Study protocol

NEPOMUC

Noninvasiv**e** characterization of **po**stprandial intestinal blood flow using **mu**ltispectral optoa**c**oustic tomography

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1. Study title, version number, version date

Study title

Noninvasiv**e** characterization of **po**stprandial intestinal blood flow using **mu**ltispectral optoa**c**oustic tomography

Version number

Version 1.3

Version date

27/07/2021

Protocol versions

Date	Version	Status	Changes
22/06/2021	1.0	Outline	Lars-Philip Paulus, Ferdinand Knieling
22/07/2021	1.1	Outline	Lars-Philip Paulus, Ferdinand Knieling
26/07/2021	1.2	Outline	Lars-Philip Paulus, Ferdinand Knieling
27/07/2021	1.3	Outline	Lars-Philip Paulus, Ferdinand Knieling, Adrian Regensburger

2. Project summary

Inflammatory activities in the gastrointestinal tract are accompanied by an increase in blood flow in the intestinal wall layers of the respective organs. Also in chronic inflammatory bowel diseases, the release of vasoactive inflammatory mediators leads to vasodilation and consecutive increase of blood flow in the bowel wall. So far, these changes in blood flow can be detected by power Doppler sonography without being part of routine clinical diagnostics. Another promising option for non-invasive measurement of blood flow in the intestinal wall is Multispectral Optoacoustic Tomography (MSOT). Previous studies have shown that MSOT can be used to quantitatively measure hemoglobin in the bowel wall and thus provide information on blood flow and inflammatory activity in the intestines of patients with Crohn's disease. This is currently being further investigated in a pivotal study (Euphoria, H2020) and could lead to the possibility of non-invasive assessment of disease activity in inflammatory bowel disease (IBD) in the future.

The regional blood flow in the intestinal wall and the distribution of gastrointestinal blood flow are also subject to strong postprandial changes. During absorption of food components, blood flow increases sequentially in the respective sections of the gastrointestinal tract, leading to postprandial hyperemia. Because postprandial hyperemia is particularly regulated locally by the presence of dietary components, there is a relationship between the sequential increase in blood flow in the intestinal wall and the peristaltic transport of chyme through the gastrointestinal tract. Postprandial hyperemia could also lead to an increase in the optoacoustic hemoglobin signal of the intestinal wall and thus have an impact on the assessment of inflammatory activity in IBD using MSOT. Additionally MSOT allows the identification of non-absorbable exogenous chromophores, such as indocyanine green (ICG), which could allow co-localization of the chyme in the intestinal lume after oral application of ICG.

The purpose of this pilot study is to investigate in healthy volunteers whether, postprandial blood flow changes can be quantitatively measured using MSOT and whether these changes occur simultaneously with the gastrointestinal passage of the chyme as measured by the ICG signal in the intestinal lumen.

3. Responsibilities

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Sponsoring

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4. Scientific Background and derivation of the question

The gastrointestinal tract essentially fulfills two major functions: digestion and absorption of food, and physical and immunological barrier against environmental influences. These basic functions are critically dependent on splanchnic blood flow at both the macrovascular and microvascular levels.[1] In particular, advances in vascular biology have revealed a central and intricate role of blood circulation in inflammatory bowel disease (IBD).[2]

Until now, changes in blood flow have been used as surrogate markers for altered inflammatory activity in the intestine, e.g., by Doppler sonographic detection.[3] Since 2017, a DFG-funded Multispectral Optoacoustic Tomograph (MSOT) has been available at the University Hospital Erlangen (Medical Clinic 1, Pediatric and Adolescent Clinic). This allows non-invasive, quantitative imaging of the molecular composition of target tissues. In MSOT, similar to conventional sonography, a transducer is placed on the skin but energy is delivered to the tissue by means of laser light in the near infrared spectrum instead of ultrasound waves. This leads to a constant alternation of minimal expansions and contractions (thermoelastic expansion) of individual tissue components or molecules.[4-7] The resulting ultrasound waves can subsequently be detected by the same examination unit. Previous studies have shown that quantitative determination of hemoglobin can provide information on blood flow and inflammatory activity in the intestine of adult patients with Crohn's disease.[8,9] In particular, the distinction between the activity levels of the disease (remission/low/moderate/high) is promising for saving invasive measures in the future when evaluating the progression of the disease. To investigate this further, an EU-funded pivotal study (Euphoria, H2020) in adults is currently being conducted in the Medical Clinic I of the University hospital Erlangen. A CE-certified version of the MSOT system now exists.

