Chang et al. [23]	Pros. blinded	110	Colorectal: Laparoscopic Robotic and Open	SPY-Elite system[a]	SPY-Q[a]	*Fmax*	5 mg	–
Dinallo et al. [24]	Retro. control cohort	554	Colorectal: Laparoscopic and Robotic	SPY-Elite system[a]	SPY-Q[a]	*Fmax*	2 mL	–
Son et al. [13]	Pros	86	Colorectal: Laparoscopic	Karl Storz[b]	Tracker 4.97[d]	*ΔFmax, $t_{1/2}$max,* and *TR*	0.25 mg/kg	–
Wada et al. [33]	Retro	112	Colorectal: Laparoscopic	PDE-neo System[c]	ROIs System[c]	*Fmax, ttp, Slp, T0 and $t_{1/2}$max*	5 mg	–
Wada et al. [32]	Retro. Propensity matched	149	Colorectal: Laparoscopic	PDE-neo[c]	ROIs System[c]	*Fmax*	5 mg	–
Hayami et al. [26]	Pros	22	High risk colorectal: Laparoscopic	Karl Storz[b]	ROIs Software[c]	*Fmax, slp, $t_{1/2}$max, and T0*	5 mg	–
Kim et al. [30]	Retro. Control cohort	657	Colorectal: Robotic	Firefly[e]	R0G160B0[i]	*R-Fmax* (25% increments)	10 mg	–
Ishige et al. [27]	Pros	20	Esophagectomy: Open	Olympus[f]	ROIs Software[c]	*ttp and Fmax*	1.25 mg	–
Kamiya et al. [29]	Retro	26	Esophagectomy: Open	PDE-Neo[c]	ROIs Software[c]	*Fmax and $t_{1/2}$max*	N/A	–
Amagai et al. [22]	Pros	71	Colorectal: Laparoscopic and open	Olympus[f]	ImageJ software[g]	*Fmax, ttp, and Tmax*	0.2 mg/kg	–
Iwamoto et al. [28]	Pros	25	Colorectal: Laparoscopic	PINPOINT[h]	ROIs Software[c]	*T0 and ttp*	7.5 mg	–

Pros Prospective cohort studies; *Retro* Retrospective cohort studies, *n* No. of patients

[a]LifeCell Technologies, Mississauga, Ontario, Canada; [b]Karl Storz GmbH and Co., KG, Tüttlingen, Germany; [c]Hamamatsu Photonics, Japan;[d] Douglas Brown, Open Source Physics, Boston MA, USA; [e]Intuitive Surgical, Sunnyvale, USA; [f]Olympus corporation, Tokyo, Japan; [g]National Institutes of Health, Maryland, USA; [h]STRYKER, Kalamazoo, Michigan, U.S; [i]Microsoft PowerPoint, Santa Rosa, USA

lower *Fmax* in colorectal patients who had re-resection (51.7 vs. 82AU., $p < 0.05$) [32].

Clinical endpoint–anastomotic leakage

Seven studies (n = 652) analyzed Q-ICG parameters in patients with AL and compared them with patients without AL [13, 22, 26, 28–30, 33]. The studies analyzed the following Q-ICG parameters: *Fmax, slp, ttp, $t_{1/2}$max, TR, Tmax, and T0*. Six studies investigated colorectal AL [13, 22, 26, 28, 30, 33], and 1 looked at venous anastomotic failure during esophagectomy [29] (Table 3).

The *Fmax* parameter was examined in five studies (n = 317) [13, 26, 29, 33]. However, only one study (n = *112*) had a significant difference in *Fmax,* comparing AL patients with non-AL patients (38.14 vs. 91.1AU, $p < 0.001$) [33].

The *slp* parameter was examined in three studies (n = 220). Two studies found a significantly different *slp* value between the AL and no-AL group (0.98 vs. 3.6 AU/s, $p = 0.009$) [33] and (0.7/s AU vs. 2.5AU/s, $p = 0.001$) [13]. However, Hayami et al. (n = *22*) who selectively included only patients with a high risk of AL did not find a significant difference (3.4 vs. 5.5 AU/s, $p = 0.27$) [26].

