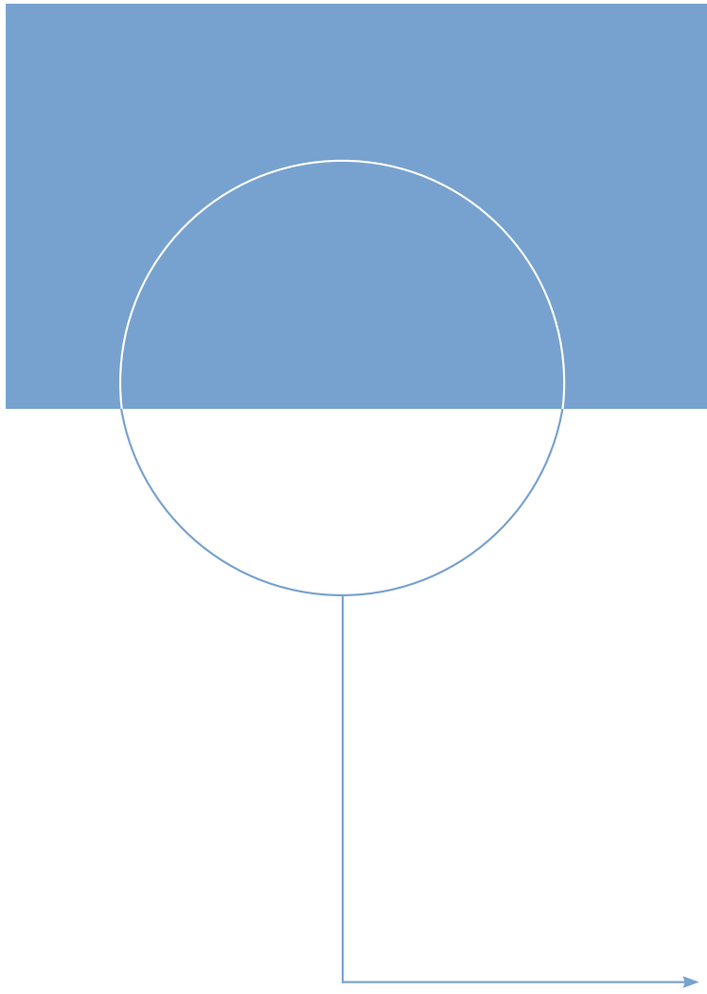

Master's thesis

2023

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Investigating Changes in Spasticity in Response to Botulinum Toxin A in Children with Cerebral Palsy: A Post Hoc Exploratory Study

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Investigating Changes in Spasticity in Response to Botulinum Toxin A in Children with Cerebral Palsy: A Post Hoc Exploratory Study

Maria Jose Lequerica

Master Thesis in Physical Activity and Health
Submission date: May 2023
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Abstract

Introduction: A common treatment for lower leg spasticity amongst children with cerebral palsy (CP) is Botulinum Toxin A (BoNT-A) injections. While the effectivity of treatment is typically assessed clinically, kinematic and neuromuscular measurements can provide insight on a more objective and physiological level.

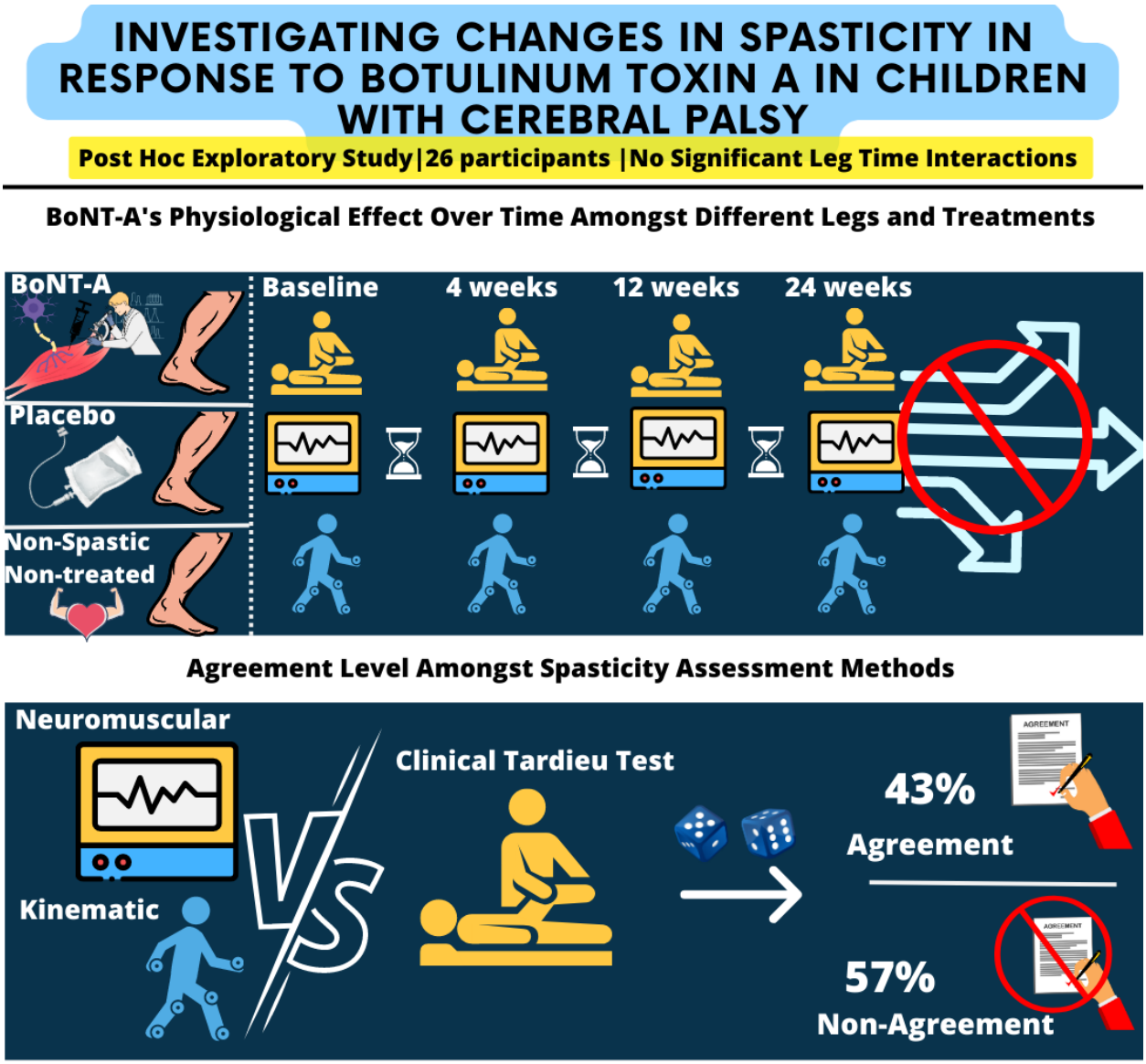
The *aim* of this study was to gain a better understanding of the physiological responses of spastic lower leg muscles in children with CP to BoNT-A treatment. Furthermore, the physiological responses were evaluated against clinical assessments to determine agreement in spasticity grading.

Methods: Twenty-seven children with CP and lower leg spasticity participating in the WE study were included. Assessing each leg individually, 16 legs received BoNT-A injections in the m. gastrocnemius and m. soleus, 13 received saline (placebo), and 25 were non spastic and not treated. Kinematic (positional markers) and neuromuscular (surface electromyography: sEMG) data was collected during the clinical assessment, being passive stretch of the calf muscles at slow and fast velocity in bent and straight knee positions prior to and 4-, 12-, and 24- weeks after treatment. Outcome measures were clinical catch, range of motion (ROM), peak angular velocity, ROM to peak angular velocity, and peak of the linear envelope of sEMG of the 2 calf muscles and m. tibialis anterior.

Results: Linear mixed model analyses did not show significant changes within the BoNT-A treated spastic leg over time, nor a time-leg interaction for any of the outcome measures. When the spasticity decision of the clinical and physiological testing methods were compared, there was a lack of consensus.

Conclusion: In this study, BoNT-A did not affect any of the spasticity measures. Moreover, there was no agreement in spasticity grading between clinical assessment and physiological responses. This could be caused by the relatively low spasticity level of the participants included, methodological issues of the assessment and measures, or a low number of participants.

Infographic:



Reference: Lequerica. M.J.(2023) Investigating Changes in Spasticity in Response to Botulinum Toxin A in Children with Cerebral Palsy: A Post Hoc Exploratory Study [Master thesis, NTNU]

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Abbreviations

Acetylcholine (ACh)

Ashworth Scale/ Modified Ashworth Scale (MAS)

Biomechanical Tool Kit (BTK)

Botulinum Toxin A (BoNT-A)

Cerebral palsy (CP)

Electromyography (EMG)

- surface Electromyography (sEMG)

Gross Motor Function Classification System (GMFCS)

Muscles:

- Gastrocnemius (GM)
- Gastrocnemius EMG peak amplitudes (GMpeak)
- Soleus (SOL)
- Soleus EMG peak amplitudes (SOLpeak)
- Tibialis Anterior (TA)
- Tibialis anterior EMG peak amplitudes (TApeak)

Range of motion (ROM)

Tardieu Scale / Modified Tardieu Scale (MTS)

- Dynamic muscle range (R1)
- Static muscle length (R2)

Timepoints:

- Baseline (B) - 0 weeks
- Post 1 (P1)- 4 weeks
- Post 2 (P2) -12 weeks
- Post 3 (P3) - 24 weeks

Units:

- Degrees (°)
- Degrees per second (°/sec)
- Hertz (Hz)
- Microvolts (uv)

Randomized clinical trial to investigate whether botulinum toxin A makes Walking Easier in children with Cerebral Palsy: (The WE-study)

1. Introduction

Cerebral Palsy (CP) is a neurodevelopmental non progressive disorder. Broken down, the term means “paralysis” [palsy] that is “relating to the brain” [cerebral]. CP is the most common disorder in children, reported in 1.5 - 3 children per 1000 live births (1). Three categories of CP (spastic, dyskinetic and ataxic) were created to distinguish the affected brain regions. Of these, spastic CP is the most common, composing 80-90% of the cases, and is due to a lesion in the upper motor neurons (2,3). Defined as a static disorder, brain damage will not degrade after the point of cerebral development (4). However, the symptoms, such as spasticity, are progressive and will affect a child’s biomechanics later in life.

