Statistical Analysis Plan

Trial Short Title	MSOT_PAD
Trial full Title	Cross-sectional study of muscle perfusion in patients with PAD by non-invasive Multispectral Optoacoustic Tomography.
ClinicalTrials.gov Identifier	NCT04641091
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Document Version	Version 1.1
Document Version Date	01/20/2022

Table of Contents

1 Version History

Version	Changes	Authors
1.1	See summary_of_changes_SAP.pdf	PD Dr. med. Ulrich Rother, Josefine Günther, Anna Träger

2 List of Abbreviations and Definition of Terms

AAA ..... *Siehe* abdominal aortic aneurysm
ABI ..... *Siehe* Ankle-Brachial Index
CCDS ..... *Siehe* color-coded vascular duplexsonography
CFA ..... *Siehe* Common femoral artery
CHD ..... *Siehe* coronary heart disease
CHF ..... *Siehe* chronic heart failure
CIA ..... *Siehe* common iliac artery
CKD ..... *Siehe* chronic kidney disease
CLTI ..... *Siehe* chronic limb threatening ischemia
DGA ..... *Siehe* Deutsche Gesellschaft für Angiologie (German Society for Angiology)
EIA ..... *Siehe* external iliac artery
Hb ..... *Siehe* deoxygenated hemoglobin
HbO2 ..... *Siehe* oxygenated hemoglobin
Hbtot ..... *Siehe* total hemoglobin
IIA..... *Siehe* internal iliac artery
MSOT ..... Siehe Multispectral Optoacustic Tomography
PAD ..... *Siehe* Peripheral aterial occlusive disease
PSV ..... *Siehe* Peak-Systolic-Velocity
SFA ..... *Siehe* superficial femoral artery
TASC ..... *Siehe* Trans Atlantic Intersociety Consensus
TS ..... *Siehe* triceps surae muscle

3 Introduction

3.1 Preface

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

Up to now, only the measurement of macrocirculation in the form of the CCDS, the 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 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 success of the therapy performed 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 now provides a new non-invasive diagnostic option that may be able to fill this diagnostic gap. The technique is based on the photoacoustic effect whereby energy by means of light flashes is applied to the tissue. 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. 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. 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.
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 have already been routinely examined in angiography in advance, will be included. In parallel, we will investigate a healthy control group, whereby these volunteers do not need a prior angiography. A validation of the results will be performed on a group of patients and healthy volunteers to be examined and analyzed afterwards.

3.2 Purpose of the Analysis

This analysis will assess capability of MSOT in comparison to the gold standard (angiography) and the clinical PAD stage as a reference standard to predict the severity of PAD. If necessary, the angiographic findings will be combined with the CCDS measures in order to define a flow relevant stenosis.

4 Hypothesis, Study Objectives and Endpoints

4.1 Hypothesis to be statistically tested

The hypothesis is that MSOT can predict clinical and angiographic disease severity in patients with PAD.

 H0: MSOT parameters are not associated with angiographic manifestations

 H0: MSOT parameters do not differ between healthy subjects and patients with PAD (graded according to angiographic classifications)

4.2 Objectives

Primary objectives

- Study group 1: Derivation of optimal diagnostic MSOT thresholds for hemoglobin through correlation with TASC classification[1] for angiographic imaging (and CCDS if necessary) as references for relevant stenosis/occlusion in patients with PAD
- 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 for 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 TS 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 TS determined by MSOT with the TASC-classification in angiography
- Comparison of the difference between oxygenated and deoxygenated hemoglobin in the area of the TS before and after gait exposure determined by MSOT
- Correlation of the difference between oxygenated and deoxygenated hemoglobin in the area of the TS before and after gait exposure determined by MSOT with the TASCclassification in angiography

[1] Angiographic images are reviewed by two independent and experienced individuals to determine the type of TASC.

