MOSAIC: **M**ultispectral **O**ptoacou**s**tic Tomography for **A**dvanced **I**maging of **C**entronuclear Myopathy

16.04.2025

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1. Study Title, Version, Date of document

Study title

Multispectral Optoacoustic Tomography for Advanced Imaging of Centronuclear Myopathy

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Version

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Date	Version	Status	Changes
21.03.2025	1.0	First draft	Lina Tan
21.03.2025	1.1	Corrected figure	Lina Tan
16.04.2025	1.2	Amendment	Lina Tan

2. Project Summary

Centronuclear myopathy (CNM) belongs to the group of congenital myopathies. It is named after its histopathological feature: the nucleus is located in the center of the muscle cell instead of its physiological position at the periphery. CNM is extremely rare; only epidemiological data for the broader group of congenital myopathies are available. The incidence is estimated at 0.06 per 1,000 live births.

CNM is genetically and clinically heterogeneous. Identified gene mutations affect, among others, genes encoding proteins involved in membrane remodeling, transport, and excitation-contraction coupling (Reumers et al. 2021).
Patients with CNM typically present in early childhood with muscle weakness and hypotonia. The severity of the disease varies depending on the underlying genotype and ranges from reduced exercise tolerance and ptosis to floppy infant syndrome and respiratory failure. One example of a severe disease course is the X-linked form caused by an MTM1 nonsense mutation (XL-MTM). Affected individuals become symptomatic in the neonatal period and usually die during childhood or adolescence. In contrast, patients with DNM2 mutations show a later onset and a milder progression. Among the few reported patients with BIN1 mutations, the onset occurs in infancy or adulthood, with a moderate disease course. RYR1-associated CNMs also vary in terms of age of onset and disease severity (Reumers et al. 2021; Fattori et al. 2015).

Diagnosis is based on molecular genetic testing and muscle biopsy. However, these methods are not widely available, time-consuming, and invasive. In young patients, anesthesia is usually required. Multispectral optoacoustic tomography (MSOT) allows the detection of specific endogenous chromophores such as collagen, myoglobin, and hemoglobin using a non-invasive approach comparable to conventional ultrasound. Instead of sound waves, MSOT uses near-infrared light pulses, which are absorbed by the tissue, leading to thermoelastic expansion of specific molecules. This expansion generates ultrasound waves that are detected by the same device. Multispectral illumination and signal unmixing enable precise localization and quantification of muscle-specific subcellular structures. MSOT has already demonstrated the potential to visualize muscle structure and the clinical extent of muscle disease in patients with Duchenne muscular dystrophy, spinal muscular atrophy, and late-onset Pompe disease (LOPD), and to differentiate these patients from healthy volunteers. To date, no optoacoustic data on CNM are available.

The aim of this study is to gain molecular insights into muscle degeneration in CNM patients for the first time. To obtain a comprehensive picture of the optoacoustic characteristics of CNM, patients of different ages and disease severities will be recruited. Due to the rarity and small number of CNM cases, recruitment is challenging. Therefore, the upcoming patient meeting in Bad Nauheim (May 29 – June 1, 2025) will be used as an opportunity to offer study participation to as many patients as possible.

As part of the study, in addition to non-invasive, radiation-free imaging, clinical-functional tests will be performed. These include muscle strength testing using the Medical Research Council (MRC) sum score and the Timed Up and Go test (TUAG; measured in seconds). For MSOT imaging, various accessible muscle groups will be scanned, including the paraspinal muscles and trapezius muscle, as well as the following bilateral limb muscles: deltoid muscle, biceps brachii, forearm flexors, quadriceps femoris, adductor muscles, hamstrings (ischiocrural muscles), triceps surae, and tibialis anterior.

3. Responsibilities

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Funding

Internal funds of the Department of Pediatrics and Adolescent Medicine Erlangen until application for third-party funding.

Provision of the examination device by Ithera Medical GmbH

Screen4Care

4. Scientific background

CNM is a neuromuscular disease characterized by the clinical features of congenital myopathy. They have the histopathological feature of a central nucleus and are characterized by generally progressive muscle weakness and atrophy (Reumers et al. 2021).

