Statistical Analysis Plan
Incl. deviations to trial protocol

Muscle Strain in Multiple Sclerosis Patients Measured by Ultrasound Speckle Tracking (MUST)

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Administrative Information

This is an overview of the deviations to the original trial protocol.
We have not been able to perform the study as planned on account of i.e. the Covid-19 pandemic.
The inclusion has been interrupted for long periods due to:

- Recruitment of patients for research projects has been forbidden due to Covid-19 lockdown.
- The departments, from which patients were recruited, stopped all non-required visits from patients, including all Fampridine patients, due to work pressure following the Covid-19 lockdown.
- Just prior to, and a long period following the Covid-19 lockdown, patients did not wish to participate in our project. This, due to fear of the extra exposure to Covid-19, which the additional visits to the hospital and contact with healthcare personnel, entailed.
- A long national strike in nurses stopped all non-required visits from patients, including all Fampridine patients.

The original sample size has not been obtained and the 52-weeks follow-up visit has been cancelled.

Data have been collected as planned at baseline and at 14-days follow-up from the original cohort (including 48 participants). Data collection was completed in October 2021. Based on these data, four sub-studies have been prepared.

Consequently, the primary study, as outlined in the original trial registration, aiming to use ultrasound speckle tracking (STU) to monitor muscle contractility in multiple sclerosis (MS) patients receiving vs. not receiving fampridine, as well as to relate these results to performance-based measures and biomarkers to explore disease progression and muscle activity, has been cancelled. The following pages outline the statistical analysis plan regarding four exploratory studies that originate from the initial trial registration but with a revised aim, outcome measures, and time points.

Study 1

Objective

To investigate whether Speckle Tracking Ultrasonography (STU) can quantitatively evaluate muscle function in participants with MS, by means of correlations to conventional clinical assessments.

Participants

The participants who performed STU analyses at baseline.

Outcome measures

Demographic measures – at baseline

- Age
- Gender
- Expanded Disability Status Score (EDSS) [1]
- Multiple Sclerosis Impairment Scale (MSIS) [2]
- Disease duration
- Sub-diagnosis (MS)
- Medical treatment (MS)

- Walking aids

- Muscle strain of the Soleus muscle (SOL), at different percentages of Maximal Voluntary Contractions (%MVC) – baseline data:
    - SOL20 (20 %MVC)
    - SOL40 (40 %MVC)
    - SOL60 (60 %MVC)

- Muscle strain of the Biceps muscle (BB), at different %MVC – baseline data
    - BB20 (20 %MVC)
    - BB40 (40 %MVC)
    - BB60 (60 %MVC)

- Muscle strain of the Supraspinatus muscle (SS) at different %MVC – baseline data
    - SS40 (40 %MVC)
    - SS60 (60 %MVC)
    - SS80 (80 %MVC)

- Clinical performance tests, legs – baseline data
    - The Timed 25-Foot Walk (T25FW) [3]
    - The Six Spot Step Test (SSST) [4]
    - 2-minute Walk Test (2MWT) [5]
    - 12-Item MS Walking Scale (MSWS-12) [6].

- Clinical performance tests, arms – baseline data
    - 9-Hole Peg Test (9HPT) [7]
    - Oxford Shoulder Score (OSS) [8]

Statistical analysis

Descriptive statistics – baseline data

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Data will be reported as numbers (n) and percentage (%), mean values and standard deviations (SD), and/or median values and interquartile range (IQR), as required, according to assumption of Gaussian distribution.

Criterion validity – baseline data

- Non-bilaterally tests: STU test-side will be used for analyses.
- The Timed 25-Foot Walk Test (T25FW) is appointed gold standard for leg function.
- 9-Hole Peg Test (9HPT) is appointed gold standard for arm function.
- Criterion validity of strain and clinical performance tests will be performed as follows:
    - Normality and assumptions will be examined by means of scatterplots and linear regressions for strain and clinical performance test using gold standards as the dependant variables.

