Study Protocol

Clinicaltrials.gov Identifier:

MSOT_IC

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

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1 Title of the study, version number, version date, authors

1.1 Title of the study

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

1.2 Version number

Version 1.0

1.3 Version date

09.05.2022

1.4 Authors

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2 Summary of the project

Peripheral arterial occlusive disease (PAD) is one of the most common diseases in the elderly with an overall prevalence of approx. 3-10% and a prevalence of 15-20% among the >70-year-olds [1][2]. Given the increasing life expectancy there is a great need to revise and improve the existing diagnostic procedures and treatment concepts [2][3].

The only independent non-invasive validation measures for the various treatment options are currently the measurement of macrocirculation in form of color-coded duplex sonography (CCDS), the ankle-brachial index (ABI) and the measurement of the walking distance. The S3 guideline on diagnosis, therapy and follow-up of PAD published in 2015 by DGA (Deutsche Gesellschaft für Angiologie und Gefäßmedizin [German Society for Angiology and Vascular Medicine]) recommends follow-up care including clinical examinations, especially for patients after vascular interventions. However, for the validation measures already mentioned, there are significant patient collectives in which these methods provide only insufficient or unusable results (e.g. diabetes mellitus, end-stage renal failure). In these cases, the success of the therapy performed would have to be validated independently using angiography (digital subtraction angiography, CT angiography or MR angiography). However, due to the associated risks (e.g. exposure to radiation, administration of contrast agent, invasiveness), this is not done routinely. With multispectral optoacoustic tomography (MSOT) a new, non-invasive diagnostic method is now available that may help to close this diagnostic gap.

A previous study (MSOT_PAD) confirmed the hypothesis that data collected via MSOT can be used for PAD diagnostics. The concentration of oxygenated hemoglobin (HbO2) proved to be the most suitable measurement parameter. A connection could be established between the measured HbO2 concentration and the clinical stage of PAD. Differentiation improved after a standardized exercise of a walking distance of 150 meters, with patients in the stage of intermittent claudication (IC, Fontaine stage II) being more difficult to differentiate in comparison to patients in stages III and IV.

The aim of this cross-sectional study is to increase the sensitivity and specificity of the procedure for IC patients by using a more suitable exercise between first and second MSOT measurement, namely repeated heel raises until the occurrence of claudication pain.

Patients with intermittent claudication are especially interesting to be examined more closely because it could be crucial for early diagnosis, choice of therapy and monitoring of the success of the chosen therapy, to be able to directly examine the circulatory situation of the muscle as the primary target organ. So far there are no diagnostic options for this.

In order to more directly gain insights into a possible monitoring of therapy success, a subgroup of IC patients having been included in the study will be asked to undergo the study protocol a second time after interventional/surgical revascularization and will be examined again by MSOT.

3 Responsibilities

3.1 Study leader

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3.3 Funding

Department for Vascular Surgery; internal funding up until the possible granting of third-party funding.

4 Scientific background

Since 2021, the University Hospital Erlangen (department of pediatrics) disposes of a CE-certified multispectral optoacoustic tomograph (MSOT). Similar to sonography, the MSOT technology allows for non-invasive, quantitative imaging of the composition and also oxygenation of target tissues such as muscle.

Just like with conventional sonography, the MSOT transducer head is placed on the skin of the examined person above the target organ. But instead of sound waves, energy is applied to the tissue via flashes of laser light. This leads to constant changes of minimal expansions and contractions (thermoelastic expansion) of different tissue components or molecules. The same examination unit that sends in the laser flashes can also detect the emitted ultrasound waves. In the newly configured device (Acuity Echo, iThera Medical GmbH, Munich, CE-certified), an extended spectrum of laser light can be used, enabling among others the derivation of values corresponding to hemoglobin and its oxygenation levels. As hemoglobin concentration and oxygenation status are markers for perfusion, MSOT-based imaging of these parameters could be a highly sensitive and reliable method to analyze muscle perfusion. [5] Figure 1 shows example recordings of the MSOT technique of calf muscles.

These parameters have already been successfully measured, validated and reproduced in preliminary studies. Furthermore, a first internal study (MSOT_PAD, NCT04641091) confirmed the hypothesis that data collected via MSOT can be used for PAD diagnostics. The derived concentration of oxygenated hemoglobin (HbO2) proved to be the most suitable measurement parameter. Correlations could be established between the measured HbO2 concentration and the radiological and clinical severity of the PAD.