There is genetic and clinical heterogeneity, as well as a genotype-phenotype correlation (Fattori et al. 2015). Mutations are known in the key genes MTM1 gene, which codes for myotubularin, DNM2 gene, which codes for dynamin-2 gene, BIN1 gene, which codes for amphiphysin-2 (or bridging integrator-1), as well as RYR1 gene, which codes for the ryanodine receptor of skeletal muscle, and TTN gene, which codes for titin. The pathogenesis of the disease is still poorly understood. It is currently assumed that the mutations lead to impaired membrane transport and deficient autophagy (Jungbluth and Gautel 2014).

Depending on the genotype, CNM presents as a spectrum disease with different onset, course and clinical features. Onset is possible at any age: more than half of patients become symptomatic within the first year of life, a quarter develop the disease in childhood or adolescence, and around a fifth in adulthood. The main symptom of this disease is muscle weakness, which can either be generalized or isolated to the respiratory, swallowing and eye muscles. Patients with a mutation in the MTM1 gene usually have an early onset of the disease, a rapidly progressive course and a high mortality rate. A common symptom in these patients is ophthalmoparesis. In contrast, DNM2 patients show a milder course overall. These patients usually reach all motor milestones. The first symptoms include exercise-induced muscle pain and slowly progressive muscle weakness. As the disease progresses, orthopaedic complications as a result of the long-term muscle weakness come to the fore: scoliosis, pes cavus and contractures. RYR1 patients, on the other hand, are characterized by delayed motor milestones, whereby the severity of muscle and respiratory involvement varies greatly. Mutations in the TTN gene are rare, but affected patients often suffer from severe cardiomyopathy (Fattori et al. 2015). Respiratory muscle involvement is common and initially occurs at night. In addition, respiratory complications are the main cause of morbidity (Smith et al. 2016).

The diagnosis of central nucleus myopathy (CNM) is based on typical histopathologic findings in muscle biopsy, complemented by characteristic clinical features. Magnetic resonance imaging (MRI) of the muscles can support clinical assessment and suggest genetic testing in cases with non-specific features. Genetic counseling should be offered to all patients and families diagnosed with CNM. Muscle biopsy shows centronuclear myopathy, i.e. centrally

located nuclei surrounded by a perinuclear halo of glycogen accumulations. These central nuclei are present in all muscles. Another common feature is type 1 fiber hypotrophy, which may precede the development of central nuclei. In a few fibers, there may be compensatory hypertrophy of type 2 fibers. Histopathologic changes may worsen over time, with marked increases in adipose and connective tissue described as prominent features (Jungbluth, Wallgren-Pettersson, and Laporte 2008).

DNA sequencing of the exons and exon-intron boundaries of the affected genes is used for molecular confirmation of the diagnosis. Depending on whether a particular gene is suspected, the mutation analysis may first examine these suspected gene regions. If genetic segregation is uncertain and in cases where there is no clear genotype-phenotype correlation, all genes should be analyzed. RNA sequencing can be used if tissue or cultured cells from the patient are available, as the genes in question are ubiquitously expressed in most cell types. In addition, examination of RNA integrity or protein levels could reveal mutations in introns or regulatory sequences that may not be detected by DNA sequencing (Jungbluth, Wallgren-Pettersson, and Laporte 2008). It is important to note that in some patients with histopathological findings, no causative gene mutation has yet been discovered (Smith et al. 2016).

In MRI studies, the musculature in CNM with DNM2 and RYR1 gene was examined with regard to muscular involvement. A characteristic progressive sequence was found in patients with DNM2 mutations: early involvement of the plantar flexor muscles (plantar flexors) subsequently leads to changes in the hamstrings and anterior thigh muscles (Jungbluth, Wallgren-Pettersson, and Laporte 2008). This sequence does not occur in cases with mutations in the RYR1 gene. Instead, there is earlier involvement of the muscles of the anterior thigh compartment and relatively selective changes in the soleus muscle (Fischer et al. 2006). Few to no MRI findings have been published for the other CNM mutations.

Multispectral optoacoustic tomography (MSOT) is a promising imaging method that offers better availability and scan time compared to MRI.

