Clinical Study Protocol

MRI-quantified Changes in Perfusion and Diffusion in Spinal Segments after High-Velocity, Low-Amplitude Spinal Manipulation: A Randomized Controlled Trial

Spinal Segment MRI Perfusion and Diffusion Response to Spinal Manipulation in Low Back Pain Patients

Study Type:	**Category A clinical trial. The trial includes an intervention that is neither a drug nor a transplant.**
Study Categorisation:	**A**
Study Registration:	**Clinical.trials.gov and SNCTP**

Sponsor, Sponsor-Investigator or Principal Investigator:	Petra Schweinhardt, MD, PhD Balgrist Campus Lengghalde 5 CH-8008 Zürich Tel +41 44 510 73 81 E-Mail petra.schweinhardt@balgrist.ch

Investigational Product:	**-**
Protocol Version and Date:	**V3, 22.11.2018**

SIGNATURE PAGE

Study number

Study Title **MRI-quantified Changes in Perfusion and Diffusion in Spinal Segments after High-Velocity, Low-Amplitude Spinal Manipulation: A Randomized Controlled Trial**

The Sponsor-Investigator and trial statistician have approved the protocol version 3, 22.11.2018, and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor/ Petra Schweinhardt, MD, PhD

Principal Investigator Head of Research Department of Chiropractic Medicine

Balgrist Campus

Lengghalde 5

CH-8008 Zürich

Tel +41 44 510 73 81

E-Mail petra.schweinhardt@balgrist.ch

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Place/Date	Signature

TABLE OF CONTENTS

STUDY SYNOPSIS

Sponsor-Investigator	Petra Schweinhardt, MD, PhD, Head of Research Department of Chiropractic Medicine University Hospital Balgrist
Study Title:	MRI-quantified Changes in Perfusion and Diffusion in Spinal Segments after High-Velocity, Low-Amplitude Spinal Manipulation: A Randomized Controlled Trial
Short Title / Study ID:	Spinal Segment MRI Perfusion and Diffusion Response to Spinal Manipulation in Low Back Pain Patients
Protocol Version and Date:	V3, 22.11.2018
Trial registration:	http://www.clinicaltrials.gov and http://www.snctp.ch
Study category and Rationale	Clinical Study Category A, other clinical trials, this study includes an intervention that is neither a drug nor a transplant.
Clinical Phase:	**n/a**
Background and Rationale:	As a non-invasive treatment method, high-velocity, low-amplitude spinal manipulative therapy (SMT) is used in the conservative treatment of non-specific low back pain (LBP). Several studies suggest an association with favourable outcome. However, the overall effect size of clinical improvement following SMT remains modest[1-7]. Most research on the effectiveness of SMT on back pain is conducted on patients suffering from “non-specific” LBP, the diagnosis of which is usually based upon the criterium that pain is not attributed to a recognizable pathology (e.g., infection, tumor, inflammatory diseases, fractures, disc herniations with nerve compression)[8]. In most of these studies, it is unclear whether patient’s diagnoses were based upon more than history and clinical examination[1,2]. We argue that in the majority of cases, back pain labelled as non-specific might be more accurately labelled as non-specified and does have an attributable source and cause, whether we are able to recognize it or not. Therefore, if the diagnosis of non-specific LBP comprises heterogenic conditions, it is unlikely that patients will respond to one therapeutic modality equally[8-11]. Diffusion-weighted MR-imaging (DWI) is an application of magnetic resonance imaging that allows the measurement of water movement within and between tissues and is increasingly being used to study musculoskeletal physiology[12]. Because DWI is sensitive even to small changes in fluid flow, it is potentially valuable in the study of altered diffusion in various diseased or injured spinal tissues[13,14]. Studies using DWI have suggested that changes in diffusion within lumbar intervertebral discs occur in response to joint mobilization[15], prone press-ups[16], spinal traction[14] and spinal manipulative therapy[17,18]. Intravoxel incoherent motion (IVIM) is a method proposed in 1988 by Le Bihan et al. which additionally extracts information on microvascular blood flow (capillary perfusion) as “pseudo-diffusion” from diffusion-weighted imaging acquired at different b-values[19,20]. It is used to generate separate images of diffusion and perfusion without requiring the injection of exogenous contrast agents. Originally developed in brain studies, it has been used in recent years to study perfusion in musculoskeletal tissue[21,22]. There is a paucity of research addressing physiological events following a SMT intervention for patients with low back pain[17]. A deeper understanding of local biomechanical and neurophysiological effects of SMT interventions will help refine its utilization and improve its effectiveness[23].

Objectives:	The main objective of this study is to quantify changes in diffusion and perfusion in the intervertebral disc and adjacent spinal muscle tissue of a spinal segment receiving SMT or control intervention and to evaluate whether those changes differ in spinal segments with or without degenerative changes in low back pain patients. The second study objective is to evaluate the repeatability of IVIM-MRI for quantifying perfusion changes in musculoskeletal tissues and to assess diurnal changes in perfusion and diffusion parameters in spinal tissues as a positive control.
Outcomes:	The primary study outcome is the change in diffusion (ΔADC) and perfusion (Δ perfusion fraction, Δ pseudodiffusion coefficient) parameters measured on DWI and IVIM MRI in the intervertebral disc and adjacent spinal muscle tissue of a lumbar spinal segment following a lumbar SMT or control intervention to that segment, or, for controls, after 20 minutes of lying in supine resting position. Secondary outcomes are the repeatability and diurnal changes of diffusion- and perfusion-weighted MRI parameters in musculoskeletal tissue in controls, the severity of degenerative changes classified according to the Pfirrmann- and Weishaupt-Classification on MRI, clinical baseline parameters, pro- and anti-inflammatory markers in the obtained blood samples and changes in pain intensity on a numerical pain rating scale after the intervention.
Study design:	Randomized controlled clinical trial

Inclusion / Exclusion criteria:	Inclusion criteria: Patients: (a) Persons over 18 years and below 75 years of age with low back pain of any duration clinically not attributable to “red flags” (i.e., infection, trauma, fractures, inflammatory spondylarthropathies). (b) Source of LBP clinically at the L4/5 or L5/S1 segment (c) Duration of LBP is longer than 4 weeks (d) Obtained informed consent. Controls: (a) Persons over 18 years and below 75 years of age who have not suffered from low back pain in the last year and have never experienced low back pain for longer than 7 consecutive days. (b) Obtained informed consent. Exclusion criteria: Subjects are excluded from enrolment if they a) have undergone spinal surgery in the lumbar spine b) have undergone facet joint, epidural or periradicular injections in the last 6 months c) had a spinal manipulative therapy intervention in the past 2 weeks d) have spinal abnormalities (benign or malignant tumors, congenital anomalies, isthmic spondylolisthesis) e) have any contraindication to spinal manipulative interventions or are deemed unable to tolerate SMT to both body sides (e.g. pain attributable to above mentioned red flags, inability to perform sidelying without pain, radiculopathy with motor deficits <M4-, severe spinal canal stenosis) f) have any contraindication to MRI (e.g. heart pacemaker, metallic foreign body or claustrophobia) g) are pregnant h) have neurological deficits related to their back pain

Measurements and procedures:	45 minutes prior to the first round of imaging, patients and controls provide baseline information on current pain and function (painDetect questionnaire[24], Oswestry Disability Index (ODI)[25]). Additional information is collected on the localization of pain (pain drawing), whether back or leg pain is dominant, and whether there is a preference in movements or activities (e.g. walking, sitting for a long time, rising from sitting, forward bending or standing) [2627-29]. Patients and controls then undergo a clinical examination including assessment of blood pressure and pulse, lower extremity neurological examination[30], nerve tension tests, active range of motion of the lumbar spine, facet joint provocation test [31,32], and palpatory segmental examination of the lumbar spine[33,34]. In patients, the skin above the interspinous ligament causing the most pain upon palpation by the examiner is marked with pen. A blood sample of approximately 10-15 ml is obtained from participants agreeing to additionally undergo venipuncture by a medical professional trained in venipuncture. This procedure is not mandatory and is agreed to separately in the informed consent form. The examination is followed by a rest period of 10 minutes. Patients and controls are then evaluated in the MRI-Scanner lying supine. Upon completion of this scan, patients will be randomized either to receive either a total of two spinal manipulative interventions, first in right, then left side-lying position at the segment of the marked interspinous ligament, or receive only pre-SMT positioning by the chiropractor administering the intervention, who places his/her hand on the site of the marked skin above the interspinous ligament without applying pressure, preload or thrust in right and left side-lying position. For patients, three sequential post intervention MRIs are performed immediately after the intervention. Once imaging is complete, the patients exit the scan room and provide post-intervention information on current pain-intensity. Blood pressure and pulse are reassessed. Patients will be contacted after 1 week, 1 month and 3 months and follow-up data will be collected on pain and disability. Controls are reassessed in a second MRI after 20 minutes of supine lying to assess the repeatability of IVIM imaging, and will undergo a third imaging sequence approximately 10 hours later (after partaking in activities of daily life as normal, but excluding strenuous physical activity) in order to assess diurnal changes in perfusion and diffusion parameters on IVIM and DWI imaging. This scan will serve as a positive control that diurnal changes in diffusion parameters described in the nucleus pulposus can be detected[35].