The $t_{1/2}max$ parameter was examined in four studies (n = 246). Three studies found significantly different $t_{1/2}max$ values between the AL and non-AL group ($p = 0.001; < 0.001; < 0.001$) [13, 29, 33]. However, again,

Table 2 Quality assessment of clinical studies according to the Newcastle Ottawa Scale

Studies	Selection					Comparability		Outcome				Total score
	1)	2)	3)	4)	Score	1)	Score	1)	2)	3)	Score	
Green et al. [25]	c	c	a	a	☆☆★★	–	☆☆	b	b	d	★☆☆	Poor
Chang et al. [23]	a	c	a	a	★☆★★	a,b	★★	b	a	a	★★★	Good
Dinallo et al. [24]	a	a	a	a	★★★★	–	☆☆	b	b	a	★☆★	Poor
Protyniak et al. [31]	a	c	a	a	★☆★★	–	☆☆	b	b	a	★☆★	Poor
Son et al. [13]	a	c	a	a	★☆★★	a,–	★☆	b	a	a	★★★	Good
Wada et al. [32]	a	c	a	a	★☆★★	–	☆☆	b	b	a	★☆★	Poor
Wada et al. [33]	c	a	a	a	☆★★★	a,–	★☆	b	b	a	★☆★	Good
Hayami et al. [26]	c	c	a	a	☆☆★★	a,-	★☆	b	b	a	★☆★	Poor
Kim et al. [30]	a	a	a	a	★★★★	a,b	★★	b	a	d	★★☆	Good
Ishige et al. [27]	a	c	a	a	–	–	☆☆	b	a	a	★★★	Poor
Kamiya et al. [29]	a	c	a	a	★☆★★	–	☆☆	b	b	a	★☆★	Poor
Amagai et al. [22]	a	c	a	a	★☆★★	–	☆☆	b	b	a	★☆★	Poor
Iwamoto et al. [28]	a	c	a	a	★☆★★	a,–	★☆	b	b	a	★☆★	Poor

★ Star awarded, ☆ No star awarded

Hayami et al. did not find a significant difference (13.3 vs. 7.8 s, $p=0.12$).

The *ttp* parameter was examined in six colorectal studies (n = 674) [13, 22, 26, 28, 30, 33]. Three studies found significantly different *ttp* values between AL and non-AL patients ($p=0.04$; 0.001; 0.01) [13, 22, 33]. The remaining three studies did not find a significant differences ($p=0.09$; 0.85; 0.33) [26, 28, 30]. Unlike the other studies, one study only performed FA before the creation of the anastomosis [28].

The *T0* parameter was examined in three studies (n = 159) [26, 28, 33]. One study did not find any statistical differences between patients with or without AL; neither did they report any numerical *T0* values [33]. Another study found a significant longer *T0* in patients with AL (64.3 s) compared with non-AL patients (18.3 s, $p=0.002$) [26]. Finally, one study found a significant longer *T0* in patients with AL (37.5 s) compared with non-AL patients (11.0 s, $p=0.03$) [28]. Data for the remaining Q-ICG parameters are listed in Table 3.

Q-ICG cut-off values–anastomotic leakage

Three studies performed a ROC analysis as a method to establish Q-ICG cut-off values associated with AL [13, 29, 33]. One study examining colorectal resections (n = 86) calculated an area under the curve (AUC) of 0.12, 0.96 and 0.93 at the following cut-off values $slp<0.7$ AU/s, $t_{1/2}max>18$ s and $TR>0.6$ for AL [13]. Subsequently, the authors performed a multivariate analysis and calculated an odds ratio of 130.8 for AL if $TR>0.6$ ($p=0.002$). Another colorectal study (n = 112) also performed a ROC analysis and found that *Fmax* and *slp* performed better than both *ttp* and $t_{1/2}max$ (no statistical data published) [33]. Furthermore, this study performed a sensitivity analysis and proposed the following cut-off values for anastomotic leakage: $Fmax>52$AU, $ttp>57$ sec, $t_{1/2}max>14$ s, *and* $slp<2.1$ AU/s [33]. Finally, an esophageal study (n = 26) found an AUC of 0.82 for venous anastomotic failure at a cut-off value of $t_{1/2}max>9.6$ s [29].

