RESEARCH

Journal of Surgery & Practice

Assessment of microvascular perfusion of the stomach in oesophageal surgery using full field laser perfusion imaging - Preliminary Study

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Abstract

Background: *After oesophagectomy, oesophageal replacement most commonly involves a transposed gastric tube.Perfusion of the transposed gastric tube may be sub-optimal and this may delay healing of the oesophago-gastric anastomosis with leakage of gastric contents.*

Setting: *This study was carried out in a teaching hospital in Scotland.*

Aim: *The aim of this study is to measure gastric micro-vascular blood perfusion during four stages of oesophagectomy using a full field laser perfusion imager FLPI.*

Patients and methods: *Twelve patients (median age: 68) were recruited into the study. All patients were diagnosed with localised oesophageal cancer and were suitable for oesophagectomy. A standard protocol was followed to measure blood perfusion in 5 regions of the stomach at 4 different stages of the procedure. Blood perfusion metrics were analysed using the moor FLPI analysis software.*

Results: *There was a significant reduction in micro-vascular blood perfusion (38%) of the whole stomach between the initial and end steps of the procedure (p<0.05). The reduction of blood perfusion was most significant at the fundus of the stomach which is the site used for anastomosis.*

Conclusion: *The FLPI device provides a reliable method of measuring gastric blood perfusion during oesophagectomy.*

Keywords: *stomach, oesophageal cancer, oesophagectomy, anastomosis, gastric tube, microvascular blood perfusion, Full Field Laser Perfusion Imager (FLPI)*

Introduction

Worldwide oesophageal cancer is estimated to be the fourth most common type of cancer and the second most frequent cause of cancer related mortality [1].Oesophagectomy remains the standard of care in most centres for fit patients with resectable (localised) oesophageal cancer. Oesophagectomy is however, associated with considerable morbidity and mortality [2].

In oesophagectomy, the oesophagus is partly or entirely removed. In most cases, the mobilised stomach is used as an oesophageal replacement conduit [3].Mobilising the stomach necessitates severing some of its blood supply. Although the stomach is a highly perfused organ, it can become hypoxic as most of its vascular supply is compromised [3].

The major complication associated with oesophagectomy is anastomotic leak [1].Anastomotic leak is thought to be due to many factors including delayed healing due to an inadequate blood supply to the anastomotic site and/or venous congestion in the rest of the stomach [2].In order to reduce anastomotic failure, an effective method to accurately assess gastric micro-vascular perfusion during surgery is ideal.

During the past two decades, many techniques have been investigated for assessing gastric perfusion [4]. There is no gold standard method widely accepted as the test of choice in clinical practice because these methods are either time consuming, invasive, operator dependent or not validated. Recent technological advances have resulted in the development of laser speckle contrast imaging for monitoring of the microvasculature. The Moor Instruments full field laser perfusion imager (FLPI) is a novel device allowing real-time measurements of micro-vascular perfusion to be made with high reproducibility [5], and is thus ideal for intra-operative application. The FLPI is highly suitable for the surgical field and has the potential of providing instant information on organ perfusion at the site of the anastomosis.

The objective of this prospective cohort pilot study is to evaluate the use of full field laser perfusion imaging in assessing micro-vascular perfusion changes during selective devascularisation of the stomach to create a gastric tube to replace the oesophagus after oesophagectomy.

Patients and methods

This is a single-centre observational study. The protocol for this study was reviewed and approved by the local Research Ethics Committee (REC). All recruited participants received an explanatory information sheet and informed consent was obtained in every case.

Study participants

Twelve patients (were recruited consecutively to this study over a 12-month period during February 2014 to January 2015 at Ninewells Hospital, Dundee. All patients were diagnosed with localised oesophageal cancer suitable for surgical resection and were sufficiently fit to have the surgical procedure (Ivor-Lewis oesophagectomy). The option of surgery was based on the recommendation of the local multi-disciplinary team and discussed with the patient by a member of the surgical team. The date for surgery was planned in advance. The study was discussed with the patients 24 hours before surgery when informed consent was obtained.

