Study Protocol

MSOT_PAD_3D

Pilot Study of Topographic Imaging of the Gastrocnemius Muscle in Patients with PAD Using Non-invasive Multispectral Optoacoustic Tomography (MSOT) as a 3D Reconstruction Based on Longitudinal 2D Measurements

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1 Study Title, Version Number, Version Date

1.1 Study title

Pilot Study of Topographic Imaging of the Calf Muscle in Patients with PAD Using 3D Reconstruction of MSOT Images

1.2 Version number

Version 1.0

1.3 Version date

11/02/2021

1.4 Protocol establishment

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2 Summary

Functional imaging diagnostics is becoming increasingly important due to the steadily growing knowledge of physiological processes in many diseases. Likewise, in peripheral arterial occlusive disease (PAD), new insights into the pathomechanism of the disease are continuously being gained [1]. Similarly, this increases the need for new non-invasive imaging methods that are able to visualize the functional level of the disease progression and thus enables the diagnosis at an early stage.

Recent studies indicate that it may be feasible to use multispectral optoacoustic tomography (MSOT) to visualize hemodynamics and the fibrotic muscle remodeling process in PAD [2]. For a better understanding of the distribution pattern, the exploration of a 3D technique is a next necessary step in imaging. This may show a possible existing heterogeneity of these molecules.

The aim of this exploratory pilot project is to image muscle perfusion of the lower extremity in three dimensions using the MSOT method and to verify its feasibility. The advantage of 3D imaging is, beyond the anatomical topography of the muscle, to map a three-dimensional representation of the perfusion situation based on muscle oxygenation.

For this purpose, six patients of different symptomatic PAD stages and a healthy control of two volunteers will be included and examined by the means of longitudinal MSOT scans in the area of the gastrocnemius muscle. To evaluate the current stage of the disease or to exclude relevant PAD in the healthy control population, non-invasive examination measures commonly used in routine diagnostics of PAD will be applied. In addition to the relevant risk factors, coexisting comorbidities and the current medication, these include the recording of the ankle-brachial index (ABI), color-coded duplex sonographic vascular imaging (CCDS) and a treadmill examination.

3 Responsibilities

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3.4 Funding source

Vascular Surgery Department; internal funding pending application for external funding.

4 Scientific Background

Starting in 2021, a CE-certified Multispectral Optoacoustic Tomograph (MSOT) will be available at the University Hospital Erlangen (Children's and Adolescent's Hospital). It will allow transcutaneous in-vivo measurements of soft tissue. Comparable to sonography, this allows noninvasive, quantitative imaging of the constitution and oxygenation of target tissues such as muscles.

In MSOT, similar to conventional sonography, a transducer is placed on the skin and instead of sound, energy is delivered to the tissue through flashlights. This leads to a constant alternation of minimal expansions and contractions (thermoelastic expansion) of individual tissue components or molecules. The resulting sound waves can be detected by the same examination probe. Previous studies have shown that quantitative determination of hemoglobin can provide information on blood flow and inflammatory activity in the intestine of patients with Crohn's disease [3, 4]. In the CE-certified MSOT device (Acuity Echo, iThera Medical GmbH, Munich), a spectrum of laser light can be used, which enables the detection of hemoglobin and its oxygenation levels as well as the detection of other markers like collagen and lipid, which has already been successfully measured, validated and reproduced in 2D imaging in pre-studies. Figures 1 and 2 show examples of the feasibility of this imaging method with selected MSOT parameters as examples.

Holzwarth et al. were already able to gain knowledge about the 3D reconstruction from 2D photoacoustic image slices using an optical pattern and the method's feasibility using phantoms as well as in-vivo measurements of the forearm in healthy volunteers [5]. This leads to the evaluation of the method as a diagnostic application for muscular perfusion imaging in patients with vascular dysfunction.

Until now, there are few noninvasive measurement methods besides MSOT that can provide transcutaneous information about 3D muscle perfusion. Even topographic muscle imaging from computed tomographic (CT) or magnetic resonance imaging (MRI) reconstructions carries certain risks despite its routine in daily clinical use. For example, CT examinations require radiation exposure, and MRI examinations exclude patients with certain comorbidities or previous performed surgeries (loose metal parts such as pacemakers) and patients with restlessness.

