The short-term impact of Botulinum Neurotoxin-A on muscle morphology and gait in children with spastic cerebral palsy

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Study protocol

This study will include participants of two different projects. Both projects are approved by the local ethics committee of the University Hospitals Leuven, Belgium (s59945 TAMTA project, s62187 and s62645 3D-MMAP project) (Ethische Commissie Onderzoek KU Leuven/UZ Leuven). Written informed consent will be obtained from the parents or legal guardian. Participants will be included between May 2019 and June 2022.

Participants

Children aged between 3-11 years, GMFCS levels I-III with SCP, scheduled for BoNT-A in the MG and/or ST, as part of a multilevel treatment, will be included. Participants will be recruited via two parallel prospective studies. Both studies will investigate the effect of integrated BoNT-A treatment on muscle morphology and gait, while one study will also evaluate the effects on spasticity (s59945 TAMTA project). The selection criteria for the participants are similar in both studies. The exclusion criteria are: any signs of dystonia or ataxia in the lower limbs, severe muscle weakness or poor selectivity in the plantar flexors or knee flexors and cognitive problems that would impede the measurements, intrathecal baclofen pump, previous muscle surgery in the lower limbs or selective dorsal rhizotomy, previous bony corrections in the lower limb within two years before assessments, previous BoNT-A treatment within six months before the baseline assessment or within six months before the pre-BoNT-A 3DGA. Additionally, the physical therapy frequency has to be consistent for at least three months before inclusion. The participants will be measured twice, before and 8-10 weeks after BoNT-A treatment, to evaluate the short-term effects. All children (of both studies) will receive 3DfUS assessments and a 3DGA with clinical examination of the lower limbs. A subset of children (i.e. the participants of one of the two studies) will undergo an additional instrumented spasticity assessment in order to evaluate the BoNT-A-induced tone reduction. During the follow-up, all children will receive usual care including intensive physical therapy, orthotic management, and oral tone reduction if needed.

An age- and functional severity-matched (group-matching based on age and GMFCS level) control group, meeting the same inclusion criteria as the intervention group, but not receiving any intervention apart from their usual care, will be included. The participants of the control group will be recruited via Cerebral Palsy Reference Center of the University Hospitals Leuven, Belgium and the Inkendaal Rehabilitation Hospital Vlezenbeek, Belgium. All children eligible for inclusion will be measured, however the final data selection will be based on accurate matching with the participants in the intervention group. The participants in the control group will undergo 3DfUS measurements only, with an interval of 8-10 weeks between the two assessments.

Procedure

The most affected leg, based on the most recent clinical examination (highest modified Ashworth score for the ankle and knee joints), will be assessed. Measures of body mass, height, total leg length and lower leg length will be taken. A standardized anamnesis will be performed to request treatment history and usual care details.

Muscle selection for BoNT-A injection will be primarily based on the most recent clinical examination and multidisciplinary evaluation of the 3DGA data. BoNT-A, Onabotulinum toxin A (Botox®, Allergan) injections will be applied by a pediatric orthopedic surgeon under general (mask) anesthesia, guided by ultrasonography or palpation. A dose of 100 units of BoNT-A will be diluted in 5ml of saline. The BoNT-A dilution will be injected at multiple sites, in areas with the largest concentration of motor endplates in the muscles selected for treatment. A maximum of 50 units will be injected per site. BoNT-A is suggested to be more effective when part of an integrated approach, i.e., combined with casting, orthotic wear and physical therapy. Therefore, lower leg casts and removable upper leg casts will be applied as indicated.

All additional study assessments (3DfUS, clinical examination, instrumented spasticity assessment and 3DGA) will be performed by three trained assessors. The pre- and post-assessments will always be performed by the same assessor.

Assessments

1. Muscle morphology

Muscle morphology of the MG and distal compartment of the ST muscle will be assessed by 3DfUS. This technique, combining B-mode ultrasonography with three-dimensional motion tracking, is proven valid and reliable in muscle morphology assessments in children with SCP in previous studies.

Ultrasound assessments will be performed while the participant will be placed in prone position. The lower leg will be supported by a triangular cushion to allow slight knee flexion (approximately 25 degrees) and avoid bi-articular stretch on the MG and ST. The ankle will be in resting position without fixation. The joint angles will be measured by goniometry.

