

Evaluation of Therapeutic Response in Spinal Muscular Atrophy using Multispectral Optoacoustic Tomography (MSOT) and Magnetic Resonance Imaging (MRI)

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Study protocol

TheraponSMA

Evaluation of therapeutic response in spinal muscular atrophy using multispectral optoacoustic tomography (MSOT) and magnetic resonance imaging (MRI)

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2. Study titel, version number, version date

Study title

Evaluation of therapeutic response in spinal muscular atrophy using multispectral optoacoustic tomography (MSOT) and magnetic resonance imaging (MRI)

Version number

Version 1.3

Version date

21.02.2020

Protocol versions

Date	Version	Status	Changes
17.12.2019	1.0	Final application	
14.01.2019	1.1	Amendment	Specification of recruitment for control patients
11.02.2020	1.2	Final	
21.02.2020	1.3	Amendment	Specification of clinical routine testing

3. Project summary

Neuromuscular diseases are caused by numerous pathologies of the central nervous system (brain and spinal cord), the peripheral nervous system or the skeletal muscle. Spinal muscular atrophy (SMA) is characterized as a pathology of the central nervous system characterized by the degeneration of motor anterior horn nerve cells in the spinal cord or lower brain stem, leading to progressive muscle weakness and atrophy. The clinical spectrum of all subtypes of the disease is broad, but in almost all cases the therapeutic options are limited to supportive therapy and palliative measures to prevent or alleviate complications such as spinal deformities or respiratory insufficiency. In the course of the disease, patients lose their motor neurons and thus their original skeletal muscle mass. In the muscle itself, a histological loss of the nerve and muscle fibers as well as a fatty remodeling can be observed. Most patients show normal cognition and consciously experience the course of their disease.

Since 2016 (USA) and 2017 (European Union), the drug Nusinersen (Spinraza®, Biogen) has been available for the treatment of children and adults with SMA. It contains an antisense oligonucleotide, which is supposed to increase the expression of the usually in SMA reduced "survival motor neuron protein". In the ENDEAR study, the study used for FDA approval, 40% of children in the treatment arm achieved significant improvements in the area of motor milestones, while no child in the control group achieved this. Further positive effects were also reported in older children and adolescents as a result of the CHERISH study. The drug is administered intrathecally (in the spinal fluid). The first three doses were administered at intervals of 14 days and the fourth at intervals of 30 days. Subsequently, maintenance therapy is administered at intervals of 4 months. According to the Joint Federal Committee, the annual therapy costs currently amount to 310,877.58 € to 310,942.95 € - internationally, Spinraza® ranks second among the most expensive drugs in the world. In addition, the first gene therapy (Zolgensma®) has already been approved in the USA at a price of over \$2,000,000. Whether patients benefit from this therapy can only be assessed clinically so far. Our preliminary work shows that new methods such as multispectral optoacoustic tomography (MSOT) and magnetic resonance imaging detect tissue changes very sensitively. With both methods, the molecular composition of muscle tissue can be determined non-invasively and quantitatively at the same time. This first pilot study will now investigate whether these differences can also be displayed in the course of therapy.

4. Responsibilities

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5. Scientific background

Spinal muscular atrophy (SMA) is an autosomal-recessive disorder, characterized by progressive muscle weakness and atrophy. Spinal muscle atrophy has an incidence of 1/10,000 with an estimated carrier frequency of 1/50 (Sugarman 2012, McAndrew 1997, Pearn 1978). The condition is caused by a homozygous deletion or mutation in the survival motor neuron 1 (SMN1), resulting in reduced expression of the survival motor neuron (SMN) protein. This leads to the degeneration of motor neurons in the spinal cord and brain stem (Lefebvre 1995, Prior 2010). A nearby related gene, survival motor neuron 2 (SMN2), also produces the SMN protein, but due to aberrant splicing around 85-90% of translated protein are truncated and non-functional and rapidly degraded in the cell (Mailman 2002); remaining 10-15% are functional (Butchbach 2016), partially compensating the loss of SMN1 by SMN2 protein synthesis (Hsieh-Li 2000). Individuals with a higher copy number of SMN2 do in general have a milder phenotype (Mailman 2002, Butchbach 2016).

The various forms of SMA are differentiated according to distribution pattern, onset of disease, disease severity and hereditary pattern and are generally designated according to the muscle groups mainly affected (Pearn 1973, <https://www.dgm.org/muskelerkrankungen/spinale-muskelatrophie>). A large number of similar, much rarer forms are known that can be associated with additional dysfunctions. The non-proximal spinal muscle atrophies are also very rare and do usually not lead to significant limitations of vital functions at the onset of adulthood. However, the vast majority (approx. 90%) of patients belong to the group of so-called proximal SMA, which is characterized by the onset of muscle weakness in muscle groups close to the trunk (above all thigh and hip muscles, later also arm and shoulder girdle involvement). Proximal SMA is divided into different subtypes, which are primarily defined according to the onset of the disease, the learned motor skills and life expectancy.