In this explorative pilot study, a 3D reconstruction of the calf muscle using MSOT techniques to visualize muscle perfusion will be investigated for the first time in a PAD patient group. Patients with different PAD stages and a control collective of healthy volunteers as a comparison group will be included. Different optical patterns offer diverse possibilities for the calculations of the 3D reconstruction. Therefore, this study will also evaluate the feasibility of the two options and analyze the measurements to optimize the 3D procedure.

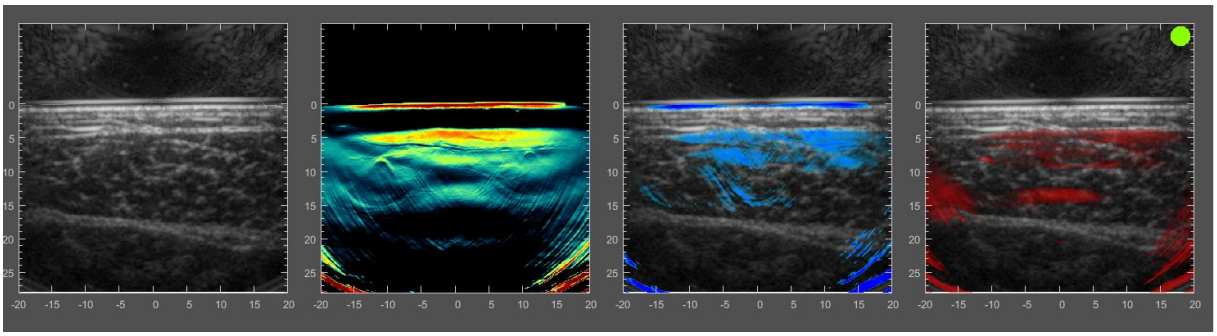

Figure 1: Visualization showing optoacoustic signal of perfusion parameters in 2D scans, from left to right: B-scan ultrasound (b/w RUCT), signal at 800 nm wavelength, Hb signal (blue), HbO2 signal (red) of the medial muscle head of the gastrocnemius muscle (transversal plane). Unpublished original data.

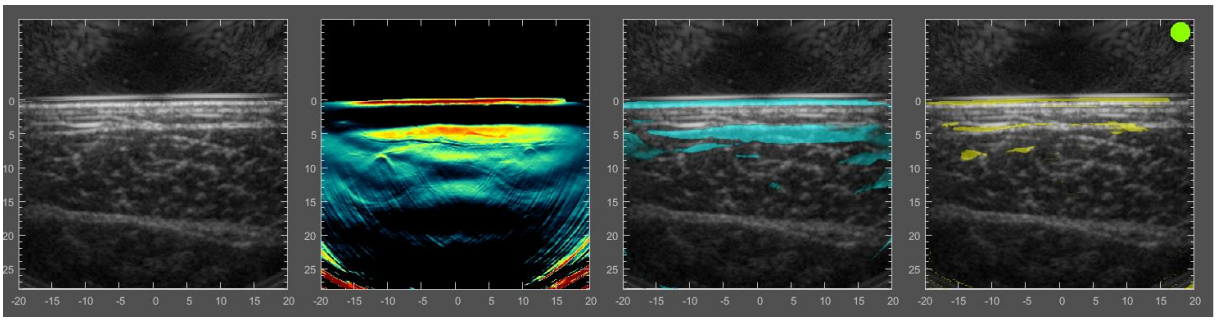

Figure 2: Visualization showing optoacoustic signal of connective tissue structures in 2D scans, from left to right: B-scan ultrasound (b/w RUCT), signal at 800 nm wavelength, collagen signal (turquoise), lipid signal (yellow) of medial muscle head of gastrocnemius muscle (transversal plane). Unpublished original data.