For MSOT, an ultrasound transducer is applied to the patient's skin, similar to a conventional ultrasound. Instead of sound waves, MSOT illuminates the tissue with light of transient energy, typically light pulses in the near infrared range, which are absorbed by the tissue and lead to thermoelastic expansion. This expansion generates ultrasound waves that are detected by the same device. Studies have already shown that MSOT-based assessment of hemoglobin levels in the intestinal wall has the potential to assess Crohn's disease activity (Knieling 2017,

Waldner 2016). With a newly configured device (Acuity Echo, iThera Medical GmbH, Munich, customized platform), an extended spectrum of laser light can be used, which enables the detection not only of hemoglobin, but also of other biomarkers such as collagen or lipids. In previous study, we were able to show the molecular composition of muscles in Duchenne muscular dystrophy using MSOT and propose to use the non-invasively measured collagen content as a new biomarker for disease severity (Regensburger et al. 2019). In a study on Pompe's disease, we were able to show that glycogen and the corresponding water binding can be visualized with the help of MSOT, as well as a change in the collagen and lipid profile of the muscle (Tan et al. 2024)

In this study we want to investigate the use of MSOT imaging for disease detection and monitoring of patients with CNM for the first time and compare it with healthy volunteers.

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5. Study objectives

Hypotheses

The optoacoustic spectrum of the muscles of patients with CNM differs compared to healthy subjects.

The quantitative proportion of hemoglobin/myoglobin in muscles, determined by MSOT, differs in patients with CNM compared to healthy subjects.

The quantitative proportion of oxygenated/deoxygenated hemoglobin in muscle, as determined by MSOT, differs in patients with CNM compared to healthy volunteers.

The quantitative proportion of lipid signal in muscle, as determined by MSOT, differs in patients with CNM compared to healthy subjects.

The quantitative part of the collagen signal in the muscles, determined by MSOT, differs in patients with CNM compared to healthy subjects.

There is a correlation between the signal derived by MSOT and the clinical condition of patients with CNM.

Primary objectives

- Comparison of the optoacoustic spectrum of MSOT measurements in different subgroups of patients with CNM and healthy controls

Secondary objectives

- Comparison of the quantitative fraction of hemo/myoglobin signal determined by MSOT in patients with CNM compared to healthy subjects and between subgroups.
- Comparison of the quantitative proportion of oxygenated/deoxygenated hemoglobin determined by MSOT in patients with CNM compared to healthy subjects and between subgroups.
- Comparison of the quantitative lipid signal determined by MSOT in patients with CNM compared to healthy subjects and between subgroups.
- Comparison of the quantitative part of the collagen signal determined by MSOT in patients with CNM compared to healthy subjects and between subgroups.
- Correlation of hemoglobin/myoglobin content determined by MSOT with disease duration/patient age.
- Correlation of oxygenated/deoxygenated hemoglobin determined by MSOT with disease duration/patient age.
- Correlation of lipid content determined with MSOT with disease duration/patient age.

- Correlation of collagen content determined with MSOT with disease duration/patient age.
- Correlation of the hemoglobin/myoglobin content determined with MSOT with age-dependent functional muscle function tests (MRC).
- Correlation of the oxygenated/deoxygenated hemoglobin content determined with MSOT with age-dependent functional muscle function tests (MRC).
- Correlation of the lipid content determined with MSOT with age-dependent functional muscle function tests (MRC).
- Correlation of the collagen content determined with MSOT with age-dependent functional muscle function tests (MRC).

6. Study design

Study type

This is a cross-sectional study with a control group.

Study arms

Control groups

No interventions are planned. In the evaluation, a distinction is made between the control group and subgroups of disease severity. All groups will receive the examinations described above.

Randomization

No randomization is planned.

Blinding

Due to the study setting, blinding of the study is not possible. The ¬evaluation of the data will subsequently be carried out in a blinded manner. Blinding of the test subjects is not necessary.