Spinal muscular atrophy type 0, also called prenatal onset SMA, describes the most severe form of SMA as it presents before birth with decreased fetal movements. Neonates are born with severe hypotonia and weakness, areflexia, facial weakness, joint contractures, difficulty swallowing and respiratory failure on examination. Life-expectancy is greatly reduced, most patients die within the first 6 months of age (Dubowitz 1999, MacLeod 1999).

Spinal muscular atrophy type 1, also called Werdnig-Hoffman disease or infantile SMA, is the most common type of SMA. Symptoms usually exhibit at birth or before 6 months of age. Infants present with hypotonia (proximal more than distal limbs, legs more affected than arms) with poor head control (“floppy baby”), reduced or absent tendon reflexes and are never able to sit unassisted. Patients may show a bell-shaped thorax, paradoxical breathing, swallowing

weakness and tongue fasciculations. Cognition is not affected. Disease is usually fatal prior to 2 years of life due to respiratory failure (Finkel 2014, Thomas 1994).

Spinal muscular atrophy type 2, also called Dubowitz disease or intermediate SMA, presents usually between 6-18 months of age. This form tends to manifest with progressive leg weakness, which is worse than weakness in the arms, hypotonia and in a great percentage of patients areflexia. Comorbidities are related to muscular weakness and include scoliosis and joint contractures, as well as possible restrictive lung disease due to intercostal muscle weakness. Cognition is normal in affected patients (von Gontard 2002). Children with SMA type 2 are able to sit unassisted but are never able to walk independently. Life expectancy is reduced, but most patients live until adulthood (Fried 1971, Hausmanowa-Petrusewicz 1985, Imai 1995).

Spinal muscular atrophy type 3, also called Kugelberg–Welander disease or juvenile SMA, usually manifests after 12 months of age. Patients suffer from a proximal muscle weakness, which is more pronounced in the legs than in the arms. Individuals are able to walk unassisted, however at some point a wheelchair may be needed. Unlike SMA type 2, these patients do usually not suffer from orthopedic comorbidities or respiratory muscle weakness. Cognition and life expectancy are normal within this group. (Kugelberg 1956, Meadows 1969, Zerres 1997).

Spinal muscular atrophy type 4, is the mildest form of disease with an onset in adulthood, often at age 30 or later. Patients present with mild muscle weakness in the legs, that may proceed to the arms. A small number of patients may require wheelchair assistance. Cognition and life expectancy is normal (Pearn 1978, Tsukagoshi 1965, Piepers 2008).

Extensive research has been done over the last two decades to develop an effective treatment strategy for SMA. Nusinersen (Spinraza®, Biogen), the first approved drug for the treatment of SMA, has been available since December 2016. This is an antisense oligonucleotide drug that leads to an increased production of full-fledged functional SMN protein. It is administered directly intrathecally as it does not cross the blood-brain barrier. Several studies showed improvements in motor function and survival in patients treated with Nusinersen, where early treatment was necessary to maximize the benefit of the drug. Long-term data are still pending. Several other promising therapeutic approaches, including gene therapy for SMA, are currently under development.

There are currently no prospective markers available that can predict early muscle degeneration, disease progression, therapy response or therapeutic changes. Since 2017, the University Hospital Erlangen (Department of Medicine 1, Department of Pediatrics) has a multispectral optoacoustic tomography (MSOT) funded by the DFG. This allows non-invasive, quantitative imaging of the composition of target tissues.

In MSOT, similar to conventional sonography, a transducer is placed on the skin and instead of sound, energy is supplied to the tissue by means of pulsed laser light. This leads to a constant change of minimal expansions and contractions (thermoelastic expansion) of individual tissue components or molecules. The resulting sound waves can then be detected by the same examination unit. Previous studies have shown that the quantitative determination of hemoglobin can be used to obtain information on blood flow and inflammatory activity in the intestine from patients with Crohn's

disease (Waldner, Knieling et al. 2016, Knieling, Neufert et al. 2017) (Waldner 2016, Knieling 2017). This principle has already been successfully used in a follow-up study on pediatric patients with Duchenne muscular dystrophy (Regensburger 2019, ESMI Young Investigator Award 2019). In addition to this technology, MRI imaging has advanced in the field of muscle diseases. The first example is the ^{23}Na -MRI: while standard MRI is based on the imaging of protons (^1H), mainly water and hydrocarbon compounds in the human body, the ^{23}Na -MRI gets its signal from the sodium atoms of the human body. With this measurement technique, valuable information about cellular sodium homeostasis in muscular sodium channel diseases can be obtained,

which has meanwhile contributed to the development of successful interdisciplinary therapeutic concepts for some of these channelopathies (e.g. (Amarteifio, Nagel et al. 2012).

