PROTOCOL TITLE:

Physiologic interindividual variability of volume and atrophy in central nervous system structures with focus on spinal cord as measured by quantitative magnetic resonance imaging.

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Version 1

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1. SYNOPSIS

This is a brief summary. For details refer to the body of the protocol.

Protocol Number:	
Protocol Title:	**Physiologic interindividual variability of volume and atrophy in central nervous system structures with focus on spinal cord as measured by quantitative magnetic resonance imaging.**
Version Number:	<1>
Study Indication:	Healthy controls
Rationale for the Study:	Spinal cord (SC) involvement is prevalent in multiple sclerosis (MS) and contributes importantly to disease progression. To be able reliably evaluate spinal cord volume and its changes in MS patients we need to understand variability of these parameters in sex and age matched healthy controls (HC). To date, no generally available data about these parameters in HC are available. The objective of this study is to investigate age and sex matched HC by MRI to get the basic set of the data representing both cross sectional values and its longitudinal changes. The present study will also investigate different strategies how to normalize the absolute spinal cord and brain volume data, what is a relationship between spinal cord volume and brain volume and what is the best protocol to be used in a routine clinical practice.
Study Objectives and	**Objectives**

Endpoints:

Primary objective

To examine 102 healthy controls on MRI to get data about spinal cord volume. This data will be used to compare spinal cord volume in HC with spinal cord volume of age and sex matched multiple sclerosis patients.

Secondary Objectives

- Test several normalization strategies and choose the best variable or set of variables to normalize spinal cord volume
- Determine inter-subject variability in spinal cord volume in healthy controls
- Determine inter-subject variability in different whole and regional brain volumes in healthy controls

Tertiary objectives

- Determine relationship between spinal cord volume and whole and differenet regional brain volumes in healthy controls.
- Optimize MRI protocol (different sequences) for separation of white and gray matter in cervical spinal cord.

Endpoints

Primary Endpoint

Establishing pathological cut-offs for spinal cord volumes. By using those we will be able to discriminate with the highest sensitivity and specificity HC from MS patients. The analysis will be done both cross-sectionally and longitudinally.

Secondary Endpoints

- Description of results of different normalisation strategies, using different variables, e.g. sex, age, body height, height of C3 vertebra (or other vertebra

derived metrics), total intracranial volume (TIV).

- Description of absolute and normalised spinal cord volume and its variability in predefined spinal segments in different age and sex groups of HC.
- Description of absolute and normalised brain volumes in HC measuring whole brain, TIV and different regional brain volumes, especially – gray and white matter compartment, corpus callosum, thalamus, and cerebellum.

Tertiary Endpoints

- Correlation between spinal cord volume (total and GM, WM separately) and several brain metrics (total brain volume, GM, WM, lateral ventricles, thalamus, corpus callosum, brainstem and cerebellum) in HC.
- Comparison of results from different MRI protocols

Study Design:	Prospective, longitudinal, monocentric, interventional study
Study Location:	Czech Republic
Number of Planned Subjects:	102 subjects – intended as healthy controls (HC) from general population
Study Population:	This study is conducted in healthy subjects. Detailed criteria are described in the protocol.
Statistical Methods:	Binary or categorical variables will be presented as frequency distributions. Continuous variables will be reported by summary statistics: mean and standard deviation (SD) or median and range. The relationship between age and spinal cord volume will be determined separately for female and male patients in different decades to determine sex- and age-related effects on spinal cord volume. The spinal cord volume (and eventually also spinal cord gray- and white matter volumes) will be compared in three age-specific groups: 18-30 years, 30-40 years and 40-60

years.

Annualised change of spinal cord volume will be calculated for each participant as the slope of a regression line fitted to all four timepoints (M0, 12, 24 and 36) with the assumption of linear change over time for each participant and for all participant (in a decade-specific manner) together.

2. INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory and neurodegenerative disease that affects central nervous system. Magnetic resonance imaging (MRI) is the main paraclinical examination used to monitor disease activity and response to treatment. However, there is only limited correlation between the clinical symptoms and findings seen on the conventional MRI – a phenomena called clinical-radiological paradox. Measuring whole and regional brain atrophy provides additional information to MRI and seems to correlate better with clinical course and prognosis. In the last two decades, brain atrophy in MS has been extensively studied, cut-offs for brain volume loss over time has been established and it is likely that it will become an important outcome measure in both clinical studies and routine practice.