- 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 determined by treadmill examination with the acquired MSOT parameters
- Correlation of the clinical severity of PAD according to the Fontaine and Rutherford classifications with the acquired MSOT parameters

4.3 Endpoints

Primary endpoint

- Optimal diagnostic MSOT thresholds [Time Frame: single time point (1 day)]: Optimal diagnostic thresholds for hemoglobin values in muscle tissue determined by MSOT in patients with PAD

Secondary endpoints

- Muscular oxygenated/deoxygenated/total hemoglobin content and MSOT-values at a wavelength of 800 nm before and after gait exposure [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin signal and signal at a wavelength of 800 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with PAD before gait exposure compared to the signals after gait exposure
- Correlation of oxygenated/deoxygenated/total hemoglobin content and MSOT-values at a wavelength of 800 nm with the TASC-classification (angiography) [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin signal and signal at a wavelength of 800 nm(Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with PAD correlated with the TASC-classification (angiography)
- Difference between oxygenated/deoxygenated hemoglobin before and after gait exposure [Time Frame: single time point (1 day)]: Difference between quantitative oxygenated hemoglobin amount (Units: arbitrary units (a. u.)) and quantitative deoxygenated hemoglobin amount (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with PAD before gait exposure compared to the amount after gait exposure
- Correlation of difference between oxygenated/deoxygenated hemoglobin with the TASC-classification (angiography) [Time Frame: single time point (1 day)]: Difference between quantitative oxygenated hemoglobin amount (Units: arbitrary units (a. u.)) and

quantitative deoxygenated hemoglobin amount (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with PAD correlated with the TASC-classification (angiography)

Muscular quantitative lipid/collagen signal fraction and the MSOT-values at a wavelength of 930 and 1064 nm [Time Frame: single time point (1 day)]: Quantitative lipid/collagen signals and signals at 930 and 1064 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with different clinical and angiographic PAD stages

- Correlation of acquired MSOT parameters with the CCDS flow profile and PSV [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin content and MSOT-values at a wavelength of 800 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT in patients with PAD correlated with the flow profile and PSV of A. CFA and A. poplitea determined by CCDS
- Correlation of acquired MSOT parameters with the ABI [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin content and MSOTvalues at a wavelength of 800 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT correlated with the ABI
- Correlation of acquired MSOT parameters with the walking distance determined by treadmill examination [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin content and MSOT-values at a wavelength of 800 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT correlated with the walking distance determined by treadmill examination
- Correlation of acquired MSOT parameters with the clinical severity of PAD [Time Frame: single time point (1 day)]: Quantitative oxygenated/deoxygenated/total hemoglobin content and MSOT-values at a wavelength of 800 nm (Units: arbitrary units (a. u.)) derived by transcutaneous MSOT correlated with the clinical severity of PAD according to Fontaine and Rutherford

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 patients with different PAD stages with TASC-classification in angiographc imaging in order to define thresholds for MSOT in these patients. MSOT data will be correlated with clinical disease severity (Fontaine; Rutherford; walking distance; ABI), CCDS and TASC II classification in angiographic imaging.

Patients with PAD will be recruited from the consulting hour of the Department of Vascular Surgery, University Hospital Erlangen. Healthy volunteers will be acquired via posters in the clinic. Following careful information about the study and after providing written consent, relevant clinical data will be collected from the electronical patient file, if available. Otherwise an anamnesis interview for relevant data is performed. The CCDS, ABI and measurement of the walking distance will be performed. Afterwards the MSOT parameters will be collected before and after an active gait exposure. If it is reasonable, both sides will be examined for all participants. The focus, however, is on the more affected leg. 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 approx. 1.5 hours. Angiographic imaging will be reviewed and analyzed if the interval between index test 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.

5.2 Study Population

At the moment we anticipate a number of approx. 200 subjects in total. We also anticipate similar group sizes for healthy control group and the four groups of PAD patients. Age should be similar in all investigated groups.