Spasticity, a primary symptom of spastic CP, is defined as “a velocity dependent increase in stretch reflex” (5). In a typically developed individual, a stretch reflex is activated when a muscle stretches too rapidly (and unexpectedly). This change in muscle length results in adaptations of muscle tone. The altered muscle tone is a physiologically calculated change that will then assure postural maintenance and movement control. Atypically, as seen with individuals with spastic CP, an overstimulation of the stretch reflex will present with excessive muscle activation and hypertonia is a resultant outcome.

Assessing the different possible levels of spasticity allows for proper treatment. The two most common clinical assessments of spasticity are the Modified Ashworth Scale (MAS) and the Modified Tardieu Scale (MTS). In 2019’s Cochrane systematic review by Blumetti et al., the two methods compose 92% of the RCT’s analyzing the effect of BoNT-A injections to the lower limb in children with CP (67% and 25% respectively) (6). An accepted form of assessment for trained raters, resulting in inter and intra-reliability of 74-90% (7) the MTS recognizes the Lance et al. definition of spasticity (27). Incorporating the idea of velocity dependent changes of the tonic stretch reflex, MTS assesses the spastic muscle during both slow and high velocity passive stretches. The MAS test fails to distinguish between these static and dynamic components of spasticity. The remaining 8% of the RCT’s within the systematic review used computerized and electromechanical methods to assess for spasticity (6). Development of these methods, their reliability and ability to determine clinical decision is of current studies.

Treatments decided upon after a full understanding of each child's CP presentation include but are not limited to physical therapy, occupational therapy, casting, surgical interventions, and pharmacological methods or a combination of the aforementioned. The ideal treatment is patient specific and can reduce spasticity, as well as lengthen a muscles range of motion (ROM), improve limb function, gait mechanics, and comfort, while reducing pain. A primary treatment for spasticity, a pharmacological method, is a neuromuscular paralyzing agent called Botulinum toxin A (BoNT-A). This locally delivered injection is currently prescribed to two thirds of the child population diagnosed with spastic CP in Norway (2). For children with a Gross Motor Function Classification System (GMFCS) level I-III the main goal of this treatment, when addressing lower extremity musculature, is to assist with functional gait through muscle tone and pain reduction. The GMFCS is a grading system which assesses the function of daily living and is reliable in "predicting motor function in children with CP" (8). With a focal effect on a targeted muscle, the suggested effect of BoNT-A on spasticity level, is perceived muscle relaxation through both reduced muscle tone and reduced stretch reflex excitability. Contributing to improved daily function for children with CP, BoNT-A is indicated for those that are ambulatory (GMFCS 1-3). Non ambulatory children with CP pose more contraindications towards this treatment, given their common complex medical comorbidities.

To further understand the physiological responses researched within this study a deeper explanation of mechanisms at the cellular level for the previously stated (a stretch reflex, the pathophysiology of spasticity, and the BoNT-A treatment) will follow, in addition to the ins and outs of the Tardieu test and finally the aim of this thesis.

1.1 The Stretch Reflex Arc and Skeletal Muscle Innervation

1.1.1 Healthy Physiological Mechanism

In a typically developed individual, the stretch reflex works to regulate skeletal muscle length. As a monosynaptic reflex, the response is quick with minimal delay between the sensory stimulus and the motor response. In healthy physiology (figure 1 A-C) a change in muscle length (stimulus) activates the muscle spindle (a sensory receptor). Through a monosynaptic reflex loop, the sensory neuron travels the information up the chain passing the central nervous system and in the form of an action potential the response reaches the synaptic terminal at the neuromuscular junction allowing the release of acetylcholine (ACh). ACh undergoes exocytosis

into the synaptic cleft, which allows for the remainder of the muscle innervation process and initiates muscle contraction (9).

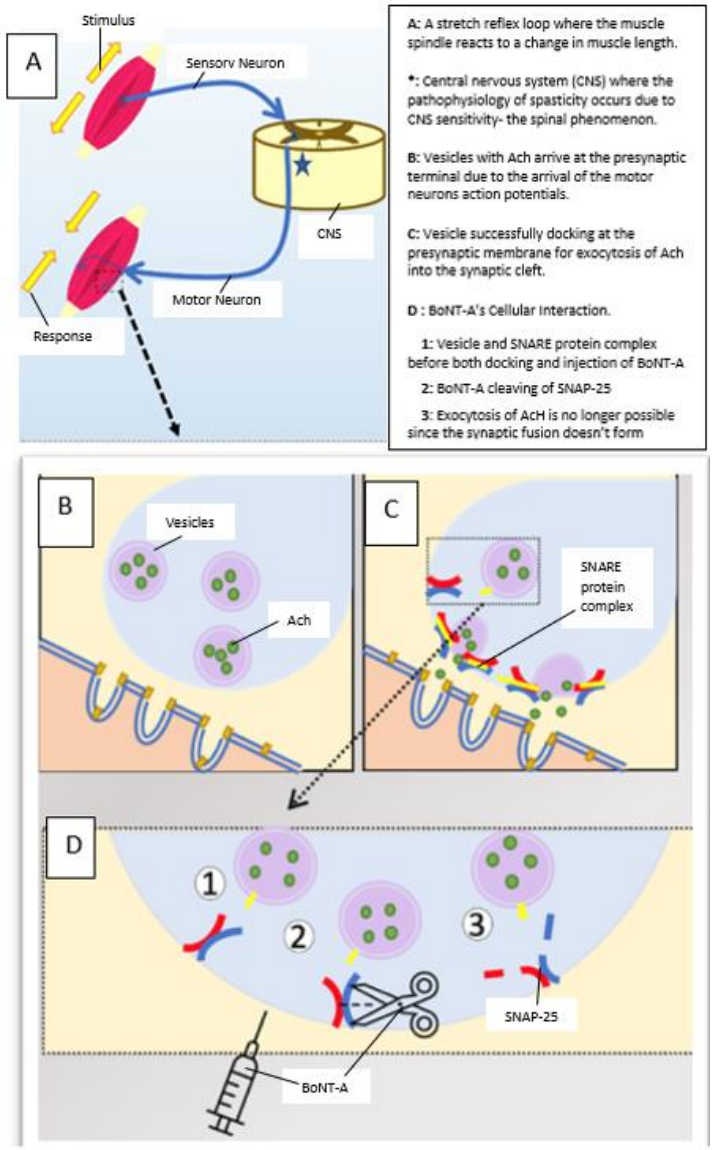


Figure 1: Non-spastic, Spastic and Spastic Pharmacological Treatment Physiology -

The cellular physiology of a stretch reflex (A) and its relation to muscle activation via acetylcholine (ACh) (B, C) along with its corresponding pathophysiology (spasticity) (*) and its cellular interaction with BoNT-A treatment injections. (D) (8,9)

1.1.2 The Pathophysiology of Spasticity

When properly seen as a spinal phenomenon rather than the previously thought peripheral phenomenon, the muscle overactivity characterization of spasticity is easily explained (10). The upper motor lesion, the root cause of the disorder, allows for an increased change in central

excitability. In a stretch reflex arc (Figure 1A) where the sensory neuron reaches the central nervous system for signal processing, the signal interpretation is abnormal and the "reflex is enhanced within the spinal cord (Figure 1A*)." Therefore, the overexcitability starts where the sent reply is an activated motor neuron despite the sensory stimulus received by the receptor in the initial part of the reflex loop (9). Rejecting the blame placed on the muscle spindle receptor, as the sensory fiber (primary afferent) of this stretch receptor is equally reactive in typically developed and spastic individuals (peripheral phenomenon) (10). This pathophysiology results in the muscles overactivity, also known as hypertonia and is due to the central nervous system sensitivity to velocity dependent increase in stretch reflex.

1.2 Spasticity Assessments

Health care professionals depend on clinical assessments to match the spasticity grade of the child to an indicated treatment. The Tardieu test and its modified scale are already used in the clinical setting for diagnosis, treatment selection and mapping of spasticity progression. The MAS and its original scale are not recommended for use of spasticity measurement, since it's rather a representation of resistance during passive movements (11,12). The use of kinematic and neuromuscular responses of spasticity as a form of assessment is a less common practice.

The MTS reports on multiple parameters via different velocities of the spastic muscle being tested. This allows for the detection of "subtle intervention phases" (13). The variables include joint ROM (in degrees) and an ordinal scale representing the type of muscle reaction and level of resistance felt by the examiner. The examiner tests the target muscle in both a slow and a fast movement. The slow movement is performed at a velocity low enough to not elicit a stretch reflex response and looks to assess the total passive ROM the joint can undergo when the targeted muscle is isolated for. When the fast movement is performed in the 'fastest possible' velocity, it is to elicit said stretch reflex response (14). Outcome measures for the fast movement include: the ROM to the instance of examiner identified resistance and an ordinal scale (Table 1) of the type of muscle reaction (15). Numanoglu et al. found MTS to hold a moderate to very high (0.54-0.95) ICC, stronger than that for MAS (0.26-0.66) (16).