These results are very promising in terms of using the MSOT technique in diagnosis and monitoring of PAD. In particular, the establishment of this method would be of great value for the examination of PAD patients in whom, due to previous illness such as severe chronic renal insufficiency or diabetes mellitus, the measurement of the ABI or the CCDS examination do not provide valid results due to progressive stiffening of the vascular wall (Mönckeberg mediasclerosis). [3][4] This also applies to patients who, due to previous illness or operations, cannot perform treadmill exercise to determine the absolute walking distance. With MSOT, an objectifiable measurement method for non-invasive monitoring of the actual target organ muscle could be established for the first time, which would be especially helpful in the mentioned patient groups. Up until now there are no non-invasive

measurement methods other than MSOT that can make transcutaneous statements about muscle perfusion.

In the previous study (MSOT_PAD, NCT04641091), differentiability of clinical PAD stages improved after a standardized exercise of a walking distance of 150 meters, with patients in the stage of intermittent claudication (IC, Fontaine stage II) being harder to differentiate compared to patients in stage III and IV. For diagnostic methods that address macrocirculation (especially ABI and FKDS), provocation tests are routinely used in PAD diagnostics and already standardized. [6] As a rule, patients with PAD are stressed up to their personal stress limit (ischemia pain). The respective examination is then repeated afterwards. This increases the ability to differentiate macrocirculatory variables, especially for minor pathologies. We assume that this also applies to the hemoglobin concentrations (at the microcirculation level), even though up until now there are no measurement methods for this.

The MSOT technique, with its non-invasive, direct measurement of muscle perfusion, could prove to be particularly helpful for IC patients in terms of early diagnosis and follow-up. Where previously conservative therapy monitoring was based mainly on anamnesis, the MSOT technique could enable objective control and evaluation of the conservative and interventional/surgical treatment approaches. In the future this might entail further comparative outcome studies which might then even result in indication changes for the various therapeutic measures – which would improve the overall care situation of PAD patients.

In this cross-sectional study, a large group of IC patients will be examined, while sensitivity and specificity of the procedure shall be improved compared to the preliminary study by more suitable exercising of the calf muscle, namely repeated heel raises until the occurrence of claudication pain. A subgroup of the IC patients having been included in the study will be asked to undergo the study protocol a second time after interventional/ surgical revascularization. This is to investigate whether and how the improved blood flow situation translates to the measured MSOT parameters.

As an additional target variable, the PAD-specific quality of life will be recorded via the VASCUQOL-6 questionnaire. [7]

A healthy control collective is included as a comparison group. Validation of the findings will be done in a separate validation cohort.