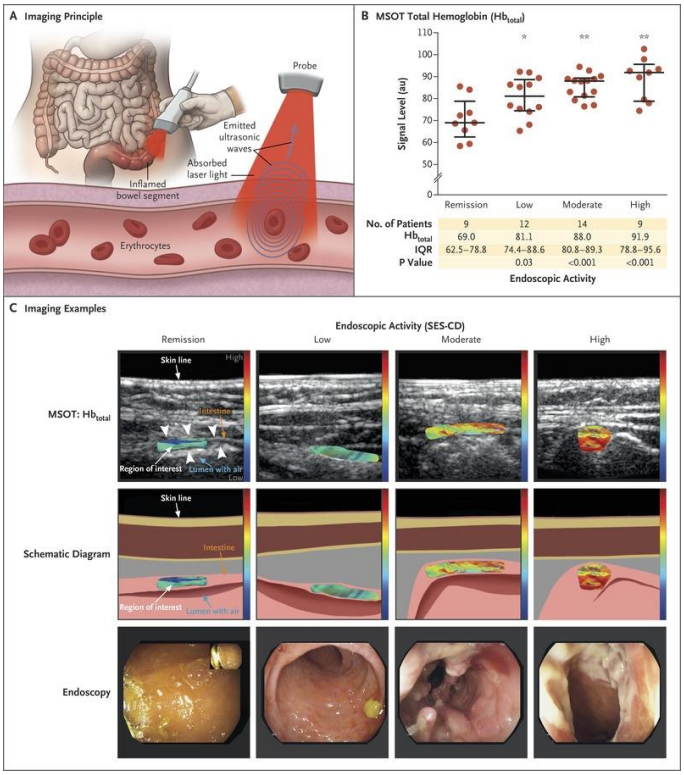

Figure 1: MSOT in Crohn´s disease, Knieling et al., NEJM 2017

In addition to inflammatory processes, food intake also causes fluctuations in regional blood flow in the gastrointestinal tract. This manifests as postprandial hyperemia, which occurs sequentially in the different sections of the gastrointestinal tract from oral to aboral. It is unclear whether this postprandial hyperemia can lead to a change and potential increase in the optoacoustic hemoglobin

signal of the intestinal wall, resulting in falsely high MSOT signals in the determination of inflammatory activity. Therefore, in this study, influences of a standardized dietary[10] on the MSOT signal of the intestinal wall will be investigated in a longitudinal design and optoacoustic signals will be compared between subjects in fasting and postprandial states. Because the postprandial increase in intestinal blood flow is predominantly a result of the local presence of chyme in the intestine,[11] a simultaneous determination of intestinal transit of chyme during MSOT measurement would be helpful to validate whether postprandial changes in MSOT signals are attributable to hyperemia in the corresponding bowel segment. With MSOT, in addition to the determination of hemoglobin, the detection of exogenous chromophores is also possible. Therefore, in this study, oral administration of the nonabsorbable dye ICG will be used for noninvasive identification of the chyme.[12] We believe that the combination of exogenous and endogenous chromophores thus allows accurate co-localization and registration of intestinal wall blood flow patterns and chyme transit. This information enables accurate anatomical mapping of interfering influences on the determination of hemoglobin using MSOT.