References

1. Signorelli, S.S., et al., *Pathophysiology of chronic peripheral ischemia: new perspectives.* Therapeutic Advances in Chronic Disease, 2020. **11**: p. 1-15.
2. Karlas, A., et al., *Multispectral optoacoustic tomography of muscle perfusion and oxygenation under arterial and venous occlusion: A human pilot study.* Journal of Biophotonics, 2020. **13**(6): p. e201960169.
3. Knieling, F., et al., *Multispectral Optoacoustic Tomography for Assessment of Crohn's Disease Activity.* The New England Journal of Medicine, 2017. **376**(13): p. 1292-1294.
4. Waldner, M.J., et al., *Multispectral Optoacoustic Tomography in Crohn´s Disease: Noninvasive Imaging of Disease Activity.* Gastroenterology, 2016. **151**(2): p. 238-40.
5. Holzwarth, N., et al., *Tattoo tomography: Freehand 3 D photoacoustic image reconstruction with an optical pattern.* International Journal of Computer Assisted Radiology and Surgery, 2021. **16**: p. 1101-1110.

5 Study Objectives

The aim of this pilot study is to implement a three-dimensional reconstruction of the perfusion situation based on two-dimensional longitudinally measured MSOT scans to visualize the topographical distribution of oxygenated hemoglobin in the calf muscle using MSOT.

5.1 Primary/secondary objectives and hypotheses

Primary hypothesis:

- MSOT provides topographic perfusion imaging of the gastrocnemius muscle using 3D reconstruction.

Secondary hypotheses:

- Collected 3D MSOT parameters in muscles of patients with PAD differ visually depending on the clinical severity of PAD, classified according to Fontaine and Rutherford.
- Collected 3D MSOT parameters in muscles of patients with PAD differ quantitatively depending on the clinical severity of PAD, classified according to Fontaine and Rutherford.
- Collected 3D MSOT parameters in muscles of patients with PAD show improved diagnostic accuracy than 2D MSOT measurements.

Primary objective:

- Visualization of a perfusion topogram based on oxygenated hemoglobin in the calf muscle of patients with PAD using 3D MSOT.

Secondary objectives:

- Acquisition of different MSOT values at multiple wavelengths in the range from 660 nm up to 1100 nm in static and longitudinal measurements.
- Acquisition of the quantitative hemoglobin signal (oxygenated/deoxygenated) as unmixed spectrum.
- Acquisition of the quantitative lipid signal as unmixed spectrum
- Acquisition of the quantitative collagen signal as unmixed spectrum
- If an MRA is already available regardless of study participation, validation of the accuracy of the MSOT 3D reconstruction using an overlay.

5.2 Study type

Exploratory pilot study

6 Outcome Measures

All measurements with MSOT are performed before and after gait exposure in the area of the medial part of the gastrocnemius muscle. Different MSOT wavelengths in the range from 660 nm to 1100 nm are assessed. Various parameters calculated from the measured wavelengths are also taken into account for the evaluation (Hb, HbO2, Hbtot, collagen, lipid, sO2).

6.1 Primary outcome measures:

- MSOT topogram showing perfusion (oxygenated/deoxygenated/total hemoglobin) of the gastrocnemius muscle
    - *These outcome measures are collected non-invasively using MSOT.*

6.2 Secondary outcome measures:

- Topographic imaging of lipid signal and collagen signal using MSOT
    - *These outcome measures are collected non-invasively using MSOT.*
- 3D reconstruction of the longitudinal measurements using two different pattern designs
    - *These outcome measures are collected non-invasively using MSOT.*
- Acquisition of static 2D MSOT measurements before and after gait exposure
    - *These outcome measures are collected non-invasively using MSOT.*
- Acquisition of the flow profile of the common femoral artery and popliteal artery using CCDS
- Acquisition of the Ankle-brachial-index
- Acquisition of the current walking distance standardized by treadmill examination
- Acquisition of the current walking distance standardized by treadmill examination (Excluding patients with PAD in chronic critical stage III or IV according to Fontaine)
- Acquisition of the PAD stage according to Fontaine and Rutherford
- Recording of relevant secondary diseases from the patient's file
- Recording of relevant previous operations from the patient's file
- Recording of current medication from the patient's file

7 Study Design

7.1 Monocentric/multicentric

This is a monocentric exploratory pilot study with prospective data collection.

7.2 Study arms: intervention/control

Interventions are not planned. The study will be conducted in two stages. First, the presentability of 3D reconstruction of the calf muscle will be tested in two healthy volunteers. Subsequently, a representative patient collective of different PAD stages will be examined with the method.