Discussion

This systematic review found that Q-ICG can identify patients with significantly increased risk of AL; however, only if performed with the correct methodology and Q-ICG parameters. On one hand, studies that analyzed the *inflow* parameters (*slp, T0,* and $t_{1/2}max$) could significantly differentiate between AL patients and non-AL patients in most of the studies. The *ttp* parameter, while also an *inflow* parameter, had conflicting results as three studies found statistical evidence for an association with AL [13, 22, 33], and three did not [26, 28, 30]. On the other hand, the *intensity* parameters (*Fmax* and R-*Fmax*) were only significantly associated with AL in 1 of 6 studies [33]. Besides, the *Fmax* parameter could not consistently differentiate between patients with re-resection and those without re-resection. Finally, a few studies performed a ROC analysis and found the *inflow* parameter ($t_{1/2}max$) performed best with AUCs of 0.96 [13] and 0.82 [29] at identifying AL and venous anatomic failure, respectively.

The optimal parameter(s) and methodology for Q-ICG for visceral perfusion evaluation

The foundation of Q-ICG rests upon the hypothesis that Q-ICG parameters accurately reflect the underlying visceral tissue perfusion.

This hypothesis is supported by the results of several exploratory animal studies [12, 34–37]. These studies

Table 3 Comparison of Q-ICG parameters in AL and non-AL patients

Studies	*Fmax* (AU)			*ttp* (s)			*slp* (AU/s)			$t_{1/2}max$ (s)			Other parameters			
	AL	Non-AL	*P* value	AL	Non-AL	*P* value	AL	Non-AL	*P* value	AL	Non-AL	*P* value		AL	Non-AL	*P* value
Wada et al. [33][a]	38.1 (±11.4)	91.1 (±31.9)	**0.001**	52.1 (±28.5)	32.8 (±15.9)	**0.01**	0.98 (±0.7)	3.6 (±2.2)	**0.009**	26.1 (±18.9)	12.5 (±7.6)	**0.001**	*T0* (s)	Not shown	Not shown	NS
Son et al. [13]	34.9 (±7.4)	58.0 (±3.4)	0.07	64.0 (±11.7)	30.3 (±2.3)	**0.001**	0.7 (±0.2)	2.5 (±0.2)	**0.001**	40.4 (±7.8)	11.7 (±0.8)	**0.001**	*TR*	0.6 (±0.0)	0.4 (±0.0)	**0.001**
Hayami et al. [26]	79.9 (±28.5)	87.6 (±33.2)	0.42	26.4 (±8.4)	18.6 (±6.2)	0.09	3.4 (±2.0)	5.5 (±2.8)	0.27	13.3 (±4.9)	7.8 (±2.9)	0.12	*T0* (s)	64.3 (±27.6)	18.2 (±6.6)	**0.002**
Kim et al. [30]	N/A	N/A	N/A	57.0 (±45)	44.0 (±18)	0.33	N/A	N/A	N/A	N/A	N/A	N/A	*R-Fmax* (AU)	<50%	N/A	1.00
Amagai et al. [22][b]	Not shown	Not shown	NS	52.8 (±30.3)	30.3 (±15.0)	**0.04**	N/A	N/A	N/A	N/A	N/A	N/A	*Tmax* (s)	74.3 (±42.3)	45.4 (±21.4)	**0.02**
Iwamoto et al. [28][c]	N/A	N/A	N/A	11.5 (±7.3)	12.0 (±9.3)	0.85	N/A	N/A	N/A	N/A	N/A	N/A	*T0* (s)	37.5 (±17.1)	11.0 (±13.1)	**0.03**
Kamiya et al. [29][d]	Not shown	Not shown	NS	N/A	N/A	N/A	N/A	N/A	N/A	16.2 (±10.6)	5.8 (±4.3)	**0.001**	N/A	N/A	N/A	N/A

NS Not significant, *AU* Arbitray units. Statistically significant values are given in bold

[a]*P* values are calculated with a two-sided T test by the authors based on published data; [b]Q-ICG values from a ROI in distal intestine with the lowest fluorescence intensity; [c]FA was performed carried out before creation of the anastomosis; [d]Venous anastomotic failure following esophagectomy

have found a significant linear correlation between various Q-ICG parameters (*ttp, slp, and Fmax)* and levels of local lactate or radioactive microspheres under both normal [12] and compromised perfusion conditions [34–37]. One study directly comparing several Q-ICG parameters in a porcine model found that the *Fmax* parameter had less robust correlation than the *slp* and *norm slp* parameters (*Fmax* r = 0.78, $p = 0.037$; *slp* r = 0.97, $p = 0.001$, and *norm slp* r = 0.96, $p = 0.004$) [12].

