Comparison of Optical Imaging Techniques to Quantitatively Assess the Perfusion of the Gastric Conduit during Oesophagectomy

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Abstract: In this study, four optical techniques—Optical Coherence Tomography, Sidestream Darkfield Microscopy, Laser Speckle Contrast Imaging, and Fluorescence Angiography (FA)—were compared on performing an intraoperative quantitative perfusion assessment of the gastric conduit during oesophagectomy. We hypothesised that the quantitative parameters show decreased perfusion towards the fundus in the gastric conduit and in patients with anastomotic leakage. In a prospective study in patients undergoing oesophagectomy with gastric conduit reconstruction, measurements were taken with all four optical techniques at four locations from the base towards the fundus in the gastric conduit (Loc1, Loc2, Loc3, Loc4). The primary outcome included 14 quantitative parameters and the anastomotic leakage rate. Imaging was performed in 22 patients during oesophagectomy. Ten out of 14 quantitative parameters significantly indicated a reduced perfusion towards the fundus of the gastric conduit. Anastomotic leakage occurred in 4/22 patients (18.4%). At Loc4, the FA quantitative values for “T1/2” and “mean slope” differed between patients with and without anastomotic leakage (p = 0.025 and p = 0.041, respectively). A quantitative perfusion assessment during oesophagectomy is feasible using optical imaging techniques, of which FA is the most promising for future research.

Keywords: optical imaging; fluorescence imaging; fluorescence angiography; indocyanine green (ICG); optical coherence tomography (OCT); laser speckle contrast imaging (LSCI); esophagectomy; gastric conduit; Sidestream Darkfield Microscopy (SDF); multispectral

1. Introduction

Surgical morbidity related to inadequate perfusion is a major challenge after oesophagectomy with gastric conduit reconstruction for oesophageal cancer. This type of surgical morbidity includes anastomotic leakage and graft necrosis, and remains high even after recent developments in minimally invasive surgery, enhanced recovery programs, and the centralisation of cancer surgery, occurring in up to 20% of the patients [1–4].

To achieve digestive continuity after oesophagectomy, the replacement of the native oesophagus by a constructed gastric conduit is a potential solution. The perfusion of the gastric conduit is mainly...
based on the right gastroepiploic artery, which usually terminates before the future anastomotic site, causing the future anastomotic site to be at risk for inadequate perfusion. Surgeons assess the perfusion of the gastric conduit by observing the colour of the serosal surface, the bleeding of resection margins, or arterial palpation. To aid surgeons' decision-making, different optical imaging techniques have been introduced to assess perfusion intraoperatively [5,6].

Optical imaging, which is established by the interaction between light and tissue, is potentially able to image changes in perfusion real-time and at high resolution. These characteristics of optical imaging have the potential to analyse a perfusion intraoperatively and in a quantitative manner [7,8]. Some optical imaging techniques are already Food and Drug Administration (FDA)-approved and Conformité Européenne (CE)-marked systems, but others lack clinical evaluation [5,9]. Not all techniques have an adequate specificity for patient outcomes and are so far unable to objectify perfusion in quantitative values for clinical usage [5]. The comparison of techniques in the literature is hampered by the large range of available imaging systems and the heterogeneity of quantitative parameters and perfusion endpoints [5]. Therefore, there is scant evidence on which optical imaging technique and related quantitative parameter is the most predictive for inadequate perfusion and patient outcomes.

In this explorative study (IDEAL phase 2a), we aim to compare four optical techniques using different wavelengths of light on performing a quantitative perfusion assessment of the gastric conduit in patients undergoing oesophagectomy with gastric conduit reconstruction for oesophageal cancer. The four optical techniques are Optical Coherence Tomography (OCT), Sidestream Darkfield Microscopy (SDF), Laser Speckle Contrast Imaging (LSCI), and Fluorescence Angiography (FA). We hypothesised that the quantitative parameters of the optical techniques show decreased perfusion (A) towards the fundus in the gastric conduit and (B) in patients with anastomotic leakage. By comparing these four techniques, we aimed for a recommendation of an optical imaging technique for a quantitative perfusion assessment of the gastric conduit for future studies and clinical intraoperative use.

2. Materials and Methods

2.1. Study

This is a single-centre, prospective, observational study in patients undergoing elective oesophagectomy with gastric conduit reconstruction for oesophageal cancer. This study adhered to the STARD (Standards for Reporting of Diagnostic Accuracy Studies) [10] and STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) [11] guidelines.

