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Multisensor perfusion assessment cohort study: Preliminary evidence toward a standardized assessment of indocyanine green fluorescence in colorectal surgery

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ABSTRACT

Background: Traditional methods of assessing colonic perfusion are based on the surgeon's visual inspection of tissue. Fluorescence angiography provides qualitative information, but there remains disagreement on how the observed signal should be interpreted. It is unclear whether fluorescence correlates with physiological properties of the tissue, such as tissue oxygen saturation. The aim of this study was to correlate fluorescence intensity and colonic tissue oxygen saturation.

Methods: Prospective cohort study performed in a single academic tertiary referral center. Patients undergoing colorectal surgery who required an anastomosis underwent dual-modality perfusion assessment of a segment of bowel before transection and creation of the anastomosis, using near-infrared and multispectral imaging. Perfusion was assessed using maximal fluorescence intensity measurement during fluorescence angiography, and its correlation with tissue oxygen saturation was calculated.

Results: In total, 18 patients were included. Maximal fluorescence intensity occurred at a mean of 101 seconds after indocyanine green injection. The correlation coefficient was 0.73 (95% confidence interval of 0.65–0.79) with $P < .0001$, showing a statistically significant strong positive correlation between normalized fluorescence intensity and tissue oxygen saturation. The use of time averaging improved the correlation coefficient to 0.78.

Conclusion: Fluorescence intensity is a potential surrogate for tissue oxygenation. This is expected to lead to improved decision making when transecting the bowel and, consequently, a reduction in anastomotic leak rates. A larger, phase II study is needed to confirm this result and form the basis of computational algorithms to infer biological or physiological information from the fluorescence imaging data.

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Introduction

Anastomotic leak after colorectal surgery still occurs in approximately 9%[1] of unselected cases but can reach up to 19% in low rectal anastomoses.[2] Surgeon predictions of the probability of leak are unreliable.[3] Intraoperative methods to prevent anastomotic leak are based on technical considerations as well as perfusion and anastomosis assessment. The most important determinant for a successful anastomosis is adequate tissue perfusion, which provides an optimal environment to promote tissue healing and preserve tissue integrity postoperatively.[4] Currently, the most common method to assess perfusion is through clinical judgment and indirect signs such as marginal artery pulse, bleeding from the edge of the transected colon, and tissue color. This is subjective and hugely variable.

Intraoperative fluorescence angiography (FA) is an emerging technique that aims to improve clinical decision-making with respect to anastomoses.[5] A fluorescent dye such as indocyanine

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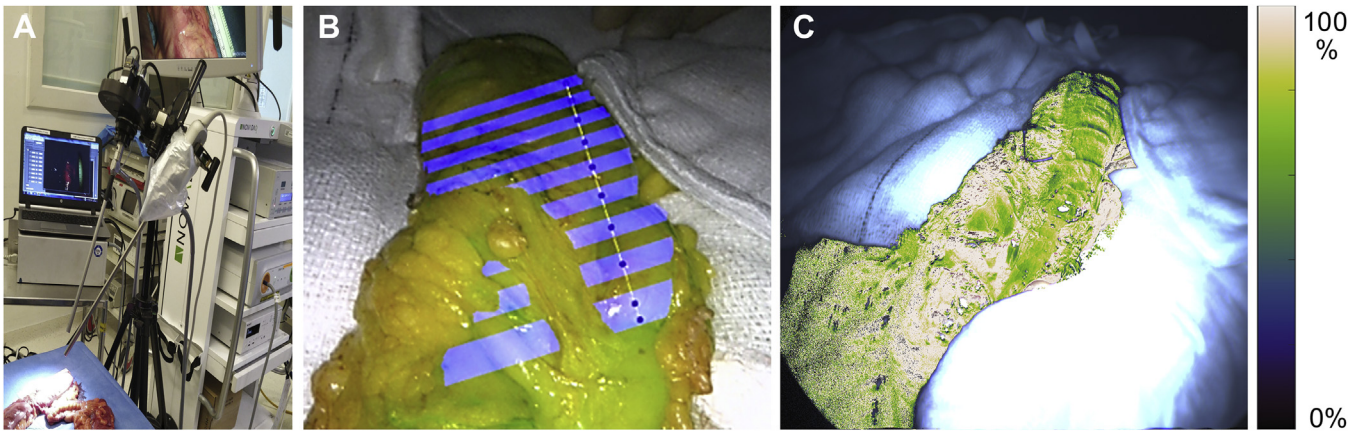