Study Product / Intervention:	A change from supine position to right-side lying position. The patient is positioned by the chiropractor (licensed by the swiss federal authorities) administering the intervention, and receives a spinous push-pull SMT intervention as described by Bergman et al. at the previously marked segment[36]. Specifically, the patient lies in side-lying position, with the hip of the up-side leg flexed to ca. 90° and the foot of the flexed leg hooked behind the popliteal space of the down-side leg. The chiropractor stands facing the subject at a 45°-angle, fixating the flexed knee with his own knee/thigh. The fingertips of the cephalic hand reach under the patient's up-side arm to contact the up-side lateral surface of the superior spinous process. The fingertips of the caudal hand hook the inferior spinous process, contacting the down-side aspect of the spinous process, while the forearm contacts the patient's posterolateral buttock and thigh. The cephalic hand thrusts lateromedially and caudocranially, from up-side toward down-side ("push"). The caudal hand thrusts lateromedially in the opposing direction, from down-side toward up-side, toward the chiropractor ("pull"). The SMT intervention is repeated after the subject turns to left side lying. The subject then resumes supine position.
Control Intervention (if applicable):	A change from supine position to right-side lying position. The subject is positioned with the hip of the upper leg flexed to ca. 90° and the foot of the flexed leg hooked behind the popliteal space of the down-side leg. The chiropractor stands facing the subject at a 45°-angle, fixating the flexed knee with his own knee and placing his hand on the previously marked segment. No pressure, preload or thrust is administered. This intervention is repeated after the subject turns to left side lying. The subject resumes supine position.
Number of Participants with Rationale:	After calculating the sample size based on an analysis of existing data[17,18], 50 patients and 20 controls are expected to be included in this study within a recruiting period of 12 months.
Study Duration:	The estimated duration for the main investigational plan is 24 months
Study Schedule:	September 2018: Application for ethical approval December 2018: Inclusion of first participant December 2019: Inclusion of last participant June 2020: Statistical analysis, Report
Investigator(s):	Dr. med. chiro. Malin Mühlemann, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich, Tel +41 44 386 57 12 malin.muehlemann@balgrist.ch M. chiro. med. Luana Nyirö, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich, Tel +41 44 386 57 28 luana.nyiroe@balgrist.ch PD Dr. med. Reto Sutter, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich, Tel +41 44 386 11 11 PD Dr. med. Lukas Filli, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich, Tel +41 44 386 11 11 Dr. Stefan Dudli, PhD, University Hospital Zurich Center of Experimental Rheumatology, Balgrist Campus, Lengghalde 5, CH-8008 Zürich PD Dr. Daniel Nanz, Balgrist Campus, Lengghalde 5, CH-8008 Zürich Prof. Dr. med. Mazda Farshad, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich, Tel +41 44 386 11 11

Study Centre(s):	**University Hospital Balgrist** **Forchstrasse 340** **CH-8008 Zürich** **and** **Balgrist Campus** Lengghalde 5 8008 Zürich
Statistical Considerations:	Parametric and non-parametric statistical tests will be performed as appropriate. After an analysis of existing data[17,18], 50 patients and 20 controls are expected to be included in this study within a recruiting period of 12 months.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

Abbreviations

AE	Adverse Event
ADC	Apparent Diffusion Coefficient
ASR	Annual Safety Report
BASEC	Business Administration System for Ethical Committees
CA	Competent Authority (e.g. Swissethics)
CEC	Competent Ethics Committee
CRF	Case Report Form
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin)*
eCRF	Electronic Case Report Form
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
DWI	Diffusion-weighted imaging
EP	End plate
FSE	Fast Spin Echo
GCP	Good Clinical Practice
HRA	Federal Act on Research involving Human Beings
HVLA	High-velocity, low-amplitude
ISO	International Organisation for Standardisation
IVIM	Intravoxel incoherent motion
MD	Medical Device
MRI	Magnetic resonance imaging
ODI	Oswestry Disability Index
PI	Principal Investigator
SMT	Spinal Manipulative Therapy
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

STUDY SCHEDULE

Study Periods	Screen-ing	Intervention Period				Follow up 1 week	Follow up 1 month	Follow up 3 months
Visit	1	2	2	2	2	3	4	5
Time (minutes)	-45	-15	0	+5	+10 hours	+7 days	+30 days	+ 90 days
Patient Information and Informed Consent	x							
Demographics	x							
Medical History	x							
In- /Exclusion Criteria	x	x						
Physical Examination	x							
Optional Blood Sample	x							
Pain Questionnaires	x					x	x	x
Vital Signs	x	x	x	x				
Pregnancy Test (if needed)	x							
Randomisation		x						
Diffusion- and Perfusion-weighted MRI for Patients		x		x				
Diffusion- and Perfusion-weighted MRI for Controls		x		x	x			
Administration of Intervention/ Control Intervention for Patients			x					
Supine Lying for Controls			x					
Primary Variables: MRI		x	x	x				
Secondary Variables	x	x						
Concomitant Therapy, Intervention						x	x	x
Adverse Events	x	x	x	x		x	x	x

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor and Principal Investigator

Petra Schweinhardt, MD, PhD, Balgrist Campus AG, Lengghalde 5, CH-8008 Zürich, is going to supervise data collection and management as well as analysis and interpretation.

1.2 Investigator(s)

Dr. med. chiro. Malin Mühlemann, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich
Tel +41 44 386 57 12, malin.muehlemann@balgrist.ch

M. chiro. med. Luana Nyirö, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich
Tel +41 44 386 57 28, luana.nyiroe@balgrist.ch

PD Dr. med. Reto Sutter, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich reto.sutter@balgrist.ch

PD Dr. med. Lukas Filli, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich lukas.filli@balgrist.ch

Dr. Stefan Dudli, PhD, University Hospital Zurich Center of Experimental Rheumatology, Balgrist Campus, Lengghalde 5, CH-8008 Zürich

PD Dr. Daniel Nanz, Balgrist Campus, Lengghalde 5, CH-8008 Zürich, daniel.nanz@balgristcampus.ch

Prof. Dr. med. Mazda Farshad, Universitätsklinik Balgrist, Forchstrasse 340, CH-8008 Zürich mazda.farshad@balgrist.ch

1.3 Statistician ("Biostatistician")

Petra Schweinhardt, MD, PhD, Balgrist Campus, Lengghalde 5, CH-8008 Zürich
E-Mail petra.schweinhardt@balgrist.ch, Tel +41 44 510 73 81

1.4 Centre

University Hospital Balgrist

Forchstrasse 340

CH-8008 Zürich

Balgrist Campus AG

Swiss Center for Musculoskeletal Imaging (SCMI) and

Swiss Center for Musculoskeletal Biobanking (SCMB)Lengghalde 5

8008 Zürich

1.5 Monitoring institution

Monitoring will be performed by internal staff not involved directly in data collection and data management.

1.6 Data Safety Monitoring Committee

Not applicable.

1.7 Any other relevant Committee, Person, Organisation, Institution

Not applicable.

2. ETHICAL AND REGULATORY ASPECTS

2.1 Study registration

The study will be registered at http://www.clinicaltrials.gov and http://www.snctp.ch.

2.2 Categorisation of study

This study is categorized as a Clinical Study Category A, This study includes an intervention that is neither a drug nor a transplant. It entails only minimal risks and burdens for participants and the intervention is recognised as standard.