This study was approved by the Institutional Review Board of the Amsterdam UMC, location AMC, (NL52377.018.15) and submitted to the clinicaltrials.gov database (NCT02902549) [12]. Written informed consent was obtained from all patients.

2.2. Procedure

All the procedures were performed totally minimally invasively with laparoscopy and thoracoscopy. A McKeown (cervical anastomosis) or Ivor Lewis (intrathoracic anastomosis) procedure was performed by two specialised upper-gastrointestinal surgeons (MlvBH, SSG), as previously described [13]. The stomach was reconstructed into a 3–4 cm wide gastric conduit using a powered linear stapler. Directly after gastric conduit reconstruction, perfusion measurements were performed using four optical techniques in the following order: OCT, SDF, LSCI, and FA. Perfusion measurements were performed at four locations (Loc1–4): 3 cm proximal of the level of (Loc1), at the level of (Loc2), 3 cm distal to (Loc3) at the level of the watershed between the right and left gastroepiploic artery, and at the level of the gastric fundus (Loc4) (Figure 1). A sterile gauze was placed to point out the watershed area (the end of the right gastroepiploic artery). Subsequently, a cervical or intrathoracic anastomosis to the proximal oesophagus was constructed. The anastomotic site of the gastric conduit was determined by a conventional white light assessment, independent of imaging outcomes, and was located between Loc3 and Loc4.
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Figure 1. Qualitative images by all four imaging techniques: upper left Optical Coherence Tomography (OCT), lower left Sidestream Darkfield Imaging (SDF), upper right Laser Speckle Contrast Imaging (LSCI), and lower right Fluorescence Angiography (FA). Perfusion measurements were performed at four locations (Loc1–4): 3 cm proximal of the level of (Loc1), at the level of (Loc2), 3 cm distal to (Loc3) the level of the watershed between the right and left gastroepiploic artery, and at the level of the gastric fundus (Loc4).

2.3. Imaging Modalities and Software

OCT was performed by a commercial 50 kHz IVS 2000 swept source system (Santec, 5823 Ohkusa-Nenjozaka, Komaki-City, Aichi, Japan), an FDA-approved and CE-marked system, using a centre wavelength of ∼1300 nm. Measurements were performed as previously described [14]. Data analysis was performed using custom-made scripts written in MATLAB (Mathworks, Natick, MA, USA) [14].

SDF is a hand-held, in-contact, green light (530 nm) imaging system that visualises microcirculation. Image contrast is based on the absorption of green light by oxyhaemoglobin in red blood cells (RBCs) [15]. Therefore, RBCs will appear as dark spots in the image. SDF measurements were performed by the Microscan (Microvision Medical, Amsterdam, The Netherlands), an FDA-approved system to monitor the microcirculation of critically ill patients. To stabilise the image, a custom-made stabiliser based on the design by Balestra et al. [16] was used to adhere the tissue to the imaging system. The SDF obtained images were analysed using the AVA3.2 software (Microvision Medical BV, Amsterdam, The Netherlands).

LSCI is a near infrared imaging system with a laser operating at 785 nm and measures speckle fluctuations induced by the motion and change in number of RBCs. The LSCI was used as previously described [17]. Imaging was performed by the MoorFLPI-1™ (Moor instruments, Devon, UK), an FDA-approved and CE-marked system, positioned at 40 cm above the gastric conduit, as measured by a laser distance meter (Leica Geosystems D110, Leica Camera, Wetzlar, Germany).

FA is a near infrared imaging modality that images the spatial distribution of the contrast agent indocyanine green (ICG), detecting at an emission wavelength of approximately 800 nm. FA
was performed by the Artemis system® (Quest Medical Imaging, Middenmeer, The Netherlands), an FDA-approved and CE-marked system, after the intravenous administration of 2.5 mg of ICG. The Artemis system was placed under a 90-degree angle and also at a distance of 40 cm above the gastric conduit, as measured with the laser distance meter. A metric ruler was placed in the field of view for image calibration. The anaesthesiologist administered 2.5 mg of ICG intravenously. The perfusion was imaged in continuous video recordings of 2–3 min. In-house software was developed to plot the ICG in- and outflow in a fluorescence–time curve. A ruler in the field of view was used for calibration, and for every location a circular region of interest (ROI) with a diameter of 1 cm was used to measure the ICG signal.

