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Computer-assisted quantification and visualization of bowel perfusion using fluorescence-based enhanced reality in left-sided colonic resections

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Abstract

Background Fluorescence-based enhanced reality (FLER) is a computer-based quantification method of fluorescence angiographies to evaluate bowel perfusion. The aim of this prospective trial was to assess the clinical feasibility and to correlate FLER with metabolic markers of perfusion, during colorectal resections.

Methods FLER analysis and visualization was performed in 22 patients (diverticulitis $n=17$; colorectal cancer $n=5$) intra- and extra-abdominally during distal and proximal resection, respectively. The fluorescence signal of indocyanine green (0.2 mg/kg) was captured using a near-infrared camera and computed to create a virtual color-coded cartography. This was overlaid onto the bowel (enhanced reality). It helped to identify regions of interest (ROIs) where samples were subsequently obtained. Resections were performed strictly guided according to clinical decision. On the surgical specimen, samplings were made at different ROIs to measure intestinal lactates (mmol/L) and mitochondria efficiency as acceptor control ratio (ACR).

Results The native (unquantified) fluorescent signal diffused to obvious ischemic areas during the distal appreciation. Proximally, a lower diffusion of ICG was observed. Five anastomotic complications occurred. The expected values of local capillary lactates were correlated with the measured values both proximally (3.62 ± 2.48 expected vs. 3.17 ± 2.8 actual; rho 0.89; $p=0.0006$) and distally (4.5 ± 3 expected vs. 4 ± 2.5 actual; rho 0.73; $p=0.0021$). FLER values correlated with ACR at the proximal site (rho 0.76; $p=0.04$) and at the ischemic zone (rho 0.71; $p=0.01$). In complicated cases, lactates at the proximal resection site were higher (5.8 ± 4.5) as opposed to uncomplicated cases (2.45 ± 1.5; $p=0.008$). ACR was reduced proximally in complicated (1.3 ± 0.18) vs. uncomplicated cases (1.68 ± 0.3; $p=0.023$).

Conclusions FLER allows to image the quantified fluorescence signal in augmented reality and provides a reproducible estimation of bowel perfusion (NCT02626091).

Keywords Fluorescence-based enhanced reality · Anastomotic perfusion · Indocyanine green · Fluorescence angiography · Fluorescence quantification · Capillary lactates · Mitochondria respiration

Fluorescence angiography (FA) is a real-time optical imaging modality, which is currently investigated as a means to improve the intraoperative appreciation of bowel perfusion and to potentially decrease the rate of anastomotic

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complications in colorectal surgery [1]. FA is obtained using near-infrared (NIR) camera systems, which are optimized to elicit and detect the fluorescent signal emitted by a fluorophore molecule, and most frequently indocyanine green (ICG) [2, 3]. In the current setting, a detectable ICG fluorescence signal, which is converted into a visible piece of information displayed on the monitor, is considered a positive marker of perfusion and could well define the level of bowel division. In the PILLAR II multicenter trial, the FA-enhanced indication of perfusion induced an 8% revision of the clinically chosen level of bowel resection, with no anastomotic leaks (AL) occurring in the revised cases [4]. Likewise, one of the largest multicenter prospective trials performed so far has reported a 6% surgical strategy change when using FA guidance in over 500 colorectal resections, and no subsequent leaks [5].

ICG-based FA seems to hold a significant potential, as highlighted in a recent meta-analysis of 5 non-randomized comparative studies [6–10], in which FA-guided colorectal resections were associated with a lower incidence of AL [1].

Recently, the first prospective multicenter randomized clinical trial (RCT) comparing ICG ·guided ($n = 118$) versus clinically guided ($n = 122$) left-sided colonic or rectal resections has been published [11]. The authors reported a change of bowel resection in more than 10% of cases based on ICG-FA, but did not find any statistically significant difference in the AL rate (5 vs. 9% in ICG vs. control, respectively) [11]. The metrics used in the RCT to categorize FA was a subjective description of fluorescence distribution as good (= uniform), poor (= non-uniform) or absent (= no fluorescence), without a standardized quantification.

A quantitative method to categorize the fluorescence signal is currently lacking widespread adoption, which is an obstacle to having reproducible and comparable data in both single and multicenter studies. The need for a quantitative and stable metric has been advocated by multiple authors [12–15] and is more apparent when considering some technical aspects of fluorescence imaging. First, fluorescence intensity is inversely correlated to the source-to-target distance following a quadratic law [16]. In other words, a perfused area observed from further away could appear less intensively fluorescent than a marginally perfused area, which is observed from more closely. Secondly, perfusion is a dynamic process, with the fluorophore which tends to distribute even to ischemic zones over time, subsequently underestimating the extent of the ischemic area. This translates with the non-reliability of the fluorescence signal presence/intensity, in the absence of a dynamic evaluation and after standardization of the distance between the NIR-endoscope and the surgical scene and/or by using a reference calibration tool [16, 17]. On the other hand, the temporal pattern (rate or speed) of dye distribution depends on the integrity of the vascular network and, as a result, the analysis of the dynamic angiographic uptake of the fluorophore over time seems a robust and reproducible metric [13, 14, 18], which is independent of distance [16, 17, 19–23].

Fluorescence-based enhanced reality (FLER) is a software analysis and display of the dynamic evolution of the fluorescent signal during angiography to evaluate tissue perfusion. FLER provides a quantitative and reproducible estimation of the fluorescence signal and its accuracy has been largely established experimentally in the animal model [16, 17, 21–26]. This study aims to clinically translate the FLER approach in humans by validating the feasibility and establishing the accuracy of ICG-FA complemented with computer analysis in documenting the intestinal perfusion during left-sided colorectal resections and correlating the results with intestinal metabolic markers of perfusion.

Patients and methods

This prospective, non-interventional, feasibility trial was approved by the National Ethical Committee (*Comité de Protection des Personnes*; CPP) and registered under the reference No. IDRCB: 2015-A01223-46. Written informed consent was obtained by the included patients. The trial was registered on *ClinicalTrial.gov* with the reference NCT02626091 and the following title: Perfusion Evaluation with Real-time Fluorescence-based EnhanCed realiTy of anastomotic site (PERFECT). The original interventional design of the study was reclassified by the Ethical Committee because of the absence of CE marking of the proposed software solution.