2.3 Competent Ethics Committee (CEC)

The investigator ensures that approval from an appropriately constituted Competent Ethics Committee (CEC) is sought for the clinical study.

All changes in research activity and all unanticipated problems involving risks to humans (including in case of planned or premature study end and the final report) will be reported, and no changes will be made to the protocol without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 2.10

2.4 Competent Authorities (CA)

CA (swissmedic) approval is only necessary for category B and C studies. Category A studies do not require a CA approval. The CA is entitled to carry out inspections of all clinical trials.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance with the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, in case of medical device: the European Directive on medical devices 93/42/EEC and the ISO Norm 14155 and ISO 14971, the Swiss Law and Swiss regulatory authority's requirements. The CEC will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

There is no conflict of interest.

2.7 Patient Information and Informed Consent

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

The participant will be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation

in the study.

The patient information sheet and the consent form will be submitted to the CEC and to the competent authority (as applicable) to be reviewed and approved. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and is going to be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the CEC.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.

All non-substantial amendments are communicated to the CEC within the Annual Safety Report (ASR).

3. Background and Rationale

3.1 Background and Rationale

Non-specific low back pain (LBP) is a very common condition in developed countries, with a point prevalence between 15% and 30% and a lifetime prevalence of about 70%[37]. As a non-invasive treatment method, high-velocity, low-amplitude spinal manipulative therapy (SMT) is used in the conservative treatment of non-specific low back pain (LBP). Several studies suggest an association with favourable outcome. However, the overall effect size of clinical improvement following SMT remains modest[1-7]. Most research on the effectiveness of SMT on back pain is conducted on patients suffering from "non-specific" LBP, the diagnosis of which is usually based upon the criterium that pain is not attributed to a recognizable pathology (e.g., infection, tumor, inflammatory diseases, fractures, disc herniations with nerve compression)[8]. In most of these studies, it is unclear whether patients' diagnoses were based upon more than history and clinical examination[1,2]. We argue that in the majority of cases, back pain labelled as non-specific might be more accurately labelled as non-specified and does have an attributable source and cause, whether we are able to recognize it or not. Therefore, if the diagnosis of non-specific LBP comprises heterogenic conditions, it is unlikely that patients will respond to one therapeutic modality equally[8-11].

Diffusion-weighted MR-imaging (DWI) is an application of magnetic resonance imaging that allows the measurement of water movement within and between tissues and is increasingly being used to study musculoskeletal physiology[12]. Because DWI is sensitive even to small changes in fluid flow, it is potentially valuable in the study of the influence of therapeutic interventions such as manual therapy, exercise, and physical agents on musculoskeletal tissues[38]. Studies using DWI have suggested that changes in diffusion within lumbar intervertebral discs occur in response to joint mobilization[15], prone press-ups[16], spinal traction[14] and spinal manipulative therapy[17,18].

Intravoxel incoherent motion (IVIM) is a method proposed in 1988 by Le Bihan et al. which extracts microvascular blood flow information from diffusion-weighted imaging acquired at different b-values[19,20]. It is used to generate separate images of diffusion and perfusion without requiring the injection of exogenous contrast agents. Originally developed in brain studies, it has been used in recent years to study perfusion in musculoskeletal tissue[21,22]. There is a paucity of research addressing physiological events following a SMT intervention for patients with low back pain[17]. A deeper understanding of local biomechanical and neurophysiological effects of SMT interventions might help refine its utilization and improve its effectiveness[23].

The purpose of our study is to quantify changes in diffusion and perfusion parameters within the intervertebral disc and paraspinal muscle tissue of a spinal segment receiving a spinal manipulative intervention and to evaluate whether those changes differ in spinal segments with or without degenerative changes in symptomatic and asymptomatic subjects.

Additionally, it is of interest to determine the relationships between baseline parameters and changes in diffusion and perfusion as well as the degree of degenerative changes. Post-intervention changes in segmental spinal muscle perfusion will also be investigated.

The repeatability and diurnal variation of IVIM imaging in quantifying perfusion in musculoskeletal tissues will be assessed by comparing perfusion parameters of 20 controls who will not receive any intervention. We will perform a diffusion- and perfusion-weighted MRI at the beginning of the visit in the morning, which is repeated after approximately 20 minutes of rest (lying supine). Controls will present for an additional MRI approximately 10 hours later in the evening of the same day.

In participants consenting to venipuncture, approximately 8 ml of venous blood will be withdrawn using serum collection tubes, allowed to clot, and then centrifuged. The resulting serum supernatant is stored in aliquots at Balgrist Campus at -80°C. It will be analysed at the end of data collection for pro- and anti-inflammatory markers (e.g. C-reactive Protein, Interleukin 1-beta, Interleukin 6 and Tumor Necrosis Factor) in order to determine the relationship between inflammatory markers and degenerative changes, pain duration, and pain and disability outcomes[39].

3.2 Investigational Product (treatment, device) and Indication

Not applicable.

3.3 Preclinical Evidence

Not applicable.

3.4 Clinical Evidence to Date

Diffusion-weighted imaging is an MRI procedure that generates images of biological tissues in which water diffusion is exponentially related to signal intensity (brightness)[40,41]. Originally used only for brain imaging to detect early signs of stroke and other brain abnormalities, diffusion-weighted MRI now has many applications within the musculoskeletal system that are increasing the knowledge of diagnosis and intervention[38].

Intravoxel incoherent motion (IVIM) is a method to separate microcirculatory effects from thermal diffusion effects in diffusion-weighted MRI.[19] IVIM offers a way to study microcirculatory blood-flow properties directly, without intravenous contrast injection. In the past years, studies validating the IVIM method to measure perfusion have accumulated due to major advancements seen in MR hardware, pulse design and post-processing capabilities. The role of IVIM in the muskuloskeletal tissue is not yet entirely clear.

A study by Beattie et al.[17] suggests that differences in a patient's reported pain intensity following a single SMT intervention are related to changes in the rate of diffusion of water in the intervertebral discs of the upper lumbar spine and the lumbosacral junction. The results of the study are consistent with previous observations by the same authors of a significant interaction between subject responses to a single treatment of manual pressures followed by prone press-up exercises administered to patients with LBP[16].

3.5 Dose Rationale / Medical Device: Rationale for the intended purpose in study (pre-market MD)

Not applicable.

3.6 Explanation for choice of comparator (or placebo)

The intervention and control procedure differ only in the administration of a high-velocity, low-amplitude SMT intervention for the intervention group. The control procedure therefore is chosen to be identical to the intervention procedure with the exception that tissue pressure and thrust are not administered to the spinal segment. This ensures that there is minimal confounding due to position changes and human touch.

3.7 Risks / Benefits

SMT is commonly associated with transient minor musculoskeletal harms such as muscle soreness and local discomfort, although they may be equally common following non-SMT manual therapy[1]. Case reports and systematic and nonsystematic reviews have included discussion of serious harms and adverse events following SMT in general and following SMT for low back pain. Examples of reported serious adverse events following lumbopelvic SMT intervention include cauda equina syndrome, lumbar disk herniation, fracture, and spinal hematoma[42]. However, these reports were not able to assess causality or calculate the incidence of serious adverse events following SMT[1,43].

In a recent systematic review and metaanalysis, SMT interventions for acute low back pain were associated with statistically and clinically significant benefit in pain and function at up to 6 weeks[1]. The size of the effect on pain is about the same as for nonsteroidal anti-inflammatory drugs in acute low back pain, according to a Cochrane review on this topic[44]. For chronic back pain, there is evidence that SMT has a statistically significant but not clinically relevant effect on pain relief and functional status compared to other interventions[42]. All SMT interventions in this study will be performed by experienced Doctors of Chiropractic with over 10 years of clinical experience. Contraindications for SMT interventions will be strictly monitored at baseline.

There are no known health hazards from temporary exposure to the MR environment. The MR environment does involve a strong static and pulsed magnetic field, as well as radiofrequency energy. Specific safety concerns concerning these factors must be taken into account. Patients are not exposed to the harmful effects of ionizing radiation, as MR images are obtained without the use of ionizing radiation.