Table 1: Modified Tardieu Scale Scoring System:

Muscle reactions matched to an ordinal scoring system that represents characteristics of the stretch reflex (15).

Tardieu Scale	Type of Muscle Reaction
0	no resistance felt
1	slight resistance felt
2	clear catch at precise angle followed by release
3	fatigable clonus
4	un-fatigable

The kinematic and neuromuscular stretch response of the elbow flexors has been connected to the validity of the clinical scores of the Tardieu test (14). With a large degree of agreement reported amongst the two methods the possible errors support the use of this method to increase understanding and detect levels of spasticity. Studies have concluded on the need for kinematic and neuromuscular assessments in future studies to evaluate its ability to predict clinical treatment outcome and therefore be used in clinical decision making (11,14).

1.3 Botulinum Toxin-A

1.3.1 Botulinum Toxin-A Mechanism

Based on the pathophysiology of spasticity, it is to be expected that the spastic muscle will receive an increased count of motor neurons carrying action potentials to the presynaptic terminal. This *unwarranted* action potential will then release ACh into the system. (Figure 1B & C) This neuromuscular paralyzing agent when injected into a spastic muscle will interact with the SNARE protein complex in order to prevent the vesicle fusion of the ACh neurotransmitter vesicle to the presynaptic membrane, preventing the unwarranted release of ACh into the system (Figure1D) (8). The interaction involved is the clipping of the SNAP-25 protein which is responsible for docking and plays an integral role within “nerve terminal physiology” (Figure 1D) (17,18).

Hereafter, availability of the neurotransmitter ACh is due to *new* nerve endings, that take place for the first 3 months after injection (8). With the forming of new nerve endings, the release of ACh will continue but now at a reduced rate and count in which enough strength for physiological function is still available, with diminished contractions (19). The affected (BoNT-A injected) nerve endings are able to function as they did previously when the neurotoxin effect wears off 3-6 months after (8).

1.3.2 Botulinum Toxin-A as Spasticity Treatment

Several studies have been carried out to assess the effect of BoNT-A treatment in comparison to a placebo, other treatments or a combination of treatments (20,21). With varying methodologies such as treatment comparisons, timepoints, spasticity evaluation assessments, outcome variables, and target muscles the amalgamating of results was not possible. However, in respect to BoNT-A versus placebo in the lower leg, some studies showed BoNT-A effectivity in reducing spasticity.

One study reports on the MAS via passive resistance, and the MTS via R2('static muscle length'), R1('dynamic muscle range') (20). Findings showed a statistically significant reduction in spasticity for both 3 and 6 months via MAS while R1 and R2 saw similar favorable outcomes.

Another study assessed spasticity through various measurements. Finding no significant difference in MAS at any time point (3,8,12,24 weeks) and yet a significant reduction in spasticity at 8 weeks and a non-significant yet notable reduction at 12 weeks through a spasticity measurement system (SMS), measuring total path length and elastic path length at various frequencies. Deep tendon reflex, clonus assessments and quantitative electromyographic (EMG) kinesiology also noted a significant decrease but at week 3. The latter confirms a blocked neuromuscular junction, the pathophysiology of the BoNT-A treatment (21).

To better prescribe the proper treatment for children with CP an understanding of a treatment's characteristics on a physiological, clinical, and functional level is required. Using neuromuscular and kinematic testing to better understand this relationship can aid in future treatments.

1.4 Aims and Objectives

The aim of this study was to get further insight in the mechanisms behind the effect of BoNT-A treatment of spastic calf muscles of children with CP. Specifically to investigate the effect of BoNT-A treatment of a spastic calf muscle on the electromechanical response to passive stretch of the treated muscle and its synergist and antagonist. Secondarily, this study was to assess to what extent the physiological responses during clinical spasticity evaluation of the calf muscles agree with the clinical evaluation. The primary expectation was to observe improvement in spasticity at the 4-week time point with a decline returning to baseline data in the 12- and 24-week posttest points due to the treatments expected duration of effect (13,22). Secondarily we expect to see strong agreement between methods, as seen in Brændvik et al (14).

2. Methods

2.1 Study Design and Setting

This post hoc exploratory study included participants from the WE Study (Walking Easier with cerebral palsy), with available required testing data to analyze, 3D motion-capture, EMG and clinical Tardieu test measurements. As an "industry independent double blinded placebo-controlled randomized control trial" (2) the WE Study had included participants from seven sites, 5 within Norway and 2 international locations in which participants were randomized per testing site. This study includes data from three sites: St. Olav's Hospital in Trondheim, Universitetssykehuset i Nord-Norge in Tromsø, and Sykehuset i Vestfold in Stavern. These sites were the only to perform the Tardieu test with equipment and measurements necessary for this study. Given all participants from Trondheim and Stavern were collected, the sites remained randomized. However, Tromsø had incomplete and or missing data files and therefore only 4 of 13 were collected, removing randomization for this site in this study. Testing was conducted in a clinical setting within each location. This post hoc exploratory study was approved by Regional Committee for Medical and Health Research Ethics (REK) (Application#2013/1195).

2.2 Participants

The participants collected for data review consisted of 27 children with CP (15 Females and 12 males) aging from 4-17 years of age (Table 2). The participants presented with either unilateral or bilateral spastic CP, and a GMFCS level of one or two (2). Participants had no

recent (6 months) BoNT-A treatment nor orthopedic surgery in the lower extremities within the two years prior to the WE study enactment. For inclusion purposes the participants had to have all the following tested and collected from the WE study: a clinical Tardieu assessment, kinematic motion capture data and surface EMG data.

Table 2: Participant Characteristics in the Initial Recruitment

Participant Characteristics		**All (n=27)**	**Placebo (n=12)**	**BoNT-A (n=15)**
Age (years)		9.2 (±3.4)	9.2 (± 2.3)	9.2 (± 4.1)
Gender	Male	15 (56)	6 (50)	9 (60)
n (%)	Female	12 (44)	6 (50)	6 (40)
Site	Trondheim	17(63)	7 (58)	10 (67)
n (%)	Tromsø	3 (11)	2 (17)	1 (7)
	Stavern	7 (26)	3 (25)	4 (27)
GMFCS Level	Level 1	23 (85)	12 (100)	11 (73)
n (%)	Level 2	4 (15)	0 (0)	4 (27)
GMFCS Side	Bilateral	2 (7)	1 (8)	1 (7)
n (%)	Unilateral	25 (93)	11 (92)	14 (93)
Prior BoNT-A	Yes	9 (33)	5 (42)	4 (27)
n (%)	No	18 (67)	7 (58)	11 (73)
Height (cm)		132.1 (± 18.7)	127.5 (± 14.6)	135.8 (± 21.3)
Weight (kg)		31.3(± 14.8)	28.8 (± 11.0)	33.5 (± 17.6)

2.3 Study Visits

The collected data consisted of four time points: prior to and 4- , 12-, and 24-weeks after treatment, also referred to as baseline (B), post 1 (P1), post 2 (P2) and post 3 (P3). At the baseline assessment the participants were randomized and then allocated on a 1:1 between BoNT-A (Botox®; Allergan) injection treatment and a saline (0.9%) injection treatment control. The treatment was thereafter delivered via guided ultrasound by a medical professional (2). The peak effects of BoNT-A were expected at Post 1. Post 2 and 3 were collected to determine continuing electromechanical effects of BoNT-A.

2.4 Equipment and Sensor Placement

For kinematic 3D Motion Capture, 2 systems were used. Twenty participants (from Trondheim and Tromsø) were tested using the VICON Nexus system (Version 2.13.0 x64) and 7 participants (from Stavern) were tested using the Qualysis system (Version QTM: 2022.2 build 7710). Both were used to collect positional data on knee, ankle, and toe markers. The anatomical landmarks for each marker were the fibular head, the lateral malleolus, and the base of the third metatarsal, respectively. Both legs were fitted with all three markers.

For surface electromyographic data collection, again two different systems were used. In Trondheim (for Tromsø and Trondheim participants) a MYON system was used and in Stavern a Cometa system. Both systems used a sampling frequency of at least 1000 Hz, were wireless, and the electrodes were placed on the Gastrocnemius (GM), Soleus (SOL), and Tibialis Anterior (TA) muscles following the same procedure. The anatomical landmarks for each electrode are recommended to be based on SENIAM (Surface Electromyography for the Non- Invasive Assessment of Muscles) guidelines for sensor placement (23).

2.5 Clinical Testing: PROM and Spasticity Evaluation

Each participant underwent bilateral testing, independent of unilateral or bilateral spasticity. Presenting with unilateral spasticity, the non-spastic side was tested first. Presenting with bilateral spasticity the initially tested side was chosen at random. Two testers were present during the test. Tester One performed the Tardieu test, explained below, and tester two measured ROM with a manual goniometer.

2.5.1 Equipment and Participant Set up

The following equipment was used for testing: height-adjustable treatment bench, tape measure, manual goniometer, psoas pillow and adjustment pillows.

The test was performed in two knee angles in order to permit for isolation of the targeted muscles. To be tested in isolation the GM requires a straight knee (180 degrees) position and the SOL requires a bent knee (90 degrees) position.

Bent Knee Testing Set up

The participant set up consisted of a supine laying position on a treatment table. A psoas pad was placed under both of the participants legs, in such a way that resulted in hip flexion and 90-degree knee flexion. The participants' heels were checked to be free from the cushion.

Figure 2: Bent Knee Testing Set up

Straight Knee Testing Set up

The participant set up consisted of a supine laying position on a treatment table. The psoas pad was *removed* to create a 180-degree knee position. The participants' heels were again checked to be free from the cushion.

2.5.2 Tardieu Testing Procedure

A demonstration of the Tardieu test was used to familiarize the participant with passive assisted motion in both slow and fast movements while emphasizing muscle relaxation. Successful relaxation from the participant in the demonstration allowed the tester to move forward with testing.