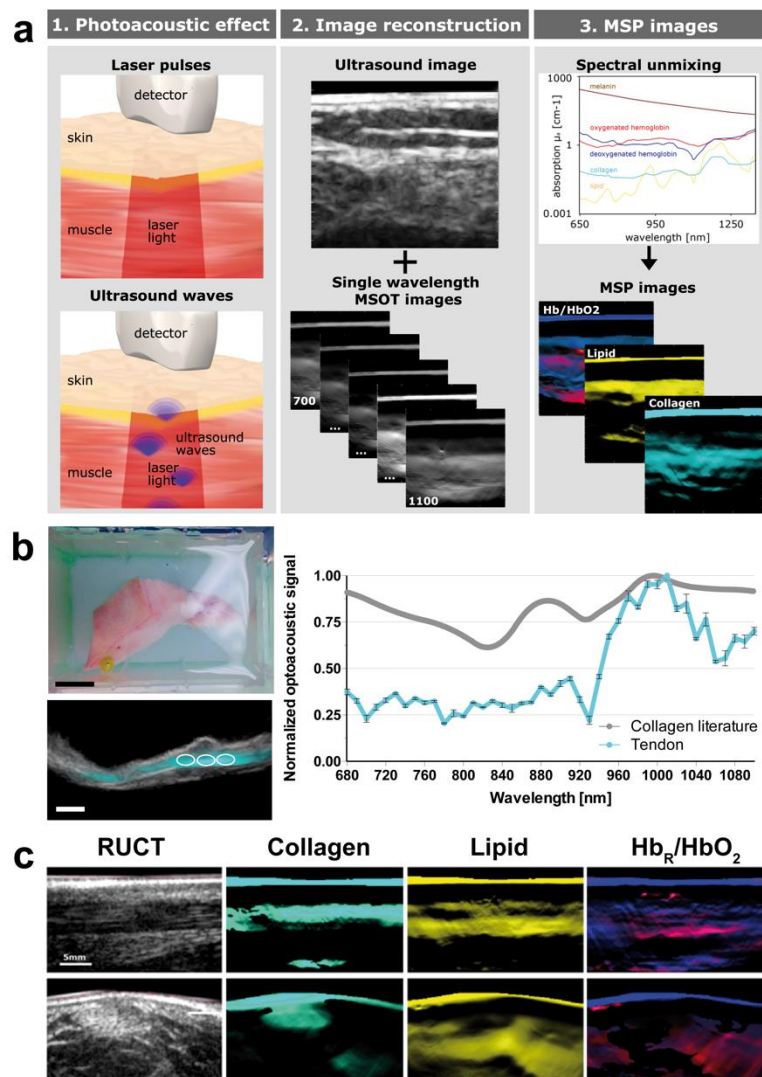


Abbildung 1 – MSOT Prinzip (a), experimentelle Vorarbeiten (b), und erstmalig Darstellung von Kollagen in vivo (c)

Hollingsworth et al (Hollingsworth, de Sousa et al. 2012) recommend the following MRI examination of skeletal muscles: T1-weighted imaging with lipid-water separation to quantify lipid infiltration and T2-mapping to examine inflammation and edema. However, many new MRI measurement techniques have not yet been used to examine rare muscle diseases. Our preliminary work shows that both technologies map different molecular structures but similar muscular pathologies (Regensburger et al. 2019). In this first pilot study on patients with SMA, we will now investigate whether the differences in muscle composition of SMA patients with or without therapy can be quantified and whether they can be used simultaneously as markers during therapy with nusinersen. Ideally, both techniques can complement or validate each other. In the future, this could generate a completely new, non-invasive method for evaluating endogenous biomarkers for therapy response.

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6. Study objectives

Evaluation of the muscle structure of patients with spinal muscular atrophy under Spinraza therapy using multispectral optoacoustic tomography (MSOT) and magnetic resonance imaging (MRI).