Figure 1. (A) Multispectral imaging camera on the left and near-infrared imaging camera on the right. (B) Near-infrared overlay data with blue squares showing the area sampled for analysis, with 1 cm spacing between regions. (C) Multispectral data showing tissue oxygen saturation according to the scale on the right.

green (ICG) is injected intravenously, where it binds to albumin and follows the vascular network.[6] When "excited" with near-infrared light, this emits longer wavelength fluorescence, which can be detected by a specialized camera with an optical emission filter. Perfused tissue is thus highlighted. There is accumulating evidence that using FA is useful in guiding proximal colonic transection and reducing anastomotic leak.[7–11] However, the observed intensity of the signal can vary considerably, making it difficult for the surgeon to interpret the relationship between signal intensity and the underlying perfusion of the tissue. Previous work to quantify the fluorescent signal has looked into measures that do not directly correlate with biological properties of tissue.[12] A notable exception is the use of fluorescence-based enhanced reality with capillary lactates as surrogate markers of tissue oxygenation.[13,14] A greater knowledge of how measured fluorescence signals relate to physiological measures of oxygenation could lead to greater diagnostic accuracy and, consequently, a reduction in anastomotic leak rates.

Multispectral imaging (MSI) is a technique that can generate spatial maps of tissue oxygen saturation (SO_2) and can be used in parallel with FA. MSI estimates SO_2 noninvasively by measuring the intensity of light reflected by the tissue at different wavelengths and using a priori knowledge of the optical characteristics of oxygenated and deoxygenated hemoglobin (HbO_2 and Hb, respectively).[15,16] This technique has previously been used to quantify organ perfusion and to detect ischemia in animal models by our group[17,18] and others.[19–21] However, the relationship between fluorescence intensity and tissue oxygen saturation has not been previously assessed in humans.

Our aim was to investigate the correlation of fluorescence intensity with tissue oxygen saturation using multispectral imaging, in patients undergoing colorectal surgery involving construction of an anastomosis.

Methods

Design

A prospective cohort study was conducted in a single tertiary referral center for colorectal surgery.

Patient selection and demographics

Patients undergoing colorectal surgery that involved construction of an anastomosis were included in the study. All cancer patients were put through a multidisciplinary tumor board after complete local and distant staging of disease and tissue confirmation of diagnosis. Patients were recruited between May and November 2019.

Anastomotic assessment and data collection

In all cases, vessel ligation and extracorporealization were performed before colonic transection. Before transection, the bowel was placed on clean white swabs to act as "background." FA and MSI cameras were connected to a stereo camera rig and mounted on a tripod to maintain a fixed, stationary position (Figure 1, *A*) during imaging. This was moved to the patient's bedside for recording, minimizing surgical workflow disruption. For fluorescence imaging, the PINPOINT camera (Stryker, MI) was used. Multispectral data were collected using an experimental laparoscopic system incorporating the SpectroCam (Ocean Insight, FL).[22]

FA was only used to inspect the proximal colon before anastomosis. Before injection of ICG, a first MSI sequence was recorded. A continuous sequence was recorded from the point of ICG injection; 10 mg of ICG was injected intravenously in all cases. The recording lasted until the operating surgeon decided fluorescence intensity was adequate to choose the point of proximal transection (maximal fluorescence intensity), as per current standard practice in FA. A second MSI sequence was recorded at this point to control for the possible effect of ICG on the reflectance measurements. After video recording, a camera calibration sequence was recorded to mathematically define the relative spatial relationship between the multispectral and near-infrared cameras and enable direct comparison of corresponding anatomical regions. This involved capturing a sequence of images of a checkerboard (8 × 8 mm squares) in different orientations.