The time course of postprandial hyperemia in the different sections of the gastrointestinal tract has been scientifically investigated in many studies. While an increase in blood flow in the stomach and duodenum can be detected after 30-60 minutes, it takes much longer for postprandial hyperemia to be detected in the areas used to measure inflammatory activity with MSOT in IBD such as the terminal ileum and sigmoid colon. An increase in blood flow in the ileum can be measured after 120 minutes at the earliest,[1] and the arrival of chyme in the colon and the accompanying local increase in blood flow occur after approximately 240-300 minutes.[13,14] Therefore in this study postprandial changes in the MSOT hemoglobin signal will be investigated over period of 7 hours.

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5. Study objectives

The objective of the proposed study is to characterize postprandial changes in blood flow in the wall of the gastrointestinal tract based on multispectral optoacoustic tomography (MSOT).

Primary/secondary objectives and/or hypotheses

Hypotheses:

- The quantitative amount of oxygenated/deoxygenated hemoglobin in the intestinal wall determined by MSOT differs between fasting persons and persons after ingestion food.
- The quantitative amount of oxygenated/deoxygenated hemoglobin in the intestinal wall determined by MSOT changes after the ingestion of food.
- The postprandial changes in the quantitative amount of oxygenated/deoxygenated hemoglobin in the intestinal wall determined by MSOT occur successively in subsequent sections of the gastrointestinal tract, from oral to aboral.

- A relationship exists between postprandial changes in the quantitative amount of oxygenated/deoxygenated hemoglobin in the wall of the gastrointestinal tract determined by MSOT and postprandial changes in Doppler sonographic signals in the major splanchnic arteries (truncus coeliacus, superior mesenteric artery, inferior mesenteric artery).

- The postprandial changes in the quantitative amount of oxygenated/deoxygenated hemoglobin in the intestinal wall determined by MSOT are associated with the presence of chyme in the lumen of the gastrointestinal tract.
- Measurement of ICG signals within the lumen of the gastrointestinal tract using MSOT after ingestion of ICG-containing food indicates the presence of chyme within the lumen of the gastrointestinal tract.
- A relationship exists between postprandial changes in the quantitative amount of oxygenated/deoxygenated hemoglobin in the wall of the gastrointestinal tract determined by MSOT and postprandial changes in the ICG signal determined by MSOT within the lumen of the gastrointestinal tract after ingestion of ICG-containing food.

Primary objectives:

- Comparison of oxygenated/deoxygenated hemoglobin signal in the wall of the gastrointestinal tract measured by MSOT between fasting subjects and subjects after ingestion of food.

Secondary objectives:

- Measurement of the postprandial change in the oxygenated/deoxygenated hemoglobin signal in the wall of the gastrointestinal tract measured by MSOT over time and over the different sections of the gastrointestinal tract.

- Comparison of the Doppler sonographic signal in the large arteries of the splanchnic area between fasting subjects and subjects after ingestion of food.
- Measurement of the postprandial change in the Doppler sonographic signal in the large splanchnic arteries over time and over the different splanchnic arteries.
- Correlation between postprandial changes in the oxygenated/deoxygenated hemoglobin signal in the wall of the gastrointestinal tract measured by MSOT and postprandial changes in Doppler sonographic signals in the large arteries of the splanchnic artery.

- Comparison of the ICG signal in the lumen of the gastrointestinal tract measured by MSOT between fasting subjects, subjects after ingestion of food and subjects after ingestion of food containing ICG.
- Measurement of the postprandial change in the ICG signal in the lumen of the gastrointestinal tract measured by MSOT over time and over the different sections of the gastrointestinal tract in subjects after ingestion of food containing ICG.
- Correlation between postprandial changes in the oxygenated/deoxygenated hemoglobin signal in the wall of the gastrointestinal tract measured by MSOT and postprandial changes in the ICG signal in the lumen of the gastrointestinal tract measured by MSOT in subjects after ingestion ICG-containing food.

Study type

Since no data exist to support the hypothesis of this study to date, it is an exploratory study.