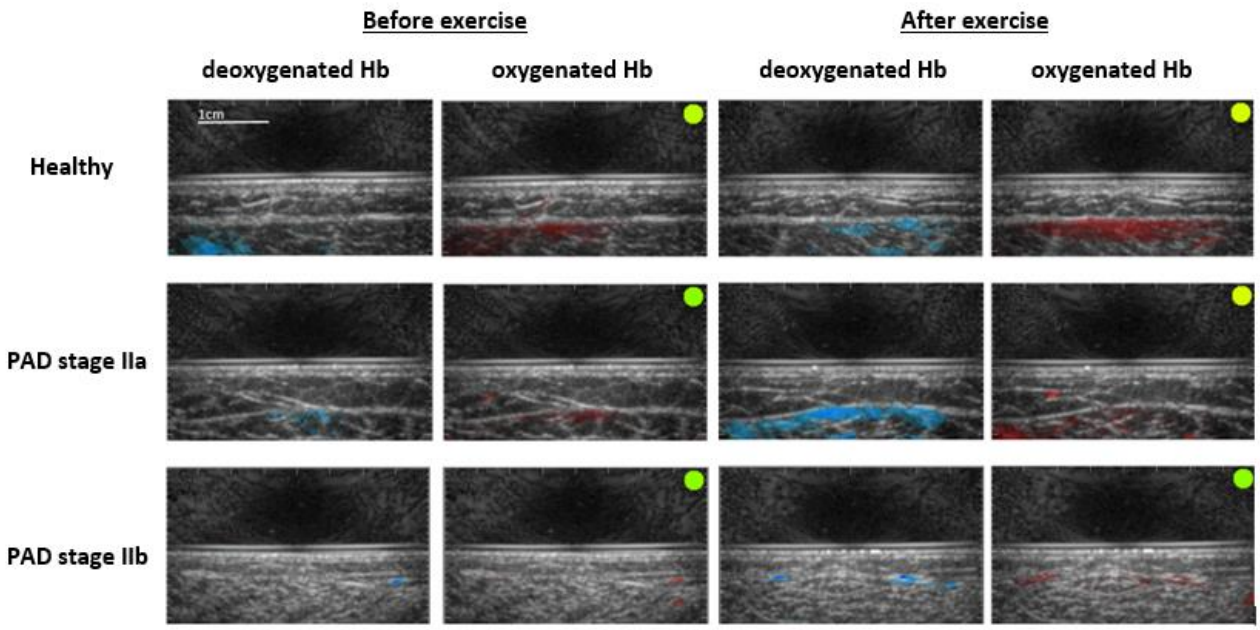


Figure 1: *Visualization of the optoacoustic hemoglobin signals (by PAD Fontaine stage)*
Visualization of the optoacoustic signals of the concentration of deoxygenated (blue) and oxygenated hemoglobin (red) before (the two columns on the left) and after exercise (the two columns on the right) in different PAD stages (top: healthy subject, middle: PAD patient in stage IIa , bottom: PAD patient in stage IIb).
The optoacoustic signals are superimposed on the B-sonography image. In the lower halves of the images, the subcutaneous layer (no optoacoustic signal) and the muscle fascia with the underlying muscle (from much optoacoustic signal to little optoacoustic signal, depending on the PAD stage) are visible. The optoacoustic HbO2 signal decreases with increasing stage, especially in the post-exercise images.
(unpublished original data Günther 2022)

Literature

1. Lawall, H., Diehm, C., Hoffmann, U., & Reinecke, H. (2015). *Periphere arterielle Verschlusskrankheit: Epidemiologie, Komorbidität und Prognose.* DMW-Deutsche Medizinische Wochenschrift, 140(24), 1798-1802.
2. Lawall, H., Huppert, P., Espinola-Klein, C., Zemmrich, C. S., & Ruemenapf, G. (2017). *German guideline on the diagnosis and treatment of peripheral artery disease–a comprehensive update 2016.* Vasa, 46(2), 79-86.
3. 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.
4. Lanzer, P. (2018). *Primary media sclerosis Mönckeberg: Diagnostic criteria*. Cor et Vasa, 60(2), e205-e208.
5. Karlas, A., Masthoff, M., Kallmayer, M., Helfen, A., Bariotakis, M., Fasoula, N. A., ... & Wildgruber, M. (2021). *Multispectral optoacoustic tomography of peripheral arterial disease based on muscle hemoglobin gradients—a pilot clinical study*. Annals of Translational Medicine, 9(1).
6. Lawall, H., P. Huppert, and G. Rümenapf. *"S3-Leitlinie zur Diagnostik, Therapie und Nachsorge der peripheren arteriellen Verschlusskrankheit."* Deutsche Gesellschaft für Angiologie und Deutsche Gesellschaft für Gefäßmedizin 30 (2015).
7. Larsen, A. S. F., Reiersen, A. T., Jacobsen, M. B., Kløw, N. E., Nordanstig, J., Morgan, M., & Wesche, J. (2017). *Validation of the Vascular Quality of Life Questionnaire–6 for clinical use in patients with lower limb peripheral arterial disease.* Health and quality of life outcomes, 15(1), 1-10.

5 Objectives of the study

The objective of the proposed study is to define independent parameters for the diagnostic assessment of the perfusion situation of the calf muscle based on multispectral optoacoustic tomography (MSOT) in a cross-sectional collective of patients with PAD in the stage of intermittent claudication (IC, Fontaine II) and a healthy control collective (study group 1). The results will be validated using an independent validation group (study group 2).

5.1 Primary/secondary objectives and hypotheses

Primary hypothesis:

- The quantitative MSOT value for oxygenated hemoglobin in the calf muscle of PAD patients in the claudication stage before and after active exercise until the onset of claudication pain is suitable for distinguishing a diseased from a healthy collective.

Secondary hypotheses:

- The MSOT reperfusion profiles (i.e. the curves of hemoglobin-associated MSOT parameters in the first ten minutes after exercise) of PAD patients in the claudication stage show certain patterns and differ significantly from the reperfusion profiles of healthy subjects.
- The collected MSOT parameters and their reperfusion profiles correlate with the perfusion parameters from the CCDS.
- The collected MSOT parameters and their reperfusion profiles correlate with the ABI.
- The recorded MSOT parameters and their reperfusion profiles correlate with the relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain) of the patient.
- The recorded MSOT parameters and their reperfusion profiles correlate with the total distance reached in the 6-minute walk test.
- The recorded MSOT parameters and their reperfusion profiles correlate with the subjectively perceived maximum walking distance achieved in everyday life.
- The recorded MSOT parameters and their reperfusion profiles correlate with the perceived PAD-specific quality of life (VASCUQOL-6 questionnaire).
- The collected MSOT parameters and their reperfusion profiles correlate with the angiographic severity of the PAD disease.
  [If angiographic imaging is already available due to routine diagnostics independently from this study.]

