Study protocol

ClinicalTrials.gov Identifier: NCT04641091

MSOT_PAD

Cross-sectional study of muscle perfusion in patients with PAD by non-invasive Multispectral Optoacoustic Tomography

Table of contents

1 Study title, version number, version date

1.1 Study title

Cross-sectional study of muscle perfusion in patients with PAD by non-invasive multispectral optoacoustic tomography

1.2 Version number

Version 1.3

1.3 Version date

01/20/2022

1.4 Creation of protocol

PD Dr. U. Rother, J. Günther, A. Träger

2 Project summary

Peripheral arterial occlusive disease (PAD) is one of the most common diseases of the elderly with an overall prevalence of about 3-10% [1]. As life expectancy increases, new treatment concepts and new diagnostic procedures are needed. In addition to the possibility of endovascular treatment and open surgery, in some cases there is also the possibility of a conservative therapeutic approach, e.g. with medication [2].

To date, the only independent non-invasive validation of these treatment options is the measurement of macrocirculation in the form of Color-Coded Vascular Duplex Sonography (CCDS), the Ankle Brachial Index (ABI) or the measurement of walking distance. The S3 guideline for diagnosis, therapy and medical aftercare of PAD published 2015 by the DGA (Deutsche Gesellschaft für Angiologie und Gefäßmedizin [German Society for Angiology and Vascular Medicine]) recommends aftercare in the sense of clinical examinations, especially for patients after vascular surgery. For the validation measures already mentioned, however, there are not infrequent patient groups for which these methods provide only insufficient or unusable results (e. g. diabetes mellitus, terminal renal failure). In these cases, independent verification of the therapeutic success would have to be performed using angiography (digital subtraction angiography, CT angiography or MR angiography). However, this is not routinely performed in the respective patient populations due to the associated risks (including radiation exposure, contrast agent administration, invasiveness).

Multispectral Optoacoustic Tomography (MSOT) now provides a new non-invasive diagnostic tool that may be able to fill this diagnostic gap.

The aim of this cross-sectional study is to define an independent parameter using the MSOT method, which allows a statement about the current perfusion situation of the lower extremity and correlates with the angiography, which is considered the gold standard. For this purpose, patients of different PAD stages, who already underwent routinely angiographies in advance, will be included. In addition, a control group of healthy volunteers (prior angiography not obligatory) will be examined.

3 Responsibilities

3.1 Study lead

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3.3 Other facilities possibly involved (e. g. laboratory, imaging, etc.)

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3.4 Sponsoring

Department of Vascular Surgery; internal financing and by grants from the ELAN Foundation.

4 Scientific background

Since 2017, the University Hospital Erlangen (Department of Medicine 1, Department of Pediatrics and Adolescent Medicine) has a Multispectral Optoacoustic Tomograph (MSOT) at its disposal, which is funded by the DFG (Deutsche Forschungsgemeinschaft [German Research Foundation]). This allows, comparable to sonography, a non-invasive, quantitative imaging of the composition and oxygenation of target tissues such as the muscles.

In the MSOT, similar to conventional sonography, a transducer is placed on the skin and instead of sound, energy is applied to the tissue by means of light flashes. This leads to a constant change of minimal expansion and contraction (thermoelastic expansion) of individual tissue components or molecules. The same examination unit can then detect the resulting sound waves. Previous studies have shown that the 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 newly configured device (Acuity Echo, iThera Medical GmbH, Munich, custom-made), an extended spectrum of laser light can be used, which ultimately enables not only the detection of hemoglobin and its oxygenation levels, but also the detection of other markers such as collagen and lipid. Figure 1 shows an example of the feasibility of these images using MSOT.

These parameters have already been successfully measured, validated and reproduced in preliminary studies.

Of special interest is the muscle perfusion in PAD patients, in whom due to previous diseases such as severe chronic renal insufficiency or diabetes mellitus the ABI measurements or the implementation of a CCDS due to a progressive stiffening of the vessel wall (Mönckeberg-Mediasclerosis) do not provide sufficiently valid values. Furthermore, there are also groups of patients who are unable to complete a treadmill examination to determine the possible walking distance due to previous illnesses or operations. To date, there are no non-invasive measurement methods besides the MSOT, which are able to make statements in a transcutaneous manner about muscle perfusion for these patient groups. Here, the non-invasive MSOT method could maintain its value in the diagnostics of PAD patients and close the diagnostic gap.

