Statistical Analysis Plan

Trial Short Title	MSOT_IC
Trial Full Title	Cross-sectional study of calf muscle perfusion in patients with intermittent claudication by non-invasive Multispectral Optoacoustic Tomography
ClinicalTrials.gov Identifier	
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1 Version History

Version	Changes	Authors
1.0		PD Dr. med. Ulrich Rother, Julius Kempf, Milenko Caranovic

2 List of Abbreviations and Definition of Terms

6MWT ..... 6 Minute Walk Test
AAA ..... abdominal aortic aneurysm
ABI ..... ankle-brachial index
CCDS ..... color-coded vascular duplexsonography
CFA ..... common femoral artery
CHD ..... coronary heart disease
CHF ..... chronic heart failure
CIA ..... common iliac artery
CKD ..... chronic kidney disease
CLTI ..... chronic limb threatening ischemia
DGA ..... Deutsche Gesellschaft für Angiologie (German Society for Angiology)
EIA ..... external iliac artery
Hb ..... deoxygenated hemoglobin
HbO2 ..... oxygenated hemoglobin
Hbtot ..... total hemoglobin
IC ..... intermittent claudication
IIA..... internal iliac artery
MSOT ..... Multispectral Optoacustic Tomography
PAD ..... peripheral aterial occlusive disease
PSV ..... peak systolic velocity
SFA ..... superficial femoral artery
TASC ..... Trans Atlantic Intersociety Consensus
TS ..... triceps surae muscle

3 Introduction

3.1 Background

PAD is one of the most common diseases of the elderly. As life expectancy increases, there is a growing need for new treatment concepts and new diagnostic procedures.

Up to now, only the measurement of macrocirculation in the form of CCDS, ABI and the measurement of the actual walking distance are available as independent validation measures of revascularization methods (endovascular/open). The S3 guideline for diagnosis, therapy and medical aftercare of PAD published in 2015 by the DGA recommends such aftercare

examinations, especially for patients that underwent vascular surgery. However, for the mentioned validation measures there are some 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 success of the chosen therapy 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).

MSOT provides a new non-invasive diagnostic method that may be able to fill this diagnostic gap. The technique is based on the photoacoustic effect. Flashes of laser light are sent through the skin into the target tissue. This leads to minimal changes in thermoelastic expansion and contraction of different tissue components or molecules, which causes the emission of ultrasound waves. 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, custom-made, 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.
A first study (MSOT_PAD, NCT04641091) confirmed the hypothesis that data collected via MSOT examination of the calf muscle can be used for PAD diagnostics. The derived 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 Fontaine stages III and IV.

3.2 Purpose of the study

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 success monitoring of the 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. Where previously conservative therapy monitoring was mainly based on anamnesis, MSOT could enable objective control and

evaluation of 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.

As additional target variables, the relative, absolute and total walking distance in a 6-minute walk test will be recorded as well as the PAD-specific quality of life (via the VASCUQOL-6 questionnaire).

A healthy control collective is included as a comparison group.

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 then be examined again with MSOT.

4 Hypothesis, Study Objectives and Endpoints

4.1 Hypothesis to be statistically tested

The hypothesis is that the MSOT parameters associated with hemoglobin in the calf muscle after a heel raise exercise can predict peripheral artery disease in the intermittent claudication stadium.

 H0: The MSOT parameters associated with hemoglobin in the calf muscle after a heel raise exercise do not differ between healthy subjects and patients with PAD in the intermittent claudication stadium.

4.2 Objectives

Primary objectives

- Study group 1: Derivation of optimal diagnostic thresholds for hemoglobin-associated MSOT parameters after a heel raise 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 hemoglobin-associated MSOT parameters after a heel raise exercise in claudication patients and a healthy control group in an independent cohort

Secondary objectives

- Recording of the reperfusion profiles of the hemoglobin-associated MSOT parameters (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 before and after the heel raise exercise 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) in the 6MWT 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

4.3 Endpoints

Primary endpoint

- Optimal diagnostic thresholds for hemoglobin-associated MSOT parameters before and after a heel raise exercise [Time Frame: single time point (1 day)]: optimal diagnostic thresholds for hemoglobin-associated MSOT parameters in calf muscle tissue in patients with intermittent claudication before and after a heel raise exercise