- Subgroup analysis: The MSOT parameters measured again after revascularization intervention show characteristic changes compared to the measurements before intervention
- Subgroup analysis: The MSOT parameters measured again after revascularization intervention show tendencies towards the MSOT parameters of healthy subjects compared to the measurements before intervention
- Subgroup analysis: The reperfusion profiles of MSOT parameters measured again after revascularization intervention show characteristic changes compared to the measurements before intervention
- Subgroup analysis: The reperfusion profiles of MSOT parameters measured again after revascularization intervention show tendencies towards the reperfusion profiles of healthy subjects compared to the measurements before intervention

Primary objectives:

- Study group 1: Derivation of optimal diagnostic MSOT threshold values regarding oxygenated hemoglobin after exercise in claudication patients and a healthy control group
- Study group 2: Validation of the diagnostic accuracy of MSOT, employing the cut-off values derived in study group 1, regarding oxygenated hemoglobin after exercise in claudication patients and a healthy control group in an independent cohort

Secondary objectives:

- Recording of all other hemoglobin-associated parameters in the calf muscle before and after repetitive heel raises using MSOT
- Recording of the reperfusion profiles (i.e. the curves of hemoglobin-associated parameters in the first ten minutes after exercise) and determination of general characteristics of the curves to distinguish between PAD legs and healthy legs
- Recording of the flow profiles and PSVs of the A. femoralis communis and A. poplitea using CCDS and correlation with the collected MSOT parameters
- Recording of the ABI and correlation with the collected MSOT parameters
- Recording of the relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain) and correlation with the collected MSOT parameters
- Recording of the maximum walking distance in the 6MWT and correlation with the collected MSOT parameters
- Recording of the subjectively perceived maximum walking distance in everyday life and correlation with the collected MSOT parameters
- Recording of the perceived PAD-specific quality of life (VASCUQOL-6 questionnaire) and correlation with the collected MSOT parameters

- Recording of the radiological degree of severity and correlation with the collected MSOT parameters

  [If angiographic imaging is already available due to routine diagnostics independently from this study.]

- Subgroup analysis: re-recording of all parameters listed here after revascularization intervention

5.2 Study type

Monocentric diagnostic cross-sectional study.

6 Target parameters

The measurements with MSOT are each carried out before and after exercise, in the area of the M. triceps surae. MSOT wavelengths are examined in the range from 700 to 1300 nm.

6.1 Primary target:

- Quantitative signal of oxygenated hemoglobin (in arbitrary units)

  [This target is measured non-invasively via MSOT.]

6.2 Secondary targets:

- Detection of oxygenated and deoxygenated hemoglobin, total hemoglobin, oxygen saturation and the wavelengths 700 to 1300 nm (in arbitrary units)

  [This target is measured non-invasively via MSOT.]

- Recording of the flow profiles and PSVs of the A. femoralis communis and A. poplitea using CCDS
- Registration of the ABI
- Recording of the relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain)
- Recording of the maximum walking distance in the 6-minute walk test
- Recording of the subjectively perceived maximum walking distance in everyday life
- Recording of the currently perceived PAD-specific quality of life (VASCUQOL-6 questionnaire)
- Recording of relevant secondary diseases from the patient file
- Recording of relevant previous operations from the patient file
- Recording of the current medication from the patient file
- Recording of the radiological severity (MRA, CTA, DSA)

  [If angiographic imaging is already available due to routine diagnostics and independently of the study described here.]

- Subgroup analysis: re-recording of all parameters listed here after revascularization intervention

7 Study design

Graphic depiction of the study design in Figure 2 on page 13.

7.1 Monocentric / multicentric

This is a monocentric diagnostic 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 any interventions. The study will examine two study groups in series. First, 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 determined. For a subgroup analysis, some of the IC patients having been included in the study will be reexamined after revascularization intervention.

7.3 Randomization

There is no randomization planned.

7.4 Blinding

The examiner is blinded when performing the MSOT measurement and evaluating the MSOT data. The examiner is blinded by the fact that the examiner only carries out the measurements, but does not know the results and in particular does not know the classification of the angiography (in case there is angiographic imaging available independent from this study). Blinding of the patients/subjects is not necessary.

8 Study population

8.1 Inclusion and exclusion criteria

Statistical analysis of the data of the previous study (MSOT_PAD, NCT04641091) suggests a necessary number of approximately 100 participants in total (minimum 48 participants, maximum 124 participants, see below). This is planning with similar group sizes for IC patients and healthy volunteers. Age should be similar in the investigated groups.