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 values between 1.0 and 1.4

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

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)

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)[2]	X	Before and after gait exposure
Angiography[3]		Less than 12 months to the date of index test was considered for statistical analysis, from electronic patient file by two independent readers

[2]2 **Index Test (MSOT):**
Signal levels for oxygenated hemoglobin (HbO2), deoxygenated hemoglobin (Hb), collagen and lipids in arbitrary units (a.u.)

3 **Angiography**:
TASC II classification femoro-popliteal PAD:
Type-A:

- *circumscribed 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 theoretically 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

CCDS[4]	X	Whether a flow-relevant stenosis is present in the examined vessels is decided by an independent reader
ABI[5]	X	
Clinical scores[6]	X	

CCDS:

Morphological flow profile and PSV of CFA and A. poplitea

4 **ABI**:

Designation	ABI value
no PAD	>0.9-1.3
mild PAD	0.75-0.9
moderate PAD	0.5-0.75
severe PAD	<0.5
mediasclerosis	>1.3

5 **Clinical scores**:

Relative and absolute walking distance

Fontaine classification

I=No symptoms

IIa=painless walking distance >200 m, IIb=painless walking distance <200 m

III=pain while resting

IV=trophic disorder

Rutherford classification

0=No symptoms

1=mild claudication

2=moderate claudication

3=severe claudication

4=ischemic rest pain

5=minor tissue loss (nonhealing ulcer, focal gangrene and diffuse pedal ischemia)

6=major tissue loss (extending above transmetatarsal level, foot no longer salvageable)

6 Determination of Sample Size

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 realised 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).

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 into 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 the reference standard as follows: clinical according to Fontaine and TASC classification (and 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.

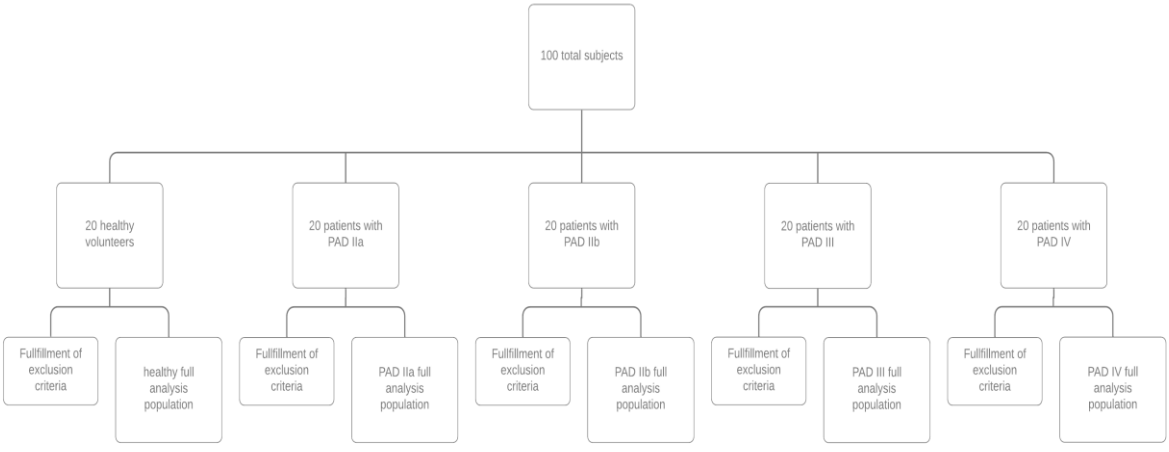
8 Summary of Study Data

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

Diagnostic performance will be examined in different successive steps: first, distribution of single MSOT readouts within the different PAD stages used as external criterion (gold standard) will be assessed. Second, the difference in means of the (MSOT) readouts between patient subgroups will be assessed and statistically tested. If significant, these readouts will be subject to a ROC analysis to determine the optimum cutpoint; further details see below.

9.1 Correlations

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

9.2 Differences in Severity of PAD

Overall differences of MSOT readouts vs. the PAD stages will be statistically tested using ANOVA including a post hoc Tukey test, comparing all three pairs deriving a 95% family-wise confidence level of differences between means.