Image calibration and video analysis

Camera calibration was performed using the captured checkerboard images and a standard mathematical model.[23] The calibration model was used to correct distortions in the imaging systems and to transform the data from the 2 different perspectives to a common plane for subsequent analysis and measurement. Calibration was carried out using the Computer Vision System Toolbox in MATLAB (Math Works).[24]

The video recorded was separated in the relevant frames. Given the aim of the study was in correlation of maximal fluorescence intensity with tissue oxygenation, the frames corresponding to maximal tissue oxygenation according to the operating surgeon were analyzed for each case. These were aligned and corresponding regions of interest were selected, as can be seen in Figure 1, *B*.

Fluorescence intensity was analyzed through pixel intensity. To obtain pixel intensity in the regions of interest, the pixel values of the near-infrared image on the RGB color space were transformed to grayscale and normalized.

The laparoscopic MSI system floods the scene with white light spanning the full visible range of wavelengths, whereas the camera sequentially captures 8 images in 0.3 seconds, in different wavebands, using a spinning filter wheel. A spectral calibration step is used to correct for wavelength-dependent characteristics of the device itself, and to convert the recorded pixel intensities to reflectance values. At a particular pixel location, the variation of reflectance as a function of wavelength, the so-called reflectance spectrum, depends on the relative concentration of absorbers in the tissue. In the gastrointestinal tract these are predominantly hemoglobin and, to a lesser extent, fat.[25] The absorption properties of Hb, HbO_2, and fat have been well characterized, and thus it is possible to estimate their relative concentrations using a linear spectral unmixing model.[17] A pixelwise calculation of SO_2 can then be performed using the relative concentrations Hb and HbO_2. The 8 captured bands can also be used to generate a conventional color image by combining those covering the red, green, and blue spectral regions. The resultant SO_2 map is displayed as an overlay on this color image, as seen in Figure 1, *C*. The accuracy of the linear model fit to the data is expressed by the coefficient of determination (CoD), which quantifies the fraction of the experimental data that is explained by the model. Pixels with CoD values under 0.9 were excluded from quantitative analysis. Common reasons for low CoD include saturated pixels due to specular reflections or excessive illumination, low illumination intensity, motion artifacts, and the presence of objects in the field of view not accounted for in the model (eg, skin, metal surgical instruments).

Correlating fluorescence intensity and tissue oxygen saturation

For each of the imaged colon segments a line was drawn along the length and divided into 1-cm intervals. The line was identified in both the MSI and FA images, using the geometric camera calibration, and regions of interest (ROIs) were defined at each interval spanning the full visible width of the colon. The mean values from each ROI in both cameras (normalized fluorescence intensity and SO_2) were calculated. Global and patient-specific correlation coefficients were calculated using the Spearman correlation coefficient assuming a non-Gaussian data distribution.[26] Confidence intervals were calculated based on the bootstrapping method.

Sensitivity analyses were performed to assess the impact of variation in specimen positioning and ambient lighting. To control for these factors, time averaging and pixel intensity normalization were performed. Time averaging was performed by calculating the mean image from a group of successive frames over a short interval (<1 s), after removing breathing motion artifacts, before the reference frame. This served to improve the signal-to-noise ratio in acquisitions where the detected fluorescence intensity was low. Intensity normalization was performed by dividing by the maximum pixel intensity in the red channel intensity in a representative area of the reference frame. This aimed to reduce the effect of varying illumination intensity between patients. Reporting of results followed the STROBE guidelines.[27]

Ethical approval

All patients provided written informed consent for video collection for this study before the procedure according to institutional guidelines for data collection. No change to standard clinical care occurred in this study.