Ferromagnetic objects brought into the static magnetic field may cause damage to the scanner or injury to the patient or medical professionals, as those objects may become projectiles. Careful screening of people and objects entering the MR environment is obligatory[45]

3.8 Justification of choice of study population

Patients included in this study are persons over 18 years and below 75 years of age with low back pain of a duration of more than 4 weeks clinically not attributable to “red flags” (i.e., infection, trauma, fractures, inflammatory spondylarthropathies). This corresponds to the patient population typically treated in chiropractic practices in Switzerland[46]. Controls are healthy participants over 18 and below 75 years of age who have not suffered from low back pain in the last year and have never experienced low back pain for longer than 7 consecutive days. Patients and controls provide informed consent before inclusion into the study. No vulnerable participants will be included in this study. Study Objective

3.9 Overall Objective

The purpose of our study is to assess and quantify changes in diffusion and perfusion within the intervertebral disc and adjacent spinal muscle tissues of a lumbar spinal segment receiving spinal manipulative intervention.

3.10 Primary Objective

The primary objective is to measure the change in diffusion and perfusion on diffusion-weighted and IVIM MRI in the intervertebral disc and adjacent spinal muscle tissue of a lumbar spinal segment following a lumbar SMT intervention or control intervention to that segment.

3.11 Secondary Objectives

Secondary objectives are to determine if diffusion and perfusion changes in DWI and IVIM-MRI are dependent on the severity of degenerative changes classified according to the Pfirrmann- and Weishaupt-Classification; to establish the relationship between clinical baseline parameters and diffusion and perfusion changes in DWI and IVIM-MRI and to determine the repeatability of IVIM-MRI in assessing perfusion changes, as well as assessing diurnal changes in perfusion and diffusion parameters in spinal tissues of healthy controls. Additionally, the presence of several pro- and anti-inflammatory markers in blood will be assessed in patients and controls.

3.12 Safety Objectives

Not applicable as the methods are used according to their certification and labelling, respectively.

4. STUDY OUTCOMES

4.1 Primary Outcome

Changes in diffusion in intra- and extracellular compartments (D), apparent diffusion coefficient (ADC), perfusion in capillaries (D*) and volume fraction of capillary perfusion (f) in the intervertebral disc and adjacent spinal muscle tissue in patients before and after an SMT- or control intervention and in controls before and after 20 minutes of lying supine.

4.2 Secondary Outcomes

Secondary outcomes are the repeatability and diurnal changes of diffusion- and perfusion-weighted MRI parameters in musculoskeletal tissue in controls.

4.3 Other Outcomes of Interest

The severity of degenerative changes classified according to the Pfirrmann- and Weishaupt-Classification on MRI, clinical baseline parameters, pro- and anti-inflammatory markers in the obtained blood samples, change in disability and pain intensity on a numerical pain rating scale after the intervention.

4.4 Safety Outcomes

Not applicable.

5. STUDY DESIGN

5.1 General study design and justification of design

This study is designed as a prospective randomized controlled clinical trial. 50 persons over 18 and below 75 years of age with low back pain of more than 4 weeks without any "red flags" (i.e., infection, trauma, fractures, inflammatory spondylarthropathies) as well as 20 asymptomatic controls meeting the inclusion criteria are included. Study visits will take place in the morning at the same time.

Patients and controls report baseline information and receive a standardized baseline examination. Patients' skin above the interspinous ligament causing the most pain upon palpation by the examiner is marked with a pen. The examiner is blinded to the intervention that follows.

Patients and controls are evaluated in the MRI-Scanner lying supine prior to intervention. The radiologist will perform a preliminary viewing of the MRI sequence to screen for contraindications to SMT (e.g. tumors, infection, fractures, severe nerve compression). Patients with contraindications for SMT will be excluded from the study.

Patients will be randomized using stratified randomization to receive either an SMT intervention in right and left side-lying position at the segment of the marked interspinous ligament, or receive a control intervention in right and left side-lying position. The chiropractor administering the intervention is blinded to clinical and radiological information. After this, patients undergo 3 sequential MR-imaging rounds. Once imaging is complete, the subjects exit the scan room and provide information on post-intervention pain-intensity.

MRI-quantified changes in diffusion and perfusion of intervertebral discs with and without degenerative changes and of adjacent spinal muscle tissue after SMT intervention are assessed and compared between patients and controls.

In order to assess the repeatability of IVIM imaging in quantifying perfusion in musculoskeletal tissues, 20 controls will undergo a separate procedure consisting of a baseline diffusion- and perfusion-weighted MRI, which is repeated after 20 minutes of lying in a supine position. In order to assess diurnal changes in perfusion and diffusion parameters in musculoskeletal tissue, controls will present for a third MRI approximately 10 hours later, after partaking in activities of daily life as normal, but excluding strenuous physical activity.

Patients will be contacted by telephone at one week, one month and three months after the intervention. Their global impression of change will be assessed. Type and quantity of concomitant therapies and interventions will be assessed. The Oswestry Disability Index and painDetect questionnaires will be repeated.

5.2 Methods of minimizing bias

5.2.1 Randomization

Patients are subdivided into strata in order to balance for age and sex, then permuted block randomization is used for each stratum to assign patients to receive either SMT or control intervention.

5.2.2 Blinding procedures

The examiner and radiologist will be blinded to the intervention. The intervention administrator will be blinded to the result of the patient examination and MRI. Patient blinding to intervention is not possible due to the nature of SMT.

5.2.3 Other methods of minimizing bias

The use of standardized protocols for data collection and training of study personnel will minimize interobserver variability. Validated questionnaires (painDetect and ODI) will be used to assess pain qualities and pain-related disability to minimize wording bias.

5.3 Unblinding procedures (Code break)

Unblinding will be done only for the purpose of data interpretation after the inclusion of the last partic-

ipant.

6. STUDY POPULATION

6.1 Eligibility criteria

Inclusion criteria:

Patients:

- **a) Persons over 18 years and under 75 years of age with low back pain of any duration clinically not attributable to "red flags" (infection, trauma, fractures, inflammatory illnesses).**
- **b) Source of LBP clinically at the L4/5 or L5/S1 segment.**
- c) Duration of LBP is longer than 4 weeks
- **d) Obtained informed consent.**

Controls:

- a) Persons over 18 years and under 75 years of age who have not suffered from low back pain in the last year and have never experienced low back pain for longer than 7 consecutive days.
- b) Obtained informed consent.

Exclusion criteria:

Subjects are excluded from enrolment if they

- **a) have undergone prior spinal surgery**
- **b) have undergone facet joint, epidural or periradicular injections in the last 6 months**
- c) had a spinal manipulative therapy intervention in the past 2 weeks
- **d) have spinal abnormalities (benign or malignant tumors, congenital abnormalities, isthmic spondylolisthesis)**
- **e) have any contraindication to spinal manipulative interventions or are deemed unable to tolerate SMT to both body sides (e.g. pain attributable to above mentioned red flags, inability to perform side-lying without pain, radiculopathy with motor deficits <M4-, severe spinal canal stenosis)**
- **f) have any contraindication to MRI (e.g. heart pacemaker, metallic foreign body or claustrophobia)**
- g) have started a new prescription medication targeting blood circulation within the last 3 months
- **h) are pregnant or nursing**
- i) Subjects are excluded from venipuncture if they are known to be HIV-positive or have Hepatitis A, B, or C or have another systemic infection (excludes subjects from venipuncture). No study specific testing for HIV or Hepatitis A, B or C is performed.

6.2 Recruitment and screening

Patients will be recruited from within the Department of Chiropractic Medicine at the University Hospital of Balgrist.

Asymptomatic controls will be recruited by flyers displayed on the hospital's internal website (intranet) and notice boards.

6.3 Assignment to study groups

Patients are subdivided into strata in order to match for age, sex, and degenerative changes, then permuted

block randomization is used for each stratum to assign patients to receive either SMT or control intervention. Randomization is performed by an investigator not involved in the examination using sequentially numbered sealed opaque envelopes.

6.4 Criteria for withdrawal / discontinuation of participants

Subjects are excluded from enrolment due to

a) **withdrawal of informed consent**
b) non-compliance
c) the safety of the participant being doubtful or at risk, respectively
d) contraindications to SMT determined on the first study MRI
e) the necessity of a spinal manipulative intervention, spinal corticosteroid or anaesthetic infiltrations or spinal surgery after inclusion into the study and before the study visit

If pregnancy cannot be ruled out with certainty, female patients of child-bearing age are tested for pregnancy prior to the MRI examination using a urine pregnancy test.
If consent is revoked, the health-related personal data of the person concerned will be anonymized after data evaluation has been completed.

