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The effect of chemotherapy on gastric perfusion in patients with gastric cancer

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Content

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2. Introduction

2.1 Summary of the study

A study from our group (Osterkamp et al. *in preparation*) used ICG to evaluate intraoperative changes in gastric perfusion when reducing the circulating blood volume by blood withdrawal in pigs. We saw a significant reduction in gastric perfusion with decreased blood volume, and this reduction of gastric perfusion was detectable with ICG. As data from a previous trial (PRESET phase 2 Protocol nr: H-15014904) has shown that chemotherapy decreases the circulating red blood cell volume in patients with gastroesophageal cancer, we wish to evaluate if standard care neoadjuvant chemotherapy also influences gastric perfusion. Gastric perfusion will be assessed during a screening laparoscopy (before chemotherapy) and then compared with a second assessment during gastric resection (after chemotherapy). The gastric perfusion will be measured using fluorescence-guided surgery with Indocyanine Green.

2.2 Gastric perfusion after neoadjuvant chemotherapy treatment

In the preoperative period during chemotherapy, there is a risk of disease progression in non-responders, all the while physical deterioration and chemo-toxicity can increase the risk of severe peri- or postoperative complications due to poor performance status. Indeed, strong observational evidence supports that poor muscular function, defined as either a low muscle mass/area (sarcopenia) or low functional strength, is a strong independent predictor of post-surgical outcomes including mortality and surgical complications in patients undergoing surgery for gastrointestinal cancers [1]. Thus, it remains a critical double-sided challenge to ensure that patients diagnosed with resectable gastric cancer attain surgery while in a sufficient physiological condition [2].

Impaired blood perfusion of an anastomosis is a significant risk factor for anastomotic leakages after surgery [3–7]. Inadequate perfusion appears to be related to intraoperative events such as low arterial oxygen saturation, metabolic acidosis, and physical tension on the anastomosis, which all may lead to impaired anastomotic healing [8–10]. However, ways to improve tissue blood perfusion during surgery are poorly understood, but maintaining the total blood volume may be a key factor for preventing marked drops in organ perfusion during surgery. Blood volume - including hemoglobin mass, red blood cell volume, plasma volume, and total blood volume - is a complex physiological feature which is not captured by “just” measuring hemoglobin concentration [11]. In order to evaluate if chemotherapy leads to impaired blood perfusion is, we aim to investigate the gastric perfusion before and after oncological treatment.

In a collaboration between Center for Physical Activity Research (CIM/CFAS) and the Departments of Surgical Gastroenterology and Oncology, Copenhagen University Hospital, Rigshospitalet (from April 2016 to September 2017), a phase 2 study (PRESET phase 2 Protocol nr: H-15014904) was conducted. Patients (n = 21) presenting with cancer of the gastroesophageal junction were allocated without randomization (based on geographical residence) to standard care or a 12 weeks exercise training intervention during neoadjuvant chemotherapy. Various clinical outcomes were collected to investigate if improvements in exercise capacity, muscle strength, or patient-reported symptoms, translated into overall improved treatment tolerability. Results showed a potentially lower risk in the exercise-group for treatment failure, defined as patients precluded from surgery due to death, disease progression or physical deterioration, treatment toxicities, and/or hospitalization [12]. These results have led to the initiation of a more extensive phase 3 randomized controlled trial (Protocol nr: H-18009502) of preoperative exercise training vs. standard care during neoadjuvant chemotherapy in patients with gastroesophageal cancer. Preliminary data from this trial show a substantial (up to 25%) reduction in red blood cell volume after chemotherapy. Importantly, the corresponding changes in hemoglobin concentration (as measured by standard hematological assessment) overestimate the loss of red blood cells and has a poor level of agreement with total Hb mass. For example, patients experiencing a median of approx. 15% drop in Hb concentration range showed a reduction in Hb mass from 0% to 25%.

Such a reduction in total oxygen-carrying capacity may reduce the peripheral tissue perfusion and oxygenation, e.g. in the tissue used for the construction of the anastomosis. Thus, giving rise to a risk of anastomotic leakage as other types of postoperative complications. Yet, this association has not been examined previously.

2.3 Fluorescence guided surgery with Indocyanine Green.

Fluorescence guided surgery enables visualization of tissue circulation and structures that otherwise are hidden from the human eye. A fluorescent contrast agent is used, often Indocyanine Green (ICG), and by illuminating the tissue with near-infrared light, the excited ICG can be detected by a camera with an optical filter. ICG is a tricarbocyanine dye with few adverse events, a short half-life, it is metabolized exclusively in the liver, and excreted unchanged in the bile [13,14]. The safety of ICG is well established, and it is used routinely in surgical clinics worldwide and also has gained popularity in oncological surgery in recent years [13–15].