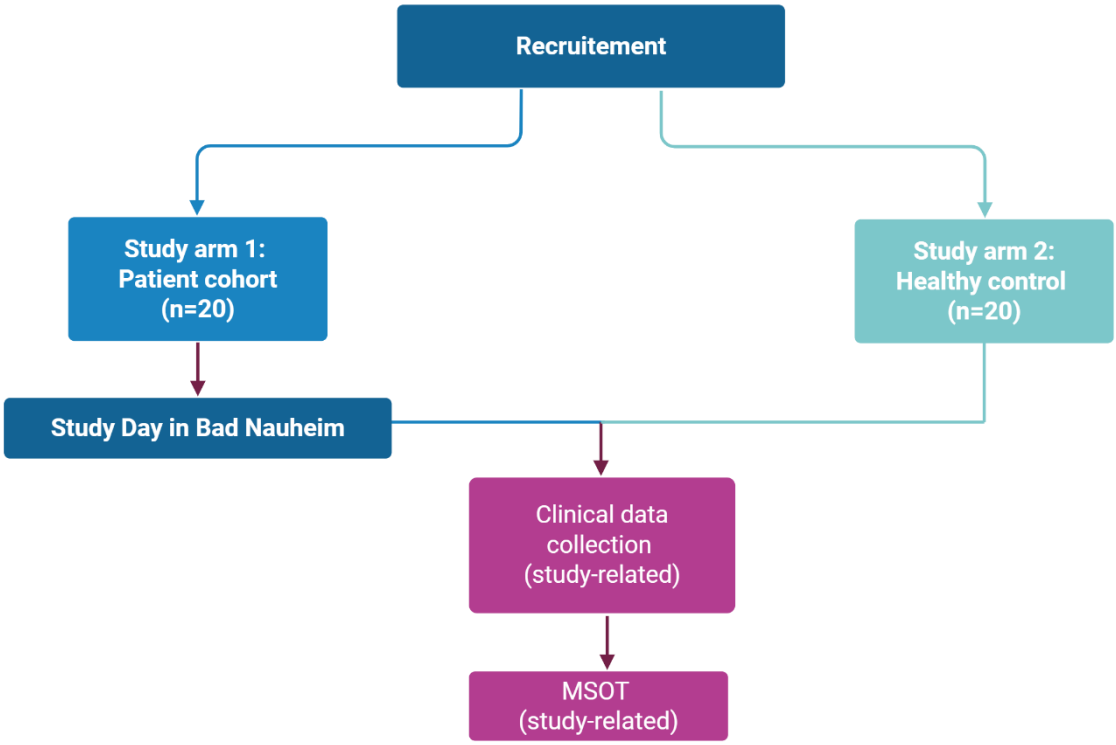

Abbildung 1: course of study.

7. Target values

All measurements with the MSOT and ultrasound are performed on the paraspinal muscles, trapezius muscle and on both sides of the deltoid muscle, biceps brachii, forearm flexors, quadriceps femoris muscle, ischiocrural muscles, triceps surae and tibialis anterior.8. Studienpopulation

8. Study Population

Inclusion Criteria

Patients with CNM

- Confirmed diagnosis
- age: at least 2 years of age

Healthy controls

- age: at least 2 years of age

The inclusion of minors is necessary because centronuclear myopathy is very rare and usually begins in childhood. The onset of the disease in childhood is associated with a different and rapidly progressing course of the disease compared to adults. Therefore, the inclusion of children and adolescents is important in order to do justice to this age-specific course of centronuclear myopathies. The study will predominantly examine adolescents. The MSOT has CE certification from the age of 2 years.

The healthy control group will be recruited from patients of the Pediatric and Adolescent Clinic Erlangen who have been hospitalized for a non-muscular disease. These patients are clinically stable.

Exclusion criteria

CNM patients and healthy controls

- pregnancy
- breastfeeding mothers
- Tattoo in the area of the examination field
- Subcutaneous fat > 3 cm

Additionally for healthy controls

- Other neuromuscular diseases (anamnestic)

Number of patients

As this is a pilot study, it is not possible to calculate the exact number of patients. The plan is to include a total of around 20 patients with CNM (adults, adolescents and children). However, this number is only a rough estimate. All patients who wish to participate should be included,

as refusal would not make sense from an ethical point of view. In addition, approximately 20 subjects should be included as a control group.

Patient recruitment

Patients (and parents) are informed about the possibility of participating in the study. For this purpose, they will receive an e-mail from the study coordinator before the start of the study with the relevant information sheet and the contact details of the study physicians. Patients willing to participate can confirm their participation in advance and send the information sheet to the study coordinator or bring it with them on the day of the examination. At the patient day, the topic will be presented again by one of the study physicians. During the course of the day, patients (and parents) also have the opportunity to register for participation with the study staff. The study coordinator will then contact them so that patients (and parents) can discuss the study and ask questions. On the day of the examination, there will also be a personal information session before each examination with the opportunity to ask questions. If the patient is willing to participate, he/she (and parents) will be fully informed about the objectives and methods (in particular about the scientific/exploratory nature of the study), the benefits and risks as well as the revocability of participation in the study. Patients in childhood and adolescence are informed and educated about the study and its procedures in an age-appropriate manner.

In the event of incidental findings, the study participants are informed and further diagnostic measures are initiated if necessary.