Compared to brain atrophy, spinal cord (SC) atrophy and its relation to MS-related disability has been given less attention. This is partly due to technical challenges such as inhomogeneous magnetic field in this region, small physical dimensions of SC and artefacts caused by motion of the SC within the spinal canal together with the flow of cerebrospinal fluid and periodic motion due to respiratory and cardiac cycles. Moreover, focal spinal cord MS lesions may cause both swelling and shrinking of SC that influence its absolute volume, resulting in problems with interpretation of absolute SC volume in MS. Despite all these challenges, it is possible to identify SC lesions and reliably measure SC volume, but there is no „gold standard" (standardized) software for SC volume measurement and no agreement on the SC level (segments) that is most suitable for SC volume loss measurement.

During 2016 we have developed an in-house semiautomatic pipeline for measurement of cervical spinal cord volume that is a part of Scanview program. The segmentation is performed on T2-weighted images. In the first step, a marker is manually placed in a center of an intervertebral disc C3-4 (sagittal plane). Subsequently a cord straightening (manual rotation of the cord to achieve a perpendicular orientation of spinal cord to dorsal part of C3 vertebral body), that enable to reduce partial volume due to the cord orientation. After this centering and straightening,transformation matrix is saved and all subsequent steps are fully automatized. These steps include: 1. Sub-pixel division, 2. Reversing the contrast of T2-weighted images and smoothing by applying a set of median, Gaussian and edge-enhancing filters and 4. Cubic spline interpolation to find a curve that represents a border of a spinal cord with highest probability. Finally, a sum of mean areas of 21 1-mm slices is calculated (1 center slice fixed at center of a intervertebral disc C3-4 and 2 x 10 slices in cranial and caudal direction.

Using this new software we have assessed 1,036 MS patients during 2016 and 2017. The intra- and inter-rater variability of SC volume assessment was done by using the intraclass correlation coefficient (ICC) with a two-way mixed absolute agreement and single-measures design. The analysis confirmed very good consistency (> 98%) of the method. The preliminary

results showed a significant correlation between spinal cord volume and different clinical phenotypes.

2.1. Study Rationale

To be able reliably evaluate spinal cord volume and its changes in MS patients we need to understand variability of these parameters in sex and age matched healthy controls. To date, no generally available data about these parameters in healthy controls are available. In addition, the result are scanner specific and using particular scanner we need to get these physiologic ranges particularly for the type of scanner where MS patients are evaluated.

The objective of this study is to investigate age and sex matched healthy controls by MRI to get the basic set of the data representing both cross sectional values and its longitudinal changes.

The present study will also investigate different strategies how to normalize the absolute spinal cord and brain volume data, what is a relationship between spinal cord volume and brain volume data and what is the best protocol to be used in a routine clinical practice

STUDY OBJECTIVES AND ENDPOINTS

2.2. Objectives

2.2.1. Primary Objective

The primary objective of the study is to longitudinally examine 102 healthy controls (HC) on MRI to get data about spinal cord volume. This data will be used to compare spinal cord volume in HC with spinal cord volume of age and sex matched multiple sclerosis (MS) patients.

2.2.2. Secondary Objectives

The secondary objectives of this study are as follows:

- Test several normalization strategies and choose the best variable or set of variables to normalize spinal cord volume
- Determine inter-subject variability in spinal cord volume in healthy subjects
- Determine inter-subject variability in different whole and regional brain volumes in healthy controls

2.2.3 Tertiary objectives

- Determine relationship between spinal cord volume and different whole and regional brain volumes.
- Optimize MRI protocol (different sequences) for separation of white and gray matter in cervical spine cord.

2.3. Endpoints

2.3.1. Primary Endpoint

Establishing pathological cut-offs for spinal cord volume able to discriminate with the highest sensitivity and specificity HC from MS patients. The analysis will be done both cross-sectionally and longitudinally.

2.3.2. Secondary Endpoints

- Description of results of different normalisation strategies, using different variables, e.g. sex, age, body height of HC, height of C3 vertebra (or other vertebra derivate metrics).
- Description of absolute and normalised spinal cord volume and its variability in predefined spinal segments in different age and sex groups of HC.
- Description of absolute and normalised brain volumes in HC measuring whole brain (total intracranial volume TIV) and different regional brain volumes, especially – gray and white matter compartment, corpus callosum, thalamus, and cerebellum.

2.3.3. Tertiary Endpoints

- Correlation between spinal cord volume (total and GM, WM separately) and several brain metrics (total brain volume, GM, WM, lateral ventricles, thalamus, corpus callosum, brainstem and cerebellum) in HC.
- Description of results from different MRI protocols (shorter and longer version, different sequences)

3. STUDY DESIGN

3.1. Study Overview

This is a prospective, longitudinal, monocentric, interventional study to examine 102 healthy subjects with MRI.