ICG binds to plasma proteins after intravenous injection, and by illuminating the tissue with near-infrared red light, a fluorescence signal time-series during first passage past tissue is considered proportional to microvascular flow, i.e., perfusion.

Assessing gastrointestinal blood supply without the use of ICG is challenging, even for experienced surgeons. Of specific concern is the vascular supply of the anastomosis since an inadequate blood supply is a significant risk factor for anastomotic leakage [3–7]. As ICG angiography allows for real-time visualization of visceral perfusion, an inadequate vascularisation of the anastomosis can be detected and addressed intraoperatively [16–19]. Of importance, the use of perioperative ICG angiography has been shown to lower the risk of anastomotic leakage [20–23].

We have developed an ICG quantification algorithm that has been validated and described earlier [24]. This algorithm has now been incorporated into a touch screen tablet, allowing for live perioperative quantitative perfusion assessments with ICG (q-ICG) (Nerup et. al Langenbeck Arch Surg 2020, *accepted for publication*). A color-coded map of the perfusion-intensity is provided as an overlay on the white light visualized tissue (Figure 1).

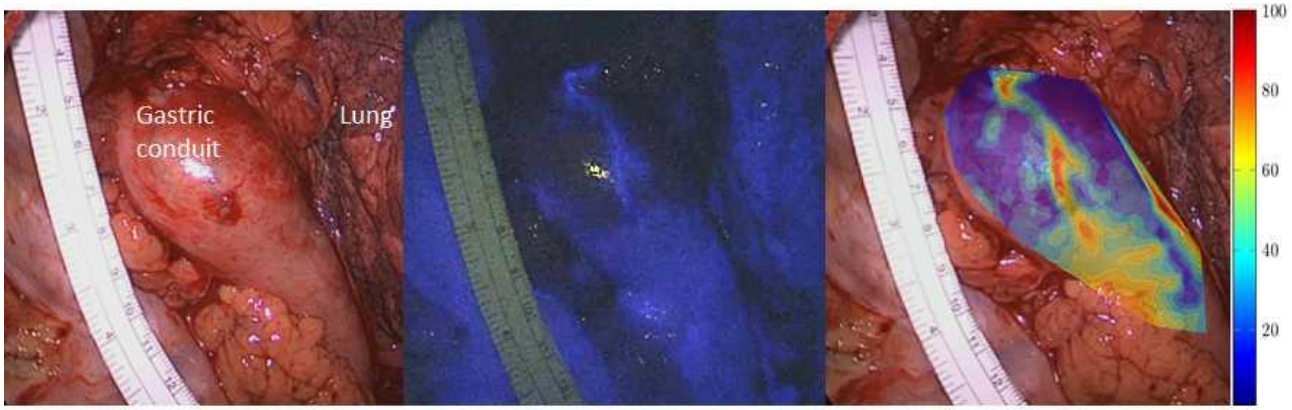

Figure 1. Gastric conduit after pull-up to the thorax viewed by white light, Near-Infrared Light (ICG), and with q-ICG overlay.

A study from our group (Osterkamp et al. *in preparation)* used q-ICG to evaluate intraoperative changes in gastric microcirculation when reducing the circulating blood volume by withdrawal of blood in pigs. We found a significant reduction in gastric perfusion as detected by q-ICG as the blood volume decreased.

By using q-ICG, we aim to assess gastric perfusion during the standardized diagnostic staging laparoscopy (before chemotherapy) in patients with gastric cancer. After treatment with neoadjuvant chemotherapy, we will re-assess the gastric perfusion in the same patients with q-ICG

during the surgical resection. The difference in perfusion before and after neoadjuvant chemotherapy will be evaluated.

2.3 Aim

We aim to evaluate if standard care neoadjuvant chemotherapy influences gastric perfusion.

2.4 Hypothesis

We hypothesize that standard care neoadjuvant chemotherapy will reduce gastric perfusion.

2.5 Primary endpoint

The primary endpoint is the difference in gastric perfusion (obtained with q-ICG) before and after neoadjuvant chemotherapy.

2.6 Secondary endpoint

Secondary endpoints are:
Short term outcome; postoperative events and complications as graded by the Dindo-Clavien classification and the Comprehensive Complication Index, length of hospital stay (data will be noted 30 days postoperatively). Complications after surgery will be defined as any deviation from the ordinary postoperative course. This definition also takes into account asymptomatic complications such as arrhythmia and atelectasis.