Healthy control recruitment

Following patient recruitment, age- and gender-matched subjects (and parents) will be informed about the opportunity to participate in the study. Subjects (and parents) willing to participate can express their interest in the study in advance. They will then be contacted by the study coordinator so that subjects (and parents) can familiarize themselves with the study in advance and ask questions. Those interested will then receive an email with the relevant information sheet and the contact details of the study doctors. Subjects (and parents) willing to participate can confirm their participation in advance and send the information sheet to the study director or bring it with them on the day of the study. On the day of the examination, an additional personal explanation will take place before each examination. There is always the opportunity to ask questions. If the subject is willing to participate, he/she (and parents) will be fully informed about the objectives and methods (in particular about the scientific/exploratory nature of the study), the benefits and risks as well as the revocability of participation in the study.

Subjects in childhood and adolescence are informed and educated about the study and its procedures in an age-appropriate manner.

In the case of incidental findings, the study participants are informed and, if necessary, further diagnostic measures are initiated.

9. Course of study

- **Procedure for providing information and obtaining consent**

Subjects can only be included in the study after written informed consent has been obtained. The written declaration of consent requires the subjects and/or parents to be informed verbally and in writing about the objectives and methods (including the scientific-exploratory nature of the study), the benefits and risks, and the revocability of participation in the study. By giving their written consent, the subjects declare that they agree to the collection and storage of study-relevant data and their review by monitoring or authorities. For participants under the age of 18, the signature of both parents is a prerequisite for inclusion in the study. If only one parent is present, the other parent can sign on behalf of the other parent in exceptional cases. In this case, it is important that information about the study has already been made available to the participants beforehand, so that the parents have already been able to discuss the study and the presumed will of the other parent to participate is therefore known. 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 are informed that this study is a purely scientific study without any current diagnostic or therapeutic benefit. The original consent form will be kept in the study folder at the study site. Subjects will be given a copy of the study participant information and informed consent form. The study participant information and the informed consent form can be found in the appendix of this study protocol. In the event of an incidental finding, the study participants and parents or legal representatives will be informed and, if necessary, further clarification will be initiated.

- **Measures and recording of target variables**

After informing the patient/control person and the parent/legal guardian and obtaining consent, clinical data will be collected. This includes age, weight, height, genetic findings, date of initial diagnosis and clinical course. The clinical assessment scales MRC muscle function test and timed up and go test are then collected. The MRC muscle function test and timed up and go test assessment scales are then collected.

MSOT imaging is then performed on 9 anatomical regions in all study participants. This includes the paraspinal musculature, as well as the trapezius muscle and additionally 7 anatomical regions of the limb muscles on both sides: left and right deltoid muscle, biceps brachii, forearm flexors, quadriceps femoris muscle, ischiocrural muscles, triceps surae, tibialis anterior. The examination is performed without further invasive procedures. The anatomical region can be

localized using the built-in B-scan sonography; the corresponding optoacoustic signals can then be measured. The duration per anatomical region is limited to 2 minutes; this corresponds to a maximum of 20 minutes. Patients can remain in a relaxed position during the examination without the need for breathing maneuvers or similar assistance.

- **Time schedule and duration of the study**

The examination of patients with CNM takes place in a designated room at the family's place of residence in Bad Nauheim. The examination of the healthy subjects will take place in a room provided for this purpose at the Children's and Youth Clinic Erlangen. A 10-minute explanation is provided. Approximately 5 minutes are allowed for the clinical function tests. The subsequent MSOT examination takes approximately 2 minutes per muscle and therefore 20 minutes in total. The total duration of the study is therefore 35 minutes per patient.

- **Total study duration**

The expected total duration consists of data collection of the patient collective in the period from 29.05.2025 - 01.06.2025, the data collection of the healthy collective in the period from 01.06.2025 - 31.07.2025 and the data evaluation of approx. 6 months. The first study results are expected 8 months after the study day.

10. Benefit Risk Assessment

Study-related risks

According to the classification criteria for medical devices (Directive 93/42/EEC, Annex IX), the optoacoustic system from iThera Medical corresponds to a Class IIa laser system:

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

The system used is CE-certified for adults and children from 2 years of age (TÜV Süd, 07.10.2024, type designation according to imprint: MSOT Acuity Echo). A conformity assessment procedure to extend the use of the system is currently not intended or planned. This is therefore a purely scientific pilot study.