7.3 Randomization

Randomization is not planned.

7.4 Blinding

The investigator will be blinded during the measurement and data analysis. The blinding of the investigator is done by the investigator only performing the MSOT measurements and not knowing the results from the previous examinations (ABI, CCDS, treadmill, PAD stage).

Blinding of the patients/healthy study participants is not necessary.

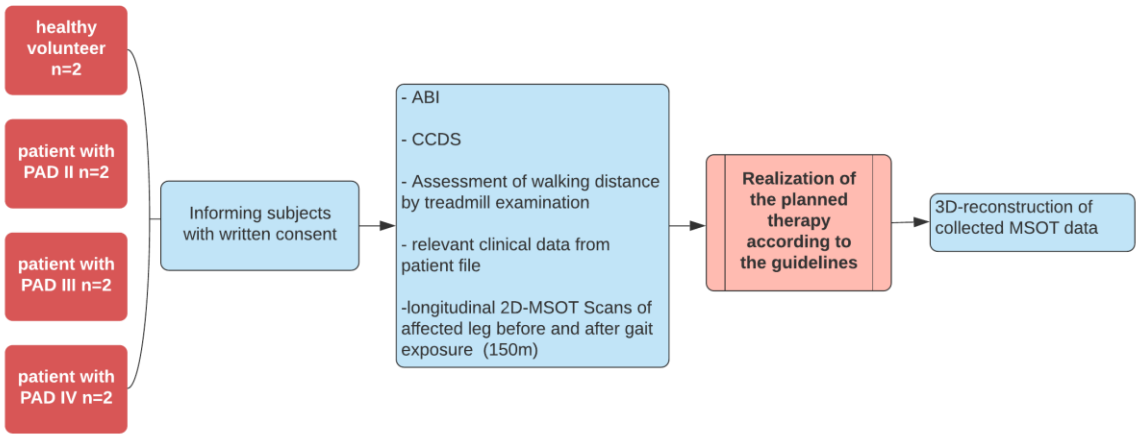

Figure 3 Graphical presentation of the study flow with the planned measurements.

8 Study Population

8.1 Eligibility criteria

Inclusion criteria:

- Patients with manifest PAD stages II-IV according to Fontaine or healthy volunteers
- Adults (>18 years) who are able to give their consent

Definition of patient group:

- Patients with PAD IIa/IIb/III/IV according to Fontaine who come in for a routine presentation of the vascular surgery consultation hours.

Definition of healthy volunteer group:

- no PAD previously known
- no diabetes mellitus previously known
- no chronic renal insufficiency previously known
- no symptoms in the sense of a Claudicatio intermittens
- ABI with normal value

Exclusion criteria:

- Patients with PAD stage I according to Fontaine
- Healthy volunteers with pre-existing diabetes mellitus, chronic renal failure, or abnormal ABI
- Underage persons
- Missing consent form
- Exclusion due to safety concerns of the study physician (patient with a physical, mental or psychiatric illness which, in the opinion of the study physician, would compromise the safety of the patient or the quality of the data and thus make the patient an unsuitable candidate for the study)

8.2 Number of cases

A valid calculation of the number of cases is not possible because of the novelty of the reconstruction procedure and due to the study design as a pilot study with explorative character. For the exploration of the planned research question, a study collective of 8

study participants is aimed for. Two healthy volunteers, two patients in PAD stage II, two patients in stage III and two patients in stage IV according to Fontaine will be examined. One male and one female will be included per stage to represent both genders.

8.3 Recruitment routes and measures

Patients will be informed of the opportunity to participate in the study during a routine consultation in the vascular surgery department. Healthy volunteers will be acquired via notices posted in the clinic. If the patient is willing to participate in the planned study, he/she will be fully informed about the aims and methods (especially about the scientific/explorative character of the study), the benefits, risks and the revocability of the participation in the study.

9 Study Procedure

9.1 Procedure of informing and obtaining consent

Patients or subjects can only be enrolled in the study after a written informed consent has been obtained. The written informed consent requires verbal and written explanation to the patients about the aims and methods (including the scientific-explorative character of the study), benefits and risks as well as the revocability of the study participation.