7. STUDY INTERVENTION

7.1 Identity of Investigational Treatment

7.1.1 Experimental Intervention

The patient turns from supine to right-side lying position, is positioned by the chiropractor administering the intervention, and receives a spinous push-pull SMT intervention as described by Bergman et al. at the previously marked segment[36]. Specifically, the patient lies in side-lying position, with the hip of the up-side leg flexed to ca. 90° and the foot of the flexed leg hooked behind the popliteal space of the down-side leg. The chiropractor stands facing the subject at a 45°-angle, fixating the flexed knee with his own knee/thigh. The fingertips of the cephalic hand reach under the patient's up-side arm to contact the up-side lateral surface of the superior spinous process. The fingertips of the caudal hand hook the inferior spinous process, contacting the down-side aspect of the spinous process, while the forearm contacts the patient's posterolateral buttock and thigh. The cephalic hand thrusts lateromedially and caudocranially, from up-side toward down-side ("push"). The caudal hand thrusts lateromedially in the opposing direction, from down-side toward up-side, toward the chiropractor ("pull"). The SMT intervention is repeated after the patient turns to left side lying. The patient then resumes supine position.

7.1.2 Control Intervention

The patient turns from supine to right-side lying position and is positioned with the hip of the up-side leg flexed to ca. 90° and the foot of the flexed leg hooked behind the popliteal space of the down-side leg. The chiropractor stands facing the subject at a 45°-angle, fixating the flexed knee with his own knee and placing both hands as described for the experimental intervention. No pressure, preload or thrust is administered. This intervention is repeated after the subject turns to left side lying. The subject then resumes supine position.

7.1.3 Packaging, Labelling and Supply (re-supply)

Not applicable.

7.1.4 Storage Conditions

Not applicable.

7.2 Administration of experimental and control interventions

7.2.1 Experimental Intervention

The experimental intervention will be administered as described in chapter 8.1.1. The experimental procedure

will take place in the University Hospital Balgrist

7.2.2 Control Intervention

The control intervention will be administered as described in chapter 8.1.2. The experimental procedure will take place in the University Hospital Balgrist.

7.3 Dose / Device modifications

Not applicable.

7.4 Compliance with study intervention

Not applicable.

7.5 Data Collection and Follow-up for withdrawn participants

The data of withdrawn participants is going to be excluded from the final analysis.

7.6 Trial specific preventive measures

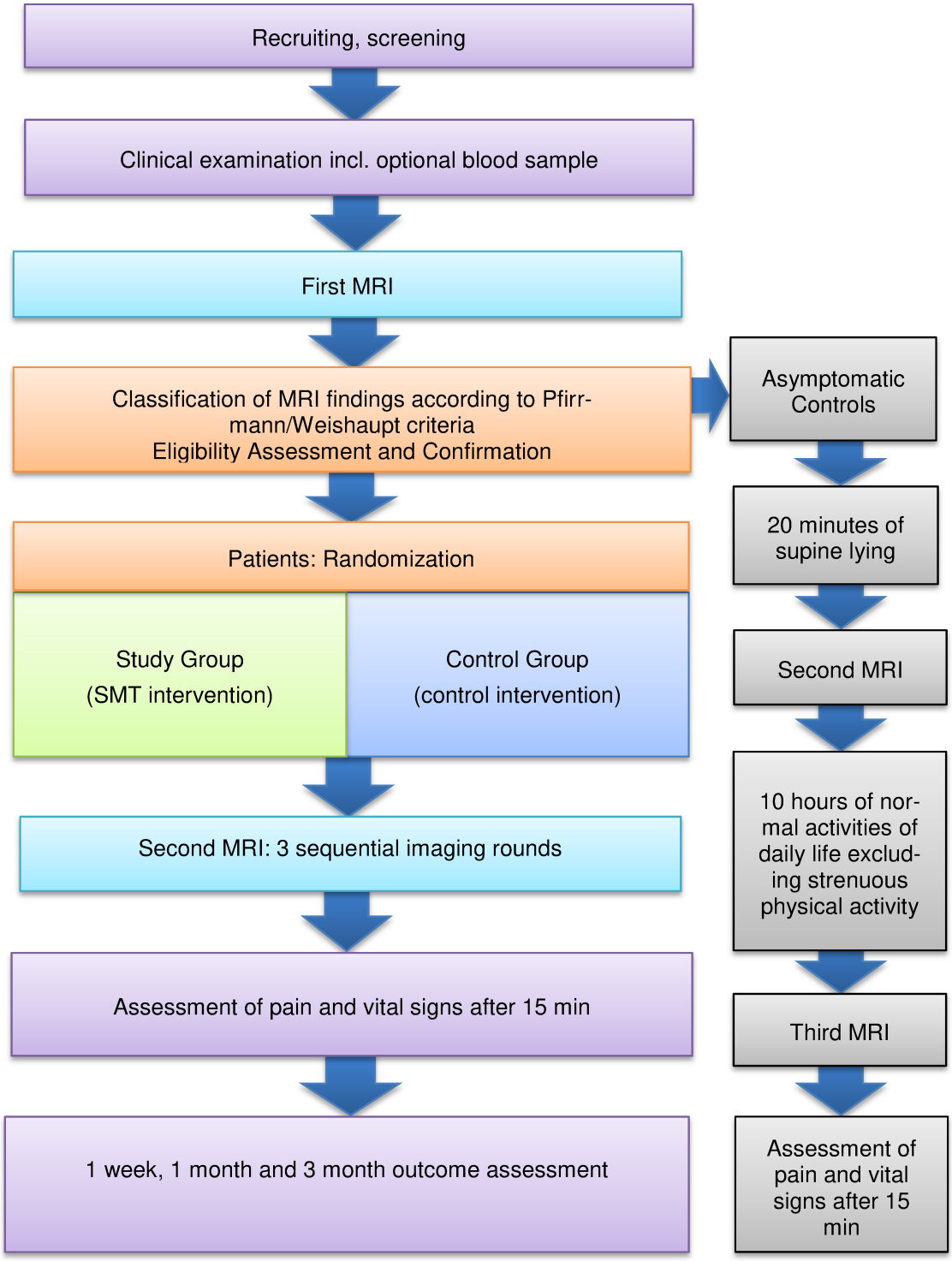
Not applicable

7.7 Concomitant Interventions

Patients requiring spinal manipulative interventions, spinal corticosteroid or anaesthetic infiltrations as well as spinal surgery between inclusion into the study and the study visit are excluded from the study. After the study visit, type and frequency of concomitant interventions will be assessed by telephone interviews at one week and one and three months after the study visit.

8. STUDY ASSESSMENTS

8.1 Study flow chart(s) / table of study procedures and assessments



8.2 Assessments of outcomes

8.2.1 Assessment of primary outcome

Diffusion and perfusion parameters on DWI and IVIM-MRI are determined by an experienced radiologist at the University Hospital of Balgrist.

8.2.2 Assessment of secondary outcomes

Diffusion and perfusion parameters on DWI and IVIM-MRI in controls are also determined by an experienced radiologist at the University Hospital of Balgrist.

8.2.3 Assessment of other outcomes of interest

Clinical baseline parameters and change in pain intensity on a numerical pain rating scale after intervention will be assessed by an investigator blinded to the radiological information and the administered intervention. Classification of degenerative changes on MRI are determined by an experienced radiologist at the University Hospital of Balgrist.

Patients who consent to giving a blood sample will have venipuncture performed by a medical professional trained in the procedure.

8.2.4 Assessment of safety outcomes

Patients' vital signs and pain intensity will be assessed before and after the experimental procedure and recorded according to clinical standards. The patient will be able to verbally communicate discomfort or pain at any time during the experimental procedure.

8.2.4.1 Adverse events

Time of onset, duration, resolution, action to be taken, intensity and relationship with SMT intervention of adverse events are going to be recorded according to clinical standards. Participants are going to be assessed for adverse events according to current standard of care after the experimental procedure.

8.2.5 Assessments in participants who prematurely stop the study

Patients who are withdrawn from the study prematurely are routinely scheduled for a follow-up visit after 4-6 weeks. Recording of adverse events, physical examination and vital signs is going to be performed according to the standards of the University Hospital Balgrist. Data of withdrawn patients is not going to be considered in the final analysis.