Twenty-seven patients (15 male patients; mean age: 59 ± 11 years; mean weight: 75.32 ± 16.82 kg) undergoing left-sided colorectal resections agreed to be enrolled in the study from January 16, 2017 to November 22, 2018. Five cases were excluded for the following reasons: technical problems with the near-infrared equipment ($n = 2$); surgery was postponed for pneumonia ($n = 1$); decision to perform a robotically assisted approach ($n = 1$) and modified procedure that lead to inappropriate inclusion (transverse colectomy, $n = 1$). The full data set was available in 22 cases (17 sigmoidectomies for diverticulitis, 3 left colectomies, and 2 low anterior resections for cancer).

Surgical procedures and fluorescence-based enhanced reality (FLER)

The procedures were performed by specialist colorectal surgeons. Sigmoidectomies for benign diseases were performed with a standard dissection of the mesocolon close to the bowel using a LigaSure™ vessel-sealing system (Valleylab, Boulder, Colorado, United States) and without ligation of the inferior mesenteric artery at its origin. A splenic flexure

takedown was performed when required. A mechanical end-to-end anastomosis was performed in all cases. Left colectomies and low anterior resections for cancer involved oncologic dissection using a medial-to-lateral approach and mesenteric vessels division. The anastomosis was an end-to-end one in all cases except in case 015, in which a side-to-side (ileosigmoid) anastomosis was performed. A protective ileostomy was made in one out of two rectal cancer cases.

After preparing for the distal resection, the surgeon identified the distal resection site based on clinical appreciation and marked it with surgical clips. Before proceeding to the stapling of the distal site, FLER imaging was performed intra-abdominally to calculate perfusion values. FLER values were recorded. However, resection was performed following the clinical decision. After exteriorization of the bowel through a Pfannenstiel incision, the surgeon marked the intended clinically based proximal resection line on the bowel, with clips or with a surgical marker, based on clinical evaluation. FLER imaging was repeated to compute perfusion values also at the proximal site.

FLER was obtained as follows: immediately before the injection of 0.2 mg/kg of indocyanine green (ICG), the near-infrared camera (D-Light *P*, KARL STORZ) was switched to a near-infrared mode, to capture the full evolution of the fluorescence signal. The camera was held still during the acquisition of the signal both intra- and extra-abdominally, by means of an articulated arm. A standard PC was connected to the laparoscopic cart to collect the video flow. The ER perfusion software built the virtual perfusion cartography, which was subsequently superimposed onto the bowel to display perfusion values (as a color code and/or as a line, Fig. 1). FLER can use multiple metrics to compute perfusion values including the time-to-peak of the fluorescence intensity and the slope of the time-to-peak (delta intensity/time). The bowel was held and exposed using a laparoscopic grasper during distal evaluation. Perfusion levels were quantified with FLER on the entire length of the bowel as it was apparent in the field of view. FLER analysis was performed in the operating room only to mark the ROIs on the bowel for further biopsies on the surgical specimen. After FLER evaluation, surgical resection was performed strictly based on the previously marked clinical decision.

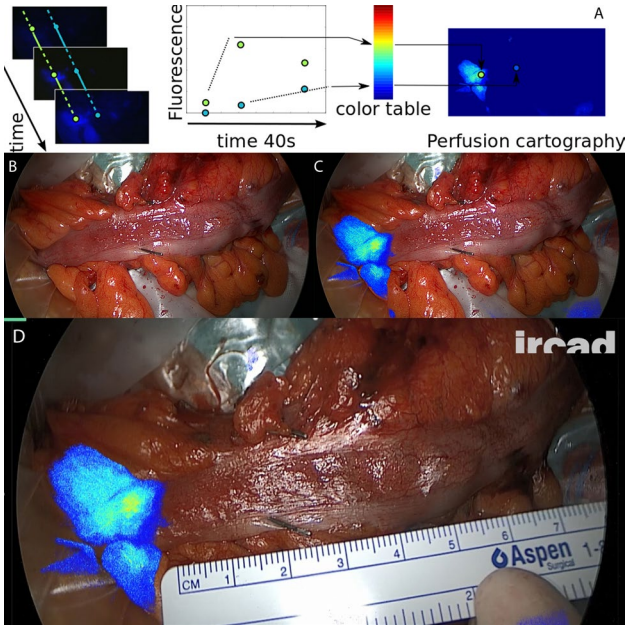

Fig. 1 FLER analysis and display. **A** A virtual perfusion cartography is obtained by averaging fluorescence signals over a 40-s video at a speed of 25 frames per second. Each single pixel of the cartogram results from the average of 200 consecutive images, which store the overtime evolution of the signal. **B** White light image during the proximal resection assessment: clips show the clinically chosen resection site. **C** The perfusion cartography is then superimposed onto real-time images to obtain a mixed reality effect and display the quantified ICG signal directly on the bowel. Alternatively, the perfusion values can be displayed directly on the bowel as virtual resection lines. **D** In this case, the distance between clinical decision and FLER50% is measured and marked under augmented reality to allow a precise sampling on the surgical specimen

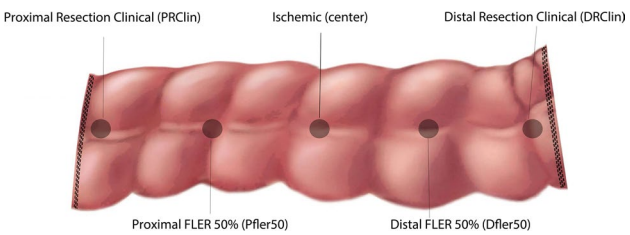

Fig. 2 Sampling sites on the bowel. Schematic representation of the regions of interest (ROIs) on the bowel in which blood for lactates and full-thickness biopsies for mitochondrial respiration were sampled. Ischemic (center of the specimen); Dfler50 (distal ROI according to FLER set at 50%); DRClin (distal resection according to clinical decision); Pfler50 (proximal ROI according to FLER at 50%), and PRClin (proximal resection according to clinical decision)

Systemic and local capillary lactates (mmol/L)

The strip-based lactate analyzer EDGE® (Apexbio, Taiwan, ROC) was used to measure systemic and local bowel capillary lactatemia. Systemic lactatemia was measured on blood samples obtained by puncturing fingertips during the following surgical stages: induction (T0), start of dissection (T1), distal resection (T2), and proximal resection with specimen removal (T3). Local bowel capillary lactatemia was measured on the surgical specimen at the 5 different regions of interest (ROIs), as displayed in Fig. 2. A prediction model based on previous experiments in the porcine model was applied to predict lactate values at the areas in which the fluorescence signal was delayed by 50% proximally (PFler50) and distally (DFler50) [27, 28].