6. Target parameters

Study related measures and target
All MSOT measurements are taken over the gastric antrum, terminal ileum, transverse colon, and sigmoid colon. **Primary targets (obligatory):** Quantitative de-/oxygenated hemoglobin signal (in arbitrary units) *These target values are collected non-invasively using MSOT.* **Secondary targets:** Qualitative and quantitative ICG signal (in arbitrary units) Quantitative single wavelengths signal (in arbitrary units) Optoacoustic spectrum (in arbitrary units, normalized) *These target values are collected non-invasively using MSOT.*
Secondary targets (*optional*): Doppler sonographic signals of the coeliac trunk, superior mesenteric artery, inferior mesenteric artery: • Doppler sonographic spectrum • Doppler sonographic curve • Velocity profile • Flow profile • Flow volume • Resistance index • Pulsatility index *These traget values are collected non-invasively using sonography.*

7. Study design

Monocentric / multicentric

It is a monocentric diagnostic study with prospective data collection (Investigator Initiated Trial, IIT).

Study arms:

A comparison is made between 3 study arms: A: Fasting, B: Standardised breakfast, C: Standardised breakfast with added ICG (250mg in 50ml aqua). As part of the crossover design, each subject will pass through all three study arms on three different days. There is a period of at least 48 hours between two consecutive study days for each subject.

Randomisation

Randomisation is not planned. As part of the crossover design, each subject passes through all three study arms. The order of the study arms is identical for each subject:

1. Study arm A: Fasting
2. Study arm B: Standardised breakfast
3. Study arm C: Standardised breakfast with added ICG

Blinding

No blinding is planned.

8. Study population

Inclusion and exclusion criteria

***Inclusion criteria*:**

- Age over 18 years
- Written declaration of consent

Exclusion criteria

Generally valid:

- Pregnancy
- Nursing mothers
- Tattoo in the field of investigation
- Subcutaneous fat tissue over 3 cm
- Chronic or acute diseases of the gastrointestinal tract or symptoms suggestive of such a disease
- Diseases requiring acute treatment
- Lack of written consent

ICG related:

- Known hypersensitivity to ICG, sodium iodide or iodine
- Hyperthyroidism, focal or diffuse thyroid autonomy
- Treatment with radioactive iodine for the diagnostic examination of thyroid function within two weeks before or after the study
- Restricted renal function
- Intake of the following drugs: Beta-blockers, anticonvulsants, cyclopropane, bisulphite compounds, haloperidol, heroin, meperidine, metamizole, methadone, morphine, nitrofurantoin, opium alkaloids, phenobarbital, phenylbutazone, probenecid, rifamycin, any injection containing sodium bisulphite.

Subject number

As this is a pilot study, it is not possible to calculate the exact number of cases. It is planned to examine a total of 10 study subjects.

Recruitment measures

Volunteers will be informed by means of a public notice and via the homepage of the University Hospital Erlangen. If volunteers are willing to participate, they will be fully informed about the aims and methods (especially about the scientific/explorative nature of the study), the benefits and risks and the revocability of participation in the study. Each participant receives an expense allowance of 150€.

9. Study course

Procedure for informing about and obtaining consent

Volunteers can only be included in the study after a written consent has been given. The written declaration of consent requires oral and written information of the volunteer about goals and methods (incl. scientific-explorative character of the study), benefit and risk as well as revocation of participation in the study. There must be a period of 24 hours between the education of the study participants and the written declaration of consent. By giving their written consent, the study participants declare that they agree to the collection and storage of study-relevant data and their verification by monitoring or authorities. The study participant must be clearly informed that the declaration of consent can be withdrawn at any time and without any disadvantage. Furthermore, all study participants are informed that this study is a purely scientific study without any current diagnostic or therapeutic benefit.

The off-label use of a drug (Indocyanine Green ICG, Diagnostic Green "Verdye" 5mg/ml) is explicitly explained.

The original of the declaration of consent will be kept in the study folder at the place of study. The study participants receive a copy of the patient information and declaration of consent. The participant information and the consent form are attached to this protocol.