Secondary endpoints

- Difference between the corresponding MSOT values before and after exercise [Time Frame: single time point (1 day)]: Difference of the values before and after exercise for hemoglobin-associated parameters derived by transcutaneous MSOT in patients with IC
- Reperfusion profiles of hemoglobin-associated MSOT parameters (i.e. the curves of hemoglobin-associated MSOT parameters in the first ten minutes after exercise)
- Correlation of acquired MSOT parameters with the CCDS flow profiles and PSVs [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT in patients with IC correlated with the flow profiles and PSVs of A. femoralis communis and A. poplitea determined by CCDS
- Correlation of acquired MSOT parameters with the ABI [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT correlated with the ABI measurements
- Correlation of acquired MSOT parameters with relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain) determined during the 6MWT [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT correlated with relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain)
- Correlation of acquired MSOT parameters with maximum walking distance in the 6MWT [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT correlated with the maximum walking distance in the 6MWT
- Correlation of acquired MSOT parameters with the subjectively perceived maximum walking distance in everyday life [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT correlated with the subjectively perceived maximum walking distance in everyday life
- Correlation of acquired MSOT parameters with the perceived PAD-specific quality of life (VASCUQOL-6 questionnaire) [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT correlated with the perceived PAD-specific quality of life (VASCUQOL-6 questionnaire)

- Correlation of the acquired MSOT parameters with the TASC-classification (angiography) [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT in patients with IC correlated with the TASC-classification (angiography)
  [If angiographic imaging is already available due to routine diagnostics independently from this study.]
- Subgroup analysis: Difference between the corresponding MSOT values before and after revascularization intervention [Time Frame: two time points (2 days)]: Difference of the values before and after revascularization intervention for hemoglobin-associated parameters derived by transcutaneous MSOT in patients with IC
- Subgroup analysis: Reperfusion profiles of hemoglobin-associated MSOT parameters (i.e. the curves of hemoglobin-associated MSOT parameters in the first ten minutes after exercise) after revascularization intervention
- Subgroup analysis: Correlation of acquired MSOT parameters after intervention with the CCDS flow profiles and PSVs after intervention [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT in patients with IC after revascularization intervention correlated with the flow profiles and PSVs of A. femoralis communis and A. poplitea determined by CCDS after revascularization intervention
- Subgroup analysis: Correlation of acquired MSOT parameters after intervention with the ABI after intervention [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT after revascularization intervention correlated with the ABI measurements after revascularization intervention
- Subgroup analysis: Correlation of acquired MSOT parameters after intervention with relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain) determined during the 6MWT after intervention [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT after revascularization intervention correlated with relative (until the first occurrence of pain) and absolute walking distance (until the first stopping due to pain) in the 6MWT after revascularization intervention
- Subgroup analysis: Correlation of acquired MSOT parameters after intervention with maximum walking distance in the 6MWT after intervention [Time Frame: single time point (1 day)]: hemoglobin-associated MSOT parameters (units: arbitrary units (a.u.)) derived by transcutaneous MSOT after revascularization intervention correlated with the maximum walking distance in the 6MWT after revascularization intervention

5 Study Methods

5.1 General Study Design and Plan

This is a monocentric, prospective cross-sectional study which aims to compare the optoacoustic signals in calf muscle after exercise in patients with intermittent claudication (PAD in Fontaine stage II) and a healthy control group in order to define MSOT thresholds. MSOT data will be correlated with CCDS, ABI, relative and absolute walking distance, maximum walking distance in 6MWT, subjectively perceived absolute walking distance in everyday life, PAD related life quality (VASCUQOL-6) and TASC II classification in angiographic imaging (only in case angiographic imaging is available independent from this study).

Patients with intermittent claudication will be recruited during the consulting hours of the Department of Vascular Surgery, University Hospital Erlangen. Healthy volunteers will be acquired via posters in the clinic. Following detailed information about the study and after providing written consent, relevant clinical data will be collected from the electronical patient file, if available. Otherwise a thorough anamnesis interview for relevant data is performed. The CCDS, ABI and the 6MWT will be performed. Afterwards the MSOT parameters will be recorded before and after a heel raise exercise until the occurrence of claudication pain in the calf muscle. In patients, the more affected leg is examined. In healthy volunteers, also only one leg is examined. All data will be adequately pseudonymized in compliance with data protection regulations before they are used for statistical analysis. The duration of the study for participants will be max. 90 minutes. The angiographic imaging will be reviewed and analyzed (if available independently from this study) and if the interval between MSOT measurement and angiography is less than 12 months. The complete study including the validation of the data by an independent study group is expected to be finished within one year. For a subgroup analysis, some of the IC patients having been included in the study will be reexamined after revascularization intervention.