2.4. Outcomes

The primary objective of this study was the comparison of the ability of the four optical imaging systems—OCT, SDF, LSCI, and FA—to obtain the quantitative perfusion parameters during surgery, and to explore the relation between the four locations (Loc1–4) and the occurrence of anastomotic leakage. The primary outcome included 14 quantitative parameters outlined below in “quantitative parameters” at four locations and anastomotic leakage. Quantitative assessment was deemed successful per technique when one or more measurements could be obtained per patient. Anastomotic leakage was defined by the Esophageal Complications Consensus Group (ECCG) classification and diagnosed by a CT-scan with contrast, upper gastrointestinal endoscopy, drainage of saliva, gastric content via wound/drains, and/or by reoperation [18].

The secondary outcomes were imaging characteristics and hemodynamic parameters. The imaging characteristics included the field of view, imaging depth, ease of use, practical limitations (overall and for quantitative analysis), invasiveness, time consumption, and quality. The quality was deemed high when no technical issues occurred to obtain qualitative images. The relations between the quantitative and hemodynamic parameters were explored at Loc1. Hemodynamic parameters were recorded at the start of imaging and included the mean arterial pressure (MAP; mmHg), heart rate (beats/min), systolic and diastolic blood pressure (mmHg), temperature nasopharynx (°C), cardiac output (L/min), stroke volume (mL), stroke volume variation (%), and cardiac index (L/min/m²).

2.5. Quantitative Parameters

Fourteen quantitative parameters were measured (OCT \( n = 1 \), SDF \( n = 6 \), LSCI \( n = 2 \), and FA \( n = 5 \)). The quantitative parameter for OCT was [14]:

1. The percentage of speckle contrast pixels (speckle %) indicative of the flow obtained in the M-mode of the scans;

Quantitative parameters derived for SDF included [15]:

2. Total vessel density (TVD): ratio between the total vessel length and the area of ROI (mm/mm²);
3. Perfused vessel density (PVD): ratio between the perfused scored vessel length and the area of ROI (mm/mm²);
4. Microvascular flow index (MFI): sum of the qualitative determination (0 no, 1 intermittent, 2 sluggish, 3 continuous) of the predominate flow in four quadrants of the SDF image, divided by four;
5. De Backer Score (DBS): number of vessels crossing a grid overlay divided by the total length of the vessels;
6. Proportion of perfused vessels (PPV): perfused vessel length divided by the total vessel length of the ROI in %;
7. Velocity (µm/s): assessed per selected vessels by a space–time diagram;

The quantitative parameters calculated for LSCI were [17]:

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8. Flux in laser speckle perfusion units (LSPU);
9. Δ%Flux defined as the percentage difference in Flux relative to Loc1 (positive number when there is a decrease in Flux);

FA was quantified by the assessment of the time-dependent change in the fluorescence signal at the ROIs in the form of the software-derived fluorescence–time curves [8,19]. The quantitative parameters for FA included:

10. Influx time point (τ): time in seconds between the occurrence of the peak fluorescence in the right gastroepiploic artery and the time point at which the fluorescence intensity in the ROI was statistically significantly larger than the background;
11. Fmax: maximal intensity in arbitrary units (AU) within 50 s after the influx time point. Fmax was corrected by subtracting the median background value measured from the start of imaging to the influx time point (τ);
12. Tmax: time in seconds after the influx time point (τ) at which the background-corrected fluorescence intensity reached Fmax, also known as the time-to-peak;
13. T1/2: time in seconds after the influx time point (τ) at which the background-corrected fluorescence intensity was half of the Fmax;
14. Mean slope: rate at which the fluorescence intensity increased (AU/s).

2.6. Statistics

The quantitative perfusion parameters were shown as the median and interquartile (IQR) or total range. All the available data were used for the analysis. The comparison of quantitative perfusion parameters between Loc1 and Loc4 were analysed using the Wilcoxon Signed Ranks Test. The results at Loc3 and Loc4 were compared between patients with or without anastomotic leakage using the Mann–Whitney U test. A p-value of below 0.05 was considered significant. Correlations between the quantitative values and hemodynamic parameters were assessed by calculating the Spearman’s rank correlation coefficient ρ. A p-value ≤ 0.05 was considered statistically significant. The imaging characteristics were summarised using simple descriptive statistics or in quantitative terms. The added surgical time was shown in the median and IQR. All the analyses were performed using IBM® SPSS® Statistics (version 26.0.0, IBM Corporation, NY, USA).