8.3 Procedures at each visit

8.3.1 Visit 1

- Patient screening and eligibility assessment in the Policlinic for Chiropractic Medicine by an Investigator
- Patient will be contacted and asked if he would like to participate 3-4 days after screening.
- Patient is sent informed consent and general consent, which he will return signed.
- An appointment is made for Study Visit 2

- Controls will contact one of the investigators by the number provided on the flyer. Control is sent informed consent and general consent, which he will return signed. An appointment for study visit 2 is made.

8.3.2 Visit 2

- Inclusion and exclusion criteria are checked.
- Demographics and baseline Information is collected by an Investigator. Oswestry Disability Index and painDetect questionnaires are administered.
- A physical examination including vital signs, lower extremity neurological examination, and lumbar spine examination is performed by an Investigator.

- Subjects consenting to venipuncture will proceed to the venipuncture site, where 10-15 ml of whole blood will be drawn from the vein (usually at the elbow) using serum collection tubes. Blood samples are allowed to clot at room temperature for 30-60 min and then centrifuged at 2500 x g for 10 min at 4°C. The resulting serum supernatant is stored in aliquots at Balgrist Campus first at -20°C and transferred within 2-20 hours to -80°C.
- Subjects will prepare for MRI, which will be performed under the supervision of a radiographer. The first sequence of MR-imaging takes approximately 20 minutes.
- Randomisation takes place.
- Patients exit the scanner and receive either an intervention or a control intervention, depending on the randomisation result.
- Controls exit the scanner and lie supine for 20 minutes.
- All subjects reenter the scanner and proceed to have a second round of imaging.
- Patients are assessed for pain and vital signs. They may then leave the study site.
- Controls may also leave the study site, and go on to lead their normal daily lives (excluding strenuous physical activity) for the next 10 hours. They then report back to the University Hospital Balgrist for their third and last MRI. After this, they are also assessed for pain and vital signs. This marks the end of the study for controls.

8.3.3 Visit 3-5

- At one week, patients are contacted by online questionnaire. Patient's global impression of change is recorded. The Oswestry Disability Index and the painDetect-questionnaire are repeated; pain parameters, and type and frequency of concomitant therapies are assessed.
- At one month, the same online questionnaire will be repeated.
- At three months, the same online questionnaire will be repeated. The three month interview marks the end of the study for patients.

9. SAFETY

Serious adverse events (ICH E6 1.2, ICH E2A) will be documented and notified as described in ClinO Art.63: If, in the course of a clinical trial, serious adverse events (SAEs) occur in participants in Switzerland, and it cannot be excluded that the events are attributable to the intervention under investigation, the investigator will document them in a standardised manner. In addition, the investigator will report these events:

a.) to the sponsor within 24 hours after they become known; and

b.) to the responsible ethics committee within 15 days

SAEs in which a relation to the study intervention cannot be excluded will be noted in the electronic case report form (eCRF) and a SAE form will be completed. During the entire duration of the study, all SAEs in which a relation to the study intervention cannot be excluded are collected, fully investigated and documented in source documents and eCRF. Study duration encompassed the time from when the participant signs the informed consent until the last investigation plan-specific procedure has been completed. Considering that the study-specific interventions are standard-of-care and performed for many decades standard safety measures of clinical routine apply and no further safety follow-up is needed.

No drugs or other products are administered during the study. Study-related severe adverse events associated to medical drugs or products are not expected.

9.1 Other Clinical Trials

9.1.1 Definition and Assessment of Safety Related Events

Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in participants, that occurs after an intervention whether in causal relation with this treatment or not.

Serious Adverse Event (SAE)

Adverse event that:

- requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
- results in permanent or significant incapacity or disability;
- is life-threatening or results in death; or
- causes a congenital anomaly or birth defect

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs are followed until resolution or stabilisation. Participants with ongoing SAEs at study termination will be further followed up until recovery or until stabilisation of the disease after termination.

Health hazards that require measures

Findings in the trial that may affect the safety of study participants and, which require preventive or corrective measures intended to protect the health and safety of study participants.

Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the intervention, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge

	(or other proof of intervention cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Assessment of Severity

The severity grading scale used for this study will be the "Common Terminology Criteria for Adverse Events CTCAE Version 4.02"[47].

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

Clinical investigators and ultimately the Principal Investigator (PI) have the primary responsibility for SAE identification, documentation, grading, and assignment of attribution to the intervention under study.

Clinical study participants will be routinely questioned about AEs at study visits. The well-being of the participants will be ascertained by neutral questioning ("How are you?").

Observed or volunteered SAEs, regardless of treatment group or suspected causal relationship to the study treatment(s) will be recorded in the patient file and subsequently in the eCRF if a relationship to the study intervention cannot be excluded.

All SAEs in which a relation to the study intervention cannot be excluded, will be fully documented in the appropriate eCRF. For each such SAE, the investigator will provide the onset, duration, intensity, treatment required, outcome and action taken with the investigational device or study related procedure.

The investigator shall report these events:

a.) to the sponsor within 24 hours after they become known; and

b.) to the responsible ethics committee via BASEC within 15 days

Periodic reporting of safety

An annual safety report is submitted once a year to the local Ethics Committee via local Investigator.

Art.37 par. 1 KlinV Safety measures

Urgently applied safety measures during the study period as well as causing factors are going to be reported to the EC within a time period of seven days.

10. STATISTICAL METHODS

This will be a Level 1 study (RCT).

10.1 Hypothesis

Intervertebral discs and adjacent spinal muscle tissue of the lumbar spine will show a change in the apparent diffusion coefficient and perfusion coefficient on MRI after an SMT intervention.

10.2 Determination of Sample Size

N = 70 (25 per patient group, 20 controls). Rationale: Based on the study results by Wong et al.[18], we expect an increase in ADC values of at least 6.5% (CI: 3.88%-9.13%) in receivers of SMT-intervention compared to no change in the control-intervention group. 25 patients per group will provide a power of 0.8 (1-beta) to detect an effect of 0.9 at an alpha-level of 0.05[48]. Additionally, the repeatability of IVIM-MRI on musculoskeletal tissue will be assessed in 20 control subjects.

10.3 Planned Analyses

Distributions of the ADC and perfusion coefficient obtained at the two time points (before and after intervention) will be tested for the assumptions of normality using the Shapiro-Wilk test. In case of non-normality, data transformation will be used to normalize data. Should this not be possible, non-parametric tests will be used. Two separate repeated measures analysis of variance (ANOVA) will be used to test for effects of time (pre- vs. post-intervention) on ADC and perfusion coefficients with intervention (experimental vs. control intervention) and group (degenerated vs. non-degenerated painful segments) as between-subject variables.

We will estimate the repeatability of IVIM-MRI in measuring perfusion in muskuloskeletal tissue using paired t-tests and the intraclass correlation coefficient ICC[49].

10.3.1 Dataset to be Analysed, analysis populations

The statistican will supervise the analysis as described in 11.4.

10.3.2 Primary Analysis

Our hypothesis is that a spinal segment receiving an SMT-intervention will show a change in the diffusion and pseudodiffusion coefficient that is significantly higher than a spinal segment receiving a control intervention. Distributions of the ADC and perfusion coefficient obtained before and after intervention will be tested for the assumptions of normality using the Shapiro-Wilk test. In case of non-normality, data transformation will be used to normalize data. Should this not be possible, non-parametric tests will be used. Two separate repeated measures analysis of variance (ANOVA) will be used to test for effects of time (pre- vs. post-intervention) on ADC and perfusion coefficient with intervention (experimental vs. control intervention) and group (degenerated vs. non-degenerated painful segments) as the between-subject variables.

The PI will supervise the primary analysis as described above. It will be analyzed using SPSS (version 23) at the end of the recruitment period and after obtaining all necessary data.

10.3.3 Secondary Analyses

We will estimate the repeatability of IVIM-MRI in measuring perfusion in muskuloskeletal tissue using paired t-tests and the intraclass correlation coefficient ICC[49].

The PI will supervise the secondary analysis as described above. It will be analyzed on SPSS (version 23) at the end of the recruitment period and after obtaining all necessary data.

10.3.4 Deviation(s) from the original statistical plan

Deviations of the original statistical plan will be reported according to chapter 2.10.

10.4 Handling of missing data and drop-outs

Intention to treat analysis is planned to account for missing data.