5.2 Study Population

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). 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)

5.3 Randomization and Blinding

Randomization is not applicable in the chosen study design. Investigators for angiography and CCDS are blinded to index test (MSOT) results. An independent reader blinded to angiographic TASC-classification and CCDS measures performs data analysis for index test (MSOT).

5.4 Study Variables

	Visit	Annotations
Index test (MSOT)[1]	X	Before and after exercise
Angiography[2]		Considered only if angiographic imaging already available in the electronic patient file due to routine diagnostics independently from this study and if less than 12 months to the date of index test; Evaluated by two independent examiners
CCDS[3]	X	Whether a flow-relevant stenosis is present in the examined vessels is decided by an independent reader
ABI[4]	X	
Clinical scores[5]	X	

1 **Index Test (MSOT):**

Signal levels for oxygenated hemoglobin (HbO2), deoxygenated hemoglobin (Hb), total hemoglobin, oxygen saturation and single wavelengths from 700nm to 1300nm in arbitrary units (a.u.)

2 **Angiography**:

TASC II classification femoro-popliteal PAD:

Type-A:
- *circumscript stenosis ≤ 10 cm or closure ≤ 5 cm length*

Type-B:
- *several hemodynamically relevant findings (stenoses or occlusions), each ≤ 5 cm*
- *single stenosis or occlusion ≤ 15 cm length, not involving the infrageniculate popliteal artery*
- *single or multiple findings in peripheral vascular occlusion to improve inflow for distal bypass*
- *heavily calcified occlusion ≤ 5 cm length*
- *single popliteal stenosis*

Type-C:
- *multiple stenoses or occlusions with a total ≥15 cm vessel involvement, with or without severe calcification*
- *Recurrent stenoses or occlusions requiring treatment after two endovascular interventions*

Type-D:
- *chronic total occlusion of the CFA or the SFA (>20 cm) with affection of the arteria poplitea*
- *chronic complete occlusion of the popliteal artery and proximal trifurcation*

TASC II classification aorto-iliac PAD:

Type-A:
- *ini-/bilateral stenoses of CIA*
- *uni-/bilateral single stenosis ≤ 3 cm of EIA*

Type-B:
- *≤ 3 cm stenosis of infrarenal aorta*
- *unilateral CIA occlusion*
- *single or multiple stenosis totaling 3-10 cm involving the EIA but not extending into CFA*
- *unilateral EIA occlusion not involving IIA or CFA*

Type-C:
- *bilateral CIA occlusions*
- *bilateral EIA stenoses 3-10 cm long but not extending into CFA*
- *unilateral EIA stenosis extending into CFA*
- *unilateral EIA occlusion that involves the IIA and/or CFA*
- *heavily calcified unilateral EIA occlusion with or without involving IIA and/or CFA*

Type-D:
- *infrarenal aortoiliacal occlusion*
- *diffuse disease involving aorta and both CIA requiring treatment*
- *diffuse multiple stenoses involvin unilateral CIA, EIA and CFA*
- *Unilateral occlusion of both CIA and EIA*
- *bilateral occlusion of EIA*
- *iliac stenoses with AAA requiring treatment (not amenable to endograft placement or other lesions requiring open aortic/iliac surgery*

Formed subgroups (aggregated TASC [aTASC]) combining TASC types according to severity and theoretical collateralization capability

aTASC 1: Healthy aTASC Type:
- *In the absence of angiographic imaging in the HV and otherwise normal findings in the medical history, ABI, and CCDS, HV are classified as aTASC 1*
- *individuals with unremarkable radiological findings according to TASC in each of the aortoiliacal (AI), femoropopliteal (FP) and infrapopliteal (IP) levels*

aTASC 2: aTASC type with good chances for collateralization:
- *due to mild findings in AI levels and any findings in FP levels which is considered a potential for collaterals through A. profunda branches.*