Mitochondrial respiration rate (pmol of oxygen/second/mg of dry tissue)

The mitochondrial respiratory rate was measured as previously reported [17, 23]. Full-thickness colonic biopsies were harvested on the surgical specimen at the same ROIs as reported for lactates assessment. The biopsies were placed in a 2 mL water-jacketed oxygraphic cell, equipped with a Clark electrode (Oxygraph-2k©, Oroboros Instruments, Innsbruck, Austria). Basal oxygen consumption (V0) was calculated in the presence of glutamate (5 mmol/L), malate (2 mmol/L), and succinate (25 mmol/L). The maximal respiration rate (Vadp) was measured in the presence of a saturating amount of adenosine diphosphate (ADP). The acceptor control ratio (ACR), which reflects the coupling between phosphorylation and oxidation (OXPHOS) [29] and mitochondrial homeostasis, was calculated as the Vadp/V0 ratio. Similarly, no biopsies were performed when FLER50 displayed a more distal and more proximal ROI than the clinical decision.

Outcomes and sample size calculation

The primary outcome to evaluate the accuracy of the FLER software technology was the correlation with biological biomarkers of perfusion. The sample size was calculated based on previous preclinical experiments and taking mitochondrial respiration ACR as the reference variable to be correlated with FLER analysis (Rho > 0.7, in vivo). However, the preclinical correlation of FLER with mitochondrial respiration was calculated in vivo and not on the specimen. For this reason, the rho coefficient was prudently assumed to be lower, for sample size calculation. Assuming a rho coefficient of 0.7, the number of sufficient samples to be correlated (alpha 0.01 and 1-β at 90%) is 17. As it was not permitted to obtain samples from the bowel surface in cases in which FLER was suggestive of resecting a larger amount of tissue (more distally and/or more proximally) than what the clinical decision indicated, in order to maximize the number of biopsies and reduce the number of patients to be included, we decided to identify an area where perfusion would drop to 50%. The rationale was that by displaying the FLER50% it would provide a ROI closer to the ischemic center when compared to the clinical resection point at both proximal (PFler50) and distal (DFler50) sites and, consequently more constantly included in the surgical specimen. Finally, the total number of paired values FLER-mitochondrial respiration was 92 (18 missing values), considering all 5 ROIs per patient. Twenty-eight of those paired values (missing 16) concerned the FLER50-mitochondrial respiration. Both V0 and Vadp could be calculated in all samples in order to obtain ACR.

Statistics

Statistics were performed with GraphPad Prism 8. ANOVA was performed, followed by Dunnett's multiple comparison tests, to calculate the differences between the mean of capillary lactates and mitochondrial respiration in the ischemic vs. the remaining ROIs. Unpaired *t* tests were performed to calculate the differences in patients presenting anastomotic leaks. Pearson's correlation coefficient (i.e., Pearson's rho) was calculated to correlate mitochondrial respiration and capillary lactates with the quantitative fluorescence analysis. A $p < 0.05$ was considered statistically significant.

Results

FLER analysis was feasible in all but two cases, due to technical problems with the light source or the optical system. Mean total time to obtain both proximal and distal FLER analysis and to allow the surgeon to mark the ROIs by means of screen display was 7.8 ± 3.34 min.

During the distal resection site appreciation, the native (unquantified) ICG fluorescence signal diffused to obvious ischemic areas within approximately one minute (video clip). During the proximal resection assessment, a lower diffusion of ICG was observed when compared to the diffusion found with the specimen still attached at the anal side.

Mean ischemia time before the first (distal) FLER was 61.8 ± 40.6 min. However, it ranged widely from 6 to 176 min (median: 52 min). Total specimen ischemia time (from the beginning of dissection until specimen removal, including proximal FLER) was 91.36 ± 47.66 min.

There were 5 anastomotic complications: 2 anastomotic leaks requiring surgical management and 3 cases presenting mild peri-anastomotic collections on CT scan, successfully managed with antibiotic treatment (Table 1).

The results of FLER time-to-peak are reported in Fig. 3. Interestingly, in cases with anastomotic complications (combining leaks and peri-anastomotic collections $n = 5$), FLER values at the clinically chosen proximal and distal resection sites were higher when compared to uncomplicated cases both distally (60.2 ± 35 frames vs. 38.6 ± 20 frames; $p = 0.06$) and proximally (112.8 ± 85 frames vs. 43.6 ± 35 frames; $p = 0.01$).

Local lactates (mmol/L) at the center (4.6 ± 3.8) were statistically significantly higher than those measured at the Dfler50 (4 ± 2.5; $p = 0.034$), DRclin (3.45 ± 2; $p = 0.012$), and PRclin (3.24 ± 2.8; $p = 0.036$), but not when compared to the values from Pfler50 (3.17 ± 2.8; $p = 0.08$). The expected values of local capillary lactates using the software at the proximal 50% site (PFler50) were highly correlated with the truly measured values (3.62 ± 2.48 expected vs. 3.17 ± 2.8 actual; rho 0.89; CI 0.58–0.97; $p = 0.0006$).

Table 1 Procedures data

Case	Diagnosis	Comments
001	Diverticulitis	Technical problem with the near-infrared light source
002	Diverticulitis	CT scan on POD 4: peri-anastomotic collection (33 × 23 mm). Antibiotic treatment
003	Diverticulitis	The surgeon spontaneously decided to change the strategy and achieved a more proximal resection, without being influenced by the FLER analysis (second case shown in the video clip)
004	Diverticulitis	The procedure was reported (pneumonia)
005	Diverticulitis	No fluorescence signal increase at the proximal site. The surgeon spontaneously decided (not on the FLER basis) to perform a more extensive proximal mobilization
006	Diverticulitis	
007	Diverticulitis	
008	Colon cancer	Wrong inclusion (transverse colon resection)
009	Colon cancer	
010	Diverticulitis	No fluorescence signal at the proximal site. A clearly ischemic segment was noticed just before the anastomosis was completed intra-abdominally. This case required a proximal re-resection of 5 cm (third case shown in the video clip)
011	Diverticulitis	Technical problem with the optical system
012	Diverticulitis	
013	Diverticulitis	CT scan on POD 1: Anastomotic leak (1 cm). Hartmann procedure using a laparoscopic approach
014	Diverticulitis	
015	Colon cancer	Open surgery
016	Rectal cancer	CT scan on POD 3: peri-anastomotic collection. Antibiotic treatment
017	Diverticulitis	
018	Diverticulitis	No fluorescence signal at the proximal site. CT scan on POD 4: peri-anastomotic collections. Antibiotic treatment
019	Rectal cancer	CT scan on POD 8: Anastomotic leak with large collections. Laparoscopic lavage and drainage
020	Diverticulitis	
021	Diverticulitis	
022	Diverticulitis	Once included in the present study, the patient opted for a robotically assisted approach (outside the protocol)
023	Diverticulitis	
024	Diverticulitis	
025	Diverticulitis	
026	Diverticulitis	
027	Colon cancer	