Time schedule

The study starts for the participants at 10 pm on the day before the first examination day with adherence to the fasting period. On the first examination day, the preprandial measurement is performed at 8 am after 10 hours of fasting in all study arms. The preprandial measurement starts with the sonographic localisation of the sections of the gastrointestinal tract that will be examined in the following measurements with MSOT and Doppler sonography. Skin markings are made with a skin-compatible marker pen for better reproducibility of the following measurements. The further procedure of the preprandial measurement and each of the following postprandial measurements will follow an identical measurement protocol. A period of 20 minutes is planned for each measurement protocol. Depending on the study arm, the subjects remain fasting throughout all the measurements of the day (study arm A) or receive a standardised breakfast with or without ICG supplement (study arm B/C) 30 minutes after the start of the preprandial measurement. After the subjects have eaten the breakfast within 15 minutes, the first postprandial measurement takes place exactly 60 minutes after the preprandial measurement in all study arms. In the following 6 hours, further postprandial measurements are taken at 60-minute intervals, so that the last measurement takes place exactly 7 hours after the start of the preprandial measurement. 4 hours after breakfast, the subjects from study arms B and C receive another standardised meal to simulate regular food intake. This meal does not contain ICG. The subjects in study arm A remain fasting throughout all the measurements of the day.

On each measurement day two subjects are examined in parallel with a time offset of 30 minutes. This educmeans that the entire examination for the second participant begins at 8:30 am.

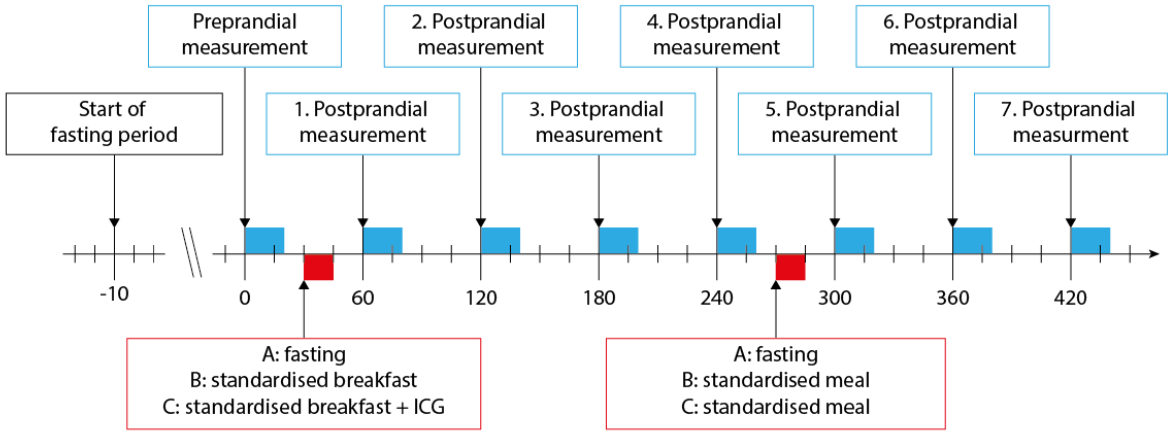

Figure 1: Course of one examination day for one participant. X-axis is tume in minutes with 0 beeing the beginning oft he preprandial measurement at 8:00 or 8:30 am..

In a crossover design, each participant is examined on three measurement days according to the three study arms. There must be a minimum time interval of 48 hours between each examination day for the participant. The order of the examination days is predetermined:

1st examination day (study arm A): Fasting
2nd examination day (study arm B): Standardised breakfast
3rd examination day (study arm C): Standardised breakfast with ICG