aTASC 3: aTASC type with poor chances for collateralization:
- *all combination of AI and FP TASC patterns with theoretically poor chances for collateralization. Here severe findings at AI level are summarized because of poor collateralization capability via A. profunda branches.*

aTASC 1	aTASC 2	aTASC 3
combination of 1. no AI TASC findings 2. no FP TASC findings 3. no IP TASC findings	combination of 1. AI no TASC or TASC A/B 2. FP no TASC or TASC A/B/C/D 3. any IP TASC findings 4. at least TASC A/B in 1. or 2.	combination of 1. AI TASC C/D 2. FP no TASC or TASC A/B/C/D 3. any IP TASC findings

3 **CCDS**:

Morphological flow profile and PSV of A. femoralis communis and A. poplitea

4 **ABI**:

Assessment of ABI value	
no PAD	0.9-1.4
mild PAD	0.75-0.9
moderate PAD	0.5-0.75
severe PAD	<0.5
mediasclerosis	>1.4

5 **Clinical scores**:

Relative and absolute walking distance determined in 6MWT
Total achieved walking distance in the 6MWT
Subjectively perceived absolute walking distance in everyday life
Perceived PAD-specific quality of life (VASCUQOL-6 questionnaire)
Fontaine classification
- I=No symptoms [not included in this study]
- IIa=painless walking distance >200 m, IIb=painless walking distance <200 m
- III=pain while resting [not included in this study]
- IV=trophic disorder [not included in this study]

Rutherford classification
- 0=No symptoms [not included in this study]
- 1=mild claudication
- 2=moderate claudication
- 3=severe claudication
- 4=ischemic rest pain [not included in this study]
- 5=minor tissue loss (nonhealing ulcer, focal gangrene and diffuse pedal ischemia) [not included in this study]
- 6=major tissue loss (extending above transmetatarsal level, foot no longer salvageable) [not included in this study]

6 Determination of 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.

7 General Considerations

7.1 Timing of Analysis

The final analysis will be performed after the last patient entered the study. Study collectives for study group 1 (derivation of thresholds) and study group 2 (validation of thresholds) will be handled separately.

7.2 Analysis Population

We will describe all screened and enrolled subjects who fulfil all inclusion criteria and fail all exclusion criteria.

7.3 Subgroups

Subpopulations are defined by the reference standard as follows: clinical according to Fontaine and TASC classification. If necessary and in order to confirm a flow relevant stenosis CCDS.

7.4 Missing Data

For the primary endpoint, single missing values will have no consequence. If missing, the individual subject will be excluded for the specific sub-analysis.

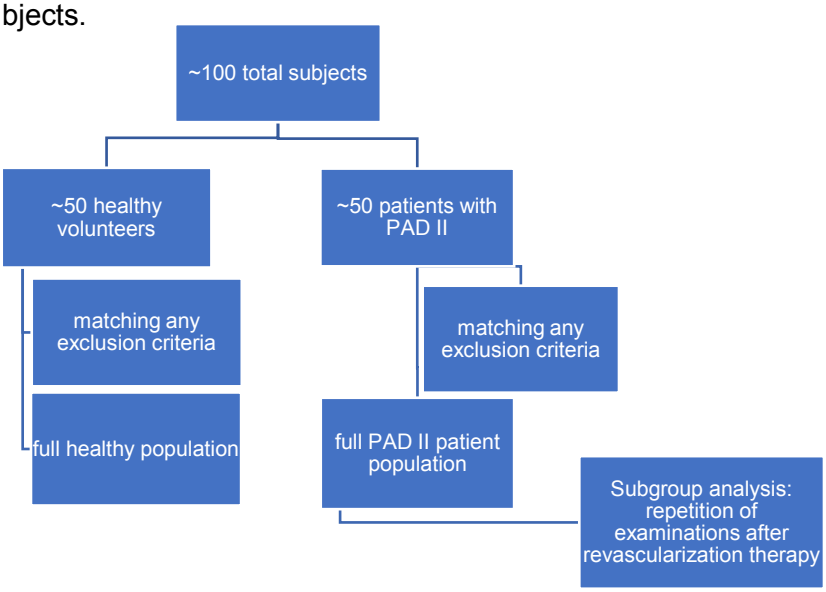
8 Summary of Study Data

Information on continuous variables will be given as means and standard deviations.

