Simultaneous computer-assisted assessment of mucosal and serosal perfusion in a model of segmental colonic ischemia

Running head: mucosal and serosal perfusion assessment

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Keywords

Bowel perfusion, quantitative fluorescence imaging, fluorescence-based enhanced reality, colon ischemia, indocyanine green (ICG)

Abstract

Background

Fluorescence-based enhanced reality (FLER) enables the quantification of fluorescence signal dynamics, which can be superimposed onto real-time laparoscopic images by using a virtual perfusion cartogram. The current practice of perfusion assessment relies on visualizing the bowel serosa. The aim of this experimental study was to quantify potential differences in mucosal and serosal perfusion levels in an ischemic colon segment.

Methods

An ischemic colon segment was created in 12 pigs. Simultaneous quantitative mucosal and serosal fluorescence imaging was obtained via intravenous indocyanine green injection (0.2mg/kg), using 2 near-infrared camera systems, and computer-assisted FLER analysis. Lactate levels were measured in capillary blood of the colonic wall at 7 regions of interest (ROIs) as determined with FLER perfusion cartography: the ischemic zone (I), the proximal and distal vascularized areas (PV, DV), and the 50% perfusion threshold proximally and distally at the mucosal and serosal side (P50M, P50S, D50M, D50S).

Results

The mean ischemic zone as measured (mm) for the mucosal side was significantly larger than the serosal one (56.3±21.3 vs. 40.8±14.9, p=0.001) with significantly lower lactate values at the mucosal ROIs. There was a significant weak inverse correlation between lactate and slope values for the defined ROIs (r= -0.2452, p=0.0246).

Conclusions

Mucosal ischemic zones were larger than serosal zones. These results suggest that an assessment of bowel perfusion from the serosal side only can underestimate the extent of ischemia. Further studies are required to predict the optimal resection margin and anastomotic site.

Introduction

Anastomotic leakage (AL) in colorectal surgery leads to increased morbidity, mortality, and worsened oncological outcomes in case of ~~(i.e. increased local recurrence and lower long-term survival rates for~~ colorectal cancer~~)~~[2]. The more distal the anastomosis level, the higher the leakage risk . Reducing AL incidence ~~AL occurs with an incidence~~ of up to 20%[6]~~, and reducing this incidence~~ is a major objective in colorectal surgery[5]. ~~The more distal the anastomosis level, the higher the risk for anastomotic leak, which means that low rectal anastomoses are at the highest risk.~~ Resection of a diseased part of the colon comprises segmental devascularization and creation of an ischemic zone. Consequently, there is a need to accurately assess the optimal level of anastomosis, as even the smallest of margins could have an impact on tension, perfusion, and anastomotic healing[7].

In addition to anastomotic technique, adequate perfusion is a necessity to promote anastomosis healing[2, 6]. An accurate perfusion evaluation has to be performed intraoperatively, and ~~to optimize the anastomosis and reduce the risk of complications such as leakage or stricture[2].~~ resection margin perfusion optimization is a prerequisite to reduce the risk of anastomotic complications [2, 8]. Clinical assessment using subjective estimates such as serosa surface color, peristalsis presence, mesenteric arteries pulsation, and bleeding from the site of bowel transection, has limited accuracy[9].

~~The distribution of a systemically injected marker into the target tissue can be considered a surrogate marker of preserved vascular supply~~ ADDIN EN.CITE

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Fluorescence angiography (FA) uses near-infrared (NIR) cameras to capture the signal emitted by means of a fluorescent dye injection, such as indocyanine green (ICG), which is injected intravenously, distributed with blood flow, and diffused into tissue. FA allows real-time bowel perfusion real-time imaging with ICG being administered at various time points, i.e. before and/or after resection or after anastomosis creation[11]. A multicenter clinical trial (Perfusion Assessment in Laparoscopic Left Sided/Anterior Resection, PILLAR II) demonstrated that assessing perfusion intraoperatively from the serosal side using ICG fluorescence angiography at the time of anastomosis creation led to a change in surgical strategy in 7.9% of patients and to absence of anastomotic leakage in this patient subgroup[6]. Despite the lack of randomized controlled evidence, FA shows promising results as a tool to decrease the AL rate in colorectal surgery[12].