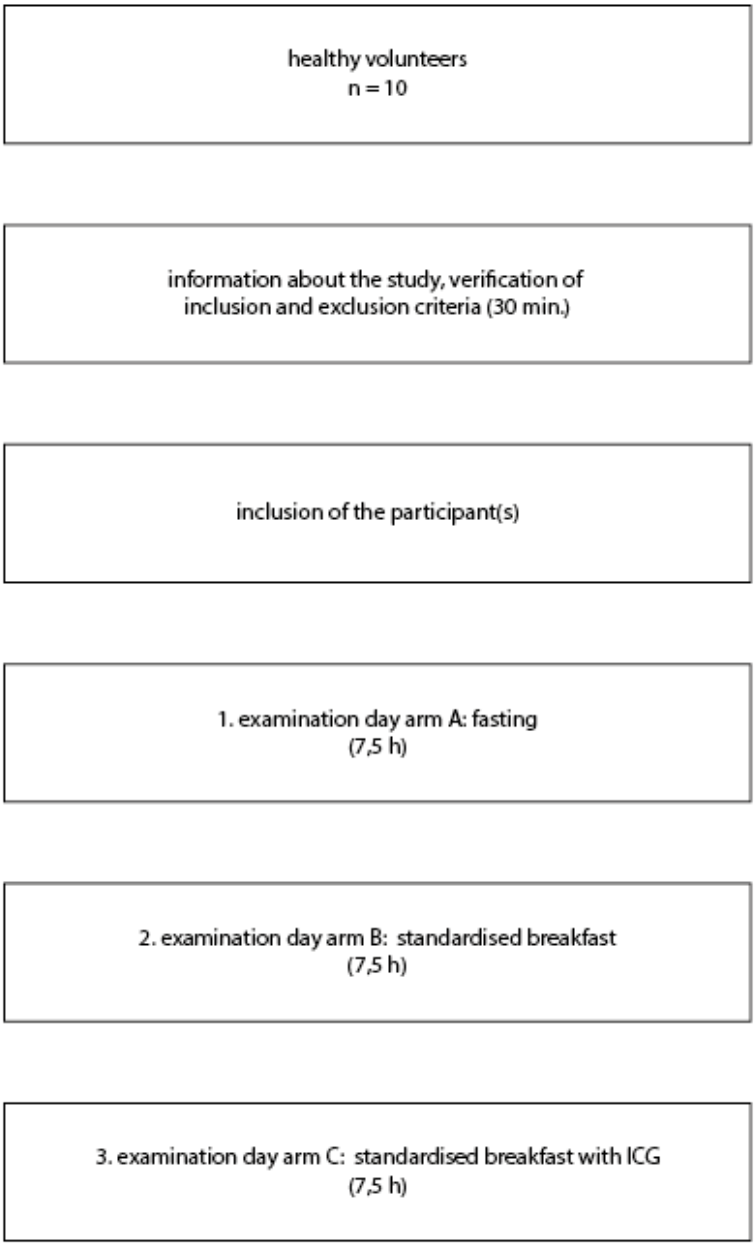

Figure 3: Flowchart

Measurement protocol

At the beginning of the preprandial measurement, the sections of the gastrointestinal tract that are to be examined in the following measurements with MSOT and Doppler sonography are localised sonographically and markings are made on the skin surface with skin-compatible marker in order to achieve better reproducibility over the following measurements.

The further procedure of the preprandial measurement and each of the following postprandial measurements are carried out according to a identical measurement protocol.

Optoakustische Messungen

a. Magenantrum
b. Terminales Ileum
c. Colon transversum
d. Colon sigmoideum

2. Doppler-sonographische Messungen
    a. Truncus coeliacus
    b. Arteria mesenterica superior
    c. Arteria mesenterica inferior

The estimated time for a complete measurment according to the protocol is 20 minutes.

At the beginning of each measurement, each subject drinks 150ml of still mineral water in order to achieve a uniform filling of the stomach during the optoacoustic measurement of the gastric antrum.

Composition of the standardised breakfast and meal

The composition of the standardised continental breakfast and the standardised meal are identical and take into account the gender and body weight of the participant. With an energy content of 400-700 kcal (8.2 kcal/kg bw), the meal each accounts for about 25% of the daily energy requirement. The nutrient composition is 55% carbohydrates, 29% fat and 16% proteins.