All but one procedures were performed using a laparoscopic approach. One left colectomy for recurrent colon cancer (case 015) was performed using an open approach. In three cases (patients 005, 010, and 018), no FLER signal could be detected during the extracorporeal assessment of the proximal site. This was due to the fact that the exposed bowel was entirely ischemic and a more extensive mobilization was required in one case and the refection of the anastomosis with a further 5 cm resection in the second one. Those changes were only made on the basis of clinical decision. In case No. 018, no change was made according to the clinical decision

Expected lactates (4.5 ± 3) were also significantly correlated with actual values (4 ± 2.5) at the distal (DFler50) site (rho 0.73; 95% CI 0.34–0.9; $p = 0.0021$).

Interestingly, in cases with anastomotic complications, the level of local capillary lactates at the proximal resection site (strictly chosen according to clinical decision) was found to be significantly higher (5.8 ± 4.5) when compared to uncomplicated cases (2.45 ± 1.5; $p = 0.008$) (Fig. 4).

Mean predicted values of ACR at PFler50 (1.38 ± 0.41) correlated well with the actual values (1.44 ± 0.4; rho = 0.85), while the predicted values at DFler50 (1.43 ± 0.44) did not correlate with the measured values on the samples (1.91 ± 1.1; rho = 0.12).

ACR was correlated with the fluorescence time-to-peak at the Pfler50 (rho 0.76; 95% CI 0.01 to 0.96; $p = 0.04$) and at the ischemic zone (rho 0.71; CI 0.09 to 0.93; $p = 0.01$). There was no correlation between ACR and time-to-peak at DFler50 (rho 0.18; CI − 0.55 to 0.76; $p = 0.32$) and DRClin (rho 0.4; CI − 0.42 to 0.86; $p = 0.16$). ACR was significantly reduced at the PRClin in patients presenting with anastomotic complications (1.3 ± 0.18) when compared to PRClin in patients without complications (1.68 ± 0.3; $p = 0.023$). Remaining results of the mitochondrial respiration rate are reported in Fig. 5 and Table 2.

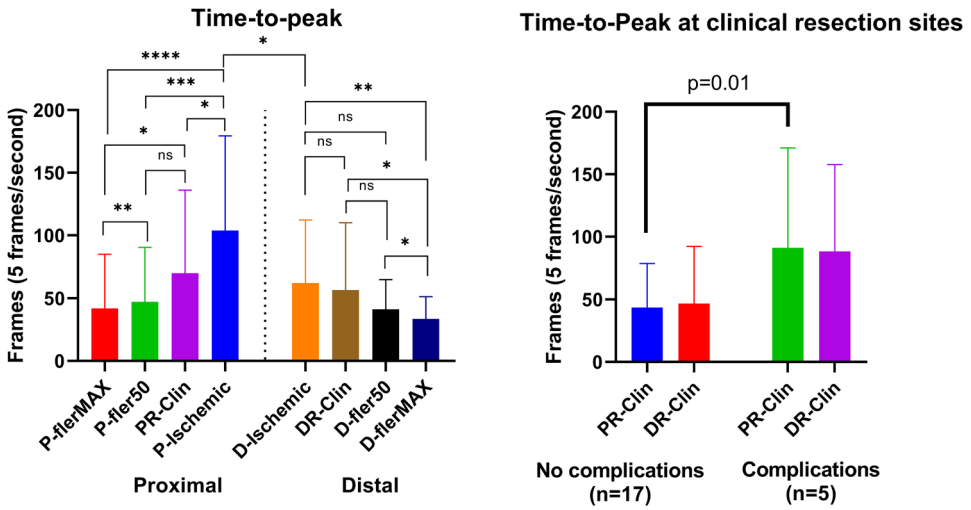

Fig. 3 Time-to-peak (TTP). During the intra-abdominal distal appreciation, the fluorescence signal at the ischemic bowel (D-ischemic) required significantly more frames before reaching the peak (62.2 ± 50) when compared to the high perfused area (Dfler-MAX = 33.62 ± 17.6; $p = 0.008$), but not when compared to Dfler50 (41.2 ± 23.7; $p = 0.06$) or to the DRClin (56.6 ± 53.44). During the extra-abdominal proximal evaluation, time-to-peak of the ischemic zone (103.9 ± 75.41) was significantly higher than the one at Pfler-MAX (41.95 ± 42.9; $p < 0.0001$), Pfler50 (47.2 ± 43.2; $p = 0.0001$), and PRClin (70 ± 66; $p = 0.014$) (Figure). The TTP was significantly longer at the ischemic area during proximal resection when compared to distal resection