Hypotheses:

- The optoacoustic spectrum of patients with and without therapy differs at 0, 2 and 12 months
- The optoacoustic spectrum of patients with therapy changes over time.
- The quantitative fraction of lipid signal in muscles determined by MSOT differs between patients with and without therapy.
- The quantitative fraction of collagen signal in muscles determined by MSOT differs between patients with and without therapy.
- The quantitative fraction of hemo/myoglobin in muscles determined by MSOT differs between patients with and without therapy.
- The quantitative fraction of oxygenated/deoxygenated hemoglobin in muscles determined by MSOT differs between patients with and without therapy.
- There are no side differences in patients with and without therapy.
- T1 relaxation time of patients with and without therapy differs at 0, 2 and 12 months
- T2 relaxation time of patients with and without therapy differs at time 0, 2 and 12 months
- Fat-water fraction of patients with and without therapy differs at the time of 0, 2 and 12 months
- Sodium concentration of patients with and without therapy differs at 0, 2 and 12 months

Primary study objective:

- Comparison of the optoacoustic spectrum determined by MSOT in patients with SMA with and without treatment over time

Secondary study objectives:

- Comparison of the quantitative lipid/collagen/haemo/myoglobin content determined by MSOT in patients with and without therapy
- Correlation of lipid/collagen/haemo/myoglobin content determined by MSOT with disease duration/patient age

- Correlation of lipid/collagen/haemo/myoglobin content determined by MSOT with clinical routine testing
- Correlation of T1 relaxation time/T2 relaxation time/fat water portion/sodium concentration with clinical routine testing
- Correlation of T1 relaxation time/T2 relaxation time/fat water portion/sodium concentration and MSOT determined lipid/collagen/haemo/myoglobin content
- Measurement of the signal differences in the right / left comparison

Study type

Since no data exists so far to support the hypothesis of this study, it is an explorative study / pilot study.

7. Target parameters

The MRT measurement are performed on the lower leg muscles.

The MSOT measurements are performed over the proximal and distal extremity muscles in left-right comparison (right - left). (Leg proximal: musculus quadriceps, distal: musculus triceps surae; arm proximal: musculus biceps, distal: forearm flexors).

Primary target:

Optoacoustic Absorption Spectrum of Muscle.

This target is measured non-invasively by MSOT.

Secondary targets:

Quantitative lipid signal (in arbitrary units)

Quantitative collagen signal (in arbitrary units)

Quantitative hemo/myoglobin signal (in arbitrary units)

Muscle oxygenation (in %)

Ratio of lipid to hemo/myoglobin signal or collagen to hemo/myoglobin signal

These target values are collected non-invasively using MSOT.

T1 relaxation time

T2 relaxation time

Fat-water percentage

Sodium concentration

These target values are collected non-invasively using a 3T/7T MRI.

Clinical scores are collected according to the recommendations as standardized examinations. These are identical tests that, according to the University Clinic for Neurology/Department of Pediatrics and Adolescent Medicine, are also performed outside of the study in the regular clinical routine in SMA patients (see below).

Clinical test	Timeline				
	Induction				Maintenance
	d 0	d 14	d 28	d 63	every 4 months
Motor function					
HFMSE	X			X	X
RULM	X			X	X
MRC-Sum*	X			X	X
ALSFRS-R*	X			X	X
6-MWT	X			X	X
Dynamometer*	X			X	X
Neurophysio.* <small>(Nn. med./uln./tib. on both sides. + F-wave)</small>	X			X	X
Lung function					
VC/FVC/FEV1*	X	X	X	X	X

Muscle function tests: Expanded Hammersmith functional motor scale (HFMSE)/Revised Upper Limb Module (RULM)/6-Minute-walking-test (6MWT)/Medical Research Council (MRC) Scale for Muscle Strength/ALS Functional Rating Scale (ALS-FRS)

Source: Department of Neurology, University Hospital Erlangen, *not in adolescents

These target values are clinically determined at presentation.

Age
Sex
Weight
Skin color
Ethnic Background
Disease duration
Current medication

These target values are either available in the electronic patient file or they are collected at presentation for the study.

8. Study design

Monocentric / multicentric

This is a monocentric study.

Study arms: intervention/control

Interventions are not planned. A comparison is made between SMA patients with and without therapy. The study procedure is identical for all patients. There is no change in the already planned therapeutic regime.