The participant’s ankle was moved to the starting position (90 degrees at the ankle joint). If the participant was unable to attain this level of dorsiflexion, the maximum dorsiflexion was noted as the starting position. The ankle was then passively assisted into maximum dorsiflexion. The second tester measured the maximum passive dorsiflexion ROM with a manual goniometer and noted it on the testing sheet. Returning to the relaxed annotated starting position, the ankle

was slowly and passively assisted into maximum plantarflexion by the tester. From maximum passive plantarflexion the tester moved slowly into maximum dorsiflexion, completing the slow movement of the MTS. From the maximum dorsiflexed position, checking for relaxation, the tester moved slowly into maximum plantarflexion again. In this second movement the tester moved the ankle joint from maximum passive plantarflexion to maximum dorsiflexion as quickly as possible. The tester scored and recorded the muscle reaction as per the MTS scoring system (Table 1). These two recorded measures were later used as clinical ROM and clinical spasticity score.

2.6 Data Analysis

VICON Nexus system (Version 2.13.0 x64) and Qualysis system (Version QTM: 2022.2 build 7710) was used for preprocessing of biomechanical data. Given the captured trial, the dynamic biomechanical positional data was reconstructed, and the identifying markers (Toe, Ankle, and Knee) were assigned to a lower leg Tardieu model. Gap filling was used when necessary. The file was converted to a MATLAB readable file (.c3d).

MATLAB Software Version 9.12 R2022a was used for overall data analysis. An open-sourced biomechanical toolkit (BTK Version M.0.4.7 (2019.11.27)), a wrapper for MATLAB, was used for the biomechanical analysis. From the processed biomechanical positional data, the identified markers (Toe, Ankle, and Knee) were used to obtain the angle of the ankle joint over time (Figure3).

Angle Formula:

Dot Product Function: $res = (x_{V_{TAnorm.}} * x_{VK_{Anorm}}) + (y_{V_{TAnorm.}} * y_{VK_{Anorm}}) + (z_{V_{TAnorm.}} * z_{VK_{Anorm}})$

Angle: $\alpha = \arccos(res)$

Figure 3: Calculations for the angle of the ankle joint over time derived from identified markers (Toe, Ankle, and Knee). V_{TA}: Toe ankle vector, VK_A: Knee ankle vector.

The raw ankle angle data was smoothed by a recursive 8^{th} order Butterworth 10 Hz low pass filter. A signal quality check in the time domain resulted in exclusion of cases based on incomplete marker data or incorrect clinical testing procedure (‘abnormal’ observations) (Figure 5). The total ROM was determined as the range of the angle during the movement.

Lance et al.'s (27) velocity dependent definition for spasticity was used to determine the peak instance of the stretch reflex response. Determined as the start of deceleration and or the peak angular velocity, the 'catch' was calculated as the maximum of the differentiated angle. The ROM to the peak angular velocity was the range of the filtered angle up until the time point of the peak angular velocity.

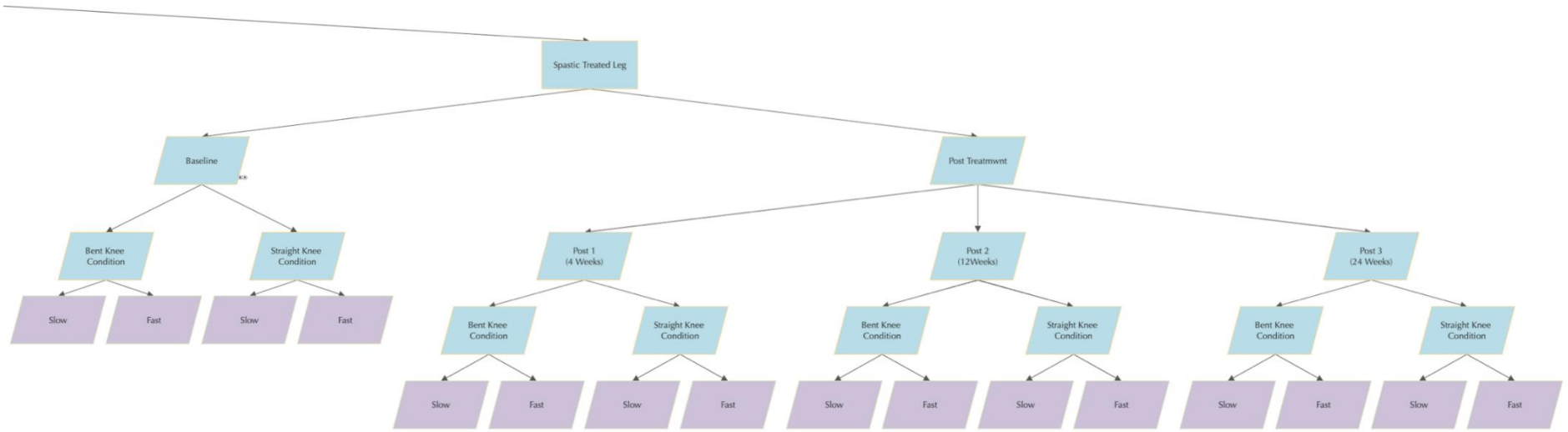
The raw sEMG (GM, SOL, TA) data was bandpass (20-250 Hz) filtered via an 8^{th} order Butterworth filter. Then a linear envelope of the rectified sEMG data was obtained via a recursive 10 Hz 8^{th} order Butterworth low pass filter. A visual inspection in the time domain of both raw sEMG data and the linear envelope resulted in per observation exclusion of EMG data based on high baseline EMG (noise) to peak EMG (signal) ratio as well as artifact noise (abnormal observations) (Figure 5).

The peak amplitude of the linear envelope of the filtered sEMG signal was used to evaluate the grade of spasticity. Existing within a one second window of the predetermined peak angular velocity, the peak amplitude was further inspected for proper selection through timing. Presenting shortly before (within a 0.5 second window prior to) the peak angular velocity indicates the peak amplitude as a possible cause for the deceleration. Presenting after the peak velocity timepoint removes this probable causation.

Furthermore, for the purpose of the secondary aim, a peak amplitude of targeted muscle that was more than three times the baseline noise was considered a significant peak and therefore could represent a 'catch' presence. The leg was determined to have a significant peak, a 'catch,' if either of the two targeted muscles had a significant peak amplitude in the EMG. This distinction in neuromuscular response between 'catch' and 'no catch' was compared with the clinical Tardieu scoring data for agreement or lack thereof between methods. A catch in the clinical Tardieu testing by testers was determined as a score of 2 or 3. No catch was defined as a score of 0 or 1 for the same variable.

2.7 Statistical Analysis

IBM SPSS Statistics (Version: 28.0.1.0 (142)) was used for statistical analysis and graph/ table production. For statistical analyses we used legs and not participants as independent cases, resulting in 27x2=54 legs. Each leg was placed under one of the following 3 categories (from now on called Leg): Spastic treated with BoNT-A, Spastic treated with saline (placebo), or non-spastic non treated (Figure 4). Each leg was tested for spasticity in 2 conditions (knee in 90 and 180 degrees) and at four time points, resulting in 27 (participants) x 2 (legs) x 2 (conditions) x 4 (time points) = 432 tests. All spasticity tests included observations from 2 passive dorsiflexion movements: one slow and one fast, resulting in a total of 864 observations prior to exclusion (Figure 5).


Spastic Treated Leg
Baseline
Post Treatmwnt
Bent Knee Condition
Straight Knee Condition
Post 1 (4 Weeks)
Post 2 (12Weeks)
Post 3 (24 Weeks)
Bent Knee Condition
Straight Knee Condition
Bent Knee Condition
Straight Knee Condition
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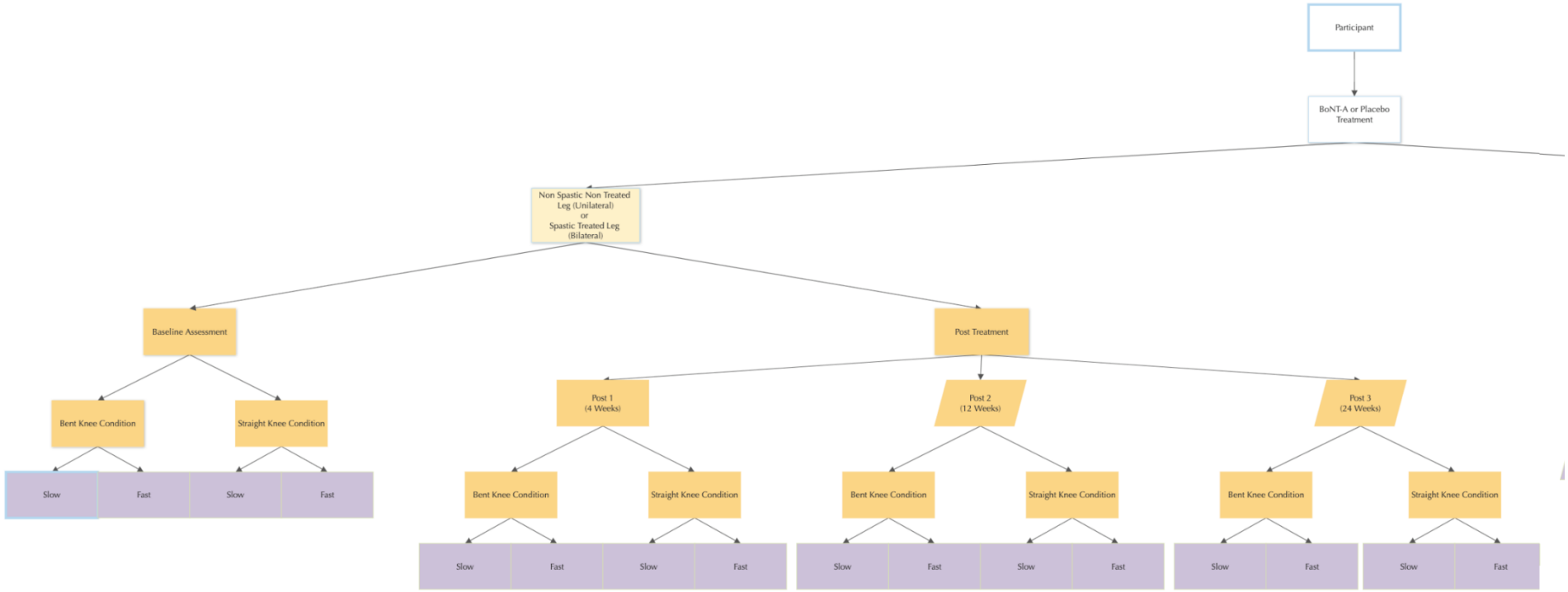
Participant
BoNT-A or Placebo Treatment
Non Spastic Non Treated Leg (Unilateral) or Spastic Treated Leg (Bilateral)
Baseline Assessment
Post Treatment
Bent Knee Condition
Straight Knee Condition
Post 1 (4 Weeks)
Post 2 (12 Weeks)
Post 3 (24 Weeks)
Bent Knee Condition
Straight Knee Condition
Bent Knee Condition
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Figure 4: *Data observations: Each participant was allocated into the placebo or BoNT-A treatment group. Both legs were tested regardless of being spastic or not. If the participant was affected in a unilateral manner, the non-spastic side received no treatment. Each leg was tested in two knee positions in which two movements were performed. This occurred across four visits. This results in 32 test observations per participant, 16 per leg.*