Adherence to energy values

Laser safety and the maximum permissible radiation dose for irradiation with laser pulses are regulated in the ANSI and IEC 60825 laser standards. The MSOT system complies with these standards and therefore remains below the MPE (maximum permissible exposure) limits for skin irradiation and is therefore considered safe.

Temperature increase due to MSOT in the tissue

Optoacoustic imaging does not lead to a significant temperature increase in the tissue. The absorption of a laser pulse in the tissue leads to a local temporary temperature increase of a few millikelvin. Depending on the duration of the examination and the patient's skin type, the temperature increases are typically in the range of less than one degree Kelvin.

Histological changes in the tissue

Histological changes in the target tissue and surrounding structures are not expected, nor have they been observed in previous preclinical and clinical studies.

Slight, reversible redness or warming may occur on very sensitive skin; such side effects may be noticed by the test person or the doctor at any time; the examination may then be interrupted or discontinued. In any case, no irreversible damage is to be expected. In general, the near-infrared light used in MSOT can lead to retinal damage if the eye is irradiated. To prevent this, test participants and examiners wear appropriate laser safety goggles during the examination.

As the data obtained will not be used to interpret the diagnostic results, there is no risk of possible misdiagnosis or misrepresentation of the data in this exploratory pilot study.

No other risks are associated with this study and were not observed based on our own preliminary data.

Study-related benefits

The data obtained in the studies could provide important insights into the course and diagnosis of centronuclear myopathies. The potentially quantifiable differences could be used in the future as an important marker for the therapeutic response or prognosis of these diseases. Potentially invasive, high-risk procedures could be replaced.

Termination criteria

Particularly in view of the inclusion of children and adolescents, participation in the study is discontinued if there is any noticeable warming or reddening of the skin. The examination time is limited to 20 minutes, so that these events are very unlikely.

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

There are no plans to discontinue the entire study.

Statement on medical justifiability

Based on previous experience, the risk of adverse events is considered to be extremely low.

To date, no serious incident has been reported by us or in the literature. Most of the reported (foreseeable) problems were related either to the use of ultrasound gel for the examination or to the need to wear eye protection. The use of filter glasses also explains the reported red vision loss. This phenomenon was reversible within seconds.

We hope that this method will provide an age-appropriate diagnostic tool, particularly in view of the inadequate non-invasive diagnostic options for assessing disease activity to date. In particular, it should help to support the diagnosis of TK2d, monitor the course of the disease and therapy and enable the initiation of early therapy. As this disease is also often progressive in childhood and is associated with major health restrictions, a scientific study of this group is essential and can only be compared with data from adults to a limited extent. Inclusion of pediatric patients in addition to the adult cohort is therefore necessary and medically justifiable.

11. Biometry

Sample size calculation

As this is a pilot study and no information is currently available regarding the expected differences between the various groups, no sample size calculation was performed. The indicated sample size represents an estimate.

Statistic

Data will be presented as mean with standard deviation. Correlations will be reported using the non-parametric Spearman correlation coefficient (Rs). Depending on the group distribution, differences in means will be statistically analyzed using a non-parametric t-test (Mann-Whitney test) or ANOVA. For all analyses, a p-value of <0.05 will be considered statistically significant.

12. Data management and data protection

Data collection and storage

The participation of individual study subjects will be documented. The principal investigator will maintain a separate list for identifying the participating subjects. This list will include names, dates of birth, examination dates, and pseudonymization codes of the subjects. The principal investigator is responsible for the quality of data collection and storage. Data will be stored exclusively on computers or specially designated network drives of the University Hospital Erlangen.

Pseudonymization

Before any scientific analysis of the data from this study, all information will be pseudonymized in accordance with the guidelines of the Federal Data Protection Act (Bundesdatenschutzgesetz).

Data sharing

Data sharing in this study is limited to the MSOT raw data. These will be shared in pseudonymized form without demographic parameters or any additional information, and only in physical form on drives, making it impossible to identify individual patients. The data will not be exchanged for analysis purposes.

The study results will be published in anonymized form; therefore, it will not be possible to draw conclusions about the identities of participating individuals. The data will be stored for 10 years and then destroyed.

Withdrawal and data deletion

In the event of withdrawal of consent, data collected up to that point may still be considered and retained. However, study participants have the right to request their destruction, unless legal provisions prevent such destruction.

13. Telemedic Procedures

No telemedical procedures will be used.

14. Biological samples

No biological samples will be collected.