3.2. Overall Study Duration and Follow-Up

The study period will be overall 4 years. During first 12 months we plan to enroll 102 HC. The enrollment period will take maximum 12 months. The study duration for a particular subject will be 3 years. During this period each participant will be examined by MRI at month 0, 12, 24, and 36.

3.2.1. Enrollment of participants

Healthy controls suitable for this study will be offered to participate in this study based on personal discussion. During this meeting a participant will receive all important information about the study and all inclusion and exclusion criteria will be checked. After signing informed consent form the basic clinical evaluation will be done and the first MRI will be scheduled. The study is planned for 3 years for an individual person. Participant will be contacted always 2-4 months before the next MRI. At this time all important conditions will be checked again.

3.2.2. MRI Assessment

All participants will be examined on the same 3T scanner, Siemens Skyra, 20 channel coil for head and spinal cord. The same standardised protocol will be used - T2WI (weighted images) fat sat (FS) 3D for spinal cord volumetry, inversion recovery (IR) sequence for gray and white matter separation of spinal cord and T1 MPRAGE 3D sequence for brain volumetry.

Each participant will be examined by MRI at month 0, 12, 24, and 36.

3.3. End of Study

The end of study will be after completion of MRI assessment for all available participants, but no longer than 4 years from the first participant date of enrollment.

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4. STUDY POPULATION

4.1. Inclusion Criteria

To be eligible to participate in this study, subject must fulfil the following eligibility criteria:

1. Each participant must provide informed consent in accordance with local regulations
2. Age 18-60 years.

4.2. Exclusion Criteria

Participant will be excluded from contributing to this study if any of the following exclusion criteria exist:

1. Not able to undergo MRI examination
2. Not able to be examined 4 times, i.e. M 0, 12, 24, 36 during the next 3 years
3. Pregnancy at the time of enrollment into the study
4. Other disease or medical condition that can influence the volume of brain or spinal cord

5. ASSESSMENTS

5.1. Prospective Acquisition of Demographics, Baseline and Follow-up clinical Characteristics, and Clinical Measures at month 0, 12, 24, and 36

- Age
- Sex
- Body weight and height at time of each MRI examination
- Regular check of medical history and changes in physical status
- Regular evaluation of adverse events including non-serious
- Neuropsychological testing
- Walking test (T25-foot walk test)

5.2. MRI Assessments at month 0, 12, 24, and 36

- Regular control of contraindication to MRI before any assessment
- Scan acquisition time
- Spinal cord volume (measured in a predefined cervical spinal cord segment in C3-C4 vertebral body)
- Antero-posterior width (APW) and transversal (or left-right) width (LRW) of spinal cord at the level of C3/C4 intervertebral disc
- Total intracranial volume
- Brain Parenchymal Fraction (BPF)
- Brain volume
- White matter (WM) volume
- Gray matter (GM) volume
- Deep gray matter volume (volume of basal ganglia, thalamus)
- Cerebellar volume
- Volume of brainstem
- Ventricular volume

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

6.1. Endpoints

6.1.1. Primary Endpoint

Establishing pathological cut-offs for spinal cord volume able to discriminate with the highest sensitivity and specificity HC from MS patients. The analysis will be done both cross-sectionally and longitudinally.

6.1.2. Secondary Endpoints

- Description of results of different normalisation strategies, using different variables, e.g. sex, age, body height of HC, height of C3 vertebra (or others vertebra derivate metrics).
- Description of absolute and normalised spinal cord volume and its variability in predefined spinal segments in different age and sex groups of HC.
- Description of absolute and normalised brain volumes in HC measuring whole brain (total intracranial volume TIV) and different regional brain volumes, especially – gray and white matter compartment, corpus callosum, thalamus, and cerebellum.

6.1.3. Tertiary Endpoints

- Correlation between spinal cord volume (total and GM, WM separately) and several brain metrics (total brain volume, GM, WM, lateral ventricles, thalamus, corpus callosum, brainstem and cerebellum) in HC.
- Description of results from different MRI protocols (shorter and longer version, different sequences)

6.2. Demography and Baseline Clinical Characteristics

Demographics and baseline data will be reported with summary statistics (mean and standard deviation [SD], median and range)

6.2.1. Analysis Population

The analysis population will be all participants with all available clinical and MRI data uploaded into the database.

6.2.2. General Methods of Analysis

In general, continuous variables will be presented with summary statistics (mean, SD, median, range) and a 95% confidence interval (CI), and categorical variables will be presented with frequency distributions.