Linear mixed models (LMM) were used to evaluate the difference in physiological responses from baseline between the 3 leg-types where the kinematic and neuromuscular outcome measures were set as dependent variables. The conditions (knee in 90 and knee in 180 degrees), the leg types (spastic treated with BoNT-A, spastic treated with saline (placebo), or non-spastic non treated) and the time points (baseline, P1, P2 and P3) and their interactions were included as fixed factors while the individualized legs were included as random factors to account for repeated measures. The observations of the 2 different passive dorsiflexion movements (slow and fast) were included in two separate LMMs. Both models were run twice, where the reference leg was the placebo in one and the non-treated in the other to have all post hoc leg comparisons.

The relationship between the two methods, clinical and kinematic/neuromuscular, was assessed through agreement (number of observations and percentage) of determined ‘catch’ versus ‘no catch.' Leg nor time were compared as scoring should not be influenced by these. Only the kinematic/neuromuscular data measured during the fast passive dorsiflexion movement was included as it is the only with an expected spastic response. The clinical scoring is also based solely on this movement.

3. Results

Of the 27 participants collected for data review (Table 2), 26 children with CP were included in the spasticity analysis based on availability of EMG and motion capture data. Of the (Participant x Leg x Knee position x Time points => 26x2x2x4=) 416 possible observations during slow and fast passive dorsiflexion, the results are based on 323 slow observations and 324 fast observations, representing 75% of the initially collected data (Figure 5).

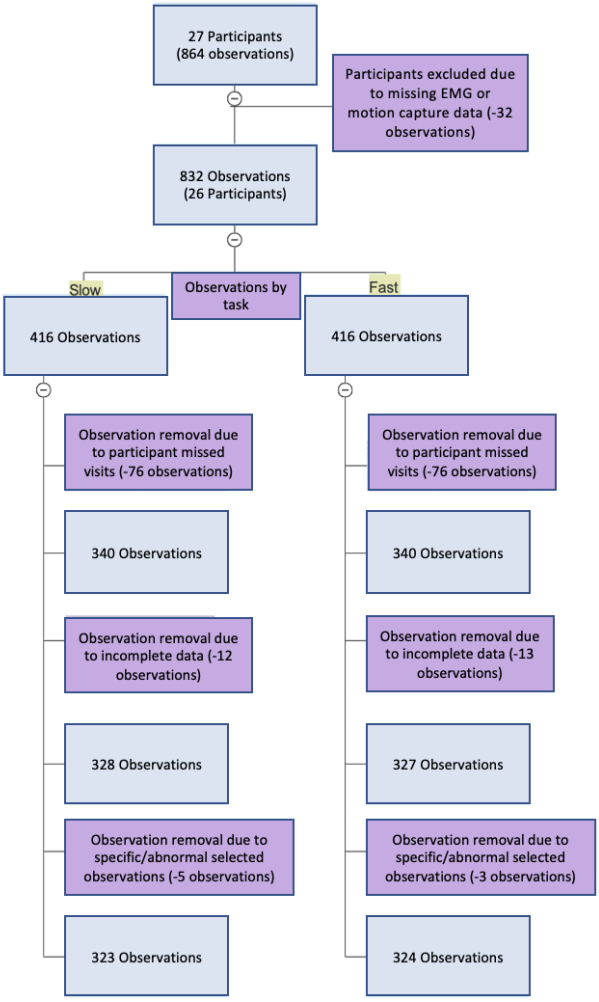

Figure 5*:* Flowchart showing the exclusion of observations and their causes.

3.1 BoNT-A's Physiological Effect Over Time Amongst Different Legs and Treatments

Range of Motion (ROM):

The total ROM during the slow movement resulted in a mean of 51° in BoNT-A legs (figure 6). This was slightly higher than placebo legs (47°) and slightly lower than the non-spastic nontreated legs (60°). During the fast movement, the ROM was very similar to the one during the slow movement (figure 6), the mean was only about 1° smaller in all leg-types. For both velocities, the LMM showed a statistical effect ($p<.001$) of leg types (table 3), where the non-spastic non-treatment leg had a significantly larger estimated ROM difference from both

placebo (10.5°, p_{fast}<.001; 9.9°, p_{slow}=.005) and BoNT-A leg (6.8°$_{fast}$, 7.8°$_{slow}$, p_{both}=.013), while the BoNT-A legs' larger estimated ROM difference was non-significant (3.7°, p_{fast}=.252; 2.1°, p_{slow}=.566) (table 4, 5). The overall effect of time and the time-leg interaction were non-significant in either movement. The testing position of the knee when bent (90-degree knee flexion) had higher ROM (6.2°$_{fast}$, 5.1°$_{slow,}$ p_{both}<.001) than in a straight position (180-degrees) (table 3,4,5).

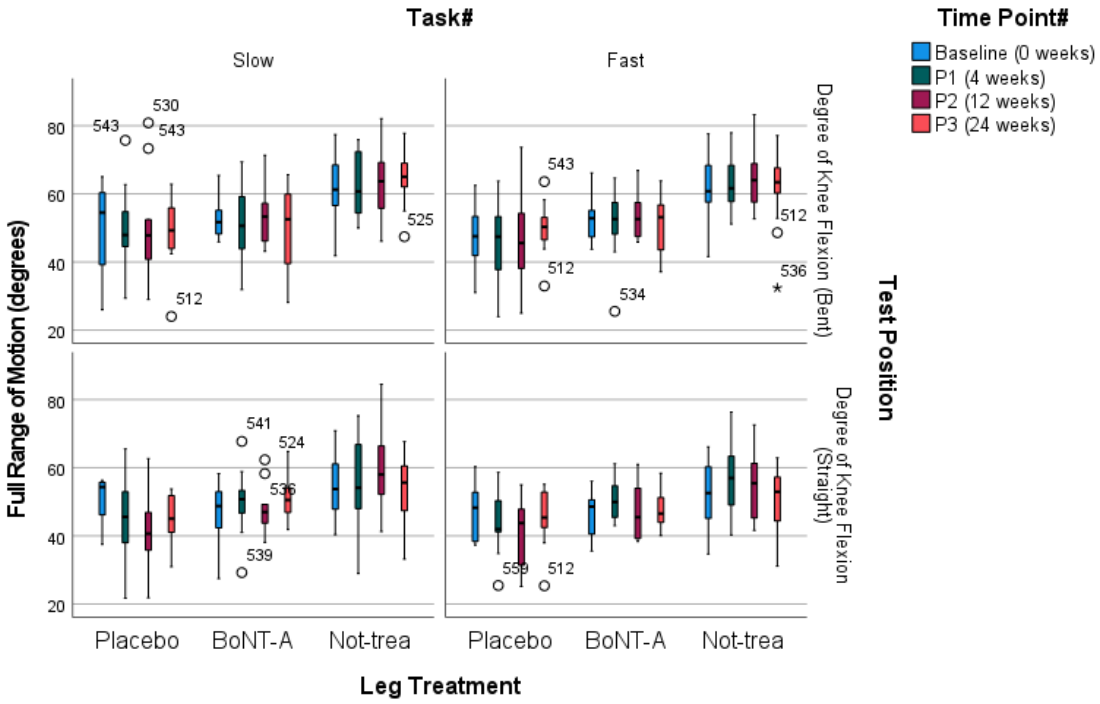

Figure 6: The Full Range of Motion, in degrees, during slow and fast movements of the Tardieu test when tested in the bent and straight knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Peak Angular Velocity:

The average peak angular velocity during the *slow* movement (46 °/sec) of the MTS was largely different from that of the fast movement (450 °/sec) (figure 6). The variance explained among the legs is about 22 times larger in the latter. The difference among the leg types is indistinguishable with estimated marginal means lying within 11 °/sec of each other in the slow movement (no leg effect, table 3). For the fast movement, the peak angular velocity for the

placebo and BoNT-A legs were 108°/sec and 69 °/sec lower than the non spastic non treated legs, with the BoNT-A legs having a difference of +39 °/sec from the placebo legs. However, none of the predictor variables (time, leg) nor their interactions reached statistical significance for either movement.