In this cross-sectional study, the MSOT method for monitoring muscle perfusion in a large group of PAD patients will be investigated for the first time to define threshold values. Patients of different stages of PAD will be examined in this study in case of previously performed angiography, which is the diagnostic gold standard and will serve as a reference. In addition, a healthy control group is included as a comparison group, but prior angiography is not mandatory in this group. A validation of the results will be performed on a group of patients to be examined and analyzed at a later time point.

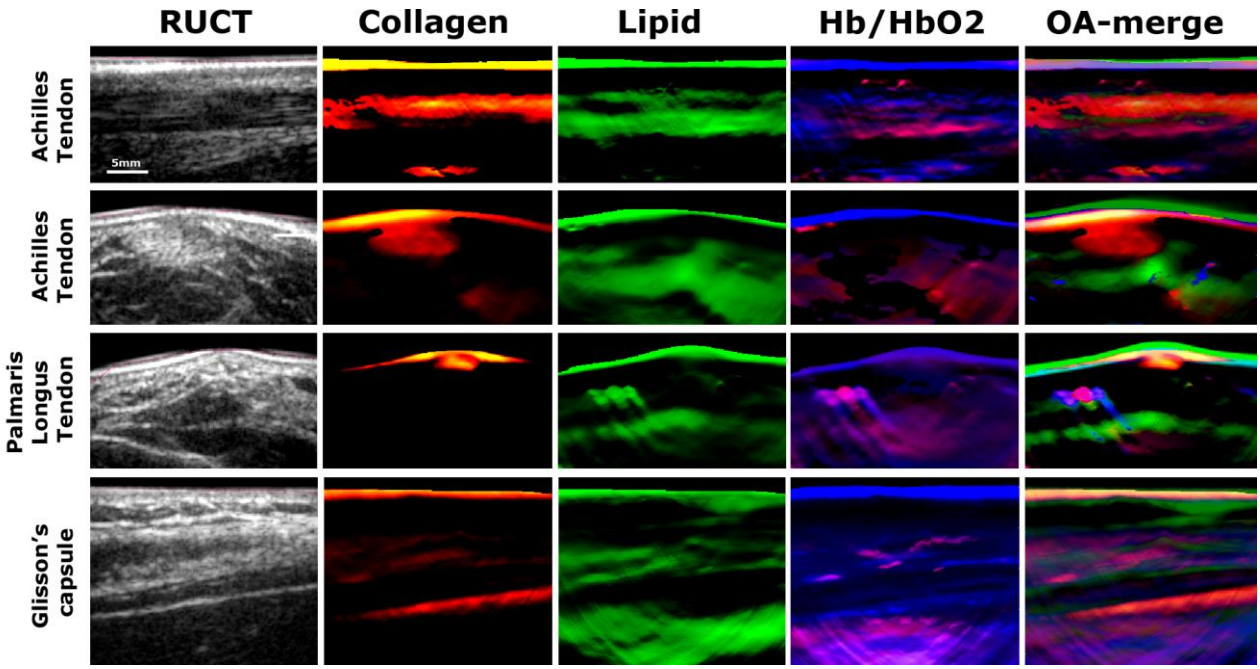

Figure 1: Visualization of the optoacoustic signal in a self-experiment

From left to right: B-scan ultrasound (b/w, RUCT), collagen signal (red, collagen), lipid signal (green, lipid), oxygenated/deoxygenated hemoglobin signal (blue-purple, Hb/HbO2), optoacoustic overlay (OA-merge). From top to bottom: Achilles tendon (longitudinal section), Achilles tendon (cross section), palmaris longus tendon, glisson capsule of liver. Note the clear signal of the connective tissue structures (tendon, capsule) in the collagen signal (red), and the surrounding fatty tissue in the lipid signal (green).

(not published original data Knieling&Waldner 2017)

Literature

1. Diehm, C., et al., *High prevalence of peripheral arterial disease and co-morbidity in 6880 primary care patients: cross-sectional study.* Atherosclerosis, 2004. **172**(1): p. 95-105.
2. Alpert, J.S., O.A. Larsen, and N.A. Lassen, *Exercise and intermittent claudication. Blood flow in the calf muscle during walking studied by the xenon-133 clearance method.* Circulation, 1969. **39**(3): p. 353-9.
3. Knieling, F., et al., *Multispectral Optoacoustic Tomography for Assessment of Crohn's Disease Activity.* N Engl J Med, 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 Study objectives

The aim of this study is to define an independent parameter for the diagnostic assessment of the perfusion situation of the calf muscle based on Multispectral Optoacoustic Tomography (MSOT) in a cross-sectional collective of healthy volunteers and patients with PAD in stages II-IV (study group 1). The gold standard is a previously performed angiography of the pelvic and femoral vessels based on routine diagnostics, this imaging is not mandatory for the healthy control collective. An independent validation group (study group 2) will validate the results found in study group 1.