Data collection

Gastric micro-vascular perfusion scans were obtained at specified and predetermined intervals as per study protocol. The scans were obtained using full field laser perfusion imaging (FLPI). In addition, data were collected on the presence or absence of an anastomotic leak in the post-operative period.

For each patient, additional relevant information was also recorded including concomitant blood pressure (BP), mean BP, central venous pressure, heart rate, O2 saturation, superficial temperature of the stomach and ambient room temperature. This set of data was collected at the same intervals as the scans. In addition, a list of all current and anaesthetic medication was collected for each patient. Anaesthesia was sustained via the administration of propofol(8 µg/ml) and fentanyl (30 µg/ml). Clinical photographs and scans taken during the surgical procedure were digitally archived with non-identifiable information for each patient.

Imaging and analysis

Study design and intraoperative testing of perfusion

Measurements of micro-vascular perfusion were made using the Moor Instrument FLPI (moorFLPI-1, Moor Instruments, Devon, UK) at the following steps of the surgical procedure:

1. Immediately after exposure of the stomach. Perfusion measurements at this step served as the baseline for comparison with subsequent steps.
2. After ligation of short gastric and the left gastric arteries.
3. When the gastric tube is created from the stomach
4. After transposition of the stomach to the chest and prior to the anastomosis (Figure 1).

Microcirculation blood perfusion assessment

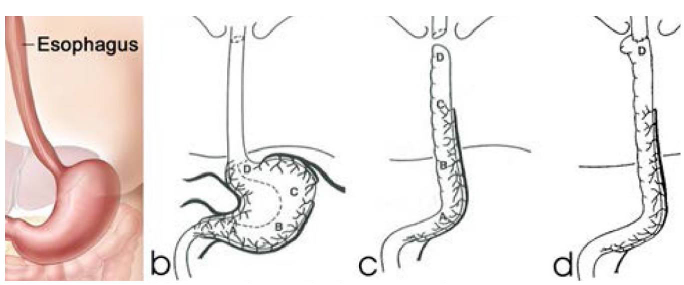

Figure 1. FLPI measurements at different times during oesophagectomy.
A. The anatomy of stomach and surrounding organs.
B. The stomach microvascular perfusion to be measured at s_{1-3} and at the sites A, B, C and D, corresponding with pylorus, antrum, body and fundus, respectively.
C. The gastric tube microvascular perfusion at the sites B, C and D to be measured at s_4.
D. The anastomosis microvascular perfusion at site D and the gastric tube microvascular perfusion at the site B & C to be measured at s_5 after oesophagectomy.

In order to obtain the perfusion scans without compromising the sterile surgical field, FLPI was mounted on an adjustable arm, which was fitted on a small trolley. FLPI was positioned `real-time in 2D'. The camera has the 1M pixels per cm2 resolution that capture blood perfusion in the micro vessels in the superficial layer of the stomach (roughly 1mm deep). The images were recorded in both grayscale and colour. Scans were obtained at the rate of 4 images per millisecond for 10 seconds. The auto gain and colour intensity were selected to reflect blood perfusion. FLPI employs the principle of laser speckle which arises upon scattering of the laser light as a result of moving particles (e.g. blood cells). FLPI and the relevant analysis software cost around £40,000. However, the device can be used multiple times and has no disposable parts

Image analysis

All scans were analysed using the moor FLPI analysis software (Moor FLPI V4.0, Moor Instruments, Devon, UK). A protocol was used to measure blood perfusion in all obtained images at 4 different stages from S1-S4. The total flux (medianblood perfusion in arbitrary units) was obtained as a reference for the blood perfusion in the marked region of interest (ROI) on the scan. Selective ROIs were marked for the pylorus, antrum, body and fundus of the stomach and the blood perfusion in these areas was compared to the total flux.