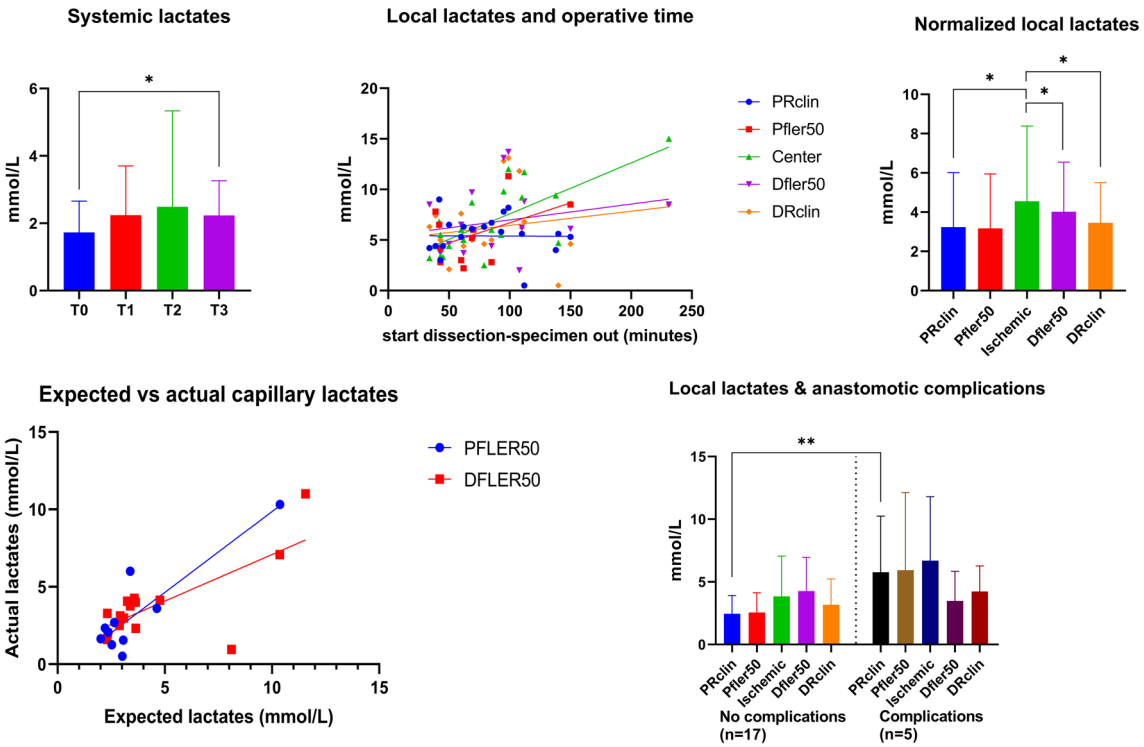

Fig. 4 Capillary systemic and local (intestinal) lactates (mmol/L). Systemic lactatemia increased during the surgical procedure when compared to baseline values (T0: 1.7 ± 0.9), reaching a statistically significant difference at the moment of specimen resection (T3: 2.2 ± 1; T0 vs. T3; $p = 0.016$). The corresponding local lactates measured on the specimen were normalized to the systemic ones. There was a statistically significant correlation between the local level of lactates measured at the ischemic center of the specimen and the operative time (rho 0.73; 95% confidence interval 0.44 to 0.88; $p = 0.0002$), but none with the ones measured at the remaining ROIs