The study participant is clearly informed that withdrawal of consent is possible at any time and without any disadvantage. All study participants will be advised that this study is a purely scientific study without any current diagnostic or therapeutic benefit.

The original informed consent form will be kept in the study folder at the study institution. The participant will be given a copy of the patient/proband information and consent form.

9.2 Measures

After informing the patients, all study participants will be asked to provide information about their existing illnesses, previous operations, and current medication. If available, the electronic patient file will be used for this purpose, otherwise these data will be collected by means of a medical history interview. Of particular interest are diseases, operations and medication related to the vascular system. This is followed by measurement of the ABI. This parameter is used in vascular medicine to estimate the

severity of PAD. For healthy study participants, the measurement of the ABI is to be regarded as a screening examination. Study participation of healthy volunteers requires an unremarkable value in the ABI measurement (the handling of incidental findings during screening is presented in chapter 13). Prior to this measurement, a rest period in lying position of at least 10 minutes should be observed. Measurements of occlusion pressures on both legs and arms are then performed. The examination of the ABI takes a maximum of 20 minutes, including the preceding rest period, and would also be recorded in PAD patients independently of study participation during the inpatient stay as part of routine diagnostics. Afterwards, a CCDS of the femoral arteries is performed at rest. The CCDS is used to determine the flow profile of the examined vessels and allows an estimation of constrictions or occlusions in these. For this purpose, the patient/proband remains lying on the examination couch. Depending on the examination conditions (individual anatomy of the vessels, body size, etc.), this examination takes about 10 minutes. This examination takes place as part of routine vascular surgery diagnostics.

The actual current walking distance is used to classify and categorize the severity of PAD and should be recorded by a treadmill examination. Under medical supervision, the patients/probands are to complete the greatest possible distance on the treadmill at a 12% incline and 3 km/h (standard setting). If the 12% incline is not practicable for the patient, the incline will initially be reduced to 6%. If this is also not practicable, it will be reduced to 0% incline. The walking distance is recorded in meters. Since the first 200 meters in particular are important for the classification of PAD, the examination will be terminated by the study physician when a walking distance of more than 500 meters has been reached. The treadmill examination takes about 15 minutes and is usually performed for patients in stage II as part of routine vascular surgery diagnostics. For patients in stage III or IV, the examination is not part of the routine diagnostics and only takes place within the framework of study participation if the study physician declares this medically justifiable.

All examinations mentioned so far take place in the facilities of the Department of Vascular Surgery.

For the MSOT measurements, the CE-certified MSOT device of the Pediatric and Adolescent Clinic of the University Hospital Erlangen is used. Since the MSOT device is located in the facilities of the Children's Hospital, the patients/probands are transported there in a wheelchair after the examinations mentioned so far. It is

important that there is no physical exertion during the transport, as this could falsify the MSOT values. The transport takes about 15 minutes. Subsequently, MSOT measurements are taken with the MSOT device over a length of about 6 cm in the longitudinal section of the medial head of the gastrocnemius muscle (in patients in the area of the affected leg). Multiple measurements of both legs are performed with different foils applied in a standardized manner.

If necessary, the area of the lower leg to be examined will be shaved to avoid artifacts from dark colored hairs. The ultrasound gel is applied to the calf. Then a foil imprinted with an "N" is applied and fixed. The proband ID is noted on the foil. Above the imprint, an initial static measurement is taken at rest and subsequently a longitudinal measurement is taken, which can be reconstructed in 3D. At least three longitudinal 2D measurements are made or repeated until a promising result is obtained. The same procedure is repeated with a foil imprinted with a trident.

The examination is performed analog to sonography above the corresponding skin layers without further invasive procedures. The anatomical region can be localized by means of built-in B-scan sonography; subsequently, the corresponding optoacoustic signals can be derived. The duration per anatomical region is limited to 5 minutes; the study participants can remain in a relaxed position during the examination; assistance, e.g., breathing maneuvers, is not necessary.

Subsequently, the patient will complete a defined walking distance of 150 meters under medical supervision. The foils printed with tridents remain fixed to the calf muscles for this purpose. Afterwards, the medial head of the gastrocnemius muscle is measured again using MSOT. Therefore, the affected leg of patients is first examined in a static 2D-measurement, then in longitudinal measurements and again in a static 2D-measurement. In healthy probands, the right leg is measured first.