Statistical analysis

The test results were statistically analysed using IBM SPSS-22. One-way ANOVA is used to check the blood perfusion significance between the different stages of ROIs. Post-hoc Bonferroni for multiple comparison test was used to see the difference between and within the different ROIs at the each individual stage. Normality and significance of the parameters checked by using the Sphiro-wilk test and ANOVA respectively. The data was analysed in the log 10 transformation for the non parametric analysis as the correlation.Pearson and Spearman correlation was checked to see the correlation of blood perfusion between the different stages and along the stomach temperature; all of these were performed at the significance level of 0.05.

Results

Over the study interval, twelve patients with lower oesophageal adenocarcinoma requiring two-stage (Ivor-Lewis) oesophagectomy, were recruited consecutively and agreed to take part in the study. The mean age of the patients was 68 Years (Range 60:76). There were 10 males and 2 females. The recorded patients' cardiovascular parameters and relevant temperatures at each stage of the procedure are summarised in Table 1. For the whole cohort, there was no statistically significant difference between any of the cardiovascular parameters or temperatures recorded at different stages of the procedure.

Analysis of perfusion Images taken by FLPI

The blood perfusion (flux) with reference to the composite value for the whole stomach at the predetermined 5 regions of interests ROIs (pylorus, antrum, body and fundus of the stomach) between stages S1-S4 were obtained (Table 2). A colour representation of the fluxes in the different regions at the predetermined stages is illustrated in Figure 2.

The whole stomach (ROI1) demonstrated a reduction in mean micro-vascular blood perfusion between stages 1 and 4, which was highly significant ($p<0.05$). The reduction in median blood perfusion between stages 1 and 2 was not statistically significant. Between stage 2 and 3, the median blood perfusion increased

	Step 1 Mean (SD)	Step 2 Mean (SD)	Step 3 Mean (SD)	Step 4 Mean (SD)
SBP (mmHg)	100.33 (10.52)	98.75 (27.76)	108.67 (20.60)	92.20 (15.37)
DBP (mmHg)	57.58 (13.64)	57.50 (7.50)	61.00 (10.46)	52.00 (8.71)
MAP (mmHg)	74.42 (15.95)	77.38 (11.89)	78.33 (13.39)	65.80 (8.61)
CVP (mmHg)	10.50 (2.65)	11.00 (3.07)	11.56 (3.21)	10.60 (2.95)
HR (beats/min)	74 (16)	71 (16)	69 (13)	75 (17)
SpO_2 (%)	99 (1)	99 (1)	99 (0)	99 (0)
Superficial stomach Temperature (oC)	33.73 (1.20)	33.44 (1.95)	32.86 (1.93)	32.44 (1.85)
Ambient Temperature (oC)	24.32 (3.23)	25.05 (4.33)	23.38 (0.35)	24.26 (3.13)

Table 1. The recorded patients' cardiovascular parameters and temperature records corresponding to the different stages of oesophagostomy. SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Pressure; CVP: Central Venous Pressure; HR: Heart Rate; SpO_2: Peripheral Capillary Oxygen Saturation.

	Stage 1	Stage 2	Stage 3	Stage 4
Median blood perfusion (IQR) for the whole stomach ROI 1 (FU)	2167(1622-4543)	1682(1455-2997)	1853(1484-2308)	1354(1230-1923)
Median blood perfusion (IQR) in ROI 2 (FU)	3052(2105-4414)	2330(2096-3006)	2439(2280-2679)	2051(1495-2405)
Median blood perfusion (IQR) in ROI 3 (FU)	2637(2077-4587)	2394(1769-2958)	2311(1747-2475)	1793(1446-2166)
Median blood perfusion (IQR) in ROI 4 (FU)	2548(1726-4524)	1712(1595-2513)	1851(1830-2051)	1644(1280-1895)
Median blood perfusion (IQR) in ROI 5 (FU)	2078(1712-4490)	1777(1391-2513)	1557(1382-2152)	1342(1274-1935)

Table 2. Median blood perfusion and Inter Quartile Range (IQR) for the whole stomach (ROI1), at the pylorus (ROI2), antrum (ROI3), body of the stomach (ROI4) and at the fundus (ROI5) with. FU: Flux units.