5.1 Primary/secondary objectives and hypotheses

Primary hypothesis:

- Hemoglobin parameters in muscles of patients with PAD determined by MSOT (before and after active exercise) are suitable to predict relevant vascular stenosis and occlusion

Secondary hypotheses:

- The MSOT parameters for hemoglobin correlate with the perfusion parameters of the CCDS
- The MSOT parameters for hemoglobin correlate with the ABI
- The MSOT parameters for hemoglobin correlate with the walking distance
- The MSOT parameters for hemoglobin correlate with the clinical stage of PAD according to Fontaine and Rutherford

Primary objectives:

- Study group 1: Derivation of optimal diagnostic MSOT thresholds for hemoglobin through correlation with TASC classification1 for angiographic imaging (and CCDS if necessary) as references for relevant stenosis/occlusion in patients with PAD.

[1] Angiographic images are reviewed by two independent and experienced individuals to determine the type of TASC.
In the TASC classification for angiographic imaging, the pelvic and femoral arteries are assessed separately based on the extent and localization of the present stenoses/occlusions. However, the perfusion situation in the target region (calf muscle), that is measured by MSOT, results from a combination of both findings. In order to represent the circulation situation in the target muscle properly and to enable a correlation with the MSOT parameters measured there, it is necessary to regard the existing aorto-iliac and femoro-popliteal constellations of findings. For this purpose, 3 categories are established (1 – 3), that result from the occurring combinations of findings of both TASC classifications. The description of the categories can be found in the Statistical Analysis Plan (version 1.1, 01/20/2022).

- Study group 2: Validation of the diagnostic accuracy of MSOT, employing the cut-off values derived in study group 1, in terms of MSOT hemoglobin values through correlation with TASC classification for angiographic imaging as a reference of relevant stenosis/occlusion in patients with PAD in an independent cohort.

Secondary objectives:

- Determination of the quantitative fraction of oxygenated/deoxygenated/total hemoglobin and the MSOT-values at a wavelength of 800 nm in the area of the M. triceps surae before and after gait exposure determined by MSOT
- Correlation of the quantitative fraction of oxygenated/deoxygenated/total hemoglobin and the MSOT-values at a wavelength of 800 nm in the area of the M. triceps surae with the TASC classification in angiography
- Comparison of the difference between oxygenated and deoxygenated hemoglobin in the area of the M. triceps surae before and after gait exposure determined by MSOT
- Correlation of the difference between oxygenated and deoxygenated hemoglobin in the area of the M. triceps surae before and after gait exposure determined by MSOT with the TASC classification in angiography
- Determination of the quantitative lipid/ collagen signal fraction and the MSOT values at a wavelength of 930 and 1064 nm determined by MSOT
- Correlation of the flow profile and PSV of the CFA and A. poplitea determined by CCDS with the acquired MSOT parameters
- Correlation of the ABI with the acquired MSOT parameters
- Correlation of the walking distance with the acquired MSOT parameters
- Correlation of the clinical severity of PAD according to Fontaine and Rutherford classifications with the acquired MSOT parameters

5.2 Study type

Monocentric diagnostic cross-sectional study.

6 Target parameters

All measurements with MSOT are performed before and after muscle exposure in the area of the M. triceps surae. Eight MSOT wavelengths (715, 730, 760, 800, 850, 930, 1000 and 1064 nm) will be measured. For the investigation five calculated parameters

extracted from the measured wavelengths will also be taken into account (hb, hbO2, hbtot, collagen, lipid).

6.1 Primary target:

- Quantitative signal of hemoglobin (in arbitrary units)
    - *This target is measured non-invasively by MSOT*

6.2 Secondary targets:

- Oxygenation of the muscle, determination of oxygenated, deoxygenated hemoglobin, determination of lipid signal, determination of collagen signal
    - *These targets are measured non-invasively by MSOT*
- Flow profile of the A. femoralis communis and A. poplitea determined by CCDS
- ABI
- Actual walking distance standardized by treadmill examination
- Stage of PAD according to Fontaine and Rutherford
- Age, sex, risk factors for PAD
    - *These target values are either available in the electronic patient file or they are collected at presentation for the study.*
- Relevant underlying diseases
    - *This target values are either available in the electronic patient file or they are collected at presentation for the study.*
- Relevant previous operations
    - *This target values are either available in the electronic patient file or they are collected at presentation for the study.*
- Current medication
    - *This target value is either available in the electronic patient file or is collected at presentation for the study.*

7 Study design

7.1 Monocentric / multicentric

This is a monocentric diagnostically cross-sectional study with prospective data acquisition and validation of the data by means of a validation group.