After a second defined walking distance of 150 meters, the unaffected leg is examined in patients and the left leg in healthy probands in the order described above.

In the context of so far unpublished preliminary studies, it was indicated that an active muscle exercise between the two examinations by MSOT causes a change of the perfusion parameters and can possibly unmask a stenosis in the supply area of the lower legs. If the patient is unable to walk 150 meters between the two MSOT measurements due to pre-existing conditions or due to the severity of the PAD stage,

the distance of walking can be reduced stepwise. In this case, an initial attempt should be made to complete a distance of 100 meters. If this is also not feasible, the distance of the walking load is first reduced to 50 meters and finally to 25 meters. In the case of complete inability to walk, standing exercises can be performed as an alternative. In this case, the patient should be instructed to alternate between tiptoe and heel stance for at least two minutes. If this form of muscle exercise is also not possible, patients can sit down and should perform extensions and flexions of the foot over a period of at least two minutes to provoke lower leg muscle exercise between the two MSOT measurements.

9.3 Acquisition of outcome measures

- Non-invasive longitudinal in vivo measurement of the gastrocnemius muscle by MSOT and subsequent 3D reconstruction of the 2D scans
- Non-invasive static 2D in vivo measurement of the gastrocnemius muscle by MSOT
- Acquisition of the flow profile of the common femoral artery and popliteal artery using CCDS
- Acquisition of the ABI
- Acquisition of the current walking distance in meters standardized by treadmill examination (3 km/h with 12% gradient)
- Acquisition of the clinical PAD stage
- Recording of relevant clinical data from the patient's file

9.4 Total duration of the study

According to the number of cases, the expected total duration of the study until inclusion of the last study participant is approximately 6 months. Only one examination session is required per patient.

10 Risk-benefit Analysis

10.1 All study-related risks

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

- Active diagnostic device
- non-invasive

- Temporary use (<60 min).

For the present research device (current type designation according to imprint: Acuity Echo), a CE certification has been available since 02/05/2021 and is used exclusively within the intended purpose without invasive measures. There is no dependency relationship with the manufacturer, all diagnostic and analytical procedures are available to the study investigators on site.

This is an "Other Clinical Trial" according to Article 82 of the Medical Device Regulation (MDR) with the aim of answering the scientific question mentioned above. No proof of conformity is provided in the process.

iThera Medical is ISO 13485:2016 certified.

The device has an IP2X rating, the handheld probe has an IPX7 rating, and the foot switch has an IPX6 rating according to IEC/EN 60529.

Compliance with energy limits

Laser safety and maximum allowable radiation dose for laser pulse irradiation is regulated in the ANSI and IEC 60825 laser standards. The MSOT system complies with 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 a significant increase in tissue temperature. Absorption of a laser pulse in tissue results in a local transient temperature increase of a few millikelvin. Depending on the duration of the examination and the patient's skin type, temperature increases typically in the range of less than one degree Kelvin.

Histological changes in the tissue

Histological changes in the target tissue and surrounding structures are not expected, nor have they been observed in previous preclinical and clinical studies.
Only slight, reversible reddening or heating is to be expected in sensitive skin.
Such adverse effects may be noticed by the subject or physician at any time; the study may then be interrupted or discontinued. In any case, no irreversible damage is to be expected.

In principle, the near infrared light used in MSOT can cause retinal damage if the eye is irradiated. To prevent this, study participants and investigators will wear safety glasses during the study.

Since the data obtained will not be used to interpret findings, there is no risk of possible misdiagnosis or misrepresentation of data in this study.

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

10.2 Benefits associated with the study

The data obtained in the study may provide essential insights into the blood flow situation in different areas of the calf muscles in PAD. The possibility of non-invasive 3D imaging without the need for administration of contrast medium could open up new aspects in the diagnosis of the disease. There is a purely scientific benefit from the study. There is no direct diagnostic or therapeutic benefit for patients/probands from participation in the study.