3. Results

Between October 2015 and June 2016, in total 26 patients that underwent minimally invasive oesophagectomy with gastric conduit reconstruction were included after written informed consent was obtained. Four patients did not receive imaging due to extended operation time and were not taken into the analysis. The baseline characteristics of 22 patients are shown in Table 1 [17]. The gastric conduit was connected to the proximal oesophagus by a cervical (n = 2) or intrathoracic (n = 20) anastomosis.

Table 1. Baseline characteristics.

| Cohort (n = 22) |          |
|----------------|----------|
| Age (years) median total range | 62 (37–79) |
| Gender (male), n (%) | 19 (86.4) |
| Body mass index (kg/m²), median total range | 25.9 (17.0–34.2) |
| Surgical procedures, n (%) |
| Ivor Lewis | 20 (91) |
| McKeown | 2 (9) |
| Cardiovascular disease, n (%) | 5 (23) |
| Diabetes Mellitus *, n (%) | 2 (9) |
| COPD, n (%) | 2 (9) |

* Only diabetes mellitus type 2. COPD = chronic obstructive pulmonary disease.
3.1. Imaging Characteristics

Qualitative imaging was successful in all the patients \( n = 22 \) with all four techniques. In Figure 1, qualitative images by all four imaging techniques are shown for one patient. The imaging characteristics are shown in Table 2. OCT and SDF had a narrow field of view, of which SDF assessed the microvasculature for a region of 1 mm \( \times \) 1 mm. LSCI and FA were wide field techniques and showed similarity in the qualitative images (Figure 1). All the practical limitations are summarised in Table 2, of which artefacts, shadowing, and focus were limitations for quantitative analysis. The motion artefacts for OCT and SDF were secondary to the respiratory rate, heart rate, peristalsis, or probe handling, and other artefacts were due to air bubbles in the surgical drape. The use of OCT, SDF, LSCI, and FA added 6 (5–7), 5 (4–7), 3 (2–4), and 4 (2–4) minutes to the surgical time, respectively.

| Field of View | Depth-Resolving | Ease of Use | Real-Time | Practical Limitations |
|---------------|-----------------|-------------|-----------|----------------------|
| OCT           | 10 mm \( \times \) 10 mm \( \times \) 2.5 mm | +           | Sterile sheet, probe handling | Motion artefacts, shadowing |
| SDF           | 1 mm \( \times \) 1 mm | -           | Sterile sheet, tissue-contact | Motion artefacts, focus |
| LSCI          | Wide-field ROI: \( \phi \) 1 cm | -           | Non-contact | OR lights off |
| FA            | Wide-field ROI: \( \phi \) 1 cm | -           | Non-contact | Invasive (ICG injection), OR lights off * |

FA Fluorescence Angiography, ICG Indocyanine Green, LSCI Laser Speckle Contrast Imaging, OCT Optical Coherence Tomography, OR Operating Room, ROI Region-of-interest, SDF Sidestream Darkfield Microscopy. * Not when used laparoscopically.

3.2. Quantitative Parameters

Of 22 patients, quantitative assessment was successful in 15, 22, 20, and 20 patients for OCT, SDF, LSCI, and FA, respectively. At the four locations (Loc1/Loc2/Loc3/Loc4), the number of obtained measurements for OCT were 12/10/8/10, for SDF 21/21/21/21, for LSCI 20/20/20/20, and for FA 17/20/20/18, respectively. The measurements for the SDF parameters were available in 21 patients at Loc4, except for MFI \( n = 14 \).

Comparing Loc1 with Loc4, a significant difference was noticed for 10 out of 14 parameters (Figure 2): the SDF parameters PVD, MFI, PPV, and velocity; the LSCI parameters Flux and \( \Delta \% \)Flux; and the FA parameters influx time point, \( F_{\text{max}} \), \( T_{1/2} \), and mean slope. Of those, PVD, MFI, PPV, velocity, Flux, \( F_{\text{max}} \), and mean slope decreased towards the fundus, while the \( \Delta \% \)Flux, influx time point, and \( T_{1/2} \) increased. No significant differences were noticed between the locations for the OCT parameter speckle %, the SDF parameters TVD and DBS, and the FA parameter \( T_{\text{max}} \).