7.2 Study arms: intervention/control

We do not plan interventions. The study will examine two study groups in series. First, the study group 1 will establish thresholds for hemoglobin values using MSOT. Then, in study group 2, the thresholds will be validated and the specificity and sensitivity will be defined.

7.3 Randomization

Randomization is not planned.

7.4 Blinding

The examiner is blinded during the measurement and evaluation of the data. The blinding of the examiner occurs because the examiner only performs the measurements but does not know the results of previous tests especially the results of the TASC classification of the previous performed angiography and the results of the CCDS.

Blinding of the patients/test persons is not necessary.

Figure 2 shows the graphic presentation of the study design.

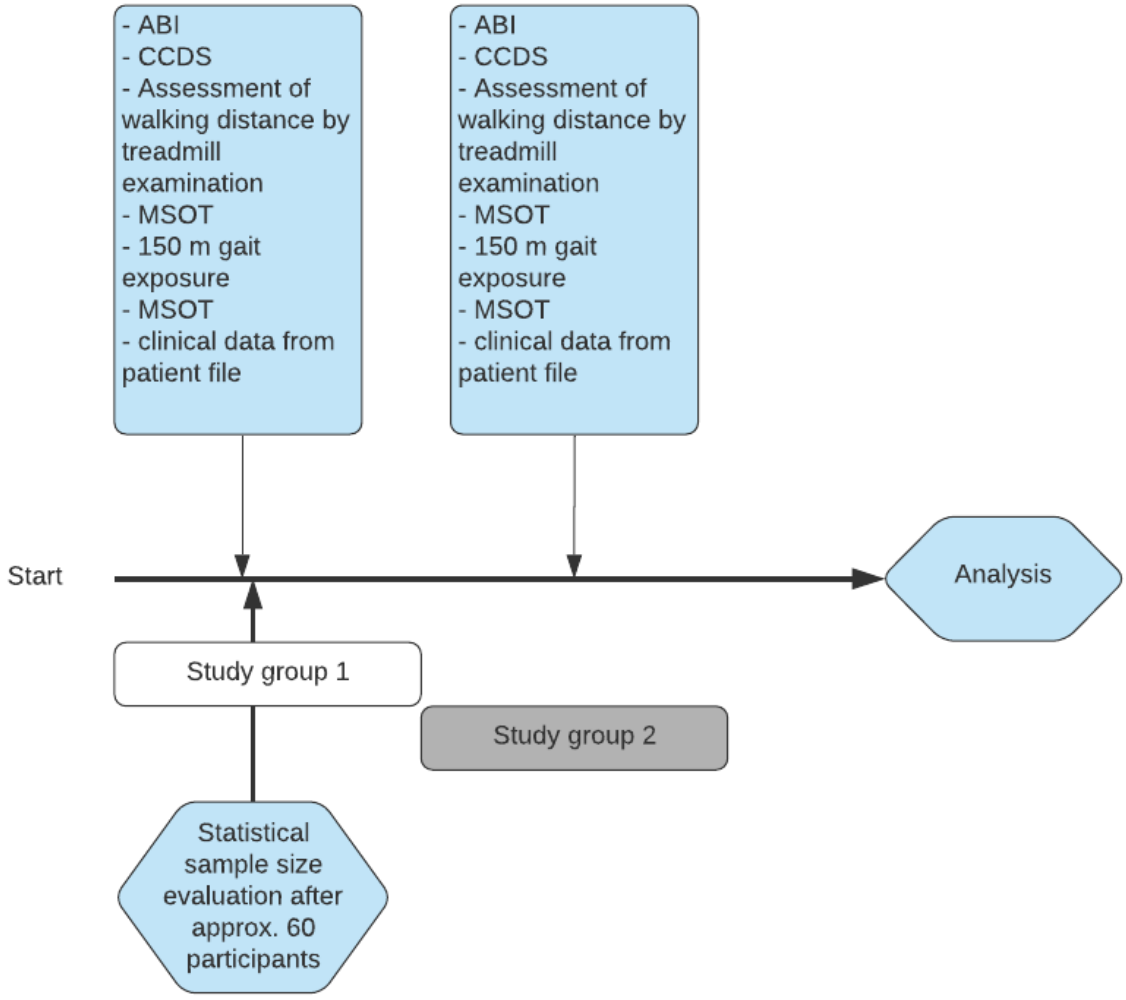

***Figure 2**: Graphical presentation of study design*

8 Study population

8.1 Inclusion and exclusion criteria

Inclusion criteria:

- Patients with manifest PAD stages II – IV according to Fontaine or category 1-6 according to Rutherford or healthy volunteer
- Adult (>18 years) persons who are able to give their consent
- Patients with manifest PAD in whom angiography has been performed as part of routine diagnostics (independent of the study) or in accordance with current guidelines, or has been indicated and the patient has given consent; healthy volunteers do not need a previous performed angiography

Definition of patient groups:

- PAD IIa with indication for angiography
- PAD IIb with indication for angiography
- PAD III with indication for angiography
- PAD IV with indication for angiography

Definition of healthy control 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 or category 0 according to Rutherford
- Healthy volunteers with previous known diabetes mellitus or chronic renal insufficiency or abnormal ABI
- Underage persons
- Missing consent form
- Patients with manifest PAD in whom angiography is not indicated
- 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 Participant number

A previous performed valid case number calculation based on the results of the pilot study was not possible due to the small number of patients and cohort studied (PAD IIb, healthy leg versus affected leg).

Based on the data collected until 05.03.2021 the case number calculation (no interim analysis) was performed in cooperation with the Institute of Biometry. Six of a total of 102 persons included up to the above-mentioned date were diagnosed with PAD stage III according to Fontaine. In the course of the study, it became apparent that a sample size calculation based on 30 patients with stage III disease and 30 healthy subjects, as initially planned, could not be realized during a realistic time period.

In order to establish a valid case number as early as possible, the two critical PAD stages III and IV were formally combined into one group to be considered together as chronic critical limb ischemia. Thus, based on the results of a total of 33 healthy study subjects and 29 subjects diagnosed with a critical PAD stage, the exemplary case number calculation could be accomplished. A power analysis, which allowed a power of 80% at an alpha of 0.05 (unilateral) was performed to determine the final sample size. For the MSOT parameter deoxygenated hemoglobin, the number of subjects was approximately 33, and for the MSOT parameter oxygenated hemoglobin, the number of subjects was approximately 11 for each of the individual groups (healthy control group, PAD stage II, PAD stage III-IV).

8.3 Recruitment routes and measures

Patients are informed about the possibility of participating in the study during a routine presentation of the vascular surgery consultation hours in case of previous performed or indicated angiography (existing signed consent form). Healthy volunteers will be acquired via posters in the clinic. If patients or volunteers are willing to participate, they will be fully informed about objectives and methods (especially about the scientific/explorative character of the study), benefits and risks, and the revocability of their participation in the study.

9 Study plan

9.1 Procedure for informing about and obtaining consent

Participants can only be included in the study after a written declaration of consent has been given. The written declaration of consent requires that the participants are informed orally and in written form about the objectives and methods (including the

scientific-explorative character of the study), benefits and risks as well as the revocability of their participation in the study.

It must be clearly communicated to the study participant that a withdrawal of consent is possible 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 original of the consent form is kept in the study folder at the study site. The participant receives a copy of the participant information and consent form. The participant information and the consent form for healthy volunteers and patients can be found in the appendix of this study protocol.

9.2 Measurements

After informing the subjects, the determination of underlying diseases, previous performed operations and current medication is performed. If available, this data is determined by the electronic patient file otherwise an anamnesis interview is performed. Underlying diseases, previous operations and medication according to the vascular system is therefore of special interest. The ankle-brachial index is then measured. This parameter is used in vascular medicine to estimate the severity of PAD. For healthy study participants, the measurement of the ABI is considered a screening examination. The participation of healthy subjects in the study requires an inconspicuous value in the ABI measurement (the handling of random findings in the screening process is described in Chapter 13). Prior to this measurement, a resting period of at least 10 minutes in a lying position should be ensured. Then, measurements of the occlusion pressures on both legs and arms are performed. The examination of the ABI lasts a maximum of 20 minutes including the preceding resting period and would also be recorded independently of the study participation during the stationary stay in the context of routine diagnostics in patients with PAD. Afterwards, color-coded duplex sonography of the femoral and popliteal arteries is performed at rest. The CCDS is used to determine the flow profile of the vessels examined and allows an assessment of constrictions or occlusions in these vessels. For this purpose the patient/proband remains lying on the examination couch. Depending on the examination conditions (individual course of the vessels, body size, etc.) this examination takes about 10 minutes. This examination takes place exclusively as part of the study participation and is not part of the routine vascular surgery diagnostics.