However, there remains a lack of randomized controlled evidence, and additionally, there is neither any consensus on technique nor on quantitative assessment of the fluorescence signal. This issue is crucial, since the fluorophore is known to diffuse even into ischemic areas over time ADDIN EN.CITE [13], providing an overestimation of vascularized areas when only fluorescence signal presence or absence is considered. In the current surgical practice, FA is evaluated mainly from the serosal side. Due to a different response to ischemia, the perfusion of the colonic wall mucosal and serosal aspects could well differ in the presence of an ischemic segment[14]. As the mucosa is more susceptible to perfusion changes, transanal assessment of the entire colon circumference is a particularly sensitive test for ischemia. Additionally, after anastomosis creation, both the proximal and distal bowel wall can be evaluated simultaneously .

Our group has proposed a fluorescence-based enhanced reality (FLER) software-assisted approach solution to allow quantitative FA, using local fluorescence signal arrival dynamics as a reproducible and precise parameter of bowel perfusion[7, 13, 17-20]. This approach is defined as fluorescence-based enhanced reality (FLER), in which a dedicated software builds a virtual perfusion cartogram, which can be superimposed onto real-time operative images, in order to obtain a mixed reality effect. Virtual perfusion cartography stores relevant angiography information related to the slope of fluorescence signal evolution over time, in a pixel by pixel manner. In previous experimental studies, FLER superimposition of a virtual perfusion cartogram onto real-time operative images showed accurate discrimination of bowel perfusion levels and provided superior accuracy in ischemia identification as compared to clinical estimation[7]. This technology is currently being tested, withshows promising preliminary results in a clinical trial at IHU-Strasbourg (NCT02626091)[10].

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<EndNote><Cite><Author>Singh</Author><Year>2009</Year><RecNum>230</RecNum>

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resection</title><secondary-title>Adv Exp Med Biol</secondary-

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The aim of the present experimental study was to quantify potential differences in mucosal and serosal perfusion levels in an ischemic colon segment with a simultaneous computer-based mucosal and serosal fluorescence dynamics assessment.

Materials and methods

Animals

Twelve male adult swine (*Sus scrofa domesticus*, ssp. Large White) were used in this experimental study, as part of a larger experimental protocol on Endoscopic Luminescent Imaging for Oncologic Surgery (ELIOS), which received full approval from the local ethical committee on animal experimentation (reference #38.2017.01.085), and from the Ministry of Superior Education and Research (MESR) (APAFIS #8721-2017013010316298v2).

The sample size was calculated based on previous works from our group in the same field of interest ([7, 13, 17, 18, 20]). The primary outcome chosen was the difference in the level increase of local capillary lactates. Using a superiority design and based on the hypothesis that the lactate value increase at the serosal 50% perfusion line is at least 25% higher than at the mucosal one, a sample size of 12 assessment points per group is sufficient to detect a significant difference with an alpha of 5% and a power (1-beta) of 90%.

All animals used in the experimental laboratory were managed according to French laws for animal use and care and according to European Community Council directives (2010/63/EU) and ARRIVE Guidelines[21]. Pigs were fasted for 24 hours with free access to water and premedicated using a 50/50mg tiletamine/zolazepam and 120mg azaperone intramuscular injection 10 minutes before surgery. Induction was achieved using an intravenous administration of 100mg propofol combined with 50mg rocuronium bromide. Anesthesia was maintained with 2% isoflurane. Rectal enemas were performed until the effluent turned clear. At the end of the experimental protocol, pigs were sacrificed using an over-therapeutic dose of isoflurane (5%) for 10 minutes, followed by an intravenous Pentobarbital (Exagon® 40mg/kg) injection.

Preparatory work

Within the framework of another open surgery study, the proof of concept to optimize the set-up for simultaneous visualization of mucosal and serosal colonic surfaces was initially established in 2 pigs (1 male, 1 female). Since the uterus covered the distal colonic region accessible to transanal endoscopic visualization, local dissection and hysterectomy were necessary to obtain sufficient corresponding serosal surface exposure. To facilitate visualization, this study was then conceived in male pigs only.