3. Methods

3.1 Study design / Sample size

We aim to assess gastric perfusion before and after standard of care neoadjuvant chemotherapy. The sample size calculation is based on the relationship between changes in blood volume and the fluorescent signal from an earlier study (Osterkamp et al. *in preparation*) as on data an ongoing trial (chemotherapy-induced reduction in whole blood volume; Protocol nr: H-18009502). The sample size required is n=25 to attain a correlation coefficient of 0.5 with a probability of a type I error (α) of 0.05 and power ($1 - \beta$) of 0.8). Taking into account an average dropout-rate of approximately 20% in clinical trial, 30 patients will be included [25]. Prospective evaluations of the surgical outcome will be recorded by means of anastomotic leakage rates (verified endoscopically or/and

radiologically), delayed gastric emptying (verified with barium swallow examination and/or endoscopy), and other postoperative complications including mortality. The trial will be conducted in accordance with the CONSORT guidelines [26].

3.2 Inclusion criteria

Patients (above 18 years) scheduled for planned open or robot-assisted resection of gastric cancer.

3.3 Exclusion criteria

- Allergy towards; iodine, indocyanine green or shellfish
- Severe liver insufficiency
- Thyrotoxicosis
- Nephropathy requiring dialysis
- Pregnancy or lactation
- Legally incompetent for any reason
- Withdrawal of inclusion consent
- Disseminated disease or other that contraindicates curative surgery

3.4 Recruitment

Upon meeting in the outpatient clinic, the patients will be informed about an ongoing study and asked if they wish further information. If accepted, the principal investigator will be called to the outpatient clinic to briefly inform the patient about the project and ask about allergy towards iodine, indocyanine green or shellfish, liver insufficiency, thyrotoxicosis, pregnancy or lactation. If there are no allergies or other contraindications for inclusion, the patient will receive written and oral information about the project from a separate office by the principal investigator. The potential participant will be asked if he or she wishes to bring a family member or other bystander to the information interview. If no bystander is present, and the patient wishes for one to be present, a new meeting will be arranged to conduct the information interview in which a bystander can participate. The meeting office is a closed office occupied by the principal investigator. After the meeting, a follow-up telephone call regarding inclusion will be held roughly one week after the first meeting. The principal investigators' telephone number and email will be handed out, allowing the patient to get in contact if necessary. If interested in participation, the patient will supply the principal investigator with written consent. All patients are informed of the possibility of withdrawal of their

consent at any time during the trial without any consequence for the operation or their treatment. First, after written consent, will inclusion in the study be possible.

3.5 Indocyanine green

Indocyanine green is a well-described non-toxic tricarbocyanine dye used for decades in ophthalmology, cardiology, and hepatology. Very few mild adverse reactions have been reported, but caution in patients with thyrotoxicosis, allergy towards iodine, or indocyanine green should be made. When injected, it binds to blood lipoproteins and is solely metabolized by the liver and excreted in the bile, with a short half-life of approximately 4-5 minutes [13,15,27]. Indocyanine green is already used routinely in many specialties, here among surgery, ophthalmology, hepatology, and cardiology [13–15,27,28].

3.6 Surgical procedure and perfusion assessment

3.7.1 Screening Laparoscopy

As part of the standard care for gastric cancer, all patients undergo a screening laparoscopy before entering neoadjuvant chemotherapy. The procedure is performed to detect overt metastases not detected on the CT/PET-CT scans. First, the patient is placed under a standardized general anesthesia, and the laparoscopic set-up is completed. After anesthesia a peripheral arterial catheter will be placed in order acquire reading of cardia output and stroke volume. The patient will then be fluid optimized using a standardized stroke volume (SV) optimization algorithm. The abdomen is inspected visually for signs of metastatic disease. The small bowel is then manipulated, allowing for visualization of the stomach. A bolus of ICG (0.2 mg/kg body weight) will be injected intravenously and flushed with 5 mL of saline. Gastric perfusion will subsequently be assessed along specific regions of interest (ROI) with q-ICG to obtain baseline perfusion values.

3.7.2 Resection of gastric cancer

The patient is placed under general anesthesia and after the stomach is visualized through surgical incision, a bolus of ICG (0.2 mg/kg body weight) will be injected intravenously and flushed with 5 mL of saline. The ROIs (the same ROIs as described in 3.7.1) will then be assessed with q-ICG. The anesthetic protocol will up to this point match that of the setting during the screening laparoscopy.