- Correlations between gold standards and strain will be assessed using Pearson's product-moment correlation coefficient *r* and/or Spearman's rank-order correlation coefficient *ρ* (rho).
- Correlations between gold standards and clinical performance tests will be assessed using Pearson's product-moment correlation coefficient *r* and/or Spearman's rank-order correlation coefficient *ρ* (rho).

Comparisons of criterion validity – baseline data

- For comparisons of criterion validity, we will use the results of the Spearman's rank-order correlation coefficient *ρ* (rho). This to ensure that the parameters are the same for all comparisons.
- Bootstrapping will be performed because independence cannot be expected, between correlation estimates from the same patient.
- Difference between criterion validity will be assessed using squared correlations on Fischer's Z-scale in combination with bootstrapping to define accelerated Bootstrap Confidence Intervals (95% BCa CI).

Study 2

Objective: To test the hypothesis that quality of gait using Gait Profile Score (GPS) based on nine different Gait Deviation Scores (GVS), as well as gait function using performance-based tests, spatiotemporal parameters, and self-perceived gait function, will improve in MS patients following two weeks of treatment with Fampridine. In addition, we hypothesize that changes in GPS are positively associated with changes in performance-based measures.

Participants

- A subgroup of participants, randomly allocated 3-timensional gait analysis (3DGA).
- Age-matched reference group consisting of healthy people, collected in our own lab.

Outcome measures

- Demographic measures – at baseline
  - Age
  - Gender
  - Height and weight (from which Body Mass Index (BMI) was calculated)
  - Disease duration
  - EDSS [1]
  - Walking aids

- Gait Quality according to Baker et al 2009 [9] – baseline and change between baseline and 14-days follow-up
  - Gait Profile Score (GPS)
  - Gait Variable Scores (GVS)
    - Pelvic tilt
    - Pelvic obliquity

        - Pelvic rotation
        - Hip flexion-extension
        - Hip abduction-adduction
        - Hip rotation
        - Knee flexion-extension
        - Ankle dorsi-/plantar flexion
        - Foot progression

- Clinical performance based tests – baseline and change between baseline and 14-days follow-up
    - T25FW [3]
    - SSST [4]
    - 2MWT [5]
    - MSWS-12 [6]
    - Spatiotemporal parameters

Statistical analysis

A priori sample size calculation

Sample size calculation was performed using GPS values formerly reported on MS patients (GPS mean ± SD) 9.1 ± 1.3 [10] and the minimal important difference of. 1.6 GPS defined by Baker et al. 2009 [9]. The SD of 1.3 from the MS population [10] was used since no SD was reported by Baker et al. 2009 [9]. A minimum of nine participants was found to be needed, based on a paired design, alpha of 0.05, statistical power of 80%, assumption of normal distribution of data.

Descriptive statistics – baseline data

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Data will be reported as numbers (n) and percentage (%), mean values and standard deviations (SD), and median values and interquartile range (IQR), as required, according to assumption of Gaussian distribution.
- Comparison of characteristics of included MS patients and patients randomised to perform 3DGA, but declined to perform the 3DGA, will be explored using used Student's unpaired *t*-test (parametric) and Wilcoxon rank sum test (non-parametric)

Changes between baseline and 14-days follow-up

- Results according to kinematic and spatiotemporal data will be reported for the participant's dominant side.
- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Change will be examined using Student's paired *t*-test (parametric) and Wilcoxon signed rank test with median values (non-parametric).
- Due to the low number of participants required, bootstrap confidence intervals (95% BCa CI) can be relevant for the non-parametric analysis.

Correlations of change between baseline and 14-days follow-up

- Correlations between change in GPS and change in performance-based tests will be examined using Pearson's product-moment correlation coefficient *r* and/or Spearman's rank-order correlation coefficient ρ (rho).