FLuorescence-based Enhanced Reality (FLER) computer-assisted analysis

Quantitative fluorescence imaging via FLER analysis was performed using the ER-PERFUSION software (IRCAD, France), to obtain a perfusion cartogram based on fluorescence dynamics analysis[7, 17-20]. The corresponding internal and external views of an ischemic colon segment were recorded and analyzed simultaneously during near-infrared fluorescence imaging of mucosal and serosal perfusion.

For each single pixel, the perfusion was computed using the slope of fluorescence signal intensity evolution over time, which represents a fluorescence signal intensity change from the first (Q1) to the third (Q3) quartile of intensity, divided by the corresponding time period. This value corresponds to the speed of fluorescence signal arrival in the target tissue, thereby representing tissue perfusion. Each imaging system's perfusion data was translated into a color code, creating a cartography which can be superimposed onto real-time laparo-/endoscopic images.

Since fluorescence signal intensity is inversely proportional to the square of the camera-to-target distance (inverse-square law)[22], the slope of fluorescence signal dynamics is biased if only absolute values are considered. Therefore, by comparing different regions' slope ratios, we used relative values in order to overcome this bias. To normalize the signals, we defined two colonic reference regions as maximum (vascularized, 100%) and minimum (ischemic, 0%) on the color-coded cartography. At this point, the software allows to visualize

any perfusion level between 0 and 100%, and to show the delineation between the influence zones of these reference regions. Arbitrarily, the delineation was computed by defining the points where the slope ratio is 50% and shown as a border called "50% perfusion line" on the enhanced reality images.

Near-Infrared Coating of Equipment (NICE)

When switching the KARL STORZ camera system to near-infrared mode, the screen turns black until arrival of a fluorescent signal. Therefore, a potential movement of the target anatomy cannot be excluded during image acquisition. A fluorescent coating material was applied onto four magnets, so they could serve as visible fiducials fixed to the colon. To This allowed to observe the magnet position during image acquisition and software analysis. a robust registration between virtual and real images during the open surgical cases, a fluorescent coating material was applied onto two pairs of magnets. Three layers of paint on the magnets created a sufficiently strong fluorescence signal to facilitate observation of their position around the ischemic devascularized segment identification in near-infrared camera mode, and allowing a robust registration before virtual FLER images superimposition onto the real-time camera views during the open surgical cases.

Surgical setting

A 5cm colonic ischemic segment was created in 12 pigs. The first 7 pigs (mean weight: 45.1 ± 3.1kg) underwent a median laparotomy. Bladder retraction was performed using a stay suture. The sigmoid colon was occluded orally to the target zone with an atraumatic bowel clamp. The Transanal Endoscopic Operations (TEO®, KARL STORZ; Germany) device was introduced and CO_2 insufflation (5mmHg) started. At a 5 cm distance, two pairs of fluorescent-coated magnets were placed oppositely at mucosal and serosal surfaces and served as landmarks (to allow a robust registration). Two near-infrared camera systems (D-LIGHT P, KARL STORZ; Germany) were used. The first one was fixed by means of an articulated arm,

in order to acquire the fluorescence signal arising from the bowel's serosal side. The second system was inserted through the TEO® device. An ischemic sigmoid segment was then created by sealing the terminal vascular branches, leaving the main inferior mesenteric vessels intact (using the LigaSure™ vessel-sealing device, Medtronic, USA). The assistant held the colon in a stable position. In order to avoid manual retraction and the need for magnetic fiducials ensuring a stable colon position during video acquisition and software analysis, we then switched to a five-port laparoscopic approach. Apart from the transition to a less invasive access technique, Tthe same procedure was then performed in 5 pigs (mean weight: 50.7±6.5kg) laparoscopically. using a five-port laparoscopic approach. An atraumatic bowel grasper was placed orally to the target region in the sigmoid colon, in order to occlude the lumen prior to transanal insufflation, as in the open surgical setting. The set-up of both camera systems and mesocolic division were performed in the same fashion as for the open approach. To replace the assistant's retraction, an additional curved bowel grasper was introduced and fixed using an articulated arm. This setting provided stability for image recording and enhanced reality display in a no-touch technique, without the need for fluorescence-coated magnets to control registration (**Figure 1**).