3.7.3 Fluorescence angiography

During the screening laparoscopy, a laparoscope (ICG-Hopkins telescope 30°, 10 mm, Karl Storz Gmbh and Co. KG, Tüttlingen, Germany) will be connected to a camera system (IMAGE1, Karl Storz Gmbh, and Co. KG, Tüttlingen, Germany) and a light-source (D-light P, Karl Storz Gmbh and Co. KG, Tüttlingen, Germany) will supply the excitatory light and record the ICG angiography. The laparoscope will be fixed in a mechanical holding arm 10 cm from the tissue of interest, ensuring a stable position throughout the experiment.

3.8 Statistics

A comparison of the gastric perfusion before and after chemotherapy will be performed using Friedman's test or a repeated measures ANOVA / linear mixed-effects depending on a non- or parametric nature of the data. A P-value < 0.05 will be considered significant. Statistic evaluation will be performed using IBM SPSS Statistics © (v 22.0 SPSS Inc. Chicago, IL, USA).

4. Adverse events, risks, and disadvantages

Adverse events associated with the use of ICG are rare and severe anaphylactic reactions extremely rare [14,27]. However, safety precautions will be made by excluding patients with allergy to iodine, ICG, or shellfish. Also, we will not include patients with severe kidney and liver insufficiency, thyrotoxicosis, and pregnant or lactating women. The total dose of dye injected will be kept well below the recommended 2 mg/kg [29]. There are no data available describing the signs, symptoms, or laboratory findings accompanying overdosage. The LD_{50} after I.V. administration ranges between 60 and 80 mg/kg in mice, 50 and 70 mg/kg in rats and 50 and 80 mg/kg in rabbits [29].

5. Patient data

Upon meeting in the outpatient clinic, the patients will be informed about an ongoing study and asked if they wish further information. If accepted, the principal investigator will be called and to briefly inform the patient about the project, and ask about allergy towards; iodine, indocyanine green or shellfish, liver insufficiency, thyrotoxicosis, pregnancy or lactation. If no allergies exist, the patient will then receive written, and oral information about the project and a follow-up

telephone call regarding inclusion will be held. If interested in participation, the patient will supply the principal investigator with written consent. First, after written consent has been provided, will the patient's electronic journal be available to the principal investigator. This, to see information about the subject's health and the course during hospitalization necessary as part of the research project. Including self-control, quality control, and monitoring, which are obligatory. Data obtained from electronic patient records are registered prospectively and pseudo-anonymized in a database and kept at the secure server provided by Region Hovedstaden (L: PerfussionVentrikel). Thus, data in this database may not be identified back to the patient without access to a patient participation list linking patient identification number and the pseudo anonymization number. This participation list will be stored in another folder on the secure server provided by the Region Hovedstaden (V: PerfussionVentrikel). Only the principal investigator and principal supervisor will have access to these folders and files. The law on the processing of personal data (databeskyttelsesforordningen and databeskyttelsesloven) will be followed. No data will be sent to other countries. Permission from the national data protection (Datatilsynet) agency will be obtained.

6. Ethical considerations

Approval from the regional ethical committee and the national data protection agency will be in place before initiating the study.

ICG is a safe and non-toxic fluorescent dye as described and has been used for decades in ophthalmology, cardiology, and hepatology. Very few adverse reactions are reported [14,27]. Patients may withdraw from the study at any time. The patients participating in the study will not be paid to participate.

7. Publication

We will publish negative, positive, and inconclusive results in international reputed surgical journals, possibly as open access with Jens Osterkamp as the primary and corresponding author. Also, we will present the work at national and/or international surgical conferences and meetings to share the findings with a large crowd of clinicians and researchers.

8. Timeframe

The study will be initiated as soon as we have approval from the ethical committee and the national data protection agency. Hopefully, starting in the fall of 2021, and completed ultimo 2023.

9. Economy

This study is a part of the principal investigators' PhD-thesis, which is covered by a grant to department C-Tx from Medtronic with a total amount of 2.255.842 DKK. in which salary and experimental costs (acquisition of ICG) will be covered. With regards to study design, realization, and presentation, the research group has complete autonomy. No remuneration will be given to patients participating in the trial. The principal investigator has in collaboration with the project group, initiated the project. There are no financial interests for the project group in this project. Equipment (tablet, video-capture device, laparoscopic equipment, surgical robot) is already present in the department. If additional support is granted, the name of the foundation(s), size of the amount, any relation between the grant provider and investigators, as well as any requirements of the grants will be announced to the committee and project participants (patients).

10. Insurance

Patients will be covered by the national Danish patient insurance.

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