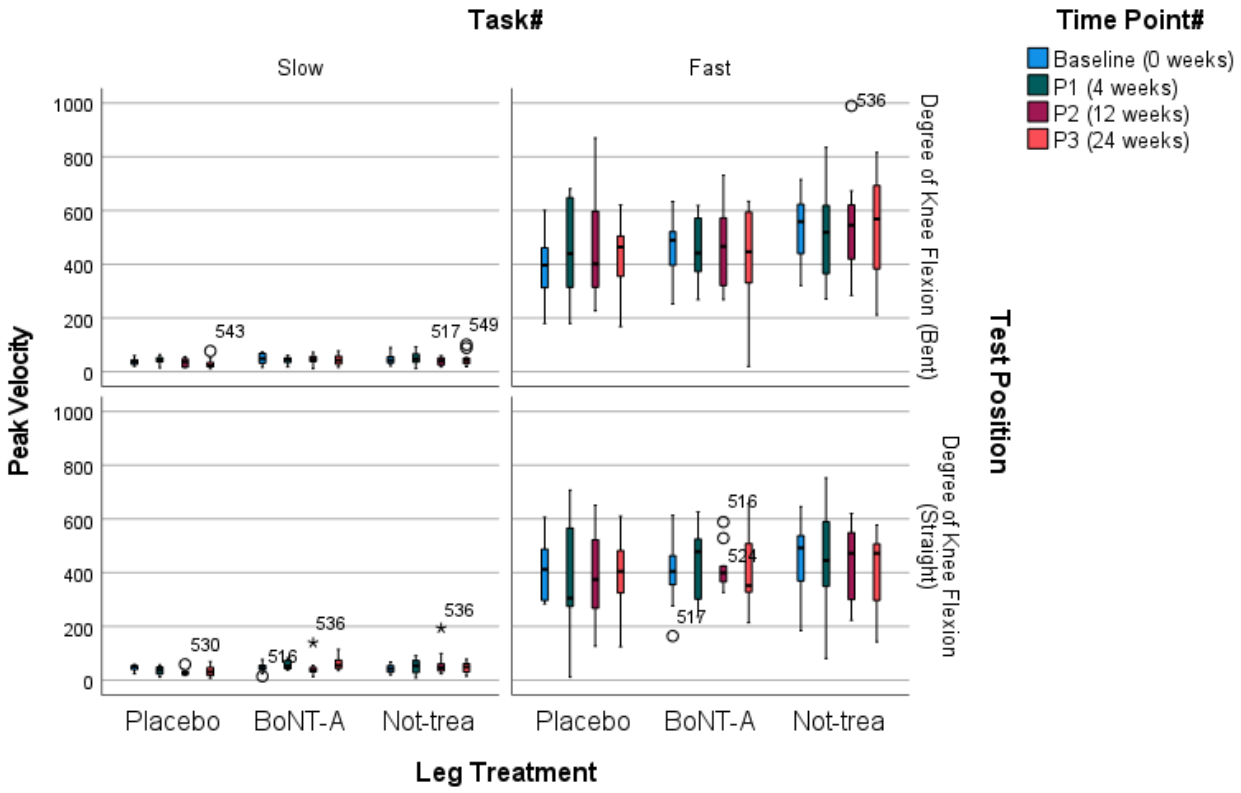

Figure 7: The peak angular velocity, in degrees per second, during slow and fast movements of the Tardieu test when tested in the bent and straight knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Range of Motion (ROM) to Peak Angular Velocity:

The average ROM to the peak angular velocity seems to be slightly larger in the fast movement (21 °) in comparison to the slow movement (15 °) (Figure 8), but this was not statistically tested. The slow movement showed no statistical effect across any of the predictor variables (time, leg) nor their interactions. The fast movement showed a statistical effect of leg types (table 3), where the non-spastic non-treatment leg had a significantly larger estimated ROM to peak velocity difference from both placebo (6.8°, p<.001) and BoNT-A legs (5.2°,

p=.002), while the BoNT-A legs' larger estimated ROM difference was non-significant (1.6°, p=.404) (table 5). The time effect for the fast movement was also significant (p=.030) though this did not translate to a significant time-leg interaction (p=.838, table3). The testing position of the knee when bent (90-degree knee flexion) had higher ROM to the peak angular velocity (p<.001, table 3) than in a straight position (180-degrees) by 3.7°, within the fast movement (Table5).

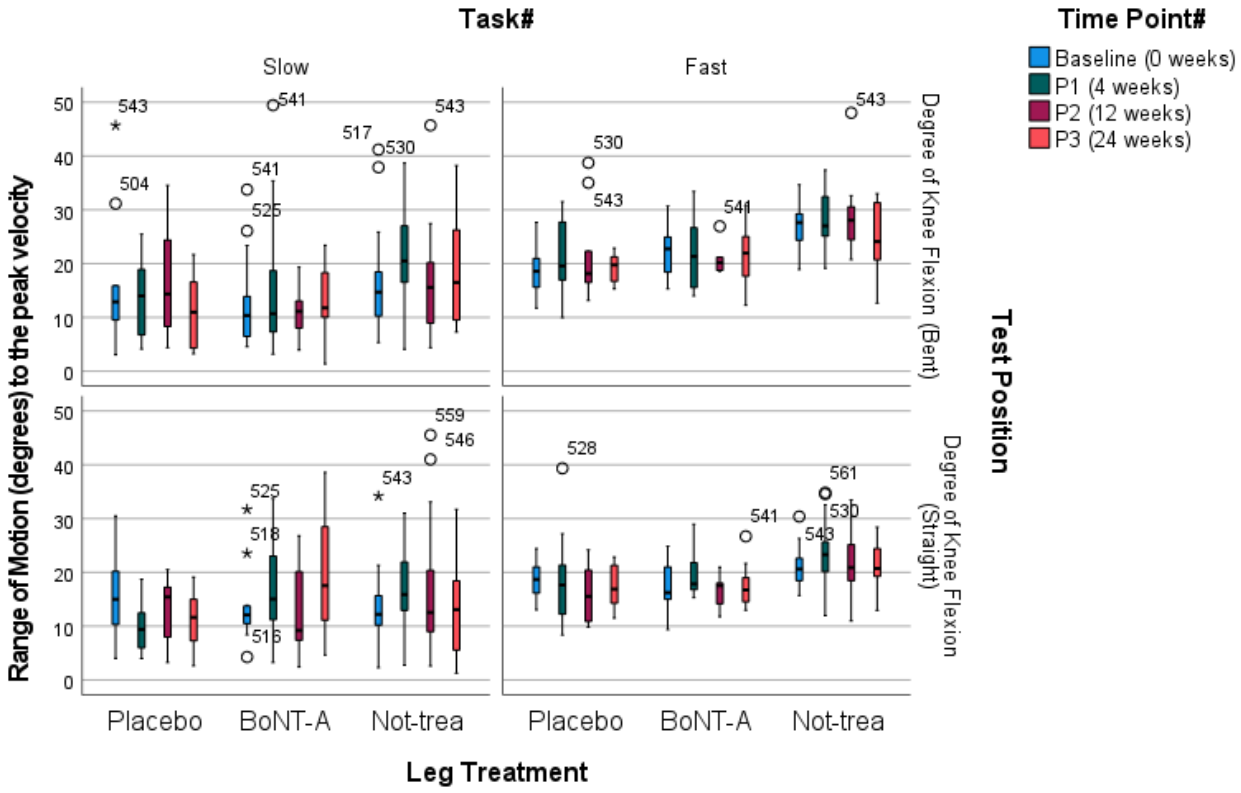

Figure 8: The range of motion to the peak angular velocity, in degrees, during slow and fast movements of the Tardieu test when tested in the bent and straight knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Gastrocnemius Muscle Activity:

The gastrocnemius EMG peak amplitudes (GMpeak), in microvolts (uv), is constant in median across all time points and leg types for the fast movement in a straight knee position (Figure 9). The BoNT-A leg has a slightly lower GMpeak at P1 (76 uv) in comparison to B (91

uv) and P2 (92 uv). However, no significant effects were observed across the time, and or the leg factors nor their interaction. The spread of the data was large with the standard deviation of the non spastic non treated leg (110) and the BoNT-A legs (139) standing around double that of the placebo legs (62), shown with the outliers and wide whiskers (Figure 9). The GM when tested in its isolated position (a straight knee) had a significantly ($p<.001$) larger GMpeak (estimated marginal mean at 74 uv) than with the bent knee (estimated marginal mean at 41 uv) (table 3). The slow movement had lower EMG activity with an overall estimated marginal mean of 12 uv for all observations (in comparison to 57 uv for the fast movement) and no statistical effects amongst factors or interactions.