Upon injection of 0.2mg/kg of indocyanine green (ICG; INFRACYANINE®, SERB; France), both camera systems were switched to near-infrared mode and the signal intensity's dynamic evolution was simultaneously recorded and analyzed using proprietary software. Both color-coded perfusion cartographies were superimposed onto the real-time images obtained by the corresponding mucosal and serosal camera systems.

The regions of interest (ROIs), as defined by predetermined degrees of perfusion calculated via the software, were superimposed onto real-time endoscopic images. The seven ROIs were as follows: the ischemic zone (I, 0% signal intensity variation rate); the four ROIs where a 50% signal intensity variation over time threshold was identified (proximally [P50M and

P50S] and distally [D50M and D50S] at both mucosal and serosal sides); and the proximal and distal vascularized areas (PV and DV respectively, 100% signal intensity variation rate) as determined with the FLER analysis (**Figure 2**).

Colonic wall transillumination with a laser pointer was used in the open setting to identify the FLER perfusion ROIs on the mucosa and translate them onto the serosal side. The mucosal and serosal 50% perfusion lines were then marked on the colonic wall with a surgical pen. In the laparoscopic setting, the intraluminal light intensity was reduced to 10% while increasing laparoscopic light intensity to 100%, to identify mucosal ROIs and translate them onto the serosal side. The shadow created by a laparoscopic instrument hovering over the colonic wall was visible from the mucosal side and replaced the laser visualization used in the open setting. The mucosal and serosal 50% perfusion lines were then marked on the colonic wall with a surgical pen and lactate levels were measured accordingly.

Lactate level measurements

Capillary lactate levels were measured using the strip-based EDGE Blood Lactate Monitoring System (ApexBio, Taiwan) in blood samples obtained by puncturing the colonic wall at all 7 ROIs one by one. Systemic lactates were assessed by puncturing the pig's snout. In the open setting, the capillary blood was directly collected. In the laparoscopic setting, a sterile 2mL Falcon tube (BD Biosciences, USA) was introduced through one of the trocars for each ROI separately, and was connected to a motorized pipette filler in order to aspirate emerging blood drops and then transfer them onto the EDGE strips.

Data analysis

To assess a possible correlation between local lactate values and the slope of fluorescence signal intensity evolution over time ("slope"), Spearman's rank correlation coefficient was calculated. One-way ANOVA with Tukey's multiple comparisons test was performed to analyze differences between the defined ROI for lactate and slope values after confirmation of

a Gaussian distribution by the Shapiro-Wilk normality test. All statistical analyses were performed using Prism 8 for macOS (GraphPad Software, Inc. USA).

An exponential regression model using Python SciPy library[23] was chosen in order to potentially predict local lactate value increases from normalized slopes obtained using FLER analysis.

Results

Perfusion level differences between mucosa and serosa were clearly identified within the colonic wall using perfusion cartographies. Superimposition of the 50% signal intensity variation over time thresholds ("50% lines") onto the real-time endoscopic image allowed to mark the ROIs on the colonic wall's serosal side with a surgical pen. To facilitate further analysis, transillumination served to locate the mucosal 50% lines to mark them on the serosal side as well **(Figures 2 and 3)**.

The mean ischemic zone as measured (mm) for the mucosal side was significantly larger than for the serosal side (56.3±21.3 vs. 40.8±14.9, p=0.001, paired t test) with a mean difference of 1.5±1.2cm.

The results of local lactate measurements and FLER analysis are summarized in Table 1. Pig number 5 was excluded from the data analysis due to elevated systemic lactate levels of 8 mmol/L (normal <5mmol/L), since the data were not comprised within the range encountered in all other pigs (PV=DV 1.1+/-0.5mmol/L).

Slopes: FLER analysis allowed to discriminate the different ROIs. The difference in slopes (fluorescence signal intensity variation over time) between the ischemic zone (I) and all other ROIs was statistically significant. Conversely, there was no statistically significant difference in slopes between vascularized zones (PV ≈ DV), mucosal (P50M ≈ D50M), or serosal (P50S ≈ D50S) zones **(Figure 4)**. This finding is suggestive of a symmetrical distribution of these zones around the ischemic center. As a result, we performed a pooled analysis by merging corresponding proximal and distal vascularized (V), mucosal (50M) and serosal (50S) zones (n=24), which showed a discrimination between all zones at a statistically significant level (50M vs. V, p<0.0001; 50S vs. V, p<0.0001; 50S vs. 50M, p=0.0016).