Definition of patient groups:

- PAD IIa
- PAD IIb

Definition of healthy control group:

- no PAD previously known

- no diabetes mellitus previously known
- no chronic renal insufficiency previously known
- no symptoms of intermittent claudication
- ABI with values between 0.9 and 1.4 (screening examination before inclusion in the study)
- palpable foot pulses

Inclusion Criteria

- Patients with manifest PAD in stage II according to Fontaine or categories 1-3 according to Rutherford or healthy volunteers
- Adults (>18 years) who are able to give their consent

Exclusion Criteria

- Patients with PAD stage I, III and IV according to Fontaine or categories 0, 4, 5 and 6 according to Rutherford or healthy volunteers with diabetes mellitus, chronic renal failure, claudication symptoms, abnormal ABI or non-palpable foot pulses
- Underage persons
- Lack of written consent
- Safety concerns of the study physician (person with physical, mental or psychiatric conditions which, by the judgement of the study physician, would compromise the person's safety or the quality of the data, thereby rendering the person an ineligible candidate for the study

8.2 Expected sample size

Statistical sample size calculation based on the results of the previous study (MSOT_PAD, NCT04641091) suggests a necessary study collective of at least 48 to a maximum of 124 subjects/patients. The two-sample t-test power calculation with a power of 0.8 gave an n of about 12 for each group (study group 1 - healthy volunteers, study group 1 - claudication patients, study group 2 - healthy volunteers, study group 2 - claudication patients). Additional sample size planning based on the AUC, which should be at least 0.7 for a relevant significance, gave an n of 31 subjects/patients for each group (with a significance level of 0.05 and a power of 0.8).

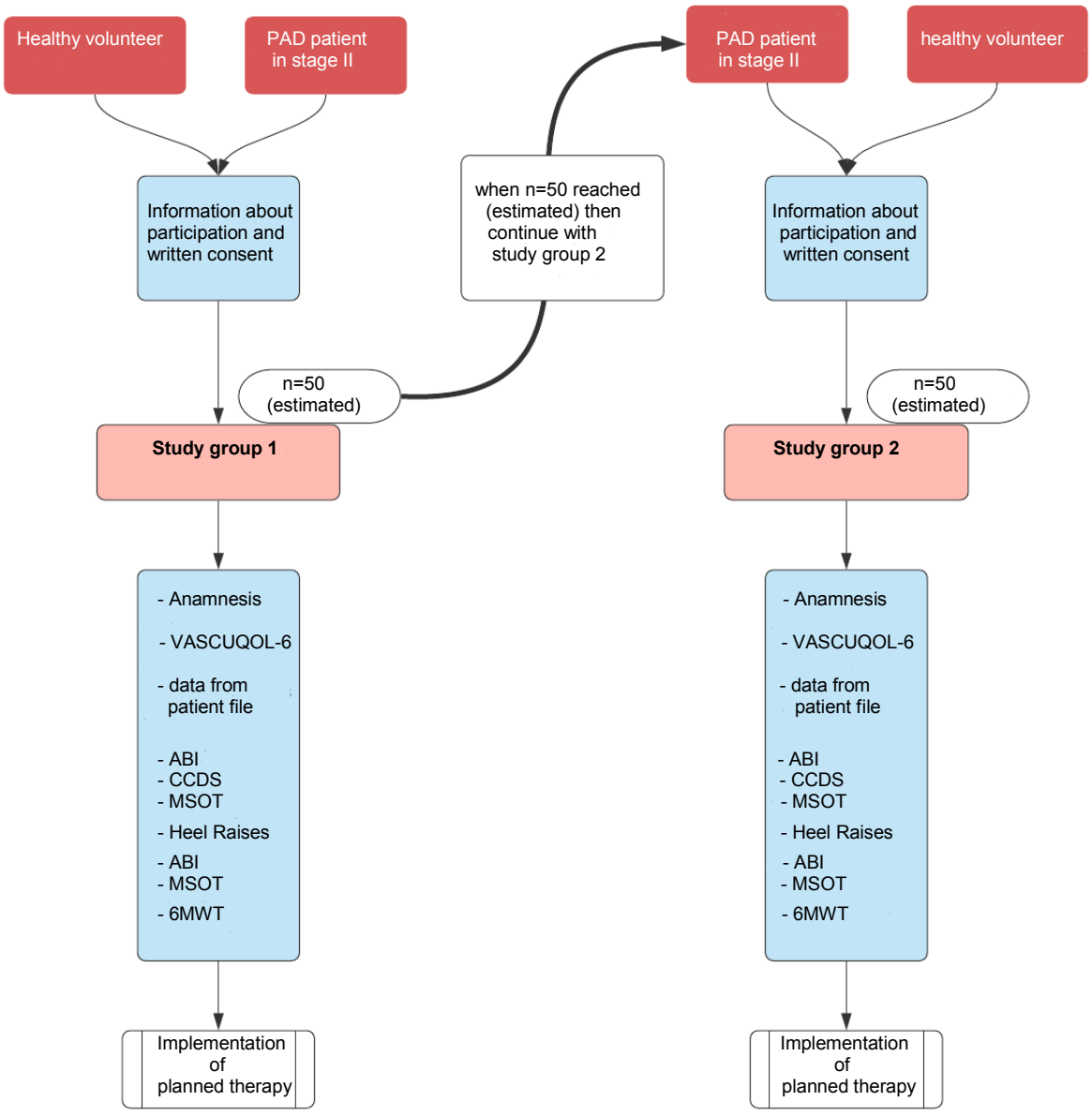
These calculations were based on data comparing healthy subjects with patients in stage IIb who, however, had not been adequately exercised (all only with the same walking distance of 150 meters). Due to the hypothesis of the study that the ability to differentiate between healthy subjects and claudication patients can be increased by adequate provocation testing, the sample size estimated here on the basis of the previous data is to be regarded as conservative. For this reason, after the inclusion of the first 25 patients and the first 25 healthy test persons, the necessary total