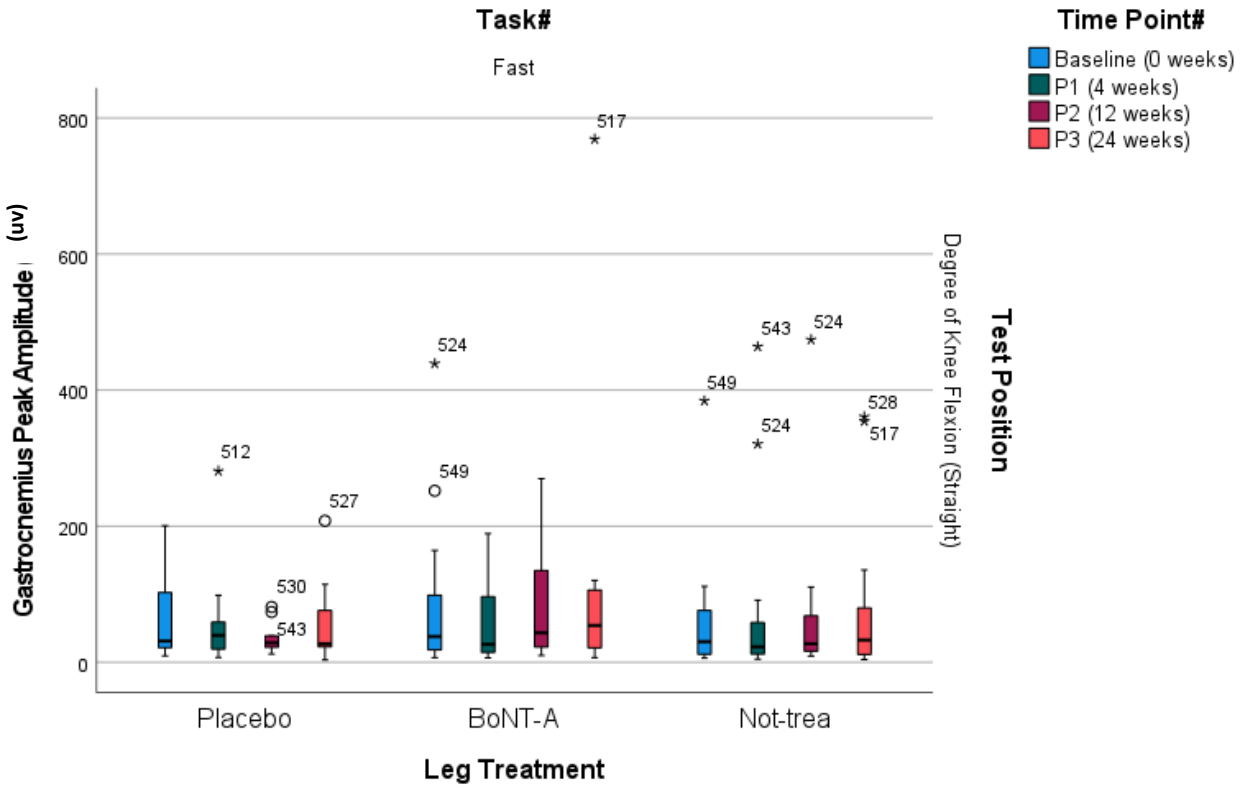

Figure 9: The gastrocnemius EMG peak amplitudes (uv) during the fast movement of the Tardieu test when tested in the straight knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Soleus Muscle Activity:

The soleus EMG peak amplitudes (SOLpeak), in microvolts (uv) during the fast movement with a bent knee testing position had an even higher difference in spread among the leg types than the GMpeak (Figure 10). The standard deviation of the BoNT-A legs (165) stood between triple and quadruple that of the non spastic non treated leg (40) and the placebo legs (53). A statistical effect of time (p=.019) showed all three posttest times to be different from baseline, by an estimate difference of 50-60 uv while similar to each other with estimate differences of no more than 9 microvolts when placebo legs were the baseline reference (Table 3 and 5). With the non-treated leg as baseline reference all four time points were within 10 uv of each other. (Table 5) The spread was visibly higher in the baseline timepoint for both the placebo and BoNT-A legs in comparison to the post test time points (Figure 10). No significant effects were observed for the leg types factor nor the time leg interactions. The SOLpeak rise of BoNT-A from baseline to P1 was a non-significant (p=.727) change in comparison to the SOLpeak response in the placebo at P1, despite Figure 10's appearance. The SOLpeak did not present significant differences between the two knee positions (p= .935) (table 3). The slow movement had lower EMG activity with an overall estimated marginal mean of 12 uv (in comparison to 56 uv for the fast movement) for all observations and no statistical effects amongst factors or interactions.

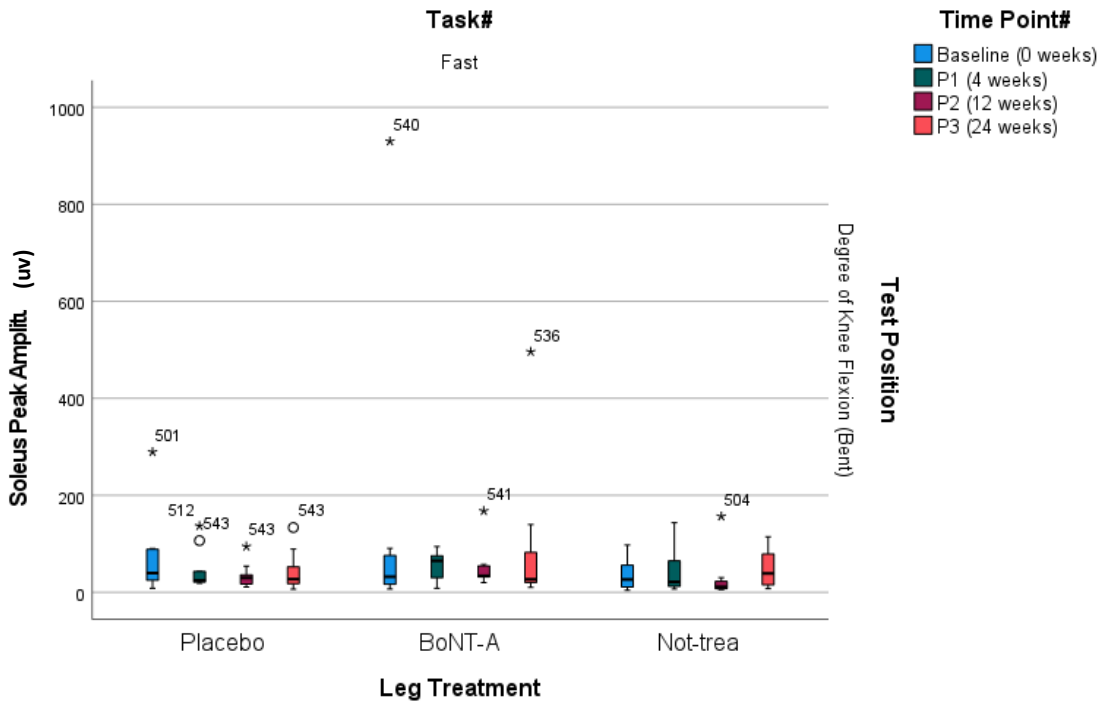

Figure 10: The soleus EMG peak amplitudes (uv) during the fast movement of the Tardieu test when tested in the bent knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Tibialis Anterior Muscle Activity:

The tibialis anterior EMG peak amplitudes (TApeak), in microvolts (uv), were analyzed primarily during the fast movement in both knee positions (Figure 11). The BoNT-A and non spastic non treated leg appear to have stable medians across time points, with some slightly higher variance shown among the time points in the placebo legs. This muscle showed no statistical effect of any factors or interactions in either movement.

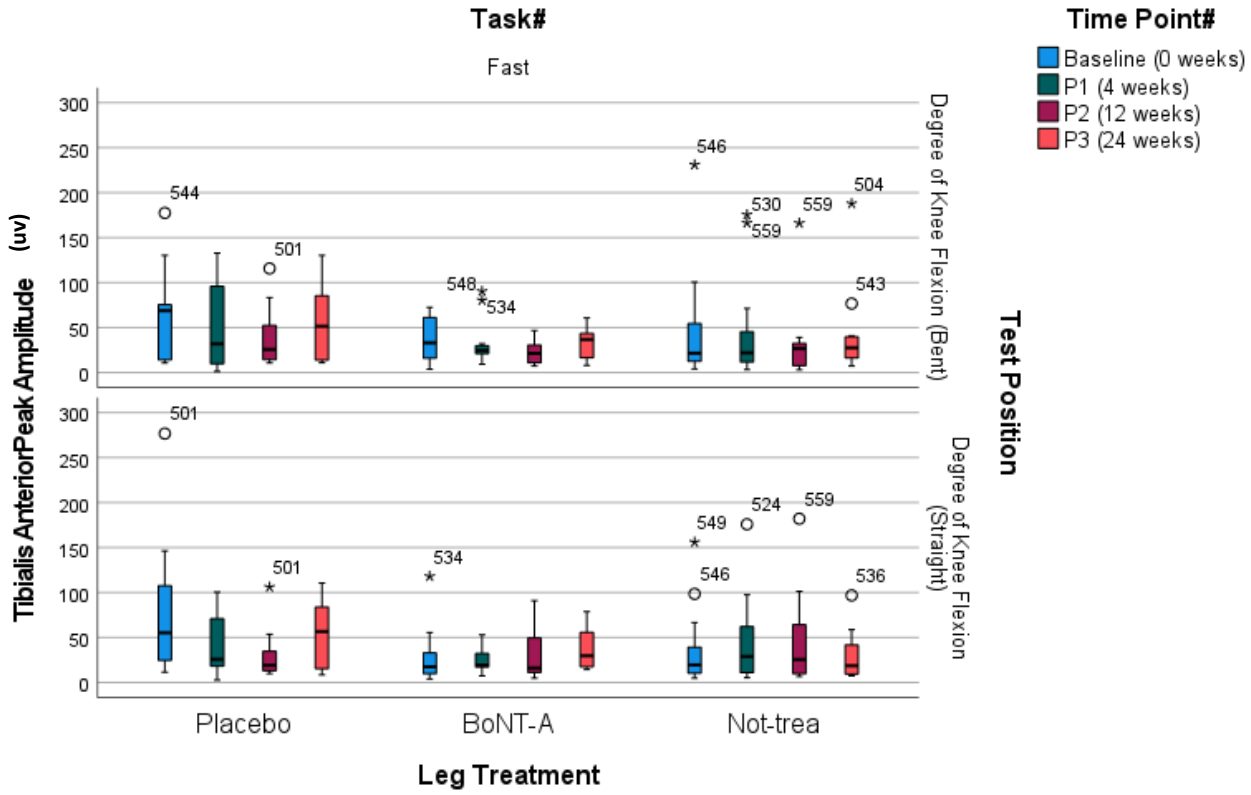

Figure 11: The tibialis anterior EMG peak amplitudes (uv) during fast movement of the Tardieu test when tested in the bent and straight knee positions for all three leg types across all four timepoints. Outliers are labeled by participant ID number.