Large amounts of acoustic transmission gel will be used to assure smooth movement of the transducer and optimal contact between the transducer and skin. An additional curved gel pad, the Portico, will be used to reduce muscle deformation during the acquisition. Muscle images will be recorded by Stradwin software (version 6.0; Mechanical Engineering, Cambridge University, Cambridge, UK) while moving the transducer from muscle origin to tendinous insertion. The acquisitions will be repeated in case of bad quality images, movement or muscle contraction or when the bony landmarks or muscle borders are missed.

After data collection, the muscle images will be processed by a trained assessor using the Stradwin software (version 6.0; Mechanical Engineering, Cambridge University, Cambridge, UK). Muscle volume (in millimeters) will be estimated by the cubic planimetry technique based on segmentations that will be manually drawn along the inside of the muscle borders in 5-10% of the transverse 2D muscle images. Additionally, echogenicity intensity, as a proxy of muscle quality, will be based on the average of the interpolated reconstruction of the muscle and will be expressed as a gray value on an 8-bit scale (values between 0-255), whereby higher values (whither images) will indicate reduced muscle quality. Muscle belly lengths will be calculated as the Euclidean distance between the muscle origin (MG: posterior surface of medial femoral condyle, ST: ischial tuberosity) and the muscle-tendon junction. The tendon length of the MG will be calculated between the muscle-tendon junction and tendinous insertion. The MTU length of the MG will be calculated by adding the muscle belly to the tendon length.

In order to compare children with different body dimensions, muscle volume will be normalized to the product of body mass*height, and muscle and tendon lengths will be expressed as a percentage of total leg length.

The absolute muscle-specific growth rates for muscle volume during the period between the two assessments will be calculated by the following formulas:

$$\textbf{growth rate, muscle volume} = \frac{muscle\ volume\ (mL)\ POST - \ muscle\ volume\ (mL)\ PRE}{age\ (months)\ POST - \ age\ (months)\ PRE}$$

The primary outcomes will include the normalized muscle volume and echogenicity intensity of the MG and distal compartment of the ST and the absolute muscle volume growth rates.

2. Gait analysis

Gait, over ground barefoot walking at a self-selected speed, will be assessed by 3DGA in a 10 to 12 camera (Oxford Metrics, Oxford, UK) gait lab (Vicon Motion Systems, Oxford, UK). Twelve reflective markers will be placed on anatomical landmarks according the Vicon Plug-In-Gait lower limb model. Ground reaction forces will be measured with force plates (Advanced Mechanical Technology Inc., USA) that are embedded in the walkway. During the 3DGA, kinematic data (joint angles for ankle, knee and hip, and segmental orientation of pelvis and foot), kinetic data (joint moments and power) and surface EMG data of the medial gastrocnemius, soleus, tibialis anterior, rectus femoris, vastus lateralis, the medial and lateral hamstrings and gluteus medius will be collected for each leg. The kinematic data of the most affected leg (same as for the 3DfUS and instrumented spasticity assessments) and a selection of spatial-temporal parameters will be included in this current investigation, but the kinetic and EMG data will not be included in any of the analyses.

Gait cycles will be identified and manually marked using the Vicon Nexus software (Oxford Metrics, Oxford, UK). All trials will be exported and imported to a custom-made Matlab script (The MathWorks,

Natick, MA, USA, 2020b). All gait cycles will be judged on quality. Trials will be excluded in case of artefacts, gaps in data or indications of inaccurate marker placement. Furthermore, the knee varus-valgus angle will be evaluated and trials with absolute knee varus-valgus ROM exceeding 15 degrees, a knee valgus angle exceeding -10 degrees, or trials that are not representative of the participants' gait pattern will be excluded. As many good-quality steps as possible, will be used to create average trials for the ankle and knee joint kinematics.

The GPS, and specific kinematic gait parameters that are found to be sensitive to detect BoNT-A-induced changes, will be extracted per joint. Additionally, the spatial-temporal parameters cadence, walking velocity and stride length will be calculated. Finally the full kinematic waveforms will be exported for pre-post comparison.

The primary outcomes will include the kinematic waveforms, BoNT-A-sensitive kinematic gait parameters, GPS and spatial-temporal parameters.

3. Spasticity assessment

A subset of participants (n=14) will undergo an instrumented spasticity assessment in order to objectively evaluate the change in the hyperactive stretch reflex during passive stretch. This method has been previously described by Bar-On and colleagues and is proven sensitive enough to detect treatment-induced changes.