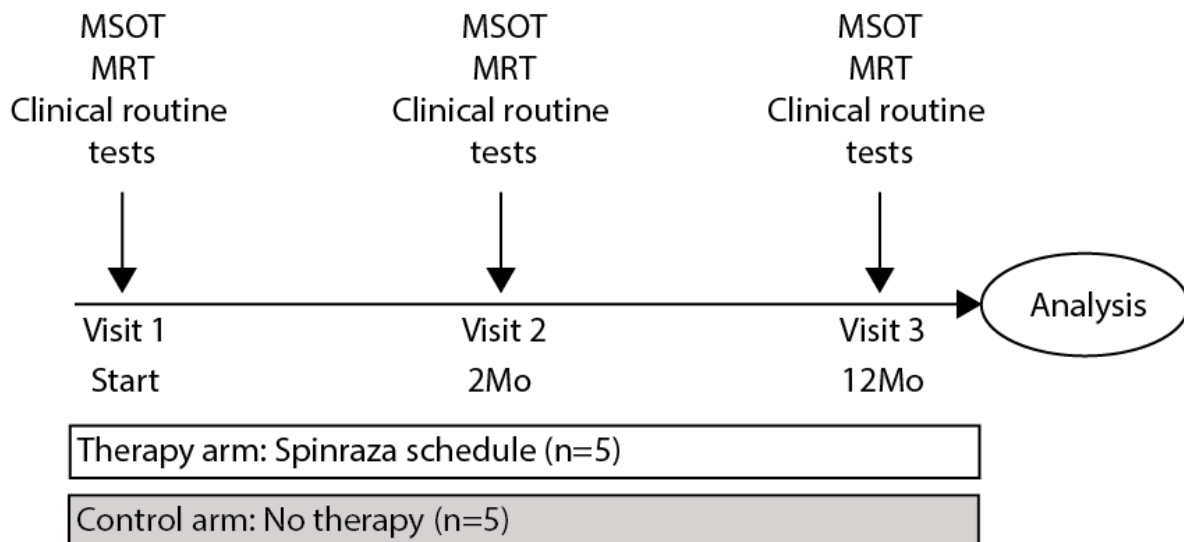
Randomization

Randomisation is not planned. The allocation to the groups is based on the known therapy.

Blinding

Blinding for the examination is not possible due to the small collective. Blinding takes place during the measurement and evaluation of the data. Blinding of the patients is not necessary.

Graphic presentation of the study design



9. Study population

Inclusion and exclusion criteria

Preliminary phase

Inclusion criteria:

SMA patients (therapy arm):

- Genetically confirmed SMA type III
- From age 14
- Willingness and ability to participate, sufficient knowledge of the german language to understand the declaration of consent, or if not possible, information of the patient in his/her mother tongue or English
- High probability that the patients will be able to fully participate in the study (defined by the ability to lie still for about 1 hour and follow any breathing commands)
- Medical indication for Spinraza® therapy; start of study before first administration Spinraza® administration

SMA patients (control arm):

- Genetically confirmed SMA type III
- From age 14
- Willingness and ability to participate, sufficient knowledge of the german language to understand the declaration of consent, or if not possible, information of the patient in his/her mother tongue or English
- High probability that the patients will be able to fully participate in the study (defined by the ability to lie still for about 1 hour and follow any breathing commands)
- **No** medical indication for Spinraza® therapy

Exclusion criteria:

SMA Patients:

- Pregnancy
- Tattoo on the skin area to be examined
- General contraindications for MRT examinations
- Electrical implants like pacemakers or perfusion pumps
- Pronounced claustrophobia

- Study participants with ferromagnetic or electrically conductive implants such as aneurysm clips, surgical clips, prostheses, artificial hearts, heart valves with metal parts, metal splinters, tattoos next to the eye, symmetrical tattoos on the extremities or steel implants must consult the study physician; they may not be able to be examined (relative contraindications for MRI).
- Non-approved concomitant medication: strongly sedating medication must be excluded, as intensive monitoring of bodily functions during ongoing imaging cannot be guaranteed and the examination is dependent on the active participation of the test person.

Patient number

As this is a pilot study, an exact case number calculation is not possible. It is planned to study a total of 5 patients with SMA with therapy and 5 patients with SMA without therapy.

Recruitment routes and measures

Patients (and parents) will be informed about the possibility of participating in the study during an elective presentation at the Department of Neuropediatrics in our Pediatric and Adolescent Clinic (children and adolescents) or at the Neuromuscular Center of the Neurology Department of the University Hospital. The primary aim is to include adult patients in this study. The medical indication for Spinraza® therapy was established in advance by the attending physicians of the Pediatric and Adolescent Clinic or the Neurology Clinic.

If patients are willing to participate, they will be fully informed about the aims and methods (especially about the scientific/explorative character of the study), benefits and risks and the revocability of their participation in the study. Patients in childhood and adolescence are additionally informed and educated in an age-appropriate manner about the study and its procedure.