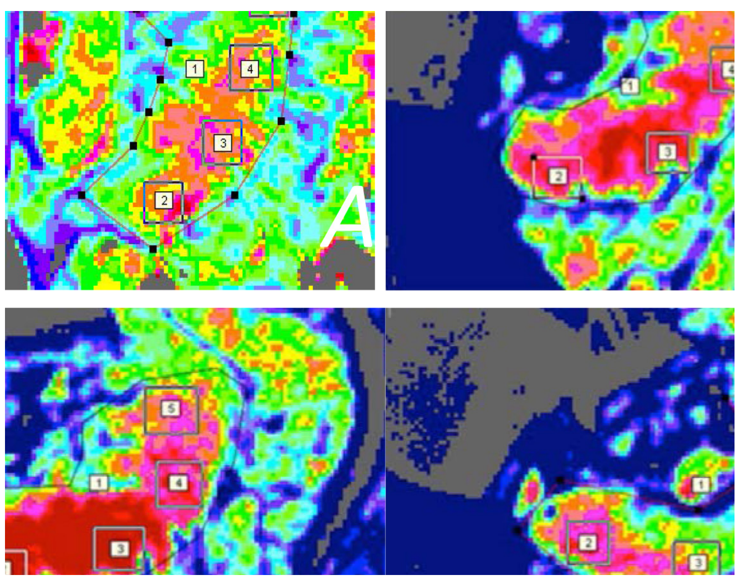

Figure 2. Representative scans obtained using FLPI device where the red colour depicts rich micro-vascular blood perfusion, the green colour shows the less vascularised tissue and the blue area indicates background noise. Figure 2A at stage 1 shows that the blood perfusion is high in the body and antrum of the stomach relative to the fundus. Figure 2B at stage 2 shows a general decrease in blood perfusion in the whole stomach particularly in the fundus. Figure 2C at stage 3 illustrates that the blood perfusion is maintained at a low level however it when compared to figure 2B it is more perfused. 2D at stage 4 shows that at the time of the gastric tube formation there is a significant reduction in blood perfusion. It also depicts the generation of 3-5cm wider gastric tube.

slightly (NS). There was no significant change in median blood perfusion between stages 3 and 4.

At the pyloric region (ROI 1) of the stomach, the median micro-vascular blood perfusion reduced between stage 1 and stage 2 (NS). Between stage 2 and stage 3, the median blood perfusion increased slightly (NS). There was no significant change in median blood perfusion between stages 3 and 4. The overall reduction in median blood perfusion between stages 1 and 4 was not significant.

At the gastric antrum (ROI 2), the median micro-vascular blood perfusion decreased between stage 1 and 2 (NS). Between stage 2 and 3 the median blood perfusion increased slightly (NS). There was no significant change in median blood perfusion between stages 3 and 4. The overall reduction in median blood perfusion between stages 1 and 4 was significant ($p<0.05$ ($p=0.04$).

At the body of the stomach (ROI 3), the median micro-vascular blood perfusion decreased between stages 1 and 2 (NS). Between stages 2 and 3, the median blood perfusion increased slightly (NS). There was no significant reduction in median blood perfusion between stages 3 and 4. The overall reduction in median blood perfusion between stages 1 and 4 was highly significant ($p<0.05$).

At the fundus of the stomach (ROI 4) (the region used for the anastomosis), the median micro-vascular blood perfusion decreased between stages 1 and 2 (NS). Between stages 2 and 3, the median blood perfusion decreased (NS). There was no significant reduction in median blood perfusion between stages 3 and 4. The overall reduction in mean blood perfusion between stages 1 and 4 was highly significant ($p<0.05$).