Table I
Patient characteristics

Patient characteristics	n
Sex, (%)	
Male	13 (72)
Female	5 (28)
Cardiovascular comorbidities, (%)	4 (22)
Indication for surgery, (%)	
Benign disease	5 (28)
Cancer	13 (72)
Procedure, (%)	
Right colectomy	4 (22)
Left colectomy	13 (72)
Total colectomy	1 (6)
Approach, (%)	
Laparoscopic	14 (78)
Open	4 (22)

Results

Eighteen patients undergoing colectomy were included in the analysis. In total, 15 patients were operated by the same surgeon, and 3 others had different surgeons. The mean patient age is 65 years. Patient characteristics can be found in Table I.

Video analysis and calibration

Paired measurements were achieved in all patients; however, calibration was unsuccessful in 3 patients. A significant misalignment between the multispectral and near-infrared cameras was present in these cases. This did not allow the multispectral results to be mapped onto the corresponding pixels in the fluorescence angiography results. Maximal fluorescence intensity was achieved after a mean of 101 seconds, with a standard deviation of 37 seconds. The length available for analysis in each case varied between 1.5 and 11.5 cm, with a mean of 6.3 cm.

Assessment of perfusion using multispectral and near-infrared imaging

The distribution of tissue oxygen saturation measured before ICG injection is shown in Figure 2, *A*. The area considered for anastomosis revealed a wide range of tissue oxygen saturation (36%–94.2%). This suggests that there is a wide variation within the optimal anastomotic site based on clinical assessment.

The distribution of fluorescence intensity at the maximal intensity as determined by the operating surgeon is shown in Figure 2, *B*. There was a wide distribution in terms of values in all but 2 cases. These measurements were excluded from the correlation analysis because they were considered to be artifacts.

Correlation assessment

The correlation coefficient between fluorescence intensity and tissue oxygen saturation was 0.73 (95% confidence interval 0.65–0.79, $P < .001$). This showed a strong positive correlation between these 2 variables (Figure 3). Correlation analysis of tissue oxygen saturation with fluorescence intensity can be modeled with a second degree polynomial ($y = 2.1031x^2 - 2.0278x + 0.5432$), which provides a R^2 statistic of 0.57. The main area where the model seems to apply is between 50% and 85% tissue oxygen saturation, which also corresponded to the area where more data had been obtained.