Woman	**Man**
60 kg	**80 kg**
500 kcal	650 kcal
1½ multigrain bread roll 1 portion of jam ½ cream cheese, lean	
½ portion of butter 1 slice of cheese (Emmental or Maasdam)	1 portion of butter 1 slice of cheese
< 65 kg: up to 2x 200 ml water >65 kg, < 70kg: 100 ml orange juice up to 2x 200 ml water >70 kg: 200 ml orange juice up to 2x 200 ml water	<85 kg: 200 ml orange juice up to 2x 200 ml water >85 kg: 300ml orange juice up to 2x 200 ml water

Study participants in study arm C drink 250mg of ICG (Verdye, Diagnostic Green) dissolved in 50ml of water with the standardised breakfast.

Total duration of the study

The estimated total duration of the study until the inclusion of the last participant is approximately 12 months.

Risk-benefit analysis

Study-related risks of MSOT

Based on the classification criteria for medical devices (Directive 93/42/EEC, Annex IX), the optoacoustic system of iThera Medical corresponds to Class IIa:

- Active diagnostic device
- non-invasive
- Temporary use (<60 min)

There is a CE certification for this medical device (type designation according to imprint: Acuity Echo). A extension of the certification or conformity assessment procedure is not planned in this study. It is therefore a purely scientific pilot study. There is no dependency on the manufacturer, all diagnostic and analytical procedures are available to the study directors on site. No data is passed on to the manufacturer.

According to the Medical Device Regulation (MDR) 2017/745 and the Medizinprodukterecht-Durchführungsgesetz (MPDG), this study does not apply as “sonstige klinische Prüfung nach Art. 1 Medizinproduktedurchführungsgesetz: §3 Abs. 4”. The medical device is used within the scope of its intended purpose, without harmful or invasive measures (omission of Art. 62 MDR).

Adherence to energy levels

The laser safety and maximum permitted radiation dose for irradiation with laser pulses is regulated in the laser standards ANSI and IEC 60825. The MSOT system meets these standards and therefore remains below the MPE (maximum permissible exposure) limits for skin irradiation and is therefore considered safe.

Temperature increases due to MSOT in tissue

Optoacoustic imaging does not result in any significant temperature increase in the tissue. The absorption of a laser pulse in the tissue results in a local transient temperature increase of a few millikelvin. Depending on the duration of the examination and the skin type of the participant, temperature increases occur typically in the range of less than one degree Kelvin.

Histological changes in tissue

Histological changes in the target tissue and surrounding structures are neither expected nor have they been observed in previous preclinical and clinical studies.

Slight, reversible redness or warming might occur in very sensitive skin.

Such side effects are to be noticed at any time by the study participant or the examining doctor; the examination can then be interrupted or aborted. In any case, no irreversible damage is to be expected.

In general, the near infrared light used in MSOT can lead to retinal damage if the eye is irradiated. In order to prevent this, participants and examiners will wear appropriate laser safety glasses during the examination.

Since the data obtained is not used to interpret diagnostic results, there is no risk of possible misdiagnosis or incorrect display of data in this exploratory pilot study.

Study-related risks of ICG

The ICG (Diagnostic Green "Verdye" 5mg/ml) used in this study, is an authorised medical product for diagnostic use. After intravenous application of ICG, nausea, hypersensitivity reactions, serious allergic reactions and coronary spasms have been described in very rare cases (<1 in 10,000). These reactions should be noticed by the participant or the investigator at any time. The study will be discontinued in such cases. No other adverse events or risks associated with ICG have been described.

Oral application of ICG is an off-label use. However, according to the product information, the dye is not absorbed and is not subject to enterohepatic circulation. Therefore, systemic side effects after oral administration of ICG are very unlikely.

The orally administered dose of 250mg ICG is below the maximum recommended daily total dose for intravenous use of 5mg/kg bw (350mg for a 70kg person).

The combination of ICG and MSOT is explicitly described in the CE label. The study aims to investigate a physiological condition (here: chyme passage) and not a medicinal product (Directive 2001/20/EC). In addition, neither safety, efficacy, pharmacokinetics nor similar aspects are investigated. An extension of the marketing licence is also not intended (no regulation according to §4 section 23). Each participant is explicitly informed about the off-label use.