11. QUALITY ASSURANCE AND CONTROL

11.1 Data handling and record keeping / archiving

Data is exclusively stored using the secured REDCap® electronic data capture tool[50]. When the study is terminated, data will be stored in the same system. Data can only be accessed by defined persons that have contributed to the project. Radiological data will be stored in the institution's PACS system according to the institutional standard at the University Hospital Balgrist.

All study data are encrypted in an automatically secured online processing system (REDCap® electronic data capture system). The principal investigator is responsible for data collection. She keeps the screening log, which guarantees the confidentiality of data by the use of participant ID numbers. The participant ID numbers are automatically assigned in consecutive ascending form by the REDCap® system. For the purpose of fulfilling their task, the principal investigator grants the necessary access authorization in the form of user log-in and password to the study staff (see Staff List). Thus, the data cannot be altered in any way by unauthorized persons. In the REDCap® system, all relevant processing operations are documented in a user-specific manner in order to ensure traceability. This is done by means of registration software, which records who has edited which data at which time. REDCap® has a database server, which is located in highly modern, secure server rooms in Rümlang and Altstetten. The RED-Cap® database is secured daily by backup. All transaction logs between two consecutive backups are kept for one week. All study programs in REDCap® are kept for an unlimited time, but at least for 10 years. Data collected in the course of this study are available for inspection or inspection by monitors, independent EC and competent authorities.

11.1.1 Case Report Forms

An electronic Case Report Form (eCRF) will be generated for every participant and all data relevant to the study is going to be recorded by authorised persons (identifiable). The REDCap® electronic data capture tool is hosted by the University Hospital Balgrist. All persons (participants, patients, excluded patients) will be documented in a screening log. Participating participants/patients will be registered in an enrolment log (patient identification key). The investigator is responsible for correct and complete data input into the study-specific database. Corrections can only be made by authorised persons and have to be justified. Older versions are not overwritten and stay accessible. All data and corrections are logged with date, time and person.

11.1.2 Specification of source documents

All data appearing in the eCRF can be found in the patient's medical record via the proprietary hospital information system. Source data only found in the CRF has to be clearly defined and labelled.

11.1.3 Record keeping / archiving

All study data is going to be archived for a minimum of 10 years (Art.45 KlinV) after study termination or premature termination of the clinical trial. Data is stored using the proprietary hospital information system and REDCap® electronic data capture tool hosted at the University Hospital Balgrist[50].

11.2 Data management

11.2.1 Data Management System

Study data is going to be recorded and managed using REDCap® electronic data capture tools hosted at University Hospital Balgrist[50].

11.2.2 Data security, access and back-up

Only authorized medical personnel are granted access to study data. Records are going to be hosted using REDCap® electronic data capture tools at the University Hospital Balgrist[50].

11.2.3 Analysis and archiving

Details of data management procedures can be found at[50].

11.3 Monitoring

11.3.1 Outline of the monitoring strategy

One monitoring visit at the investigator's site prior to the start, one visit within one year after inclusion of the first participant and approximately once visit per annum during the course of the study will be organised by the Sponsor. Furthermore, there will be a monitoring visit at the study end. During the monitoring, all documents including source data/documents will be accessible for the monitor and all questions will be answered.

Study period	Time	Monitoring
Before study start	Dec 2018	Information about study conduct concerning data sampling and safety reporting. Monitor controls if • Documents are approved by KEK • Documents are at site • Investigators are familiar with study protocol and safety reporting • Investigators know their duties and responsibilities
After inclusion of first patient	Within 1 year	All subjects: SDV for existence and informed consent First trial participant and at least 10% of trial participants recruited at the time of the visit, as far as available: eligibility, primary endpoint, SAEs
Further visits	1 per annum	All subjects: SDV for existence and informed consent At least 10% of trial participants recruited at the time of the visit, as far as available: eligibility, primary endpoint, SAEs, additional protocol-specific safety parameters.
Study end	Dec 2020	Close out visit to control for completeness of source data

11.4 Audits and Inspections

Direct access to source documents will be permitted for purposes of audits and inspections for eligible authorities (swissmedic, independent ethics committees). Auditors/Inspectors are going to be granted full access to medical records, documents as well as signed consent forms via the implemented "audit trail" of the REDCap® electronic data capture tool or the proprietary hospital information system[50].

11.5 Confidentiality, Data Protection

Direct access to source documents will be permitted for purposes of monitoring (12.3), audits and inspections (12.4). Only authorised medical personnel are going to have access to protocol, dataset and statistical code during and after the study.

11.6 Storage of biological material and related health data

Health data will be stored as described in chapters 12.2 and 12.2.

Whole blood is withdrawn from fasting patients using serum collection tubes. Blood samples are allowed to clot at room temperature for 30-60 min and then centrifuged at 2500 x g for 10 min at 4°C. The resulting serum supernatant is stored in aliquots first at -20°C and transferred within 2-20 hours to -80°C. All samples will be stored in the *Swiss Center for Musculoskeletal Biobanking (SCMB), Balgrist Campus AG,* and analysed together at the end of the recruitment period. Serum samples are taken for the assessment of selected inflammatory molecules. Serum concentrations of 8 biomarkers will be measured using electrochemiluminescent based multi-array immunoassays from MesoScale Discovery (MSD, Rockville, MD, USA) following the protocol of the manufacturer. Remaining serum will be encoded and stored for a minimum of 10

years at -80° (general consent).

12. PUBLICATION AND DISSEMINATION POLICY

The results of this trial are going to be published in a peer reviewed medical journal.

13. FUNDING AND SUPPORT

13.1 Funding

A part of the cost for the experimental intervention (MRI) is defrayed by a financial grant of CHF 15'000 provided by the Zürcher Verein Pro Chiropraktik. The study intervention will be free of charge for the study participants and their health insurances. The majority of costs (staff and standard equipment) will be covered by the participating institution (Balgrist).

14. INSURANCE

The standard Balgrist research insurance (as already presented to the KEK) is applicable.

15. REFERENCES

1. Paige NM, Miake-Lye IM, Booth MS, et al. Association of Spinal Manipulative Therapy With Clinical Benefit and Harm for Acute Low Back Pain: Systematic Review and Meta-analysis. *JAMA* 2017; **317**(14): 1451-60.

2. Dagenais S, Tricco AC, Haldeman S. Synthesis of recommendations for the assessment and management of low back pain from recent clinical practice guidelines. *Spine J* 2010; **10**(6): 514-29.

3. Ferreira ML, Ferreira PH, Latimer J, Herbert R, Maher CG. Efficacy of spinal manipulative therapy for low back pain of less than three months' duration. *J Manipulative Physiol Ther* 2003; **26**(9): 593-601.

4. Bronfort G, Maiers MJ, Evans RL, et al. Supervised exercise, spinal manipulation, and home exercise for chronic low back pain: a randomized clinical trial. *Spine J* 2011; **11**(7): 585-98.

5. Bronfort G, Haas M, Evans RL, Bouter LM. Efficacy of spinal manipulation and mobilization for low back pain and neck pain: a systematic review and best evidence synthesis. *Spine J* 2004; **4**(3): 335-56.

6. Chou R, Huffman LH, Society AP, Physicians ACo. Nonpharmacologic therapies for acute and chronic low back pain: a review of the evidence for an American Pain Society/American College of Physicians clinical practice guideline. *Ann Intern Med* 2007; **147**(7): 492-504.

7. Hadler NM, Curtis P, Gillings DB, Stinnett S. A benefit of spinal manipulation as adjunctive therapy for acute low-back pain: a stratified controlled trial. *Spine (Phila Pa 1976)* 1987; **12**(7): 702-6.

8. Brennan GP, Fritz JM, Hunter SJ, Thackeray A, Delitto A, Erhard RE. Identifying subgroups of patients with acute/subacute "nonspecific" low back pain: results of a randomized clinical trial. *Spine (Phila Pa 1976)* 2006; **31**(6): 623-31.

9. Flynn T, Fritz J, Whitman J, et al. A clinical prediction rule for classifying patients with low back pain who demonstrate short-term improvement with spinal manipulation. *Spine (Phila Pa 1976)* 2002; **27**(24): 2835-43.

10. Parkin-Smith GF, Amorin-Woods LG, Davies SJ, Losco BE, Adams J. Spinal pain: current understanding, trends, and the future of care. *J Pain Res* 2015; **8**: 741-52.