Supplementary analysis

Additional analysis has demonstrated no correlation between the changes in median blood perfusion and the changes in superficial gastric temperature, ambient temperature, or blood pressure.

Discussion

This study confirms the feasibility of using the full field laser perfusion imaging in assessing micro-vascular perfusion changes intra-operatively. The device is relatively small and can be mounted on a column out with the surgical field and used intermittently as required in a non-contact manner without compromising the sterile surgical field. The device is relatively cost efficient. The output can be displayed on a computer screen out with the surgical field. The chromatic display provides an instant indication of the extent of perfusion of the scanned structures. This enables revision of the surgical site of anastomosis if the scans indicated a significant compromise to perfusion. Although many factors determine the integrity and healing of an anastomosis, perfusion at an anastomotic site plays a significant part in healing. However, there is currently no evidence to indicate a perfusion cut off point below which the anastomosis is at a greater risk of dehiscence.

This study has shown a significant reduction in the mean micro-vascular blood perfusion of the whole stomach with selective devascularisation, which was independent of the change in superficial gastric temperature and ambient temperature. This reduction was predictable since two of the four main arteries supplying the stomach (short gastric and left gastric arteries) were compromised in the process of freeing the stomach to act as a replacement conduit for the removed section of the oesophagus. As the stomach was selectively devascularised, the mean gastric blood perfusion decreased in the whole stomach with a variable rate of reduction in different regions. At the end of step 4 of surgery, the mean blood perfusion for the whole stomach (ROI1) was reduced by 38%, the pylorus (ROI2) was reduced by 33%, the antrum (ROI3) was reduced by 32%, ($p<0.05$), the body of the stomach (ROI4) reduced by 35%, ($p<0.05$) and fundus (ROI5) reduced by 35%, ($p<0.05$). The variable reduction of gastric perfusion was published by three independent studies where blood perfusion at the pylorus was higher than blood perfusion recorded at the fundus after gastric mobilisation during oesophagectomy [4-6]. In these studies, gastric perfusion decreased by 23% to 42% during gastric mobilisation. A further study noted the drop in blood perfusion at the fundus post gastric tube formation was 75.4% with a reduction in blood perfusion at the antrum and body of 54% and 50.7% respectively [7]. The larger magnitude of the finding of that study compared with other studies including this one can be explained by differences in surgical approach, the delivery/dose of anaesthetic agents, changes in blood pressure and variability in stomach temperature at various stages of the surgery.

Spatially, the first decrease in whole stomach median blood perfusion was observed at step 2 and amounted to 21%. This is due to ligation of the short gastric and left gastric arteries. However, the effect of anaesthetic agents or epidural analgesia could not be discounted [6,8]. By the end of step 3, the median blood follow was reduced by a further 18% upon creation of the gastric tube from the stomach. Creation of the gastric tube involves resection of the lesser curvature of the stomach and in effect, disrupting the network of established vascular channels within the stomach that course around the lesser gastric curvature. The overall reduction in median gastric blood perfusion observed between steps 1 and 4 was 34%. During this surgical procedure a decrease in systolic, diastolic, and mean blood pressure was also observed between steps 1 and 2. Thereafter, it was found that the mean arterial blood pressure increased at Step 3. This may partly explain the observed increase in blood perfusion in step 3 [9]. Alternatively, adaptation of the gastric arterio-venous plexus may have occurred [10]. This may be facilitated by the body's homeostatic mechanisms through the baroreceptor reflex.However, the effect of anaesthetic agents could not be discounted [6]. Subsequently, the median gastric blood perfusion decreased again between steps 3 and 4. This may be due to a change in temperature of the stomach as it was transposed to the chest or due to stretching of the remaining blood supply to the stomach or its arterio-venous network.