Categorical variables will be summarized as frequencies or percentages of observed levels.

8.1 Subject Disposition

Flow of study subjects.



8.2 Protocol Deviations

Protocol deviations that influence the analysis and consequences:

- Not fulfilling inclusion criteria ○ Subjects will not be included in the full analysis population
- Withdrawal of consent ○ Subjects will not be included in the full analysis population
- Withdrawal of consent to read medical file ○ Subjects will not be included in the full analysis population
- Drop-out for other reasons ○ Subjects will not be included in the full analysis population
- Single missing values ○ Subjects will be included in the full analysis population

8.3 Clinical Investigation Plan Deviations

Data will be analyzed according to the Statistical Analysis Plan; any further/additional/deviation from the Statistical Analysis Plan will be reported as such.

8.4 Baseline Variables

- Age
- Gender
- Affected leg/examined leg
- Risk factors for PAD (smoking, arterial hypertension, lipid abnormalities, diabetes mellitus, obesity, positive family history)
- Relevant underlying diseases (CHD, CHF, atrial fibrillation, CKD, carotid artery stenosis, previous cerebrovascular event)
- Previous surgery or interventions on the arterial vessels of the lower extremity
- Current medication
- Date of last angiography

9 Diagnostic Performance Analysis

The distribution of single MSOT readouts after exercise and the distribution of difference values of the MSOT readouts after and before exercise will be assessed and statistically tested as possible criteria to determine PAD affected legs.

9.1 Correlations

The association and correlation, respectively, of MSOT parameters versus clinical/angiographic outcomes (secondary endpoints) is calculated by appropriate measures of association and correlation, e.g. differences in mean, or Pearson's or Spearman's rank correlation coefficients.

9.2 Sensitivity and Specificity

Using the cutpoint maximising the Youden index derived from study group 1 based on healthy volunteers vs. IC patients, the diagnostic properties of MSOT values will be illustrated by sensitivity and specificity (with 95% confidence interval) associated with a diagnostic test decision dichotomised at that cutpoint. The same cutpoint will be employed in study group 2 for validation of diagnostic properties.

10 Reporting Convention

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". Non-significant P-values (>0.05) will be reported to two decimal places. The mean and standard deviation will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to three significant figures.

11 Technical Details

All analyses are performed using IBM SPSS Statistics, version 24 or newer (IBM Corp., N.Y., USA) and R Statistics, version 3.6 or newer.

12 Sample Tables for Data Reporting

12.1 Demographic data PAD II patients in study groups 1 and 2

Baseline characteristics-patients with PAD II	
Age	Mean ±SD
Sex	n (% of total n)
Risk factors Smoking, arterial hypertension, lipid abnormalities, diabetes mellitus, obesity, positive family history	n (% of total n)
Relevant underlying diseases CHD, CHF, atrial fibrillation, CKD, carotid artery stenosis, previous cerebrovascular event	n (% of total n)
Previous vascular surgery/intervention	n (% of total n)
Current Medication	n (% of total n)
Clinical scoring ABI Pulse status lower extremity Fontaine stage, Rutherford stage	 Mean ±SD n (% of total n) n (% of total n)
CCDS Morphological flow profile, Presence of relevant stenosis PSV	 n (% of total n) Mean ±SD
Angiography TASC score	 n (% of total n)
MSOT MSOT values for oxygenated/deoxygenated/total hemoglobin, oxygen saturation and single wavelengths from 700nm to 1300nm Difference values (“deltas”) between respective MSOT values before and after exercise	 Mean ±SD Mean ±SD
Walking distances Relative walking distance in 6MWT (until first occurrence of pain) Absolute walking distance in 6MWT (until first stopping because of pain) Total walking distance in 6MWT (total walking distance within the 6 minutes)	 Mean ± SD Mean ± SD Mean ± SD
Anamnestic values Subjectively perceived absolute walking distance in everyday life Score in VASCUQOL-6 questionnaire	 Mean ± SD Mean ± SD

12.2 Demographic data healthy volunteers in study groups 1 and 2

Baseline characteristics – healthy volunteers	
Age	Mean ±SD
Sex	n (% of total n)