sample size will be reevaluated in cooperation with the Institute for Biometry – on the basis of the data collected up to that point.

Dropout is not considered because the study is performed during a single visit.

8.3 Recruiting

Patients will be informed about the possibility of participating in the study when presenting during the consultation hours of the vascular surgery department. Healthy volunteers will be recruited via notices posted in the clinic. Persons interested in participating in the planned study will be fully informed about the goals and methods (especially the scientific/exploratory nature of the study), the benefits and risks and the revocability of their participation in the study



.Figure 2: Graphical representation of study design and study flow

9 Study plan

9.1 Procedure for informing and obtaining consent

Patients or healthy volunteers can only be included in the study after having given written informed consent. The written informed consent requires an oral and written explanation to the patients about the aims and methods (incl. scientific-explorative character of the study), benefits and risks as well as the revocability of their participation.

It will be clearly communicated to the study participant that withdrawal of consent is possible at any time and without any disadvantage. Furthermore, all study participants will be advised that this study is a purely scientific study without any present diagnostic or therapeutic benefit.

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

9.2 Procedures and measurements

After having been fully informed and after having given written consent, all study participants will be asked to provide information on their preexisting conditions, previous operations and current medication. If available, the electronic patient file will be used for this purpose; otherwise, these data will be collected in the anamnesis interview. Particularly interesting are diseases, operations and medication related to the vascular system. Approximately 15 minutes are estimated for information and anamnesis interview.
Furthermore, the subjectively perceived maximum walking distance until the first stopping due to pain in everyday life is to be queried and a questionnaire on the PAD-specific quality of life is to be completed (VASCUQOL-6), which will take about ten minutes.
All other measures will take place in the sonography room in the department of pediatrics, which has been adapted for the use of the MSOT system. Participants will be brought to this room by wheelchair from the department of vascular surgery. It is important that there is no physical exertion during the transport, as this could falsify the measured values. The transport takes about 15 minutes. On site, the participant should lie down on the examination couch and rest for another 15 minutes. Then the first oscillometric measurement of the ankle-brachial index (ABI) is performed. For this purpose, blood pressure cuffs are placed on the participant's arms and legs. The ABI 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 normal values in the ABI measurement (the handling of incidental findings in screening is presented in Chapter 13). The oscillometric measurement of the ABI takes only about two minutes. In patients, the measurement of the ABI would also be performed independently of study participation as part of routine diagnostics during the inpatient stay.

After the first measurement of the ABI, color-coded duplex sonography (CCDS) of the femoral arteries is performed at rest. The CCDS is used to determine the flow profiles of the examined vessels and allows an assessment of constrictions or occlusions in these vessels. During this examination, the participant remains lying on the examination couch. Depending on the examination conditions (individual anatomy of the vessels, body size, etc.), this examination takes about ten minutes. This examination takes place exclusively for the purpose of this study and is not part of routine diagnostics for patients.

During the 15 minutes of the initial resting phase, the optimal position on the M. triceps surae muscle for the placement of the MSOT probe can already be determined (by means of built-in B-scan sonography). In case of strong hair growth in the examined area above the calf muscle, this area of approx. 5cm x 10cm will be shaved using a medical single-use razor.