Due to the thoracic position of the anastomosis, anastomotic failure would lead to contamination of the thoracic cavity with ingested material and gastric contents. The results can be catastrophic. Anastomotic healing is dependent amongst other important factors on optimal blood perfusion at the anastomotic site. In this study, a single surgical team carried out the surgery and the anastomosis used was a sutured end oesophagus to side gastric fundus. Although other factors can affect the integrity of the anastomosis, this study has focused on the vascularisation of the anastomotic site. In clinical studies it has been suggested that hypotension and blood loss are the main factors, which contribute to ischaemia and subsequent anastomotic failure [11]. However, previous chemotherapy and radiotherapy can also have a big impact on anastomotic healing. Age was also an independent risk factor for the failure of the anastomosis [12]. Animal studies have shown that blood perfusion is highest at the pylorus and gradually decreased towards the fundus [13]. Our observations are in agreement with these results. Another human study has shown that surgical processes such as tube formation and anastomosis formation had a greater effect on decreasing the microcirculation of the fundus and body of the stomach than on the antrum and the pylorus [14]. If the fundus (site of anastomosis) is poorly perfused there is a greater chance of anastomosis failure [15]. The success of an anastomosis can be enhanced by the acquisition of accurate blood perfusion measurements [12,14] at different regions in the stomach and subsequent corrective action by the surgeons if indicated. With the FLPI, assessment of microcirculation at four distinct regions of the stomach (fundus, body, antrum and pylorus) can potentially help the surgical placement of the anastomosis. This study demonstrates the potential application of FLPI in prevention of anastomosis failure. FLPI can reliably and safely assess micro-vascular gastric blood perfusion during oesophagectomy.

The limitation of this technique is that it can only depict blood perfusion in vessels up to a depth of 1mm [16]. In addition, FLPI fails to detect subtle transient alterations in blood perfusion and can only interpret stationary images taken at intervals. However, it is less likely that major long lasting and clinically significant alterations in blood perfusion of deeper vessels would not be reflected in the superficial blood perfusion. Other techniques such as indocyanine green fluorescence (ICG), Doppler optical coherence tomography (DOCT), laser assisted florescence-dye angiography (LAA) are all invasive methods and less suitable for intra-operative use. Due to inherent difficulties, some of these techniques are popular amongst enthusiasts but none of them have been used universally [17]. Laser Doppler Imaging (LDI) can also measure perfusion, but the results are less accurate for regional perfusion in different parts of the stomach [5,18]. However, FLPI is able to measure microcirculation in real-time and the analysis software enables consistent interpretation results. In addition, FLPI is a non-invasive, relatively quick, contactless method, which can assess a full field microcirculation in real-time which can be used with a remote setup in the surgical environment.

This pilot study was conducted on 12 patients undergoing oesophagectomy. No anastomotic failures were detected in this study. A larger study on more patients is required to correlate perfusion measurements with the clinical outcome of the anastomosis and to determine a perfusion cut off for anastomosis.

Conclusion

This observational cohort pilot study confirms the feasibility of FLPI use during surgery in a sterile environment and its potential in producing reliable perfusion measurements.The study has shown a reduction in gastric blood perfusion with selective devascularisation of the stomach, which was most notable in the fundus of the stomach (the site of the anastomosis).

Acknowledgement and author contributions

Faisel Khan and Sami Shimi have contributed to study conception and design; Muhammad Hussain has contributed to data acquisition, data analysis and interpretation, and writing of article; Muhammad Hussain, Professor Faisel Khan and Mr Sami Shimi have contributed to editing, reviewing and final approval of article.The authors would like to thank Fiona Adams for her technical assistance.

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Rec: Jan. 26, 2019; Acc: Feb. 15, 2019; Pub: Feb. 19, 2019

J Surg Practice. 2019;2(1):7
DOI: gsl.jsp.2019.00007

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