Lactates: The representative metabolic analysis obtained with local capillary lactate measurement showed a statistically significant difference in lactate levels between ischemic

and vascularized zones, between ischemic and mucosal 50% perfusion zones as well as between ischemic and proximal serosal 50% perfusion zones. There was no statistically significant difference between lactate levels at the ischemic center and the distal serosal 50% perfusion zone. There was no statistically significant difference in lactate levels between vascularized zones (PV vs. DV), mucosal (P50M vs. D50M), or serosal (P50S vs. D50S) zones **(Figure 4)**. This is congruent with the ROIs' symmetric distribution around the ischemic center observed in the FLER analysis.

The pooled analysis of vascularized (V), mucosal (50M) and serosal (50S) areas around the ischemic center showed a statistically significant difference between 50S and 50M (p=0.0492), between 50M and V (p=0.0041), and between 50S and V (p<0.0001).

The Spearman's rank correlation coefficient showed a significant weak inverse correlation (r=-0.2452, 95% CI -0.4418 to -0.02605, p=0.0246) between lactate and slope values for the defined ROIs. For the exponential regression model, ROI lactate values for each individual were normalized by subtraction of the corresponding vascularized lactate value to represent the local increase. The individual ROI slope values were normalized using a division by the vascularized value (ratio). The exponential regression model was then given by the equation: *local lactate* = exp(-2.37 x *normalized_slope* +1.23) + *systemic lactate*. The mean error of prediction using this equation was 0.58 ± 0.49 mmol/L, while 95% of the errors were < 1.68 mmol/L **(Figure 4)**.

Discussion

The first affected area and the initial site of injury caused by impaired blood flow is the mucosal bowel surface[17]. The mucosa and submucosa receive 70% of the mesenteric blood flow, whereas 30% goes to the muscularis and serosal layers[24, 25]. Consequently, during periods of intestinal ischemia, the mucosa reacts before the serosa, and mucosal necrosis appears within 3h, while full-thickness necrosis develops within 6h[24]. In a study on 7 patients undergoing colorectal surgery using intraoperative measurement of colonic oxygenation with lightguide spectrophotometry and laser Doppler flowmetry, there was a significantly lower baseline oxygen saturation (SO_2) in the mucosa (75%) as compared to the serosa (87%). Additionally, the authors observed a mean mucosal SO_2 decrease to 55% after IMA ligation and to 39% after complete devascularization, while the serosal SO_2 was unchanged after IMA ligation and showed a 7% decrease only after complete devascularization. The authors concluded that, due to a different response to ischemia, mucosal SO_2 measurements accurately diagnosed ischemia, as opposed to the serosal ones[14]. Since the mucosa shows a higher vulnerability to ischemic stress than the muscular layer, intraluminal measurement of anaerobic metabolites was claimed to detect early signs of ischemia, whereas their detection on the serosal side corresponded to a later stage of ischemia[26]. However, among various experimental techniques available to intraoperatively assess bowel viability, there is a lack of standardization and of precise limiting values of tissue oxygenation and flow indicative of ischemic complications[27].

In anastomosis creation, there is a full colonic wall disruption, and resection margin perfusion optimization is a prerequisite to foster anastomotic healing, and to reduce the risk of anastomotic complications such as leakage or stricture ADDIN EN.CITE ADDIN EN.CITE.DATA [2, 8]. Preservation of well-perfused mucosa might play a role to prevent

these complications, since reduced exposure of the colonic epithelium to colonic microbiota has been shown to prevent ischemia-induced intestinal inflammation ADDIN EN.CITE [28].