After ABI and CCDS the first measurement with the MSOT device is performed over the previously selected position (for patients in the more severely affected leg). The examination is analogous to sonography over the corresponding skin layers without further invasive procedures. The measurement duration per anatomical region is limited to ten minutes; the study participants can remain in a relaxed posture during the examination; assistance in the form of breathing maneuvers or the kind is not necessary.

Following these initial measurements patients are asked to do a heel raise exercise to explicitly exercise the M. triceps surae. The participants should do repeated heel raises until their calf muscles are exhausted. For both IC patients and healthy volunteers this means the occurrence of pain in the calf muscle. All participants will be motivated to continue the heel raises over at least 30 seconds to ensure a minimum exercise time for the muscle. During the exercise the participants should fix themselves with their hands to the wall or door frame for safety.

After the exercise, the participant should lie back down on the examination couch in prone position as quickly as possible and the second measurement of both the ABI and the MSOT parameters after exercise is performed. The MSOT parameters are recorded sequentially every 30 seconds over five minutes and then every minute for another five minutes to possibly record characteristic reperfusion profiles of the muscle after exercise.

In the end, after a resting pause of at least 15 minutes, a 6-minute walk test (6MWT) is performed in the hallway of the department of pediatrics. A course of 30 meters is marked, with additional markings every two meters. The participant is to walk as far as possible along this course (turning around every 30 meters) within six minutes. Breaks and changes of pace are allowed, but the patient is told that he should not take his first break until the claudication pain forces him to stop. That way, in addition to the total walking distance achieved within the six minutes, the actual maximum walking distance until the onset of claudication pain is recorded. In addition, the patient is asked to indicate the first occurrence of pain during the walking test, and the walking distance completed up to that point is also noted.

The time required for the CCDS and the three MSOT measurements, including the rest period prior to the start of the measurements, is approximately 60 minutes. These examinations take place exclusively in the context of study participation and are not part of routine diagnostics.
Together with information and written consent, medical history, quality of life questionnaire and transport to the pediatrics department, the estimated time required for all procedures performed will be max. 90 minutes.
Afterwards, regardless of the results of the study measurements, the guideline-driven planned vascular surgical therapy will take place for the respective patient (endovascular treatment/vascular surgery, conservative procedure).
Figure 2 on page 13 shows the graphical representation of the planned measures, as well as their chronological sequence.
For the subgroup analysis, for which some of the included IC patients will be re-examined after revascularization intervention, the procedures described here will be performed again in exactly the same way. Only the anamnesis and the quality of life questionnaire can be omitted, reducing the total duration of the examinations by 15-20 minutes.

9.3 Recording of target parameters

- Non-invasive MSOT measurements
- Collection of relevant clinical data from patient file
- Recording of the flow profiles of A. femoralis communis and A. poplitea using CCDS
- Recording of ABI before and after exercise
- Recording of the relative (until the first occurrence of pain) and absolute walking distance (until the stopping due to pain)
- Recording of the maximum walking distance in the 6-minute walk test
- Recording of the subjectively perceived maximum walking distance in everyday life
- Recording of the currently perceived PAD-specific quality of life (VASCUQOL-6 questionnaire)
- Recording of the radiological severity of the disease (MRA, CTA, DSA)
  [If angiographic imaging is already available due to routine diagnostics independent from this study.]

9.4 Total duration of the study

According to the expected required number of study participants, the expected total duration of the study until the inclusion of the last study participant is approximately six months. Participation in the study is approximately 90 minutes per study participant. Only one examination 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 opto-acoustic system corresponds to Class IIa:

- active diagnostic device
- non-invasive
- temporary application (<60 min)

A CE certification is available for this research device (current type designation according to the label: Acuity Echo).

Adherence to energy limits

Laser safety and maximum radiation dose for exposure to laser pulses is regulated in the ANSI and IEC 60825 laser standards. The MSOT system meets these standards and thus stays below the MPE (maximal permissible exposure) limits for skin exposure and is therefore considered safe.

Tissue temperature increases due to MSOT

Optoacoustic imaging does not result in any relevant increase in temperature in the tissue. The absorption of one laser pulse results in a transient local increase in tissue temperature of a few milli-Kelvin. Depending on the duration of the examination and the skin type of the patient, the temperature typically increases by less than one Kelvin.

Histological changes in the tissue

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

Slight, reversible reddening or warming of the skin is only to be expected in the case of very sensitive skin.