9.3 Sensitivity and Specificity

Using the cutpoint maximising the Youden index derived from study group 1 based on controls vs. PAD classes III/IV, the diagnostic properties of MSOT values will be illustrated by sensitivity and specificity (with 95% CI) 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 2 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 3 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 all stages in study groups 1 and 2

Baseline characteristics-patients with PAD	
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 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 Lipid content, collagen content	 Mean ±SD

12.2 Demographic data healthy control collective in study groups 1 and 2

Baseline characteristics-healthy collective	
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 Lipid content, collagen content	 Mean ±SD

12.3 Data correlation PAD all stages in study group 1 and 2

All MSOT parameters have two values: "*Pre*" means before gait exposure and "*Post*" means after gait exposure.

Parameters	Hbtot	HbO2	Hb	Difference HbO2/Hb	Values at 800 nm
Angiography TASC score	Correlation	Correlation	Correlation	Correlation	Correlation
Clinical scoring ABI Walking distance Fontaine stage, Rutherford stage	Correlation	Correlation	Correlation	Correlation	Correlation
CCDS Morphological flow profile, relevant stenosis PSV	Correlation	Correlation	Correlation	Correlation	Correlation

12.4 Data correlation healthy control collective in study groups 1 and 2

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

Parameters	Hbtot	HbO2	Hb	Difference HbO2/Hb	Values at 800 nm

Clinical scoring ABI Walking distance Fontaine stage, Rutherford stage	Correlation	Correlation	Correlation	Correlation	Correlation
CCDS Morphological flow profile, relevant stenosis PSV	Correlation	Correlation	Correlation	Correlation	Correlation

12.5 Samples for PAD clinical scoring

Disease Severity – grouping according to Fontaine classification

Parameters	Healthy	PAD IIa	PAD IIb	PAD III	PAD IV	pvalue	ANOVA
Angiography TASC score	*Value not collected*	n (% of total n)	n (% of total n)	n (% of total n)	n (% of total n)		
CCDS Morphological flow profile, relevant stenosis PSV	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD		
Clinical scoring ABI Walking distance	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		

Disease severity – grouping according to Fontaine classification

Parameters	Sensitivity	Specificity
Angiography TASC score	%	%
Clinical scoring ABI Walking distance	%	%
CCDS Morphological flow profile, relevant stenosis PSV	%	%
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	%	%

Disease severity – grouping according to Rutherford classification

Parameters	Healthy	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6	pvalue	ANOVA

Angiography TASC score	*Value not collected*	n (% of total n)	n (% of total n)	n (% of total n)	n (% of total n)	n (% of total n)	n (% of total n)		
CCDS Morphological flow profile, relevant stenosis PSV	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD	n (% of total n) Mean ±SD		
Clinical scoring ABI Walking distance	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		

Disease severity – grouping according to Rutherford classification

Parameters	Sensitivity	Specificity
Angiography TASC score	%	%
Clinical scoring ABI Walking distance	%	%
CCDS Morphological flow profile, relevant stenosis PSV	%	%
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm 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 Walking distance Fontaine stage, Rutherford stage	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		

Severity of stenosis – grouping according to combined TASC subgroups

Parameters	Sensitivity	Specificity
Clinical scoring ABI Walking distance Fontaine stage, Rutherford stage	%	%
CCDS Morphological flow profile, relevant stenosis PSV	%	%
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm 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 Walking distance Fontaine stage, Rutherford stage	Mean ±SD	Mean ±SD		
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	Mean ±SD	Mean ±SD		

Stenosis severity – grouping according to CCDS scoring

Parameters	Sensitivity	Specificity
Clinical scoring ABI Walking distance Fontaine stage, Rutherford stage	%	%
Angiography TASC score	%	%
Hbtot_Pre HbO2_Pre Hb_Pre Difference HbO2_Pre/Hb_Pre Values at 800 nm Pre Hbtot_Post HbO2_Post Hb_Post Difference HbO2_Post/Hb_Post Values at 800 nm Post	%	%