Inflow versus intensity parameters

The animal studies found that both *inflow* and *intensity* Q-ICG parameters do correlate with visceral perfusion given the controlled settings of animal experiments. However, when evaluating the results of the clinical studies of this review, the *inflow* parameters perform better than *intensity* parameters. The following section explores some likely reasons for the poor performance of the *intensity* parameter.

The inherent problems with the *intensity* parameters quickly become noticeable in a clinical setting. The problems arise when either the ICG plasma concentration, amount of excitatory light, camera distance, or camera angulation is changed as intensity parameters are influenced by all [13, 38]. Achieving a uniform plasma concentration across different patients is near impossible due to differences in metabolization, vascular status, and distribution volume [39]. However, using a body-mass-adjusted dosage can reduce the impact of these factors. Unfortunately, only two of the included studies did this [13, 22], while the remaining used varying static dosages (1.25–10 mg) or did not disclose their dosage.

Furthermore, *Fmax* is susceptible to timing-bias as ICG with time will begin to enter even ischemic tissue due to capillary diffusion/retrograde flow [13, 40], which may have been the case in two studies that only sampled the *Fmax* value at once at 60 s following injection [24, 31]. One might argue that a *R-Fmax* parameter would be less sensitive to changes in ICG plasma concentration, camera distance/angulation, and vascular status as each patient becomes their own reference. However, *R-Fmax* still suffers the problem with capillary diffusion/retrograde flow, and the selection of representative reference ROIs. One clinical study that analyzed *R-Fmax* could not find a difference in the AL rate between *R-Fmax* > 50% patients compared with *Fmax* < 50% patients at the anastomotic site [30]. This combination of factors is likely to be the reason for the poor performance of *Fmax* concerning clinical endpoints.

On the contrary, a significant strength of *inflow* parameters is that variables that massively impact the *intensity* parameters have far less of an impact on the *inflow* parameters. *Inflow* parameters are more resilient because they depend on the timing of "perfusion events" rather than the "numerical intensity values" determined by the Q-ICG system (Fig. 1). Another strength of the *inflow* parameters is that unlike the *intensity* parameters, they are not subject to the capillary diffusion/retrograde flow problem. It has been reported that the introduction of V-ICG initially increased the rate of surgical complications. The increase may have been a result of capillary diffusion/retrograde flow leading surgeons to misdiagnose the fluorescence angiogram as adequate perfusion even though it was judged to be deficient by traditional perfusion assessment [13].

The superiority of *inflow* parameters is supported by theoretical work in computer models. One study identified the *inflow* phase of the FA to carry most information about tissue perfusion [41]. In another study, the gastric conduit was modeled, and the *"time- to-20% of max intensity"* was the best way to discriminate between sufficient and impaired perfusion with an AUC of > 0.85 [42].

Finally, while *T0* is defined by the *inflow* phase of the fluorescence–time curve, it suffers from an ambiguous definition as "time to first fluorescence signal." Thus, it can be difficult to quantify due to background noise. Furthermore, the *T0* parameter is like to be unstable in a clinical setting as it is altered by peripheral vs. central venous catheter, infusion speed, the height of the person, and vascular status [43]. Hence, we suggest exploring 1 of the other *inflow* Q-ICG parameters (*TR, ttp, slp, and* $t_{1/2}max$).

Challenges and perspectives for Q-ICG in the future

Q-ICG has the potential to become a viable surgical tool; however, Q-ICG is faced with a couple of challenges.

Firstly, the camera and target ROI must be stationary to allow for quantification. A stationary camera can be achieved by either fixating the laparoscope/ICG camera in a mechanical holding arm or by locking the camera during robotic surgery. The target can be kept stationary by inducing a brief period of apnea. Another method would be the use of motion tracking, which could correct the image position based on the motion of the camera or the target [44]. Secondly, repeated perfusion measurements are valuable in many different clinical situations. Unfortunately, it takes approximately 15–20 min for the fluorescence signal has decreased to a negligible level. Thus, rapidly repeated Q-ICG and especially V-ICG measurements may not be viable as the increased fluorescence intensity oversaturates the image causing problems for both the naked human eye and computer software. However, this challenge might be overcome by utilizing *normalization* as one study showed that Q-ICG measurements could be reliably repeated if based on the *normalized slp* [45]. Thirdly, Q-ICG still lacks verified procedure-specific cut-off values as these are necessary for swift objective decision making. However, there was little agreement on cut-off values and what parameter

to use between the colorectal studies included in this review. Thus, we suggest a focus on the *inflow* parameters and relative *inflow* parameters. Relative Q-ICG parameters are not well explored in either animal or clinical studies. However, it offers multiple benefits as the patients become their own control, and cut-off values based on relative parameters are more valid when comparing different patients [38].