The actual current walking distance is used to classify the severity of PAD and will be recorded by a treadmill examination. Under medical supervision, the participants should walk the maximum possible distance on the treadmill at a 12% incline and 3 km/h (standard setting). Should the participant not be able to manage the incline, it will be reduced to 6%. Should that also be impossible, the incline will be reduced to 0%. The walking distance is measured in meters. Since the first 200 meters are of particular importance for the classification of PAD, the study physician terminates the examination when the walking distance exceeds 500 meters. The treadmill examination lasts about 15 minutes and is exclusively part of the study participation; it is not part of the vascular surgical routine diagnostics.

All examinations mentioned above take place in the vascular surgery department. Since the MSOT device is located in the endoscopy rooms of the internal medicine center, the participating persons are transported there by wheelchair after the previously mentioned examinations. It is important that no physical exercise is exerted during transport, as this could falsify the MSOT values. The transport takes about 15 minutes. Afterwards, the examination is performed in the area of the M. triceps surae (in patients in this area of the affected leg) with the MSOT device.

The MSOT examination is performed analogous to a sonography over the corresponding skin layers without further invasive procedures. The anatomical region can be localized by means of built-in B-image sonography; the corresponding optoacoustic signals can then be derived from this. The duration per anatomical region is limited to 5 minutes; participants can remain in a relaxed position during the examination; assistance in the form of breathing maneuvers or similar is not necessary. The position of the transducer is marked with a skin pencil so that the subsequent measurement can be taken over the same region of the muscle. Afterwards, the participant has to complete a defined walking distance of 150 meters, which takes place under medical supervision. This is followed by another measurement of the M. triceps surae using the MSOT. The pilot study showed that active muscle exercises between the two MSOT examinations causes a change in the perfusion parameters and may possibly unmask a stenosis in the lower leg area.

If the participant is unable to walk 150 meters between the two MSOT measurements due to previous illnesses or because of the severity of the PAD stage, the walking distance can be reduced in a graduated manner. The first step is to try to cover a

distance of 100 meters. If this is also impossible, the walking distance will be reduced to 50 and finally to 25 meters. In the case of a complete inability to walk, the muscle exposure can alternatively be provoked in a standing position. In this case, the participant should be instructed to change between tip-toe and heel position for at least two minutes. If this form of muscle activity is also impossible, patients can perform extensions and flexions of the foot for at least two minutes while sitting in order to provoke a muscle activity on the lower leg muscles between the two MSOT measurements.

The time required for both MSOT measurements including the muscle activity in between is about 30 minutes. This examination takes place exclusively as part of the study participation and is not part of routine vascular surgery diagnostics.

Independent of the results of this examination, the planned vascular surgery therapy for the patients with PAD (endovascular treatment/vascular surgery, conservative procedure) will be performed according to the guidelines.

Figure 3 shows the graphical presentation of the planned measures as well as their chronological sequence.