6.2.3. Primary Endpoint Analysis

Establishing pathological cut-offs for spinal cord volume able to discriminate with the highest sensitivity and specificity HC from MS patients will be done by employing cross-sectional and longitudinal logistic regression model and ROC analysis. The cross-sectional analysis will be done at each timepoint. Moreover, the pairwise comparison of results between each pair of timepoints will be done in order to analyse the robustness (stability) of results. Longitudinal analysis will be done to identify cut-offs of changes of spinal cord volume over 3 years follow-up.

6.2.4. Secondary Endpoints Analysis

For all secondary endpoints, continuous variables will be presented with summary statistics (mean, SD, median, range), and categorical variables will be presented with frequency distributions. The inference of population characteristics will be estimated by confidence intervals. The statistical testing (parametric/non-parametric tests) will be done to compare characteristics of central tendency and variability between patient's and HC's cohorts.

6.2.5. Tertiary Endpoints Analysis

Correlation between spinal cord volume (total and GM, WM separately) and several brain metrics (total brain volume, GM, WM, lateral ventricles, thalamus, corpus callosum, brainstem and cerebellum) in HC over time will be assessed using appropriate regression models or Pearson or Spearman correlation or Chi-square test as appropriate.

Results from different MRI will be described as follows: continuous variables will be presented with summary statistics (mean and SD, median and range) and 95% CI, and categorical variables will be presented with frequency distributions. The different MRI results will be compared by running proper statistical tests and correlation analysis.

6.3. Sample Size Considerations

6.3.1. Sample Size

102 healthy controls

6.3.2. Sample Size Calculation

The exact sample size calculation is difficult due to several problems – there is very limited data about spinal cord volume in both MS patients and HC. In addition to that spinal cord volume is scanner and protocol specific, so it is not possible to use directly data from other centres.

Based on current knowledge we can estimate that MS patients are losing spinal cord volume 3-5 times faster compare to HC. The problem is that this can be age and sex specific and different in different MS phenotypes. So, it is reasonable to choose the group of HC that the best copy a typical MS patient population, i.e. ratio of male : female 1:2 and age range 18-60 years.

From the statistical point of view is generally recommended to have at least 10 subjects in each age and sex specific subgroup (see Table 1). Taking into account that the study is planned for 4 years and there is a risk of drop out of some participants we recommend to increase number of participants in each group by 2.

Based on this conditions we recommend to enrol:

Table 1.

Age range / Sex	Male	Female
18-30 years	12	22
30-40 years	12	22
40-60 years	12	22
Total	**36**	**66**

7. ETHICAL REQUIREMENTS

The Investigators complies with all instructions, regulations, and agreements in this protocol and International Conference on Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines if applicable.

7.1. Ethics Committee

Participating investigators must obtain ethics committee (EC) approval of the protocol, informed consent form (ICF), and other required study documents prior to starting the study if applicable.

It is the responsibility of the Principal Investigator to ensure that all aspects of institutional review are conducted in accordance with current governmental regulations.

7.2. Subject Information and Consent

Participant consent prior to performing any study-related activities will be obtained in accordance with local regulations.

7.3. Subject Data Protection

EC and various government health agencies may inspect the records of this study. Every effort will be made to keep the subject's personal medical data confidential.

In any presentations or in publications of the study results, the participants' identities will remain anonymous and thus confidential. The participant will not be identified by name in the database or in any study reports.

8. ADMINISTRATIVE PROCEDURES

8.1. Quality Assurance

The investigators will be expected to cooperate with any audit and to provide assistance and documentation (including source data) as requested.

8.2. Publications

Scientific findings of the data analysis will be published in line with ICMJE guidelines.
Roche financial support will be transparently acknowledged. Publication plan will be in details described in the contract agreement.

9. FURTHER REQUIREMENTS AND GENERAL INFORMATION

9.1. Changes to Final Study Protocol

All protocol amendments must be submitted to the EC and Regulatory Authorities if required by local law.

In the event of a protocol modification, the subject ICF may require similar modifications.

9.2. Ethics Committee Notification of Study Completion or Termination

Where required, the Health Authorities and EC must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

9.3. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations.

10. SIGNED AGREEMENT OF THE STUDY PROTOCOL

I have read the foregoing protocol, "**Physiologic interindividual variability of volume and atrophy in central nervous system structures with focus on spinal cord as measured by quantitative magnetic resonance imaging.**" and agree to conduct the study according to the protocol and the applicable International Conference on Harmonisation (ICH) guidelines and good clinical practice (GCP) regulations, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

Investigator's Name (Print)	Investigator's Signature	Date
MANUELA VALEČKOVÁ		12 DEC 2014
MICHAELA ANDĚLOVÁ		12. December 2017
KAROLINA KUČEROVÁ		11 December 2017
JAN KRAŠENSKÝ		12. 12. 2017
LUKÁŠ SOBÍŠEK		12. 12. 2017