Clinically, the correlation of mucosal and seromuscular ischemic changes is insufficiently known to decide which factor is better to anticipate anastomotic ischemia occurrence[17]. Discussion is still underway as to whether mucosal assessment should be used as the most sensitive parameter for bowel perfusion, or whether the mucosa might be too sensitive and serosal assessment should be considered to be more indicative of a significant perturbation of blood flow[16]. In anastomosis creation, there is a full colonic wall disruption. Preservation of well-perfused mucosa might play a role to prevent anastomotic complications, since reduced exposure of the colonic epithelium to colonic microbiota has been shown to prevent ischemia-induced intestinal inflammation[28].

Fluorescence imaging is increasingly used to estimate bowel perfusion in colorectal surgery[12], by assessing the fluorescence signal from the serosal side in most cases.

A sequential evaluation including an initial assessment before resection (serosal side) followed by further assessment after anastomosis creation (mucosal side through a transanal view) was reported by several authors[6, 11, 29, 30]. A multicenter clinical trial (Perfusion Assessment in Laparoscopic Left-Sided/Anterior Resection, PILLAR II) demonstrated that intraoperative perfusion assessment using ICG-fluorescence angiography led to a change in surgical strategy in 9 patients due to serosal, and in 2 patients due to postanastomotic mucosal evaluation. None of these 11 patients (7.9%) developed AL[6]. Similarly, sequential FA assessment of serosa and mucosa resulted in a surgical strategy change in 13.3% of patients, with no AL in the FA group[11]. Transanal fluorescence imaging was proposed to visualize mucosal and anastomotic blood flow in the high-risk population of low rectal anastomoses. However, it was limited due to a subjective/qualitative assessment[16, 31]. In a case report of ileocolic resection for ischemia complicating an aortic repair, authors visualized ascending

colon hypoperfusion with FA. While macroscopically the colonic serosa appeared intact from a clinical viewpoint, the mucosa was completely ischemic in the resected specimen[24]. Interestingly, a feasibility study on intraoperative transanal fluorescence imaging reported 2 patients in which clinical judgment suggested adequate anastomotic perfusion, while an inadequate fluorescence uptake of the proximal colon was observed. In the clinical course, these 2 patients most likely had a symptomatic occult anastomotic leak with peri-anastomotic collections on CT scan treated conservatively, and their hospital stay was increased as compared to patients with a normal ICG angiogram or a diverting loop ileostomy for hypofluorescence [16].

In a previous study, the accuracy of the FLER system was validated by comparing the outcomes of anastomoses performed at two perfusion levels (25 and 75%). There was a strong correlation between measured local metabolic markers, anastomotic healing evaluated histopathologically, and the predicted perfusion level ADDIN EN.CITE [7]. In the present study, a distal colonic ischemia model was chosen to perform simultaneous transabdominal and transanal perfusion evaluation, in order to obtain synchronous image acquisition of mucosal and serosal colonic wall sides. Quantitative fluorescence imaging was performed with FLER analysis. Local capillary lactate levels served as an accurate biomarker for tissue hypoxia-ischemia, as previously demonstrated[7, 17, 18, 20, 32].

Perfusion differences between mucosal and serosal colonic wall sides could be accurately visualized and compared. Congruently, FLER analysis and lactate measurements clearly discriminated the ischemic area from all other ROIs. The computer-assisted quantitative fluorescence analysis allowed to discriminate both mucosal and serosal 50% lines from vascularized and ischemic areas. These findings were consistent with local capillary lactate measurements, with a statistically significant weak inverse correlation between lactate and slope values for the defined ROIs (**Figure 4**).

As visualized with enhanced reality via perfusion cartography, the mucosal ischemic zone was significantly larger than the serosal one. Lactate measurements showed significantly lower values at the mucosal ROI (P50M and D50M) as compared to the ischemic zone. Conversely, the findings in the serosal ROIs were ambiguous since the difference between the proximal serosal zone (P50S) and the ischemic area was not significant, whereas it reached statistical significance for the distal serosal zone (D50S). Consequently, our hypothesis is underlined, assuming that the serosal assessment might provide a less accurate depiction of the metabolic changes than the mucosal assessment.