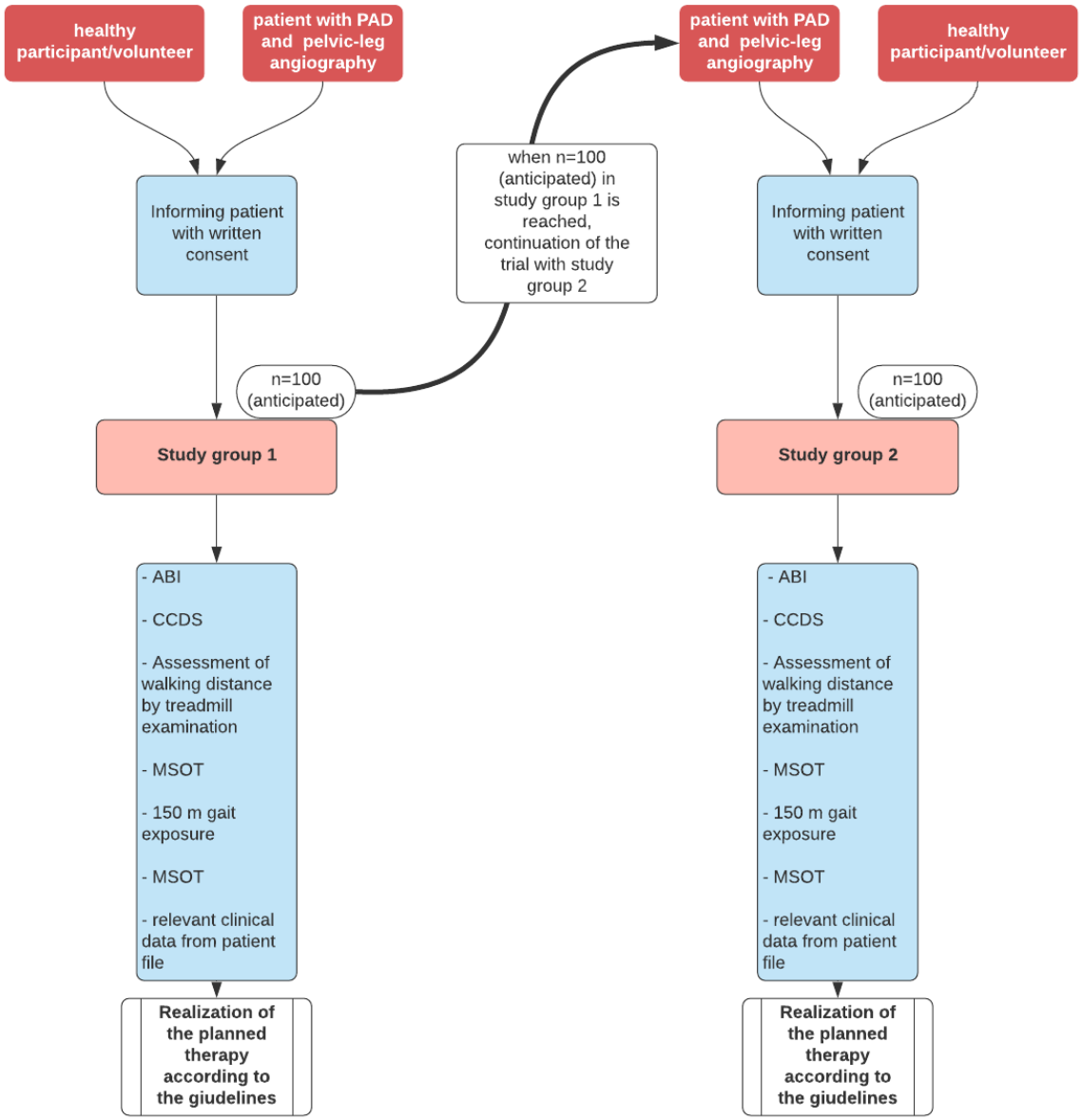

***Figure 3**: Graphical presentation of study plan*

9.3 Recording of target parameters

- Determination of flow profile of the A. femoralis communis and A. poplitea by CCDS
- Determination of ABI
- Assessment of walking distance by standardized treadmill examination (3km/h with 12% inclination)
- Determination of clinical PAD stage according to Fontaine and Rutherford
- Non-invasive in-vivo measurement by MSOT

- Determination of relevant clinical data: Age, Sex, risk factors for PAD, underlying diseases, previous operations and current medication
    - *These target values are either available in the electronic patient file or they are collected at presentation for the study.*

9.4 Total study duration

Corresponding to the number of patients, the expected total duration of the study until the inclusion of the last patient is approximately 12 months. Participation in the study takes approximately 1.5 hours per participant. For patients with PAD approximately 70 minutes are spent on examinations and transport, which only arise from participation in the study. Only one examination time point per patient will be needed.

10 Risk-benefit analysis

10.1 All study related risks

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

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

No CE certification is available for this research device (current type designation according to imprint: Acuity Echo). A conformity assessment procedure in the sense of the MPG is not intended or planned by the manufacturer as of now. It is therefore a purely scientific study. There is no dependency on the manufacturer; all diagnostic and analytical procedures are available to the study directors on site.

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 test person or 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 the MSOT can lead to retinal damage if the eye is irradiated. In order to prevent this, test 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 scientific study.

There are no further risks within the scope of this study, nor were additional risks observed based on the assessment of preliminary data.

10.2 Benefits associated with the study

The data obtained in the studies can provide essential information about the blood circulation and perfusion of the musculature in patients with PAD. It is therefore possible that these diagnostic procedures can be used in the future to better detect non-invasively critical blood flow situations and to derive therapy indications. Especially in groups of patients who can only be assessed inadequately or not at all using classical diagnostic methods.