11. Wand BM, O'Connell NE. Chronic non-specific low back pain - sub-groups or a single mechanism? *BMC Musculoskelet Disord* 2008; **9**: 11.

12. Subhawong TK, Jacobs MA, Fayad LM. Diffusion-weighted MR imaging for characterizing musculoskeletal lesions. *Radiographics* 2014; **34**(5): 1163-77.

13. Antoniou J, Demers CN, Beaudoin G, et al. Apparent diffusion coefficient of intervertebral discs related to matrix composition and integrity. *Magn Reson Imaging* 2004; **22**(7): 963-72.

14. Mitchell UH, Beattie PF, Bowden J, Larson R, Wang H. Age-related differences in the response of the L5-S1 intervertebral disc to spinal traction. *Musculoskelet Sci Pract* 2017; **31**: 1-8.

15. Beattie PF, Donley JW, Arnot CF, Miller R. The change in the diffusion of water in normal and degenerative lumbar intervertebral discs following joint mobilization compared to prone lying. *J Orthop Sports Phys Ther* 2009; **39**(1): 4-11.

16. Beattie PF, Arnot CF, Donley JW, Noda H, Bailey L. The immediate reduction in low back pain intensity following lumbar joint mobilization and prone press-ups is associated with increased diffusion of water in the L5-S1 intervertebral disc. *J Orthop Sports Phys Ther* 2010; **40**(5): 256-64.

17. Beattie PF, Butts R, Donley JW, Liuzzo DM. The within-session change in low back pain intensity following spinal manipulative therapy is related to differences in diffusion of water in the intervertebral discs of the upper lumbar spine and L5-S1. *J Orthop Sports Phys Ther* 2014; **44**(1): 19-29.

18. Wong AY, Parent EC, Dhillon SS, Prasad N, Kawchuk GN. Do participants with low back pain who respond to spinal manipulative therapy differ biomechanically from nonresponders, untreated controls or asymptomatic controls? *Spine (Phila Pa 1976)* 2015; **40**(17): 1329-37.

19. Federau C. Intravoxel incoherent motion MRI as a means to measure in vivo perfusion: A review of

the evidence. *NMR Biomed* 2017; **30**(11).

20. Le Bihan D, Breton E, Lallemand D, Aubin ML, Vignaud J, Laval-Jeantet M. Separation of diffusion and perfusion in intravoxel incoherent motion MR imaging. *Radiology* 1988; **168**(2): 497-505.

21. Nguyen A, Ledoux JB, Omoumi P, Becce F, Forget J, Federau C. Application of intravoxel incoherent motion perfusion imaging to shoulder muscles after a lift-off test of varying duration. *NMR Biomed* 2016; **29**(1): 66-73.

22. Filli L, Boss A, Wurnig MC, Kenkel D, Andreisek G, Guggenberger R. Dynamic intravoxel incoherent motion imaging of skeletal muscle at rest and after exercise. *NMR Biomed* 2015; **28**(2): 240-6.

23. Coronado RA, Gay CW, Bialosky JE, Carnaby GD, Bishop MD, George SZ. Changes in pain sensitivity following spinal manipulation: a systematic review and meta-analysis. *J Electromyogr Kinesiol* 2012; **22**(5): 752-67.

24. Freynhagen R, Tölle TR, Gockel U, Baron R. The painDETECT project - far more than a screening tool on neuropathic pain. *Curr Med Res Opin* 2016; **32**(6): 1033-57.

25. Chiarotto A, Boers M, Deyo RA, et al. Core outcome measurement instruments for clinical trials in non-specific low back pain. *Pain* 2017.

26. Hüllemann P, Keller T, Kabelitz M, Freynhagen R, Tölle T, Baron R. Pain Drawings Improve Subgrouping of Low Back Pain Patients. *Pain Pract* 2017; **17**(3): 293-304.

27. Young S, Aprill C, Laslett M. Correlation of clinical examination characteristics with three sources of chronic low back pain. *Spine J* 2003; **3**(6): 460-5.

28. Petersen T, Laslett M, Juhl C. Clinical classification in low back pain: best-evidence diagnostic rules based on systematic reviews. *BMC Musculoskelet Disord* 2017; **18**(1): 188.

29. Tonosu J, Inanami H, Oka H, et al. Diagnosing Discogenic Low Back Pain Associated with Degenerative Disc Disease Using a Medical Interview. *PLoS One* 2016; **11**(11): e0166031.

30. Al Nezari NH, Schneiders AG, Hendrick PA. Neurological examination of the peripheral nervous system to diagnose lumbar spinal disc herniation with suspected radiculopathy: a systematic review and meta-analysis. *Spine J* 2013; **13**(6): 657-74.

31. Laslett M, Oberg B, Aprill CN, McDonald B. Centralization as a predictor of provocation discography results in chronic low back pain, and the influence of disability and distress on diagnostic power. *Spine J* 2005; **5**(4): 370-80.

32. May S, Aina A. Centralization and directional preference: a systematic review. *Man Ther* 2012; **17**(6): 497-506.

33. Mainka T, Lemburg SP, Heyer CM, Altenscheidt J, Nicolas V, Maier C. Association between clinical signs assessed by manual segmental examination and findings of the lumbar facet joints on magnetic resonance scans in subjects with and without current low back pain: a prospective, single-blind study. *Pain* 2013; **154**(9): 1886-95.

34. Seffinger MA, Najm WI, Mishra SI, et al. Reliability of spinal palpation for diagnosis of back and neck pain: a systematic review of the literature. *Spine (Phila Pa 1976)* 2004; **29**(19): E413-25.

35. Katsura M, Suzuki Y, Hata J, et al. Non-Gaussian diffusion-weighted imaging for assessing diurnal changes in intervertebral disc microstructure. *J Magn Reson Imaging* 2014; **40**(5): 1208-14.

36. TF B, DH P, DJ L. Chiropractic technique. New York: Churchill Livingstone; 1993. p. 490-2.

37. Becker A, Held H, Redaelli M, et al. Low back pain in primary care: costs of care and prediction of future health care utilization. *Spine (Phila Pa 1976)* 2010; **35**(18): 1714-20.

38. Beattie PF. Diffusion-weighted magnetic resonance imaging of the musculoskeletal system: an emerging technology with potential to impact clinical decision making. *J Orthop Sports Phys Ther* 2011; **41**(11): 887-95.

39. Klyne DM, Barbe MF, Hodges PW. Systemic inflammatory profiles and their relationships with demographic, behavioural and clinical features in acute low back pain. *Brain Behav Immun* 2017; **60**: 84-92.

40. Baur A, Reiser MF. Diffusion-weighted imaging of the musculoskeletal system in humans. *Skeletal Radiol* 2000; **29**(10): 555-62.

41. Baur A, Dietrich O, Reiser M. Diffusion-weighted imaging of the spinal column. *Neuroimaging Clin N Am* 2002; **12**(1): 147-60.

42. Hebert JJ, Stomski NJ, French SD, Rubinstein SM. Serious Adverse Events and Spinal Manipulative Therapy of the Low Back Region: A Systematic Review of Cases. *J Manipulative Physiol Ther* 2015; **38**(9): 677-91.

43. Nielsen SM, Tarp S, Christensen R, Bliddal H, Klokker L, Henriksen M. The risk associated with spinal manipulation: an overview of reviews. *Syst Rev* 2017; **6**(1): 64.

44. Roelofs PD, Deyo RA, Koes BW, Scholten RJ, van Tulder MW. Nonsteroidal anti-inflammatory drugs for low back pain: an updated Cochrane review. *Spine (Phila Pa 1976)* 2008; **33**(16): 1766-74.

45. Hartwig V, Giovannetti G, Vanello N, Lombardi M, Landini L, Simi S. Biological effects and safety in magnetic resonance imaging: a review. *Int J Environ Res Public Health* 2009; **6**(6): 1778-98.

46. Humphreys BK, Peterson CK, Muehlemann D, Haueter P. Are Swiss chiropractors different than other chiropractors? Results of the job analysis survey 2009. *J Manipulative Physiol Ther* 2010; **33**(7): 519-35.

47. Institute NIoHC. Common Terminology Criteria for Adverse Events (CTCAE). 2009. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.

48. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**(2): 175-91.

49. Watson PF, Petrie A. Method agreement analysis: a review of correct methodology. *Theriogenology* 2010; **73**(9): 1167-79.

50. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; **42**(2): 377-81.