In case of these rare events, they can be noticed at any time by the study participant or the examining physician; the examination can then immediately be interrupted or aborted. In no case is there irreversible damage to be expected.

In principle, the near-infrared light used in MSOT can lead to retinal damage when the eye is irradiated. To prevent this, study participants and examiners will wear safety goggles at all times during the examination.

Since the data obtained via MSOT will not be used for diagnosis or therapy planning and also not in interpretation of other diagnostic findings, there is no risk of possible misdiagnosis, even if mistakes would occur.

10.2 Benefits associated with the study

The data obtained in the study can provide essential information about the blood circulation and perfusion in muscle of IC patients. It is therefore possible that these diagnostic procedures can be used in the future to better non-invasively detect 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 terminated if the skin becomes noticeably warm or reddened. The examination time per anatomical region is limited to three times five minutes, so that these events are very unlikely.

In addition, participation in the study will be discontinued if the exercises between the MSOT measurements which are described above cannot be carried out, e.g. due to dyspnea, orthopedic diseases, etc.

Due to the short duration of study participation, no other termination criteria are provided.

Termination criteria for the entire study:

The entire study must be terminated if previously unknown and unobserved significant and harmful side effects occur through the MSOT examination. This is considered unlikely, as more than 400 patients or volunteers have already been examined with this method at the University Hospital in Erlangen alone.

Otherwise, termination of the entire study is not provided.

10.4 Statement concerning medical responsibilty

Based on previous experience with MSOT examinations in adult patients, the risk of adverse events occurring is considered to be extremely low. No central organs are examined in this study, measurements are carried out on the extremities only - this leads to a further significant reduction in a possible residual risk.

11 Results of preliminary study, biometrics

Results of the preliminary study (MSOT_PAD):

In the preliminary study (MSOT_PAD), a total of 59 healthy volunteers and 138 PAD patients in Fontaine stages II, III and IV were examined using MSOT in a very similar study design.

Measurements were taken at rest and after a standardized exercise of a walking distance of 150 meters. For the derivation and validation of meaningful measurement parameters, the study participants were divided into a derivation cohort (31 healthy subjects and 70 PAD patients) and a validation cohort (28 healthy subjects and 68 PAD patients). The presence of angiographic imaging as part of the routine examinations was an inclusion criterion for the participating PAD patients of all PAD stages. The classification of severity of the angiographic images was correlated to the measured hemoglobin-associated MSOT parameters before and after the exercise.

It could be shown that the parameter for oxygenated hemoglobin derived by MSOT correlates with the angiographic severity of the disease and is suitable as a diagnostic biomarker for hemodynamically and clinically relevant arterial occlusions. PAD patients in stages III and IV according to Fontaine could be clearly distinguished from healthy subjects. For patients in the claudication stage (stage II according to Fontaine), however, the differentiability was not satisfyingly sufficient. Here, the present study project hopes for greater accuracy through a more suitable exercise for this patient group between the MSOT measurements.

Statistical methodology:

The data will be displayed as mean values with standard deviation. Correlations will be given using the non-parametric Spearman correlation coefficient (Rs). According to the distribution of the groups, the differences in the mean values are examined statically using a non-parametric test (Wilcoxon test). For all analyses, an error level of <0.05 is considered statistically significant.

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 healthy volunteers in the study is documented. The study leader keeps an independent list for the identification of the participating persons. This list contains the names and datess of birth as well as the dates of examination and pseudonymization codes of the patients and healthy volunteers. 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 materials obtained is not intended in this study and will not take place; in particular, the manufacturer oft the MSOT device 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 will then be destroyed

12.4 Revocation, data deletion

If the declaration of consent is revoked, data collected up to this point can be taken into account and can be further stored. However, the patient has the right to request their destruction, unless legal provisions prevent such destruction.

13 Handling of incidental findings in healthy volunteers

All findings including the evaluation of the ABI and the images from the CCDS, are only collected for the purpose of this study and not for the purpose of 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 specialist will be consulted for the review and evaluation of the data and images originally determined for research purposes. Whether reportable findings are present is decided by the study leader according to his best judgment. The participant will be informed in advance about any disadvantages of getting informed in case of such incidental findings – orally and 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 findings that may pose a health risk, even if further investigation shows that the original findings had no pathological value

After having been informed about above-mentioned possible disadvantages caused by the information about incidental findings during this study, the participant will give a written declaration whether he/she wishes to be informed about such abnormalities.

14 Handling of biomaterials

No biomaterials are obtained.

15 Insurance

A separate study participant insurance is taken out under an annual contract with the insurer HDI Global SE, Düsseldorf 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“).