Fourthly, there is a lack of commercial Q-ICG systems. This lack of industry-supported FA systems with Q-ICG options may be a consequence of legal concerns regarding their accountability for clinical decisions based on their Q-ICG systems. Fortunately, it seems that these concerns have been put to rest as newly developed FA systems do support intraoperative Q-ICG: SPY-Elite® [46] and VisionSense® [47]. However, these systems still only employ *intensity* parameters. Thus, we urge manufactures of FA systems to incorporate a method for easy and intuitive intraoperative Q-ICG measurement of *inflow* parameters. A final challenge is that some surgeons believe that surgical experience and the naked human eye are superior to technology-based techniques for perfusion assessment [48]. However, evidence suggests that surgeons assisted by a Q-ICG system can identify anastomotic sites with superior perfusion compared with unassisted surgeons [40, 49]. Furthermore, intraoperative Q-ICG might be more assessable than some surgeons believe, as it was found that a real-time intraoperative Q-ICG with a plug-in device was both feasible and had an excellent usability score during gastroesophageal resection [50].

Limitations

A limitation of this review is that only 2 of 13 studies [25, 31] performed intraoperative Q-ICG. Hence, it was not possible to directly compare Q-ICG with V-ICG or traditional perfusion assessment. However, intraoperative Q-ICG has been shown to increase sensitivity and specificity, positive and negative predictive values compared with V-ICG and traditional perfusion assessment for identifying ischemic bowel [51]. Furthermore, while unfortunate that only a few studies utilized intraoperative Q-ICG, the focus of this review was to identify the determining factors for whether Q-ICG does reflect clinical endpoints.

Another limitation was the heterogeneity between the studies with regards to the patient population, surgical procedures, ICG methodology, Q-ICG parameters, and endpoints. This is reflected in the bias evaluation, where only four of 13 studies achieved a high level of evidence (Table 2). Furthermore, most of the studies had relatively small patient populations, and only three studies included a historical control cohort [24, 30, 32]. No RTC studies were present, and only one study performed propensity matching to ensure that the historical cohort and the V-ICG cohort were comparable [32]. Propensity matching had a substantial impact on the difference in AL rate between V-ICG patients and the control cohort (AL rate in control cohort 6.9% before matching and 14.7% following matching). Q-ICG measurements were only repeated in two studies following re-resection in the studies looking et al. rates [29, 30]. Consequently, re-resected patients brought false Q-ICG values into the analysis of the AL rates, thus introducing bias. Finally, all included studies only looked at the arterial phase of perfusion. When dividing the mesentery, the venous outflow may also be impaired, leading to venous congestion, which also may contribute to poor anastomotic healing [52].

This comprehensive list of biases and differences between studies is a global challenge for fluorescence angiography during surgery. A recent meta-analysis exploring V-ICG in colorectal cancer reported similar results with a moderate level of bias and an intrinsic heterogeneity between the studies [53]. Ultimately, the authors believe that this heterogeneity is a consequence of the lack of a gold standard within the field of fluorescence angiography.

Conclusion

The results, while heterogenous, all seem to point in the same direction. Q-ICG parameters based only on fluorescence *intensity* parameters (*Fmax* and *R-Fmax*) are unstable and do not reflect clinical endpoints. In contrast, the *inflow* parameters (*ttp, slp, T0, and* $t_{1/2}max$) are resilient to much of the variance that occurs when performing Q-ICG. The *inflow* parameters also correlate better with perfusion in animal models and have a stronger association with clinical endpoints. Regarding the Q-ICG methodology, we recommend that future studies utilize a body-mass-adjusted ICG administration and a fixated camera setup. We believe that standardization of the methodology is necessary to establish a gold standard within the field of Q-ICG.

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Compliance with ethical standards

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