Anastomotic leakage occurred in 4/22 patients (18.2%). Of the measurements available at Loc3, anastomotic leakage occurred in 1/8 (12.5%), 3/21 (14.3%), 4/20 (20.0%), and 2/20 (10.0%) for OCT, SDF, LSCI, and FA, respectively. No significant differences were observed for the quantitative parameters between the patients with or without anastomotic leakage at Loc3 (data not shown). Of the measurements available at Loc4, anastomotic leakage occurred in 1/10 (10.0%), 3/21 (14.3%), 4/20 (20.0%), and 2/18 (11.1%) for OCT, SDF, LSCI, and FA, respectively. A comparison of quantitative parameters at Loc4 between patients with and without anastomotic leakage is shown in Table 3. Of all the quantitative parameters, the FA values for the \( T_{1/2} \) and mean slope significantly differed between patients with and without anastomotic leakage. The \( T_{1/2} \) was 13.0 (total range 6.3–21.3) seconds in patients without anastomotic leakage, and increased to 29.3 (total range 27.8–30.8) seconds in the case of anastomotic leakage \( p = 0.025 \). For \( T_{1/2} \), the total range of values showed no overlap between patients with and without anastomotic leakage. The mean slope decreased from 1.3 (total range 0.1–11.0) to 0.2 (total range 0.1–0.2) in patients without and with anastomotic leakage \( p = 0.041 \), respectively. All the values for the SDF parameters and for Flux were non-significantly higher (Table 3).
Figure 2. Fourteen quantitative parameters at four locations of the gastric conduit. Data are shown as medians and interquartile ranges. * p-value is shown for the difference between Loc1 and Loc4. OCT Optical Coherence Tomography: Speckle (%). SDF Sidestream Darkfield Imaging: TVD total vessel density in mm/mm², PVD perfused vessel density in mm/mm², MFI microvascular flow index, DBS de Backer score, PPV proportion (%) of perfused vessels. LSCI Laser Speckle Contrast Imaging: Flux in LSPU (laser speckle perfusion units), ∆%Flux percentage difference in Flux relative to Location 1 (right vertical axis). * Not calculated, p-value calculated on absolute data (see Flux). FA Fluorescence Angiography: Influx time point in sec, Fmax in arbitrary units, Tmax in sec, T1/2 in sec, mean slope in arbitrary units per sec.

Table 3. Comparison of the perfusion-related parameters at location 4 between patients with and without anastomotic leakage.

|          | Location 4 No Anastomotic Leakage (n = 18) | Location 4 Anastomotic Leakage (n = 4) | p-Value |
|----------|------------------------------------------|----------------------------------------|---------|
| OCT      |                                          |                                        |         |
| Speckle (%) | 21 (14–31)                                  | 17 (17)                                | 0.600   |
| TVD (mm/mm²) | 8.7 (5.1–15.9)                              | 9.8 (9.5–17.4)                        | 0.088   |
| PVD (mm/mm²) | 0.5 (0.0–7.0)                                | 2.0 (0.9–5.4)                         | 0.155   |
| MFI       | 1.1 (0.3–2.0)                                | 1.3 (1–1.5)                           | 0.646   |
| DBS       | 5.4 (3.0–10.3)                               | 7.2 (6.9–11.00)                       | 0.056   |
| PPV (%)   | 5.7 (0.0–86.4)                               | 21.3 (9.3–31.1)                       | 0.155   |
| Velocity (µm/s) | 15.2 (4.3–186.0)                             | 23.8 (14.8–60.3)                      | 0.560   |
| SDF      |                                          |                                        |         |
| LSCI     |                                          |                                        |         |
| Flux (LSPU) | 128.7 (41.1–480.5)                          | 167.1 (114.1–335.2)                   | 0.422   |
| ∆%Flux (%) | 54 (−31–87)                                 | 52 (52–87)                            | 0.072   |
| FA       |                                          |                                        |         |
| Influx time point (s) | 39.5 (21–73)                             | 44.0 (42–46)                          | 0.673   |
| Fmax (AU) | 39.8 (1.8–163.2)                            | 12.3 (10.9–13.7)                      | 0.131   |
| Tmax (s)  | 38.8 (34.2–44.6)                            | 39.8 (31.1–48.58)                     | 0.888   |
| T1/2 (s)  | 13.0 (6.3–21.3)                             | 29.3 (27.8–30.8)                      | 0.025   |
| Mean slope (AU/s) | 1.3 (0.1–11.0)                           | 0.2 (0.1–0.2)                        | 0.041   |