10. Study course

Procedure for informing about and obtaining consent

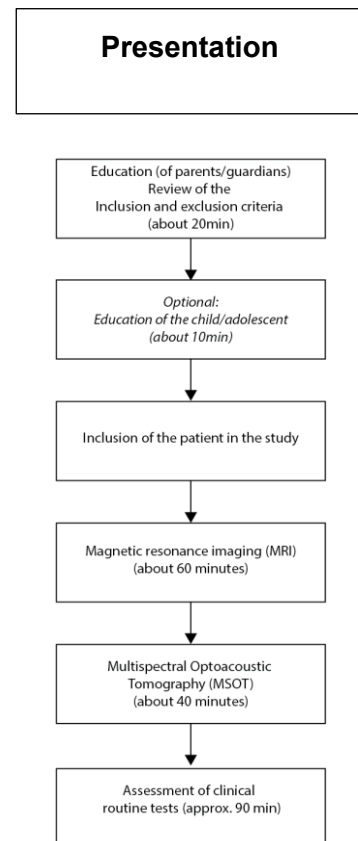
Patients can only be enrolled in the study after a written consent form has been given. The written declaration of consent requires oral and written information of the patients, as well as their parents or legal guardians, about objectives and methods (including the scientific-explorative character of the study), benefits and risks, and the revocability of study participation. Children and adolescents are informed by means of age-appropriate, comprehensible patient information sheets. By giving their written consent, the patients or their parents/guardians declare that they agree to the collection and storage of study-relevant data and their verification by monitoring or authorities. It must be clearly communicated to the study participant that a withdrawal of consent is possible at any time and without any disadvantage. Furthermore, all study participants/test persons or parents/guardians are informed that this study is a purely scientific study without any current diagnostic or therapeutic benefit.

The original of the consent form is kept in the study folder at the study location. The patient or parents/guardians will receive a copy of the patient information and informed consent form. The patient information and informed consent form are attached to this study protocol.

Measurements

After informing the patients and parents/guardians and obtaining consent, the MRI examination will be performed. This takes place in a lying position; according to the body region to be examined (lower leg), the test person is positioned with its feet first on the equipment couch. Rigid or flexible "coils" are attached directly or close to the patient. These coils serve as antennas that pick up the signals from the body and forward them directly to the computer. Hearing protection must be worn during the examination, as MRI examinations can be very loud. In order for the patient to be able to draw attention to themselves, he/she receives a bell button shortly before the examination begins. During the examination, communication via an intercom system is possible. An intravenous administration of contrast medium does not take place.

Subsequently, all study participants are imaged by MSOT on 4 anatomical regions: thigh/lower leg, forearm/upper arm at predefined muscle groups. (Leg proximal: musculus quadriceps, distal: musculus triceps



surae; arm proximal: musculus biceps, distal: forearm flexors). The examination is performed analogous to sonography over the corresponding skin layers without further invasive procedures. The anatomical region can be localized by means of built-in B-scan sonography; subsequently, the corresponding optoacoustic signals can then be conducted. The duration per anatomical region is limited to 5 minutes; this corresponds to a maximum of 20 minutes for both upper extremities and 20 minutes for both lower extremities. Patients can remain in a relaxed body position during the examination; assistance in the form of breathing manoeuvres or similar is not necessary. Clinical routine tests scores are then collected including tests for muscle strength like the Expanded Hammersmith functional motor scale (HFMS)/ Revised Upper Limb Module (RULM) / 6-minute-walking test (6MWT)/ Medical Research Council (MRC) Scale for Muscle Strength/ ALS Functional Rating Scale (ALS-FRS)/ Dynamometer as well as neurophysiologic testing and lung function testing.

Recording of target parameters

- MRI imaging and measurement of T1 relaxation time/T2 relaxation time/fat water proportion/ sodium concentration
- Non-invasive in-vivo measurement of lipid, collagen, myo/hemoglobin content and oxygenation by MSOT
- Clinical evaluation of routine testing including muscle tests, neurophysiologic testing and lung function testing
- Determination of routine data (duration of illness, current medication) from electronic patient files in Soarian/Meona

Time schedule and study duration for the individual patient/control

For the individual patient, the duration of study participation is 180 minutes. Approximately 20 minutes are allotted for the education of study participants and parents/guardians, approximately 90 minutes for the clinical routine testing, as well as 40 minutes for the MSOT and 60 minutes for MRI examination. The appointments are planned according to the therapeutic plan or the application of Spinraza®, so there is no additional travel time for the patients. The control group without therapy receives in addition to the annual controls (visit 1 and 3) one additional appointment for visit 2.

Total duration of study

Depending on the number of patients, the expected total duration of the study until the inclusion of the last patient is approximately 24 months.

11. Risk-benefit analysis

All study-related risks (for MSOT)

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

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

No CE certification is available for this research device (current type designation according to imprint: Acuity Echo). A conformity assessment procedure in the sense of the MPG is not intended or planned by the manufacturer at the present time. It is therefore a purely scientific pilot study. There is no dependency on the manufacturer, all diagnostic and analytical procedures are available to the study directors on site. The cooperation with the company is regulated in a separate contract drawn up by the legal department before the start of the study.

Adherence to energy levels

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

Temperature increases due to MSOT in tissue

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

Histological changes in tissue

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

Slight, reversible redness or warming might occur in very sensitive skin.

Such side effects are to be noticed at any time by the patient or doctor; the examination can then be interrupted or aborted. In any case, no irreversible damage is to be expected.

In general, the near infrared light used in the MSOT can lead to retinal damage if the eye is irradiated. In order to prevent this, test participants and examiners will wear appropriate laser safety glasses during the examination.