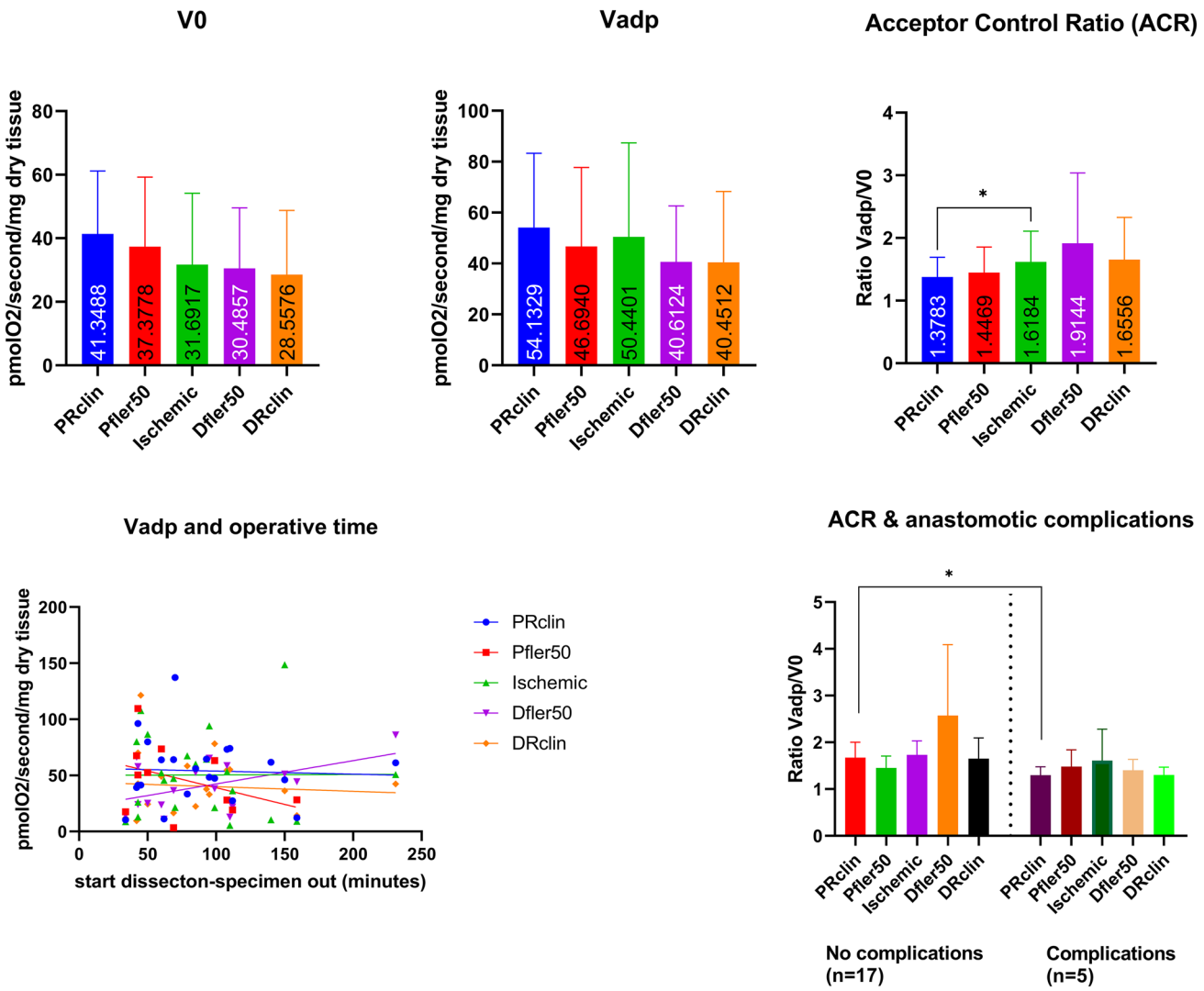

Fig. 5 Intestinal mitochondrial respiration (pmol of oxygen/second/mg of dry tissue). ANOVA (all vs. ischemic): The basal (V0) mitochondrial respiration rate was similar among the ROIs on the specimen (PRClin = 41.35 ± 19.8; PFler50 = 37.38 ± 21.87; Ischemic = 31.7 ± 22.44; DFler50 = 30.49 ± 19; DRClin = 28.56 ± 20.23). The maximal respiration rate in excess of substrate (Vadp) increased significantly in all ROIs, (PRClin = 54.13 ± 29.17; $p = 0.0003$ PFler50 = 46.7 ± 31; $p = 0.008$ Ischemic = 50.44 ± 36.9; $p < 0.0001$ DFler50 = 40.61 ± 22; $p < 0.0001$; DRClin = 40.45 ± 27.81; $p = 0.0002$). ANOVA (all vs. ischemic) showed that the mean acceptor control ratio (ACR = V0/Vadp) was significantly higher at the ischemic ROI (1.6 ± 0.5) when compared to the anastomotic site (1.33 ± 0.3; $p = 0.02$) chosen according to clinical decision. ACR increased at the ischemic area significantly more when compared to the PRClin, witnessing that the relatively short ischemia time was insufficient to induce an irreversible loss of the mitochondrial function. V0 was not correlated with ischemia time (start of dissection specimen removal), at any of the ROIs, while Vadp was significantly correlated with ischemia time only at the DFler50 (rho 0.49; confidence interval 0.01 to 0.78; $p = 0.046$)

Discussion

This observational trial allowed to confirm the intraoperative feasibility of the FLER quantification concept [17] in clinical cases during left-sided colorectal resection. The augmented reality demonstration of the computed perfusion cartography registered over the bowel can be easily integrated in the OR, with only a marginal disruption of the standard workflow (namely a few extra minutes). It was possible to document that the unquantified ICG fluorescence signal was not reliable and that the ICG diffused at clearly non-vascularized areas in both benign and malignant cases, particularly before distal resection (with the future specimen still attached at both proximal and distal ends). During proximal resection, ICG diffusion was lower. This could be related to the compression on the mesenteries through the abdominal wall and because of the absence of the backflow from the distal side. It is essential to stress that the objective of this trial was to evaluate the accuracy of the FLER quantification method and that neither a native fluorescence

Table 2 Correlations between the Acceptor Control Ratio and FLER time-to-peak

Time-to-peak vs. acceptor control ratio			
ROI	Rho (Pearson)	95% CI	*p*
PRClin	0.4	− 0.35 to 0.84	0.29
PFler50	0.76	0.01 to 0.96	0.024
Ischemic	0.71	0.09 to 0.93	0.01
DFler50	0.18	− 0.55 to 0.76	0.32
DRClin	0.4	− 0.42 to 0.86	0.16
Ischemic + PRClin	0.6	0.18 to 0.83	0.004
Ischemic + PFler50	0.74	0.4 to 0.9	0.0004
Ischemic + DRClin	0.59	0.15 to 0.83	0.006
Ischemic + DFler50	0.13	− 0.35 to 0.56	0.3
All ROIs	0.23	− 0.08 to 0.5	0.07
All ROIs except DFler50	0.57	0.28 to 0.76	0.0003

ACR was correlated with time-to-peak at the Pfler50 (rho 0.76; 95% CI 0.01 to 0.96; $p = 0.04$) and at the ischemic zone (rho 0.71; CI 0.09 to 0.93; $p = 0.01$). Combining all proximal ROIs (PRC, Pfler, and Ischemic), the rho was 0.64 (CI 0.33 to 0.83; $p = 0.0003$). Combining all ROIs, except Dfler50, the overall rho was 0.57 (CI 0.28 to 0.76; $p = 0.0003$)

signal nor FLER images were used to guide the resections, which were made strictly according to the clinical decision.

Following our previous experimental studies [16, 17, 21–25], we measured intestinal capillary lactates and mitochondrial respiratory rate at the ROIs identified via the analysis of fluorescence time-to-peak and displayed in augmented reality for the first time in humans. The study was sufficiently powered to explore the correlation between the software analysis of the fluorescent signal and the validated metabolic markers of perfusion. The results demonstrated a strong correlation of FLER with capillary lactates and with the acceptor to control (ACR) ratio of mitochondria respiration, including the ability to correctly predict their values. Interestingly, the FLER values at the clinically chosen resection sites were higher (slower signal increase) when compared to uncomplicated cases. Similarly, both capillary lactates and the ACR were significantly impaired at the clinically chosen proximal resection site in cases with anastomotic complications. However, this preliminary study was not designed to explore clinical outcomes. Consequently, it is not sufficiently powered to draw solid conclusions on the potential of FLER to predict the occurrence of an anastomotic complication.

Capillary lactates are robust markers of fine changes in perfusion that we previously cross-validated with sophisticated high-end methods, such as metabolomics profiling using magnetic resonance spectroscopy [30]. Contrarily to the experimental setting, the FLER analysis in the present clinical trial was not used to determine the resection level, which was strictly decided on clinical judgment. Capillary blood sampling and biopsies were used as surrogate markers and were performed on the specimen, directly in the OR, immediately after complete transection. In order to identify the most relevant biological data to correlate with the digital analysis of the perfusion, since the accepted study design included sampling on the surgical specimen, we did a preliminary test in 6 pigs (unpublished data) and measured capillary lactates before and immediately after complete removal at the same sites. This pilot test demonstrated a significant increase in lactate values when measured ex vivo as compared to in vivo measures. As a matter of fact, the increase was 1.75 ± 0.54 times at the ischemic area ($p = 0.002$), 1.4 ± 0.14 times at the vascular proximal area ($p = 0.05$; not significant), and 2.8 ± 2.2 times at the vascular distal area ($p = 0.0008$). Given the homogeneous increase in both ischemic and vascularized areas, capillary lactates were still considered reliable, although they were measured on the specimen and not in vivo. The ischemia time was largely variable in this series. However, it was strongly only correlated with local lactates in the center of the ischemic zone, as predictable, and not with the other ROIs. The expected values of local capillary lactates were highly correlated with the truly measured values at both the proximal (PFler50%) site (rho 0.89) and at the distal (DFler50%) site (rho 0.73). In a nutshell, FLER was correlated with local capillary lactates, and the level of local capillary lactates at the proximal resection site identified according to clinical decision and independently of FLER analysis were higher in cases with anastomotic leaks (Fig. 4). Additionally, in survival experimental models of bowel anastomosis with preset levels of perfusion determined by FLER (25 vs. 75% of perfusion), we could demonstrate that local lactates were directly correlated with the anastomotic inflammation score [23] and potentially with leaks (two leaks occurred only in the group of animals with 25% of perfusion). Again experimentally, FLER quantification could predict the anastomotic tensile strength [25], used as a surrogate marker of leak.