Risk factors Smoking, arterial hypertension, lipid abnormalities, diabetes mellitus, obesity, positive familiar history	n (% of total n)
Relevant underlying diseases CHD, CHF, atrial fibrillation, CKD, carotid artery stenosis, previous cerebrovascular event	n (% of total n)
Previous vascular surgery/intervention	n (% of total n)
Current Medication	n (% of total n)
Clinical scoring ABI Pulse status lower extremity	 Mean ±SD n (% of total n)
Fontaine stage, Rutherford stage	n (% of total n)
CCDS Morphological flow profile, Presence of relevant stenosis PSV	 n (% of total n) Mean ±SD
MSOT MSOT values for oxygenated/deoxygenated/total hemoglobin, oxygen saturation and single wavelengths from 700nm to 1300nm Difference values (“deltas”) between respective MSOT values before and after exercise	 Mean ±SD Mean ±SD
Walking distances Relative walking distance in 6MWT (until first occurrence of pain) Absolute walking distance in 6MWT (until first stopping because of pain) Total walking distance in 6MWT (total walking distance within the 6 minutes	 Mean ± SD Mean ± SD Mean ± SD
Anamnestic values Subjectively perceived absolute walking distance in everyday life Score in VASCUQOL-6 questionnaire	 Mean ± SD Mean ± SD

12.3 Data correlation PAD II patients in study groups 1 and 2

All MSOT parameters have two values: “*PRE*” means before exercise and “*POST*” means after exercise. If not explicitly mentioned, the following assumes the analysis of both values.

Parameters	Hbtot	HbO2	Hb	Difference HbO2/Hb	Oxygen saturation	Single wave-lengths from 700nm to 1300nm
Angiography TASC score	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation
CCDS Morphological flow profile, relevant stenosis PSV	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation

Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation

12.4 Data correlation healthy volunteers in study groups 1 and 2

All MSOT parameters have two values: "*Pre*" means before exercise and "*Post*" means after exercise. If not explicitly mentioned, the following assumes the analysis of both values.

Parameters	Hbtot	HbO2	Hb	Difference HbO2/Hb	Oxygen saturation	Single wave-lengths from 700nm to 1300nm
CCDS Morphological flow profile, relevant stenosis PSV	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	Correlation	Correlation	Correlation	Correlation	Correlation	Correlation

12.5 Samples for PAD II clinical scoring

Parameters	Healthy	PAD II
Angiography TASC score	*Value not collected*	n (% of total n)
CCDS Morphological flow profile, relevant stenosis PSV	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	Mean ±SD	Mean ±SD
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	Mean ±SD	Mean ±SD
Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	Mean ±SD	Mean ±SD

Parameters	Sensitivity	Specificity
Angiography TASC score	%	%
CCDS Morphological flow profile, relevant stenosis PSV	%	%
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	%	%
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	%	%

Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	%	%

12.6 Samples for PAD angiographic scoring

Severity of stenosis – grouping according to combined TASC subgroups

Parameters	I	II	III	IV	p-value	ANOVA
CCDS Morphological flow profile, relevant stenosis PSV	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		

Severity of stenosis – grouping according to combined TASC subgroups

Parameters	Sensitivity	Specificity
CCDS Morphological flow profile, relevant stenosis PSV	%	%
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	%	%
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	%	%
Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	%	%

12.7 Samples for PAD CCDS scoring

Stenosis severity – grouping according to CCDS scoring

Parameters	CCDS relevant stenosis	CCDS no relevant stenosis	p-value	ANOVA
Angiography TASC score	Mean ±SD	Mean ±SD		
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	Mean ±SD	Mean ±SD		
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	Mean ±SD	Mean ±SD		

Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	Mean ±SD	Mean ±SD		

Stenosis severity – grouping according to CCDS scoring

Parameters	Sensitivity	Specificity
Clinical scoring ABI Absolute/relative walking distance Total walking distance in 6MWT	%	%
Anamnestic scoring Subjectively perceived absolute walking distance Score in VASCUQOL-6 questionnaire	%	%
Angiography TASC score	%	%
Hbtot_PRE HbO2_PRE Hb_PRE Difference_ HbO2_Pre/Hb_PRE Oxygen_Saturation_PRE Single_Wavelengths_700nm_PRE - Single_Wavelengths_1300nm_PRE Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Oxygen saturation Post Single_Wavelengths_700nm_POST - Single_Wavelengths_1300nm_POST	%	%