Since the data obtained is not used to interpret diagnostic results, there is no risk of possible misdiagnosis or incorrect display of data in this exploratory pilot study.

No other risks exist for this study, nor have we described any risks based on our own preliminary data.

Magnetic resonance imaging

In contrast to computed tomography, MRI does not use ionising radiation, so that no permanent side effects are expected. The risk for the planned MRI feasibility study is not higher than for any clinically performed native MRI examination.

Static magnetic field

The static magnetic field exerts forces and torques on ferromagnetic objects, which can be so strong that the (mostly ferromagnetic) objects fly uncontrolled towards the magnet and can hit patients and staff (missile effect). The magnetic forces are proportional to the field strength B and the field change with location (dB/dz). To protect the patients from the dangers of magnetic attraction of ferromagnetic objects, the operating personnel is trained to ensure that magnetic objects are not brought into the examination room. Magnetic fields also have general biological effects. For example, when movement occurs in the magnetic field, electrical voltages are induced in the human body. However, these effects are very small. In a study funded by the Federal Office for Radiation Protection, the cognitive performance of 41 healthy volunteers was examined at the German Cancer Research Center (DKFZ) at different magnetic field strengths (1.5T; 3T; 7T). During this study, the test subjects were lying on the patient table of the MR system. The tests were performed at rest and during a defined movement of the patient table. For the investigated static magnetic fields strength (up to 7T) no significant effects on cognitive performance were observed.

In summary, studies in animals and humans have shown that body temperature, blood pressure, heart rate, respiration or other physiological parameters are not affected by chronic exposure to static magnetic fields. Only a small but measurable increase in systolic blood pressure with increasing magnetic field strength has been shown. The maximum increase at 8 T was about 3.6 mmHg. However, this corresponds to only half the change caused by sitting up from the supine position outside a magnetic field. At no time symptomatic physiological changes were recorded, and no complaints were reported even after 3 months. The effects of

prolonged exposure to high magnetic fields above 4 Tesla on mammals, however, have been poorly studied, so that statements on long-term safety can only be made to a limited extent. No measurable effects were observed in studies in rats exposed to a field of 9.4T for up to 10 weeks.

The gradient system

Gradient circuits can cause the appearance of magnetic phosphenes as well as nerve and muscle cell stimulation. Fast switched gradients produce high magnetic field changes per time (dB/dt), which induce voltages in the body. If a current flows through the tissue, for example via nerve endings, this can lead to so-called peripheral nerve stimulation. However, the manufacturer of the gradient system guarantees that the limits for gradient switching times and amplitudes recommended in the IEC 60601-2-33 guidelines are adhered to. Therefore, the nerve stimulation effects do not need to be further considered in the risk assessment of this study.

Another safety-relevant effect of the gradient fields are noises caused by gradient switching, which develop due to current and field strength-dependent Lorentz forces in the gradient tube. These often unpleasant loud knocking noises are especially generated in fast imaging processes where high currents flow through the gradients. The test patients always wear hearing protection during the examination, so that the noise exposure remains well below the legal limits of 99 dB.

The high frequency system

During the MR measurement, high-frequency (HF) fields are sent into the human body, which are partially absorbed by the tissue and can lead to an increase in body temperature. The thermoregulatory response of human tissue to HF pulses has been studied for 50 years. For example, it has been calculated that the body temperature of lightly dressed subjects with undisturbed thermoregulation at room temperature increases by up to 0.6 °C (63 MHz, 1.5 Tesla) at an RF exposure of 4 W/kg. The assumed specific absorption rate (SAR) of 4 W/kg body tissue corresponds to the so-called "controlled mode first level" (IEC safety guideline), which is also used as the upper limit in routine clinical imaging. The magnitude of the real temperature rise is generally even smaller because skin cooling was not taken into account in the calculations (worst case scenario).

The body's own energy production at rest is about 1.2 W/kg - this corresponds to the energy that is conserved when wearing a thin sweater. Most healthy people are able to compensate 15x this resting energy, and there will only be a minimal increase in core body temperature. Studies at 1.5T have shown that HF absorption in humans only leads to the expected cardiac adaptation and does not cause any negative health effects. Theoretically, a 63 kg person is

even able to release 1296 W to the environment through the skin by cardiac adaptation (i.e. maximum blood flow increase) - this would correspond to a SAR of 20.6 W/kg.

Benefits associated with this study

The data obtained in this study may provide important insights into the pathomechanism of SMA. The possible quantitatively determinable differences could be used in the future as a monitoring method for therapeutic response or prognosis of this disease.

There is a potential benefit for the trial participants. With low probability, the scientific evaluation of the data may yield results that indicate previously unknown pathological changes in this patient. In this case, further investigation by a specialist would be recommended to the patient.