Mitochondrial respiration assessment using an oxygraphic method is a robust and validated marker of perfusion. It provides insights into the energetic status of the tissues by calculating the amount of oxygen that is consumed by the mitochondria. The acceptor control ratio (ACR) represents the efficiency of OXPHOS coupling between the electron transfer chain (OX) and phosphorylation (PHOS). It is the key bioenergetic transduction in the mitochondria. ACR is the maximal rate of ADP-induced O_2 consumption (Vadp) to the basal rate of oxygen consumption in the absence of ADP (V0). Ischemic tissues are highly dependent on the phosphate acceptor ADP, and the addition of ADP induces an increase in mitochondrial respiration, which is more pronounced in ischemic areas than in perfused tissues, provided that the ischemic insult is still reversible with a preserved electron transport chain function [29]. As a result, a reduced

ACR represents a deficit of energetic coupling, which is an early phenomenon of mitochondrial impairment that precedes defects in the electron transport chain [29].

The ACR of the mitochondrial respiration rate was strongly correlated with the FLER quantification of the fluorescent signal expressed as time-to-peak, except for the DFler50%, probably because of the time lapse between distal resection (1st FLER analysis) and proximal resection (2nd FLER analysis).

The globally preserved bowel mitochondria function in this clinical study, with a limited mean ischemia time of less than two hours, is in line with our previous experimental observations that a substantial decrease occurs after 4 h of ischemia [21]. Nevertheless, the correlation between FLER and ACR indicates that the dynamic pattern of the fluorescence signal can discriminate between areas with early signs of bioenergetic impairment.

Of note, the ACR was lower at the clinically chosen proximal resection site (PRClin) in patients presenting with anastomotic leaks, demonstrating a more impaired mitochondrial efficiency at the anastomotic site (Fig. 5). In patients who experienced a leak, there was almost no increase from the basal O2 consumption (V0) after addition of the ADP substrate (Vadp). As a matter of fact, several metrics can be used to quantify the fluorescence signal, namely time-to-peak, the slope of time-to-peak, the difference between maximum and baseline fluorescence (ΔF), and the time ratio (the time from the first increase in intensity which allows to reach half of the maximum fluorescence). Those are related by a simple equation but they actually have a difference in terms of applications. For example, time-to-peak is completely independent of the relative fluorescence intensity and of the distance between the light source and the camera. As a result, this allows for a more accurate inter-individual comparison. The slope of time-to-peak, on the other hand, is a more precise intra-individual metric and allows for a finer discrimination of the perfusion evaluation between the different ROIs. However, the slope is influenced by the distance. In this study, the aim was to correlate the FLER analysis with intestinal metabolic changes in a series of patients with different operative times and different conditions, and consequently time-to-peak is the most adapted metric.

Several independent researchers tested the same quantification metrics used with our FLER solution, both experimentally [15] and clinically [13, 14, 31]. Wada et al. used a quite similar software analysis to compute the ICG fluorescence intensity variation over time. The authors did a retrospective analysis of 112 cases of ICG-FA during proximal resection in left-sided colorectal surgeries and they found a significant correlation between a delayed time to reach the maximal fluorescence intensity and the occurrence of anastomotic complications ($n=5$). A cut-off value predicting complications with a 100% sensitivity and a 92.5% specificity was found using the Fmax (difference between maximum and baseline fluorescence; ΔF). The slope and the Tmax (time-to-peak) were globally less performant in their retrospective analysis [13]. Subsequently, Son et al. prospectively applied the same metrics of ICG time-fluorescence curve in their series of 86 colorectal cancer patients (6 anastomotic leaks) with similar results to those obtained by Wada et al. However, in their series, the most reliable predictor of anastomotic complications was the time ratio [14], similar to the one used in our study. Recently, Hayami et al. published a prospective series of 22 colorectal cancer patients in which the time to the beginning of fluorescence (T0) was significantly longer in the 4 cases with anastomotic complications [31]. The main difference between our trial and those studies [13, 14, 31] on the quantitative analysis of fluorescence lies in that FLER allows to display the information in augmented reality, thereby providing a precise identification and co-localization on the operative image. Additionally, and for the first time in humans, the other strong point of our study lies in the use of robust biological markers of perfusion to correlate digital information to the metabolic status of the intestinal tissue, with a sufficiently powered sample size. In addition, all values (FLER analysis, capillary lactates, and ACR) are concordantly altered at the proximal resection site chosen according to clinical decision in patients experiencing anastomotic complications. There are also several limitations, including the fact that the procedures were not homogeneous with different ischemic times. Additionally, a low number of non-consecutive patients agreed to participate, mostly because of the observational design, with no direct benefits, and the recruitment was slow. The sample size is too low to draw robust conclusions on the clinical utility of FLER to prevent anastomotic complications. However, it is essential to stress that the data set analyzed comprised a total of 92 paired values for the primary outcome and that the concordance between biological and software-based parameters are promising. The next sensible step is to build a large scale, multicenter and possibly interventional trial making it possible to assess the effective role of ICG-FA, using metrics comparable to those of fluorescence signal. In this perspective, the strategy is to provide the FLER software to the members of the EURO-FIGS registry [32], a joint initiative of the Research Institute against Digestive Cancer (IRCAD, Strasbourg), the Institute of Image-Guided Surgery (IHU-Strasbourg), and the Technology Committee of the European Association of Endoscopic Surgery (EAES).

Conclusions

The native unquantified ICG fluorescence signal during fluorescence angiography (FA) is not reliable, due to the distance-fluorescence intensity relationship and to the diffusion

of ICG to ischemic parts over time, and particularly during distal transection. The FLER software allows to quantify and image the fluorescence signal using augmented reality. In this non-interventional trial, the quantified values of the fluorescence signal in terms of time-to-peak were well correlated with biomarkers of perfusion (capillary lactates and mitochondria respiration) evaluated on the surgical specimen. The FLER solution could provide a means to obtain standardized data from clinical trials evaluating fluorescence angiography, in order to effectively assess the impact of ICG-FA on anastomotic complications.