10.3 Termination criteria

Termination criteria for the individual participant:

Participation in the study is discontinued if the skin becomes conspicuously warm or reddened. The examination time per anatomical region is limited to 5 minutes, so that these events are very unlikely.

In addition, participation in the study will be terminated if the above described muscle exercises between the two MSOT measurements cannot be performed, e.g. due to dyspnea, orthopedic disorders, etc.

Due to the short duration of the study participation, no other discontinuation criteria are planned.

Termination criteria for the whole study:

Termination of the entire study must be declared if significant and unobserved serious adverse health effects, which have not been known or observed so far, occur as a result of the MSOT investigation. This is considered unlikely, since more than 200 patients or test persons have already been examined with this method at the University Hospital Erlangen alone.

Otherwise, a termination of the entire study is not planned.

10.4 Statement on medical justifiability

Based on previous experience in adults the risk of occurrence of unwanted events is stratified as extremely low. In this study no central organs are examined, but only extremities are measured – this leads to a further significant reduction of a possible residual risk.

11 Biometrics, results pilot study

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

Results pilot study

The pilot study included seven patients. One leg with collateralized superficial femoral artery occlusion (group: "*occlusion"*) and the contralateral leg with open superficial femoral artery (group: "*open"*). The macrocirculation parameters examined showed a significant difference between the two groups (Ankle-Brachial Index). Transcutaneous examination of the M. triceps surae was possible in all cases using the MSOT procedure. Oxygenated hemoglobin (oxyHb) and total hemoglobin (totalHB) were found to be suitable parameters. Both active and passive muscle loading in the form of walking load and passive muscle loading (Whobbler©), respectively, showed a strong influence on muscle perfusion. This effect was more pronounced in the case of long-distance occlusive A. femoralis superficialis. After active walking, the "*open"* group showed a significant increase in the parameters (totalHB p=0.028, oxyHB p=0.028). This effect was not significant in the "*occlusion"* group (totalHB p=0.176, oxyHB p=0.398).

12 Data management and data protection

12.1 Data acquisition and storage

All raw data, such as patient files, are source documents. Their availability is ensured for routine monitoring. The participation of the individual patients or test persons in the study is documented. The study leader maintains an independent list for the identification of the participating patients. This list contains the names and date of birth as well as the date of examination and pseudonymization codes of the patients and subjects. The study leader is responsible for the quality of data collection and storage. The data storage (complete data) takes place on computers or specially designed network drivers of the University Hospital Erlangen.

12.2 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 Bundesdatenschutzgesetz (Federal Data Protection Act).

12.3 Data transfer

A transfer of the data or biological material is not intended in this study and will not take place; in particular, the manufacturer will not have access to the data. The study

results can be published anonymously, whereby it will not be possible to draw conclusions about the identity of the participating persons. The data will be kept for ten years and then will be destroyed.

12.4 Revocation, data deletion

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

13 Dealing with Random Findings in Healthy Subjects

The collection of all findings as well as the evaluation of the ABI and the images from the FKDS, are only for the purpose of this study and not for general or specific diagnostics. Evidence of a disease unknown to the subject may escape detection in these examinations. If abnormal findings are discovered during the scientific evaluation, a corresponding specialist will be consulted for the review and reporting of the values and images originally determined for research purposes. Whether a reportable finding is present is decided by the study leader according to his best judgment. The test person will be informed in advance about any disadvantages of a report in the written declaration of consent.

Such disadvantages are to be understood as such:

- Strong psychological stress due to the knowledge or suspicion of a threatening disease
- Disadvantages of certain legally significant acts, such as the conclusion of a life or health insurance policy or an employment contract
- Further examinations linked to the notification of findings that may pose a health risk, even if further processing means that the findings have no disease value.

After clarification with regard to the above-mentioned possible disadvantages caused by the notification of random findings within the framework of this study, the test person will inform in writing whether or not he/she wishes to be informed and advised about such abnormalities.

14 Handling of biomaterials

No biomaterials are obtained.

15 Insurance

A separate proband insurance is taken out under an annual contract with the insurer HDI Global SE, Düsseldorf Representative Office. The insurance is based on the agreements in accordance with the „Versicherung für nicht der Versicherungspflicht unterliegende klinische Prüfungen“(Insurance for clinical trials not subject to obligatory insurance).