10.3 Discontinuation criteria

Discontinuation criteria for the individual participant:

Discontinuation of study participation will occur if there is noticeable heating or redness of the skin. The examination time per anatomical region is limited to 5 minutes, so that these events are highly unlikely.

In addition, study participation will be discontinued in the event of unfeasible muscle exercise described above between both MSOT measurements, e.g. due to dyspnea, orthopedic diseases, etc.

Discontinuation criteria for the entire study:

Discontinuation of the entire study must occur in the event of the occurrence of previously unknown and unobserved significant and adverse health effects from the MSOT examination. This is considered unlikely, since several hundred patients and healthy volunteers have already been examined with this method at the University Hospital Erlangen alone.

10.4 Statement on medical justifiability

Based on previous experience in adult patients, the risk of the occurrence of adverse events is considered to be extremely low. No central organs are examined in this study,

but only measurements on extremities are performed - this leads to a further significant reduction of a possible residual risk. The examination will be performed by persons who are familiar with the device and the measurement method.

11 Statistical Methodologies

Due to the explorative character of the pilot project, mainly a descriptive statistical evaluation of the collected parameters will be performed. A case number of 8 study participants will be chosen to test the feasibility of the 3D method initially. A sample size calculation will not be done. In order to quantitatively classify the results, individual parameters of the study will be compared with values from previous studies on the MSOT procedure in patients with PAD. The MSOT parameters are compared before and after muscle exercise using a paired-samples t-test. The selection of probands will be standardised with one male and one female per stage. Due to the small number of cases, probands who are suitable for MSOT measurements are selected. The criteria include little subcutaneous fat tissue, light skin colour and less hair. Therefore, the selection of probands is not representative of the normal population.

12 Data Management and Data Privacy

12.1 Data acquisition, data storage

All raw data, such as patient records, represent source documents. Their availability is ensured for routine monitoring. Participation of individual patients or probands in the study will be documented and the study director will maintain an independent list for identification of participating probands. This list includes name and date of birth as well as study date and pseudonymization abbreviation of the patients and subjects. The study director is responsible for the quality of data collection and storage. Data storage is exclusively on computers or specially designated network drives of the University Hospital Erlangen.

12.2 Pseudonymization

Before any 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.

12.3 Data transfer

There are no plans passing the data of this study to third parties and it will not be done; in particular, the manufacturer will not be given access to the data. The study results can be published anonymously, and it will not be possible to infer the identity of the participating persons. The data will be kept for 10 years and then will be destroyed.

12.4 Revocation, data deletion

In the event of revocation of the declaration of consent, data collected up to this point can be taken into account and can be continued to be stored. However, the patient has the right to request their destruction, unless legal provisions prevent destruction.

13 Handling of Incidental Findings

The acquisition of all results as well as the evaluation of the ABI and the images from the CCDS are performed for healthy probands only for the purpose of this study and not for general or special diagnostic purposes. Evidence of diseases unknown to the participant may not be detected in these examinations. Incidental findings in patients are rare (routine diagnostics), and the procedure for handling incidental findings applies equally to healthy probands and to patients. If abnormal findings are discovered during the scientific evaluation, an appropriate specialist is consulted for follow-up and reporting of the values and images that were originally determined for research purposes (healthy probands) or in the course of routine diagnostics (patients). Whether a reportable finding exists is decided by the study director according to his professional discretion. All study participants will be informed in advance about any disadvantages of reporting findings as part of the written informed consent.

Such disadvantages are to be understood as

- severe psychological stress due to the knowledge of or the suspicion of a threatening disease
- disadvantages in the case of certain legally significant actions, such as the conclusion of a life or health insurance policy or an employment contract
- further examinations linked to the notification of the findings, which may represent a health risk, even if the further work-up reveals that the findings have no pathological significance

After being informed with regard to the above-mentioned possible disadvantages due to the notification of incidental findings in the context of this study, the proband will put

down in writing whether or not he/she wishes to be informed and advised about such abnormalities.

14 Handling of Biomaterials

Not applicable

15 Proband Insurance

A separate proband insurance will be concluded within the framework of an annual contract with the insurer HDI Global SE, Düsseldorf branch. The insurance is based on the agreements according to the "Insurance for clinical trials not subject to compulsory insurance".

16 Signatures

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