Data are shown as median and total range. OCT Optical Coherence Tomography, SDF Sidestream Darkfield Imaging, TVD total vessel density in mm/mm², PVD perfused vessel density in mm/mm², MFI microvascular flow index, DBS de Backer score, PPV proportion (%) of perfused vessels, LSCI Laser Speckle Contrast Imaging, Flux in LSPU (laser speckle perfusion units), ∆%Flux percentage difference in Flux relative to Location 1, FA Fluorescence Angiography, Fmax in arbitrary units, mean slope in arbitrary units per sec.
At Loc1, the heart rate was correlated with the OCT parameter speckle % ($\rho = 0.669, p = 0.017$). Systolic blood pressure was inversely correlated with LSCI parameter Flux ($\rho = -0.451, p = 0.046$), and stroke volume with SDF parameter MFI ($\rho = -0.528, p = 0.017$). No other significant correlations were observed for the quantitative and hemodynamic parameters.

4. Discussion

Four optical techniques were tested intraoperatively for a quantitative perfusion assessment of the gastric conduit in oesophageal cancer patients undergoing resection. The perfusion restriction towards the fundus was quantitatively assessed and was significantly objectified in 10 out of 14 parameters. At the fundus of the gastric conduit (Loc4), the FA parameters $T_{1/2}$ and mean slope significantly differed between the patients with or without anastomotic leakage.

The gastric conduit is mainly vascularised by the right gastroepiploic artery, which usually terminates before the future anastomotic site, making the future anastomotic site prone to vascularisation problems. Therefore, the gastric conduit is an adequate model for perfusion, as perfusion restriction is expected towards the gastric fundus. Quantitative data from this study indeed showed perfusion restriction towards the fundus. Ten quantitative parameters significantly differed between Loc1 and Loc4, of which seven decreased towards the fundus. Comparing those to the literature, the Flux and $F_{\text{max}}$ are described to indicate perfusion restriction towards the fundus [19,20]. Three parameters ($\Delta \% \text{Flux}$, influx time point ($\tau$), and $T_{1/2}$) increased towards the fundus, indicating decreased perfusion as well. Influx time point ($\tau$) and $T_{1/2}$ measured the time-dependent change in fluorescence. In the literature, the time-dependent change in fluorescence intensity is a potential method for FA quantification, and increased $\tau$ and $T_{1/2}$ might indicate inadequate perfusion [19,21–23]. An increase in these values might be due to either diffusion through tissue instead of an intact arterial route or to resistance in the vascular network in the case of venous outflow obstruction.

Quantitative parameters were compared between patients with and without anastomotic leakage at Loc4. In patients with anastomotic leakage, a significantly higher $T_{1/2}$ and significantly lower mean slope were found. $T_{1/2}$ and mean slope provide information about the ICG influx velocity, amount of vessel density, and potentially the outflow. $T_{1/2}$ and mean slope are potentially hands-on parameters for intraoperative perfusion evaluation and anastomotic leakage prediction [19]. At Loc4, in contrast to an expected decrease, the SDF parameters and Flux appeared to be higher for patients with anastomotic leakage compared with patients without leakage. This increase might be explained by venous outflow obstruction. SDF measures the velocity of RBCs using a space–time diagram. The Flux measured by LSCI is dependent on the amount of blood particles and movement of these particles. In the case of venous outflow obstruction at the fundus of the gastric tube, veins swell to 5–10 times their original size [24,25]. The SDF parameters and Flux are possibly influenced by the number of RBCs or blood particles in swollen veins and therefore could be higher at Loc4 in the case of anastomotic leakage secondary to venous outflow obstruction. No differences were observed at Loc4 for the OCT parameter speckle %.