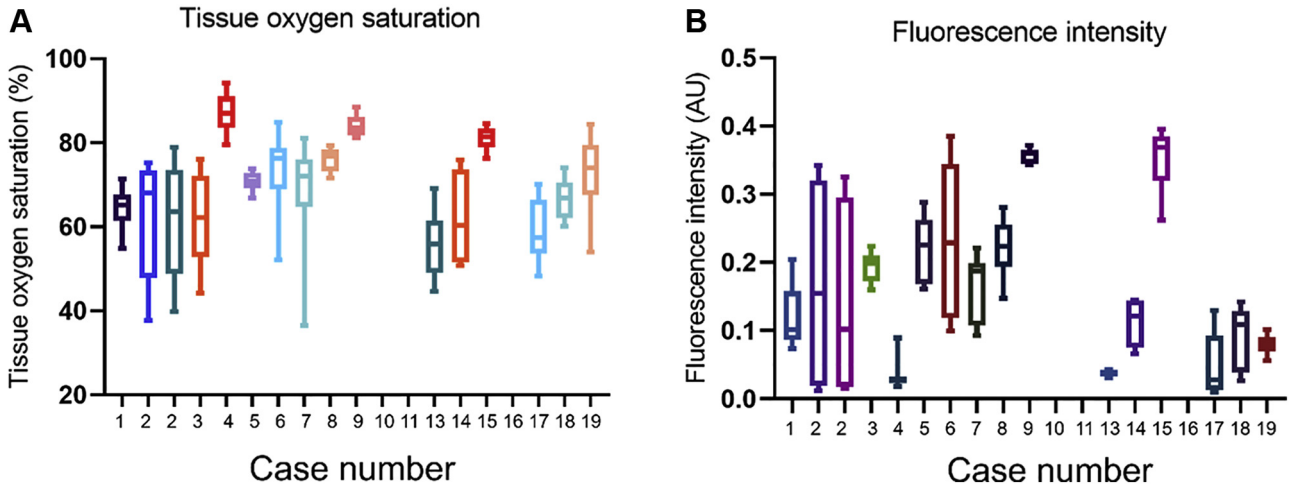

Figure 2. (A) Tissue oxygen saturation in 18 different cases measured through multispectral imaging; (B) Fluorescence intensity in 18 different cases measured in near-infrared imaging. There are 2 measurements for case 2: the first is from the colon and the second from the small bowel.

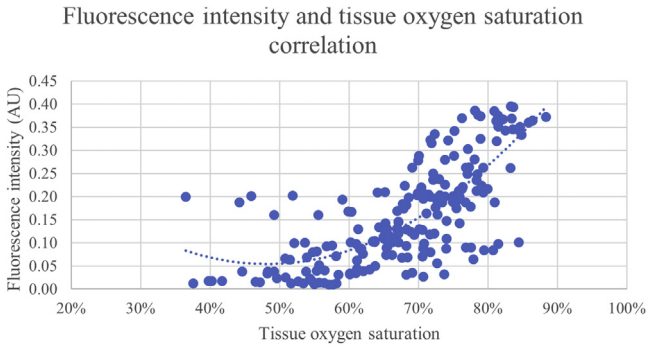

Figure 3. Scatterplot of tissue oxygen saturation versus fluorescence intensity for correlation analysis.

Sensitivity analysis

Intensity normalization was achieved in 15 (83%) patients, with only the patients in whom calibration was unsuccessful not achieving fluorescence intensity normalization. The Spearman correlation coefficient for this analysis was 0.67 (95% confidence interval 0.58−0.76).

Time averaging was achieved in 10 (55.5%) patients. Patients not included are the first 5 cases due to data collection not allowing time averaging and the 3 patients where calibration was unsuccessful. The correlation coefficient for this analysis improved to 0.78 (95% confidence interval 0.70−0.84).

Discussion

A statistically significant strong positive correlation was observed between the fluorescence intensity using fluorescence angiography and tissue oxygen saturation in this study. Although a sensitivity analysis could be performed only in a selected subgroup of patients, it was shown that time-averaging led to an improved correlation coefficient in this context. Whereas previous studies have shown an inconsistent result of fluorescence quantification and clinical outcomes,[12] we have demonstrated a strong correlation between fluorescence intensity and a noninvasive marker of tissue oxygenation. This shows that fluorescence intensity may be a surrogate marker for tissue oxygenation and therefore one of the elements to be optimized when using fluorescence angiography.

Anastomotic leak remains a hugely morbid complication of colorectal surgery with clinical, oncological, and financial consequences.[28] However, there is still a lack of understanding of how best to optimize the anastomosis beyond subjective means. FA has provided some encouraging evidence in recent years that a more objective assessment of perfusion may lead to reducing leak rates,[10,11] but there is still skepticism due to the absence of any strong randomized clinical study data. Furthermore, while the premise of FA is to provide a more objective assessment of bowel perfusion, there is still a subjective element in FA since the fluorescent signal requires interpretation by the surgeon, raising the question—when is the signal intense enough to suggest adequate perfusion? In addition, how the signal correlates to underlying tissue oxygenation is also unknown. This leaves us with a challenging situation whereby clinical decision-making is reliant on an observation that is not grounded in sufficiently robust clinical or scientific data.