Termination criteria

Termination criteria for the individual participant:

- an event occurs that could endanger the patient or the personnel,
- the patient waives his/her consent to participate in the examination
- the patient does not follow the instructions of the examiner and the operating

Termination criteria for the whole study:

- Intermediate results indicate that the investigations cannot achieve the objective of the study

An examination can be interrupted by the test person/patient at any time without giving reasons. For this purpose, the MRI provides a so-called bell button. The activation of this button gives a signal to the doctor/examiner, helping the patient to draw attention to himself/herself even while the measurement is in progress.

In addition, between the individual measurements/examinations, the patient is in contact with the doctor/examiner via microphone and headphones and can thus also verbally request the abortion of an examination.

Statement of medical justifiability

The risk of occurrence of undesired events is considered as extremely low, especially in regard of previous experiences made in children with Duchenne muscular dystrophy and in healthy volunteers. No serious event has been reported so far, neither at our site nor in the literature. The majority of the reported (foreseeable) problems were related either to the use of ultrasound gel for examination or to the necessity to wear eye protection. The use of filter glass also

explains the reported vision redness. This phenomenon was reversible within seconds. Table 1 shows the reported adverse events from our study (MSOT_DMD, 67_18 B).

	Muscular dystrophy Duchenne N=10	Healthy volunteer N=10
Reversible adverse events- no.(%)		
Pressure of safety goggles	2 (20%)	
Coolness of Ultrasound-gel		4 (40%)
Red cast view		1 (10%)
Serious adverse events- no.(%)	0 (0%)	0 (0%)

Table – Adverse events

In this study no central organs are examined, but only extremities are measured - this leads to a further significant reduction of a possible residual risk. Likewise, contrast agents are not applied during any examination.

Particularly in light of the background of completely new therapeutic approaches, we hope that this method will provide us with a child-friendly, non-invasive diagnostic tool for the treatment of these complex diseases.

12. Biometrics

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

Case number calculation:

As this is a pilot study and no information on the expected differences between the different groups is available yet, no case number calculation was performed. The number of cases given represents an estimate or is within the appropriate range for a pilot study.

Statistical methods:

Continuous variables are given as mean values with standard deviation, categorical variables as numbers with percentages if necessary. The MSOT parameters are compared using a two-sided, unpaired t-test with the same deviations. If the standard deviation is unequal, a correction according to Welch may be applied. Furthermore, ROC analyses (Receiver Operator Characteristics) between patients with and without therapy are planned. Genetics serve as the gold standard. Correlations are indicated by the Pearson coefficient. All statistical tests are performed on both sides and a p-value of <0.05 is considered statistically significant. All analyses are performed using GraphPad Prism (version 7.00 or later, GraphPad Software, La Jolla, CA, USA), RStudio (version 1.1.456 or later, RStudio Inc., Boston, MA, USA) or IBM SPSS Statistics (version 24 or later, IBM Corp., Armonk, NY, USA).

13. Data management and data protection

Data acquisition and storage

All raw data, such as patient files, are source documents. Their availability is ensured for routine monitoring. The participation of the individual patients or test persons in the study is documented. The study leader maintains an independent list for the identification of the participating patients. This list contains the names and date of birth as well as the date of examination and pseudonymisation codes of the patients and subjects. The study leader is responsible for the quality of data collection and storage. The data storage (complete data) takes place on computers or specially designed network drives of the University Hospital Erlangen. The raw imaging data (no patient-related data) are stored on dedicated servers of iThera Medical GmbH.

Pseudonymisation

Prior to a scientific analysis of the materials and data of this study, all information will be pseudonymized according to the guidelines of the Federal Data Protection Act.

Data transfer

In this study, data transfer is only intended for the MSOT raw data. The company iThera Medical GmbH will work with this data to ensure adequate recording quality and to develop algorithms for evaluation. The data will only be passed on pseudonymously on encrypted physical drives. The data will not be used for later approval of the prototype used. The cooperation is explicitly regulated in advance by the legal department (an additional agreement to the service contract can be found in the appendix).

The study results can be published anonymously, whereby it will not be possible to draw conclusions about the identity of the participating persons. The data will be kept for 10 years and then destroyed.

Revocation, data deletion

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

14. **Handling of biomaterials**

No biomaterials are obtained.

15. **Insurance**

The participants of the study are insured via the group contract of the CCS Erlangen.

16. Signatures

Dr. med. Ferdinand Knieling
Study director

Prof. Dr. med. Regina Trollmann
Study director

Prof. Dr. med. Michael Uder
Study director

Prof. Dr. rer. nat. Armin Nagel
Study director

Dr. med. Matthias Türk
Study director

Prof. Dr. med. Maximilian Waldner
Technical implementation