Four low-velocity (during 5 seconds) passive stretches of the plantar flexors and medial hamstrings will be performed over the full ROM. Next, four full ROM high-velocity passive stretches will be performed whereby the stretch will be performed as fast as possible. The interval between the stretch repetitions will be at least 7 seconds to avoid post-activation depression. During the stretches, muscle activation will be recorded by surface EMG (Zerowire Cometa, Milan, IT)

The raw EMG signals will be filtered by a 6^{th} order zero-phase Butterworth bandpass filter (20-500 Hz) The root mean square envelope of the EMG signal (RMS-EMG) will be computed after application of a low-pass 30 Hz 6^{th} order zero-phase Butter-worth filter.

All data analyses will be carried out with MATLAB Software (The MathWorks,Natick, MA, USA, 2020b). The quality of the individual stretches will be checked using custom-made software. Stretch repetitions will be excluded in case of inconsistent stretch velocities, poor surface EMG data, out-of-plane movement or indications of insufficient participant relaxation (constant EMG activity of the antagonist).

In order to quantify the alteration in muscle tone after BoNT-A treatment, the average RMS-EMG expressed in millivolts, will be calculated as the square root of the area underneath the RMS-EMG time curve, divided by the duration of the time interval, during the high-velocity stretch. The interval will start 200 milliseconds prior to the time point where maximum stretch velocity will be reached and ended at the time corresponding to 90% of the full ROM. This value will be referred to as the muscle activation during a high-velocity stretch. All BoNT-A-induced changes will be compared to previous reported SEM or BoNT-A-induced values.

The average RMS-EMG expressed in millivolts during a high-velocity stretch will be used as the primary outcome to assess alterations in muscle tone. Additionally, the increase during the high-velocity stretch will be calculated by subtracting the muscle activation during a low-velocity stretch, which will be calculated following the same method. This value will be called the change in muscle activation and will be included as a secondary outcome.

4. Clinical examination

A standardized clinical examination including assessments of ROM, spasticity, selectivity and strength will be performed during the pre- and post-BoNT-A assessments of the intervention group and during the pre-assessment of the control group. These data will be used to describe and compare both groups at baseline. The maximal dorsiflexion angle and popliteal angle (unilateral) will be assessed by goniometry. The level of spasticity will be assessed by the modified Ashworth scale and modified Tardieu scale. Muscle strength will be assessed by manual muscle testing, and selectivity will be assessed by the Selective Control Assessment of the Lower Extremity. The evaluations of strength and selectivity will not be included in the current investigation.

5. Statistical Analysis

Normality of data distribution will be checked by a Kolmogorov-Smirnov test ($p<0.05$). When most parameters are not normally distributed, non-parametric statistical tests will be performed.

Participant characteristics, maximal dorsiflexion angle (measured with the knee extended), popliteal angle (unilateral), and normalized muscle morphology parameters of the intervention and control group will be compared at baseline by a Mann-Whitney U test.

A Wilcoxon signed rank test will be used to evaluate (within) treatment effects in muscle morphology, instrumented spasticity assessment outcomes, spatial-temporal parameters of the 3DGAs (alpha = 0.05). A Bonferroni correction will be applied for the gait kinematic parameters per joint (ankle: alpha 0.05/4 = 0.0125, knee: alpha 0.05/3 = 0.0167). Gait parameters will be considered meaningful if statistically significant changes will be noted that are exceeding the SEM or MCID.

Additionally, median difference scores ($x_{post} – x_{pre}$) for muscle morphology parameters will be calculated in both the intervention and control group and between-group differences were tested by a Mann Whitney-U test. Additionally, the changes will be expressed as a percentage change of the baseline values for both groups.

Paired t-tests of the ankle and knee kinematics over the entire gait cycle using SnPM analysis will be performed to identify treatment-induced changes in these sagittal plane motions. The averaged ankle and knee kinematic waveforms, consisting of 101 points will be imported to the SPM1d software (version 0.4, available for download at http://www.spm1d.org/) in Matlab (The MathWorks, Natick, MA, USA, version 2020b). Clusters will be formed if the critical threshold, based on random field theory, will be crossed (alpha = 0.05). In case of significant clusters, the p-value, details on the location and the duration (percentages of the entire gait cycle) will be reported. Clusters will be considered meaningful when their duration was exceeding 3% of the gait cycle and whether the differences between the means of the pre- and post-BoNT-A joint kinematics will be exceeding the respective SEMs.

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