Study 3

Objective

To assess potential changes of relevant biomarkers in participants with MS following two weeks of Fampridine treatment. Furthermore, to explore the correlation between biomarkers and clinical performance measures.

Participants

The group of participants, who provided a blood sample at baseline

Outcome measures

- Demographic measures – at baseline
    - Age
    - Gender
    - Expanded Disability Status Score (EDSS) [1]
    - Multiple Sclerosis Impairment Scale (MSIS) [2]
    - Disease duration
    - Sub-diagnosis (MS)
    - Medical treatment (MS)
    - Walking aids
    - Smoking status
    - Alcohol intake

- Biomarkers – change/baseline
    - Plasma IFN-gamma (pg/mL)
    - Plasma IL-2 (pg(mL)
    - Plasma IL-4 (pg(mL)
    - Plasma IL-8 (pg(mL)
    - Plasma IL-17 (pg/ml)
    - Plasma TNF (pg/mL)
    - Plasma TNF-RI (pg/mL)
    - Plasma TNF-RII (pg/mL)
    - Neurofilament light chain (NF-L)
    - Glial fibrillary acidic protein (GFAP)

- Clinical measures – change/baseline
    - T25FW [3]
    - SSST [4]
    - 2MWT [5]
    - MSWS-12 [6]
    - 9HPT [7]
    - OSS [8]
    - STU

Statistical analysis

Descriptive statistics – baseline data

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Data will be reported as numbers (n) and percentage (%) or mean values and standard deviations (SD), and median values and interquartile range (IQR), as required, according to assumption of Gaussian distribution.

Changes between baseline and 14-days follow-up

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Change will be examined using Student's paired *t*-test (parametric) and Wilcoxon signed rank test with median values (non-parametric).

Correlations of change between baseline and 14-days follow-up

- If a change in biomarkers is demonstrated, correlations between change in biomarkers and change in clinical measures will be examined using Pearson's product-moment correlation coefficient *r* and/or Spearman's rank-order correlation coefficient ρ (rho).
- If no change in biomarkers is demonstrated, correlations between baseline data for biomarkers and baseline data for clinical measures will be examined using Pearson's product-moment correlation coefficient *r* and/or Spearman's rank-order correlation coefficient ρ (rho).

Study 4

Objective

To evaluate changes in hand and walking parameters in participants with MS following Fampridine treatment, according to the disability level.

Participants

Participants, who completed both baseline and 14-days follow-up, who followed instructions of Fampridine intake, and who did not receive Fampridine at inclusion.

Outcome measures

- Demographic measures – at baseline
    - Age
    - Gender
    - EDSS [1]
    - Disease duration
    - Sub-diagnosis (MS)

- Clinical measures – baseline and change between baseline and 14-days follow-up
    - T25FW [3]
    - SSST [4]
    - 2MWT [5]
    - MSWS-12 [6]
    - 9HPT [7]

Statistical analysis

Descriptive statistics – baseline data

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Data will be reported as numbers (n) and percentage (%), mean values and standard deviations (SD) and median values and interquartile range (IQR), as required according to assumption of Gaussian distribution.

Definition of subgroups according to their EDSS

- Subgroup 1: Moderate walking disability (EDSS 4.5-5.5)
- Subgroup 2: Severe walking disability (EDSS 6.0-7.0)
- Comparison of subgroups at baseline will be examined using Student's unpaired *t* test (parametric) and Wilcoxon rank sum test/Mann Whitney test (non-parametric).

Changes between baseline and 14-days follow-up

- Gaussian distribution will be inspected by normal probability plots and the Shapiro-Wilk test.
- Change for all included participants, subgroup 1 and subgroup 2, respectively, will be examined using Student's paired *t*-test (parametric) and Wilcoxon rank sum test (non-parametric).
- Comparison of change scores between subgroups will be examined using Student's unpaired *t* test (parametric) and Wilcoxon rank sum test/Mann Whitney test (non-parametric).

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