Other risks do not exist in the context of this study and were not observed based on our own previous data.

Benefits associated with the study

The data obtained in this study can provide essential insights into the postprandial changes in blood flow in the wall of the gastrointestinal tract and thus contribute to establishing new non-invasive diagnostic approaches for inflammatory bowel diseases and other pathologies of the gastrointestinal tract. Potentially invasive, risky procedures could be replaced.

Termination criteria

Termination criteria for the individual participant:

Termination of participation in the study takes place in the case of noticeable warming or reddening of the skin. The examination time is limited to 20 minutes per hour, so that these events are very unlikely.

Another termination criterion is adverse reactions to taking the standardised breakfast, meal or ICG such as stomach upset, nausea, vomiting, diarrhoea, abdominal cramps or signs of an allergic reaction such as itching, skin rash, rapid heartbeat, drop in blood pressure, shortness of breath.

In addition, the study will be terminated for the individual participant if, as a result of adherence to the fasting period before the measurements and during the measurements in study arm A, complaints such as malaise, weakness, a strong feeling of hunger, stomach complaints, abdominal cramps or similar occur.

Termination criteria for the whole study:

A termination of the entire study is not planned

Statement on medical justifiability

Based on previous experience, the risk of undesired events associated with is considered extremely low.

No serious event has been reported so far, neither at our own site nor in literature. The majority of the reported (foreseeable) problems related either to the use of ultrasound gel for examination or the need to wear eye protection. The use of filter glass also explains the reported red-sightedness. This phenomenon was reversible within seconds. Table 1 shows the reported events from our study (MSOT_DMD, 67_18 B, Clinicaltrials.gov Identifier: NCT03490214).

	Muscular dystrophy Duchenne N=10	Healthy volunteer N=10
Reversible adverse events- no.		
Pressure of safety goggles	2 (20%)	
Coolness of Ultrasound-gel		4 (40%)
Red cast view		1 (10%)
Serious adverse events- no.	0 (0%)	0 (0%)

Table 1 – Reported events MSOT_DMD, 67_18 B

The occurrence of adverse side effects from the systemic use of ICG (Verdye, Diagnostic Green 5mg/ml) is very rare (<1 in 10 000). According to the manufacturer, the dye is not absorbed after oral application of ICG and is not subject to enterohepatic circulation. Therefore, the occurrence of systemic side effects from oral intake of ICG is very unlikely.

10. Biometrics

Explorative study: explanation of the statistical methodology, justification of the selected number of cases

Case number calculation:

As this is a pilot study and no information on the expected differences between the different groups is available yet, no case number calculation was performed. The number of cases given represents an estimate or is within the appropriate range for a pilot study.

Statistical methods:

The data is given as mean value with standard deviation. Correlations are given with the non-parametric Spearman correlation coefficient (R_s). According to the distribution on groups the differences of the mean values are examined statically with a nonparametric T-test (Mann-Whitney test) or ANOV. In all analyses an error level of <0.05 is considered statistically significant.

11. Data management and data safety

Data acquisition and storage

The participation of the individual participant in the study is documented. The principal investigator maintains an independent list for the identification of the participating persons. This list contains the names and date of birth as well as the date of examination and pseudonymization codes of the participants. The principal investigator is responsible for the quality of data collection and storage. The data storage (complete data) takes place on computers or specially designed network drives of the University Hospital Erlangen.

Pseudonymization

Prior to a scientific analysis of the materials and data of this study, all information will be pseudonymized according to the guidelines of the Federal Data Protection Act.

Revocation and data deletion

If the declaration of consent is revoked, data collected up to this point can be taken into account and retained. However, the participant has the right to demand the data to be destroyed, provided that legal provisions do not prevent such destruction.

12. Insurance

All participants of the study are insured through the group contract of the CCS Erlangen. This will be pointed out separately in the study participant information.

13. Signatures

Dr. med. Ferdinand Knieling Principal Investigator	Prof. Dr. med. Joachim Wölfle Clinic director

Further investigators

Dr. med. Adrian P. Regensburger

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