This explorative study allowed the comparison of four optical techniques in terms of their quantitative assessment and imaging characteristics. Both OCT and SDF have a limited field of view focusing on microperfusion, while LSCI and FA are wide-field (considering the heterogeneity of the capillary network). Wide-field techniques allow the immediate overview of entire gastric conduit, and potentially show vascular confines visually, which is valuable for clinical determination of the anastomotic site. Besides, the time consumption for imaging seemed lower for wide-field techniques. OCT, LSCI, and FA are all non-contact techniques, thereby avoiding stabilising problems. Only OCT is depth-resolving (depicting individual overlying vessels). In terms of quantification, quantitative SDF, LCSI, and FA had acceptable success rates. The quantitative parameter for OCT did not significantly differ between Loc1 and Loc4, nor in the case of anastomotic leakage. This observation might be explained by the method of analysis that assessed only one transection of 2.5 mm depth for quantification, indicating that limited field of view techniques might be prone to sampling errors. SDF and LSCI show perfusion restriction towards the fundus, but both show non-significant higher values at Loc4 in the
case of anastomotic leakage. As mentioned above, the SDF and LSCI trends might be comparable due to focus on the same perfusion units and might be higher in the case of anastomotic leakage due to venous outflow obstruction. FA visualises the spatial distribution of ICG that is bound to plasma proteins, and thus assesses the patency of vessels and shows vascular confines. FA parameters were able to indicate perfusion restriction towards the fundus as well as in patients with anastomotic leakage. In the case of venous congestion, FA parameters might show resistance in the vasculature, which leads to prolonged time values and a reduced rate in the increase in fluorescence intensity. Furthermore, FA time values might show what degree of arterial diffusion (instead of direct arterial flow) or venous outflow obstruction might lead to anastomotic leakage. Although FA use is dependent on ICG injection, ICG is a safe dye and can be applied at low dosages. Moreover, FA imaging systems are available for laparoscopy, which is clinically beneficial. Therefore, and on the basis of this study, FA quantitative parameters are most promising for future research.

$T_{1/2}$ and mean slope will be promising quantitative parameters for intraoperative clinical decision-making if a threshold can be derived for patient outcomes, including anastomotic leakage. According to the threshold, change in management can include the administration of medication, to shorten the gastric conduit at the maximum extent, or no anastomotic reconstruction and oesophagostomy. The latter option is a very radical solution with a decreased quality of life and need for surgery in the future to make a new reconstruction. Most surgeons would probably choose to make an extra effort to shorten the gastric conduit and make an anastomosis at increased risk instead of choosing no reconstruction as an option. Furthermore, the threshold can be used to identify high-risk patients for anastomotic leakage.

The quantitative parameters for FA may be biased by the duration of imaging, as this might not have been long enough to reach the peak value ($F_{\text{max}}$). This study was further limited by a relatively small number of patients with a low absolute number of anastomotic leakages, and particularly measurements at Loc4 were infrequent. However, this pilot study can guide future studies, by for example serving as a base for sample size calculations. Furthermore, the exact anastomotic site was not recorded, but the anastomosis was constructed between Loc3 and Loc4. At Loc3, no differences were observed for any quantitative parameter, however at Loc4 differences were found. The site of anastomotic reconstruction may have led to bias in the observations. In addition, the system used for SDF measurements involves an older model and older software, and possibly new models (e.g., Cytocam) allow higher resolution, and new software (AVA 5, MicroVision Medical, Amsterdam, The Netherlands) might perform better. However, the SDF technique is still employed, and sampling error and increased values in the case of anastomotic leakage can still be expected. In addition, the parameters were not assessed in relation to patient risk factors. Co-morbidities such as diabetes mellitus and cardiovascular disease affect the (micro) circulation [26,27], and might therefore influence the read-out of the parameters. Furthermore, diabetes mellitus and chronic obstructive pulmonary disease are known risk factors for anastomotic leakage [28]. Owing to the small sample size, correction for co-morbidities was not performed. Nevertheless, it is difficult to compare different optical imaging systems in the literature, and this study allows comparison for the same group of patients. Furthermore, this study evaluated quantitative parameters for both perfusion and patient outcomes.

In conclusion, for future studies, FA and software-derived fluorescence–time curves are most promising to quantitatively assess decreased perfusion (A) towards the fundus in the gastric conduit and (B) in patients with anastomotic leakage. Future FA ideally has a real-time perfusion map with analysis per pixel showing perfusion thresholds. In future studies, FA data should be combined with risk factors for patient outcomes to provide additional information on FA thresholds.

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