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Compliance with ethical standards

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References

1. Blanco-Colino R, Espin-Basany E (2018) Intraoperative use of ICG fluorescence imaging to reduce the risk of anastomotic leakage in colorectal surgery: a systematic review and meta-analysis. Tech Coloproctol 22(1):15–23
2. Baiocchi GL, Diana M, Boni L (2018) Indocyanine green-based fluorescence imaging in visceral and hepatobiliary and pancreatic surgery: state of the art and future directions. World J Gastroenterol 24(27):2921–2930
3. van Manen L, Handgraaf HJM, Diana M et al (2018) A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. J Surg Oncol 118(2):283–300
4. Jafari MD, Wexner SD, Martz JE et al (2015) Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): a multi-institutional study. J Am Coll Surg 220(1):82–92
5. Ris F, Liot E, Buchs NC et al (2018) Multicentre phase II trial of near-infrared imaging in elective colorectal surgery. Br J Surg 105(10):1359–1367
6. Kudszus S, Roesel C, Schachtrupp A et al (2010) Intraoperative laser fluorescence angiography in colorectal surgery: a noninvasive analysis to reduce the rate of anastomotic leakage. Langenbecks Arch Surg 395(8):1025–1030
7. Kim JC, Lee JL, Yoon YS et al (2016) Utility of indocyanine-green fluorescent imaging during robot-assisted sphincter-saving surgery on rectal cancer patients. Int J Med Robot 12(4):710–717
8. Kin C, Vo H, Welton L et al (2015) Equivocal effect of intraoperative fluorescence angiography on colorectal anastomotic leaks. Dis Colon Rectum 58(6):582–587
9. Jafari MD, Lee KH, Halabi WJ et al (2013) The use of indocyanine green fluorescence to assess anastomotic perfusion during robotic assisted laparoscopic rectal surgery. Surg Endosc 27(8):3003–3008
10. Boni L, Fingerhut A, Marzorati A et al (2017) Indocyanine green fluorescence angiography during laparoscopic low anterior resection: results of a case-matched study. Surg Endosc 31(4):1836–1840
11. De Nardi P, Elmore U, Maggi G et al (2019) Intraoperative angiography with indocyanine green to assess anastomosis perfusion in patients undergoing laparoscopic colorectal resection: results of a multicenter randomized controlled trial. Surg Endosc 34(1):53–60
12. van den Bos J, Al-Taher M, Schols RM et al (2018) Near-infrared fluorescence imaging for real-time intraoperative guidance in anastomotic colorectal surgery: a systematic review of literature. J Laparoendosc Adv Surg Tech A 28(2):157–167
13. Wada T, Kawada K, Takahashi R et al (2017) ICG fluorescence imaging for quantitative evaluation of colonic perfusion in laparoscopic colorectal surgery. Surg Endosc 31(10):4184–4193
14. Son GM, Kwon MS, Kim Y et al (2019) Quantitative analysis of colon perfusion pattern using indocyanine green (ICG) angiography in laparoscopic colorectal surgery. Surg Endosc 33(5):1640–1649
15. Nerup N, Andersen HS, Ambrus R et al (2017) Quantification of fluorescence angiography in a porcine model. Langenbecks Arch Surg 402(4):655–662
16. Diana M, Noll E, Agnus V et al (2017) Reply to letter: "Enhanced reality fluorescence videography to assess bowel perfusion: the cybernetic eye". Ann Surg 265(4):e49–e52
17. Diana M, Noll E, Diemunsch P et al (2014) Enhanced-reality video fluorescence: a real-time assessment of intestinal viability. Ann Surg 259(4):700–707
18. Watanabe J, Ishibe A, Suwa Y et al (2019) 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 34(1):202–208
19. Diana M (2017) Enabling precision digestive surgery with fluorescence imaging. Transl Gastroenterol Hepatol 2:97
20. Mascagni P, Longo F, Barberio M et al (2018) New intraoperative imaging technologies: innovating the surgeon's eye toward surgical precision. J Surg Oncol 118(2):265–282
21. Diana M, Halvax P, Dallemagne B et al (2014) Real-time navigation by fluorescence-based enhanced reality for precise estimation of future anastomotic site in digestive surgery. Surg Endosc 28(11):3108–3118
22. Diana M, Dallemagne B, Chung H et al (2014) Probe-based confocal laser endomicroscopy and fluorescence-based enhanced reality for real-time assessment of intestinal microcirculation in a porcine model of sigmoid ischemia. Surg Endosc 28(11):3224–3233
23. Diana M, Agnus V, Halvax P et al (2015) Intraoperative fluorescence-based enhanced reality laparoscopic real-time imaging to assess bowel perfusion at the anastomotic site in an experimental model. Br J Surg 102(2):e169–e176
24. Quero G, Lapergola A, Barberio M et al (2019) Discrimination between arterial and venous bowel ischemia by computer-assisted analysis of the fluorescent signal. Surg Endosc 33(6):1988–1997
25. Gosvig K, Jensen SS, Qvist N et al (2019) Remote computer-assisted analysis of ICG fluorescence signal for evaluation of

small intestinal anastomotic perfusion: a blinded, randomized, experimental trial. Surg Endosc 34(5):2095–2102
26. Seeliger B, Agnus V, Mascagni P et al (2019) Simultaneous computer-assisted assessment of mucosal and serosal perfusion in a model of segmental colonic ischemia. Surg Endosc. https://doi.org/10.1007/s00464-019-07258-z.pdf
27. Barberio M, Longo F, Fiorillo C et al (2020) HYPerspectral enhanced reality (HYPER): a physiology-based surgical guidance tool. Surg Endosc 34(4):1736–1744
28. Barberio M, Felli E, Seyller E et al (2020) Quantitative fluorescence angiography versus hyperspectral imaging to assess bowel ischemia: A comparative study in enhanced reality. Surgery. https://doi.org/10.1016/j.surg.2020.02.008
29. Schlagowski AI, Singh F, Charles AL et al (2014) Mitochondrial uncoupling reduces exercise capacity despite several skeletal muscle metabolic adaptations. J Appl Physiol 116(4):364–375
30. Diana M, Noll E, Diemunsch P et al (2015) Metabolism-guided bowel resection: potential role and accuracy of instant capillary lactates to identify the optimal resection site. Surg Innov 22(5):453–461
31. Hayami S, Matsuda K, Iwamoto H et al (2019) Visualization and quantification of anastomotic perfusion in colorectal surgery using near-infrared fluorescence. Tech Coloproctol 23(10):973–980
32. Agnus V, Pesce A, Boni L et al (2019) Fluorescence-based cholangiography: preliminary results from the IHU-IRCAD-EAES EURO-FIGS registry. Surg Endosc. https://doi.org/10.1007/s00464-019-07157-3