Better understanding the limitations of FA by using MSI is a novel idea. MSI has been shown to allow an adequate measurement of tissue oxygenation and thus perfusion, using the physical properties of light. This technology has been applied successfully not only in the bowel[17] but also in other tissues.[29] Combining FA and MSI allows us to understand the relationship between quantitative tissue oxygenation and fluorescence, and whether signal intensity is a function of perfusion or something else. This introduces the concept of quantification of the signal, which has been studied in various ways, including time to fluorescence, rate of change of fluorescent signal, and time for signal washout, among other techniques.[12] Unfortunately, none of these methods has been validated and still rely on subjective measures of maximal signal intensity. Conversely, video analysis allows us to begin to standardize the interpretation of the fluorescent signal by studying the pixel data. One of the main challenges with FA in its existing form is that there is variability in surgeons' interpretation of the fluorescent signal. By standardizing this aspect, there is an opportunity to produce a decision-support tool, a tool that is based on underlying biological and physiological data and that uses computer vision techniques to identify the peak fluorescent signal.

The limitations of the study can be categorized into those that are intrinsically related to the methodology and those that are due to the pragmatic data acquisition in this study. In terms of intrinsic limitations, this experiment did not include a direct measurement of products of anaerobic metabolism as a direct effect of hypoxia. Also, only maximal fluorescence intensity as determined by the

operating surgeon was considered. Other studies have looked into dynamic quantification, such as time to maximal fluorescence intensity, but these parameters have not been analyzed in the present article and will be the focus of further work. Furthermore, biologic plausibility suggests a mechanistic relationship between fluorescence intensity and tissue viability. However, to be an added value in clinical practice, this measurement must allow a change in decision making that improves patient outcomes. The main clinical outcome of interest is anastomotic leak rate reduction, which is the aim of this technology. Our sample size was insufficient to allow comparisons according to different outcome results.

In terms of pragmatic limitations, the lighting conditions and distance between camera and tissue could not be completely controlled for in the operating theater setting. This means that there may be added noise to the data captured at the patient's bedside. An experimental challenge with the dual imaging setup is the differing mechanisms of MSI and FA acquisition. MSI measures reflected light intensity from a broadband white light xenon surgical source. However, the spectrum of this xenon bulb overlaps with the emission spectrum of ICG. At the same time, the FA system rapidly switches between its own white light and near-infrared fluorescence excitation sources to enable simultaneous display of standard color and ICG fluorescence images. Therefore, the MSI source must be switched off during FA acquisition to avoid washing out the fluorescence signal, and the FA source must be switched off during MSI acquisition to avoid periodic and uncontrolled variations in illumination intensity. Two patients were excluded from the correlation analysis due to an abnormal reading of fluorescence intensity. In these 2 cases there was discordance between visual inspection of fluorescence intensity that was normal and fluorescence intensity measurements that were below 0.1 with negligible variation. This discordance led us to consider these values to be artifacts and therefore not include them in the correlation analysis. The ICG dose was also fixed, which meant that the patient's body weight was not considered in the administered dose calculation. This may have an impact due to differing distribution volumes for the drug and therefore its effect in vivo. Fluorescence angiography was carried out with the specimen extracorporealized, which could have added tension and therefore become a confounder, although care was taken to avoid this.

For fluorescence angiography to make a positive impact on patient care, it will be necessary to develop a quantitative tool that improves patient outcomes in clinical trials. Unfortunately, the PILLAR III trial[30] was terminated early due to slow recruitment and did not reach the required sample size. These limitations leave the absolute effect of fluorescence angiography still not quantified. We have shown a mechanistic effect of fluorescence intensity being correlated with tissue oxygenation. Future development will require a prospective assessment of fluorescence intensity and patient outcomes and different quantification strategies.

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Conflict of interest/Disclosure

No industry funding was involved in the present study. MC received consultancy fees for Stryker unrelated to the present study. The other authors report no biomedical financial interests or potential conflicts of interest.

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