In a previous study, the accuracy of the FLER system was validated by comparing the outcomes of anastomoses performed at two perfusion levels (25 and 75%). There was a strong correlation between measured local metabolic markers, anastomotic healing evaluated histopathologically, and the predicted perfusion level ADDIN EN.CITE [7]. Our group currently translates FLER software analysis into clinical practice (NCT02626091) with quantitative fluorescence imaging on the serosal side in left-sided colonic resections for diverticular disease and colorectal cancer. Recently a study on quantitative FA in colorectal cancer patients to assess colon perfusion was published, also using the parameter of fluorescence intensity change over time (slope). A ratio (TR) of times for $T_{1/2MAX}$ (first fluorescence increase to half of maximum) divided by T_{MAX} (first fluorescence increase to maximum) was determined, and a TR of >0.6 was found to be an independent factor of anastomotic complications [34].

In conclusion, the extent of ischemia was significantly larger when evaluated on the mucosal side, and can be underestimated with serosal assessment. These results suggest considering a quantitative bowel perfusion assessment from the mucosal side or potentially performing a larger resection when only the serosal perfusion is analyzed with fluorescence angiography. Further survival studies are necessary to evaluate these preclinical findings in the clinical

setting, to determine their potential impact on AL, and to assess the role of mucosal ischemia in anastomotic healing. Eestablishing the optimal FA-based method to assess the resection margin and the anastomotic site in colonic resections. This will further help in standardizing technical protocols going forward whereby the type and number of assessments can be agreed upon.

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Figure legends

Figure 1. Surgical setting

Laparoscopic setting: the transanal and abdominal camera systems are fixed with articulated arms. Steadily positioning the colon using a bowel grasper fixed by means of another articulated arm, fluorescence angiography and FLER analysis can be performed using a stable no-touch technique. The operating room set-up shows both camera systems, i.e. the abdominal one on the left side with two screens allowing for real-time image and enhanced reality superimposition side by side, as well as the transanal one on the right side. In the background, our computer scientist can be seen working on the computer connected to both camera systems for FLER analysis.

Figure 2. Simultaneous serosal-mucosal FLER analysis

First row: Virtual perfusion cartography was computed simultaneously for both the serosal and mucosal side using the ER-PERFUSION software (see text for details). Second row: Laparoscopic view (left) and transanal view (right) of the ischemic region with superimposition of the 50% perfusion lines (white) and central ischemic area (turquoise line). For illustration purposes, the yellow circles in the laparoscopic view were added to show the 7 ROIs identified with FLER and subject to lactate analysis. Third row: Marking of the serosal 50% line as a continuous line on the colon. Using transillumination, the mucosal 50% line is translated to the serosal side and marked as a broken line.

Figure 3. Robust registration with laser pointer and fluorescent-coated magnets in the open procedures

Corresponding abdominal = serosal (left) and transanal = mucosal views (right) with the white 50% perfusion lines in the open surgical setting. A, B: FLER cartography superimposed onto real-time images. Signal intensity variation over time is translated into a color code (red

= high, blue = low). C, D: a laser pointer targeted abdominally onto the colon (serosal side) and visible intraluminally (mucosal side) was used to translate the mucosal perfusion lines onto the serosal side. E, F: the 50% perfusion lines are marked with a surgical pen on the serosal surface. Asterisk: Fluorescent coating on 4 magnets as used in this study (top) compared to 4 uncoated magnets (bottom), visualized in near-infrared camera mode. Arrows: fluorescent-coated magnets on the colon in NIR mode (C, D) and in white light mode (E, F).

Figure 4. Local capillary lactates and slopes of the fluorescence intensity evolution

Local lactate levels (A) and slopes of fluorescence signal intensity evolution over time (B) for ROIs determined with FLER analysis, with symmetrical distribution of the vascularized (PV and DV), mucosal (P50M and D50M) and serosal (P50S and D50S) ROIs around the ischemic center, and inverse correlation between lactate and slope values. Asterisks represent statistically significant differences, as determined using one-way ANOVA with Tukey's multiple comparisons test (see Table 1). C) The dotted line represents the predicted normalized lactate values from the slopes in the exponential regression model, while the colored dots represent the measured values. D) Graph representing the cumulative prediction error distribution.

Table title

Table 1.

Lactate and slope values compared between the different ROIs. **BOLD** = statistical significance, using one-way ANOVA with Tukey's multiple comparisons test.