Table 3*:* Linear mixed model (LMM) analysis of measurements during the *slow and fast* passive dorsiflexion movements. To investigate the effect of time (baseline, P1, P2, P3), Leg (BoNT-A treated, Saline-treated, non-treated), Knee (bent, straight) and time x leg interaction.

Movement Type	**-**	**Slow**				**Fast**			
Dependent Variable	**Source**	**DF1**	**DF2**	**F**	**Sig.**	**DF1**	**DF2**	**F**	**Sig.**
Full ROM	Time	3	257	0.43	.734	3	260	0.58	.629
	Legs	2	47	11.01	<.001*	2	48	14.05	<.001*
	Knee Position	1	250	38.61	<.001*	1	253	74.04	<.001*
	Time Leg Interaction	6	257	0.59	.739	6	260	1.14	.339
Peak A. Vel	Time	3	234	2.39	.069	3	261	0.13	.940
	Legs	2	34	1.60	.217	2	49	2.71	.077
	Knee Position	1	230	2.93	.088	1	254	32.82	<.001*
	Time Leg Interaction	6	234	0.86	.526	6	261	0.42	.869
ROM to the A. Vel	Time	3	256	0.80	.493	3	263	3.02	.030*
	Legs	2	46	1.02	.368	2	47	11.98	<.001*
	Knee Position	1	248	1.38	.240	1	253	52.60	<.001*
	Time Leg Interaction	6	256	2.00	.067	6	263	0.46	.838
Gastrocnemius Peak Amplitude	Time	3	236	0.37	.773	3	262	0.58	.629
	Legs	2	30	0.56	.575	2	50	0.50	.608
	Knee Position	1	219	45.71	<.001*	1	248	11.74	.001*
	Time Leg Interaction	6	236	1.15	.333	6	262	0.19	.979
Soleus Peak Amplitude	Time	3	236	1.73	.161	3	234	3.39	.019*
	Legs	2	38	1.28	.289	2	37	0.88	.423
	Knee Position	1	224	1.34	.248	1	220	0.01	.935
	Time Leg Interaction	6	235	1.70	.123	6	234	0.62	.713
Tibialis Anterior Peak Amplitude	Time	3	230	0.34	.796	3	253	2.61	.052
	Legs	2	34	0.77	.473	2	47	2.59	.086
	Knee Position	1	218	0.93	.336	1	240	0.35	.555
	Time Leg Interaction	6	230	1.66	.133	6	254	1.60	.147

ROM: Range of Motion, A.VEL: Angular velocity, DF1: numerator degrees of freedom, DF2 denominator degrees of freedom, * Significant at an alpha .05 level

Table 4**:** Linear mixed model (LMM) post hoc analyses for the significant measurements (* in Table 3) during the *slow* passive dorsiflexion movement.

Dependent Variable	Source	Comparison	Estimate	Std. Error	95% CI	Sig.
Full ROM	Legs	Placebo < NT	-9.9°	3.5	-16.8, -3.0	.005*
		BoNT-A < NT	-7.8°	3.0	-13.8, -1.7	.013*
		BoNT-A>Placebo**	2.1°	3.7	-5.2, 9.5	.566
	Knee Position	Bent > Straight	5.1°	.8	3.5, 6.7	<.001*
Gastrocnemius Peak Amplitude	Knee Position	Bent < Straight	-5.7	0.8	-7.3, -4.0	<.001*

ROM: Range of Motion, A.VEL: Angular velocity, NT: Non-Treated, * Significant at an alpha .05 level, **Model with Placebo as reference leg (All other Values have NT as the reference leg)

Table 5**:** Linear mixed model (LMM) post hoc analyses for the significant measurements (* in Table 3) during the *fast* passive dorsiflexion movement.

Dependent Variable	Source	Comparison	Estimate	Std. Error	95% CI (Lower Bound, Upper Bound)	Sig.
Full ROM (°)	Legs	Placebo < NT	-10.5°	3.0	-16.4, -4.6	<.001*
		BoNT-A < NT	-6.8	2.7	-12.1, 1.5	.013*
		BoNT-A>Placebo**	3.7	3.2	-2.7, 10.1	.252
	Knee Position	Bent > Straight	6.2	0.7	4.7, 7.6	<.001*
Peak A. Vel (°/sec)	Knee Position	Bent > Straight	62.4	10.9	41, 83.9	<.001*
ROM to the Peak A. Vel (°)	Time	P1 > B	0.8	1.1	-1.3, 2.8	.470
		P2 > B	0.1	1.1	-2.1, 2.3	.918
		P3 < B	-1.9	1.1	-4.1, 0.	.081
	Legs	Placebo < NT	-6.8	1.8	-10,3, -3.2	<.001*
		BoNT-A < NT	-5.2	1.6	8.3, -2.0	.002*
		BoNT-A>Placebo**	1.6	1.9	-2.2, 5.4	.404
	Knee Position	Bent > Straight	3.7	.5	2.7, 4.8	<.001
Gastrocnemius Peak Amplitude (uv)	Knee Position	Bent < Straight	-32.6	9.5	-51.3, -13.9	<.001*
Soleus Peak Amplitude (uv)	Time	P1< B	-50.5** (-9.8)	27.4** (19.8)	-104.4, 3.6 ** (-48.7,29.1)	.067** (.620)
		P2< B	-59.5** (-10.4)	27.4** (20.9)	-113.5, -5.5** (-51.5,30.7)	.031*,** (.619)
		P3< B	-53.7** (-9.3)	27.4 ** (20.8)	-107.8, 0.3 ** (-50.4, 31.7)	.051** (.655)

ROM: Range of Motion, A.VEL: Angular velocity, NT: Non-treated, * Significant at an alpha .05 level, **Model with Placebo as reference leg (All other Values have NT as the reference leg), SOLpeak shows both placebo** and (NT) models

3.2 Agreement Level Amongst Methods

The 324 observations from the fast movement were considered for inclusion in these results. For all the observations with GMpeak and SOLpeak only 314 observations had pairing data recorded from the clinical Tardieu test. Therefore, when the neuromuscular data used the significant peak definition (>3*baseline EMG), 177 of the GMpeak observations and 192 of the SOLpeak observations were classified under said definition. An overall presence of a significant peak in any or both of the targeted muscles was used to define a catch within the entire leg. This results in 237 observations with, and 77 without a significant P.A. in the EMG (table 6). This data includes observations from all time points, groups, and knee positions. The clinical spasticity scores are composed of 185 labeled 'No Catch' (Score of 0 or 1) and 129 labeled 'Catch' (Score of 2 or 3). The 2x2 contingency (table 6) shows that both methods agreed a catch was present in 94 (29.9%) and agreed that a catch was absent in 42(13.4%) of the observations based on the total analyzed. This results in 43.3% agreement (table 7) and 56.7 % non-agreement for the two methods.

Table 6*:* 2x2 Contingency table: Spastic catch agreement between the Clinical Tardieu Test and the surface EMG from the gastrocnemius and soleus in terms of 'Catch'

Agreement In Spastic Catch Presence		EMG: Gastrocnemius and/or Soleus		
		No Significant P. A	Significant P.A (Yes)	Total
Clinical Tardieu Test	No Catch	**42 (13.4)**	143 (45.5)	185 (58.9)
	Catch (Yes)	35 (11.1)	**94 (29.9)**	129 (41.1)
	Total	77 (24.5)	237 (75.5)	314 (100)

EMG: Electromyography, P.A: Peak amplitude

Table 7: Summed Agreement and Non-Agreement in Spastic Catch Presence Amongst Methods

Decision	**Catch or No Catch Detection** (Clinical Tardieu Test/EMG)	**Count n (%)**
Agreement	Yes/Yes	94 (29.9)
	No/No	42 (13.4)
	Total	136 (43.3) *
Non-Agreement	Yes/No	35 (11.1)
	No/Yes	143 (45.5)
	Total	178 (56.7) *
Total		314 (100)

EMG: Electromyography

4. Discussion

This studies main aim was to assess the change in electromechanical response of spastic lower leg musculature of children with CP when treated with BoNT-A over time. The results showed no significant change for any of the five outcome measures. In addition, the study secondarily aimed on reporting the agreement level between clinical assessment versus the neuromuscular physiological responses when assessing the presence of a 'catch.' No consistent agreement was shown in the spasticity grading. Expected versus observed trends for each aim and outcome measure will be discussed further to better understand the physiological responses seen in the study.

4.1 The Physiological Responses to BoNT-A

With no statistical effect for the interaction of legs over time for the BoNT-A treatment, an effect that has already been confirmed by other studies, the methodology and spasticity level of the included participants of the study comes into question. A difference between the legs overall would demonstrate a significant level of spasticity in the spastic legs and therefore a potential for improvement towards the non spastic non treated leg. However, the non spastic non treated leg only differed from the spastic legs in 2 of the 6 outcome measures: ROM and ROM to peak angular velocity. With low difference between leg types across the study overall, the spasticity level of the spastic participants may be considered too low to assess a treatment effect. The GMFCS level distribution amongst participants consisted of 85% with level 1 and 15% with level 2. Therefore, the inclusion criteria may have had to include GMFCS levels of 2 and 3 to better see treatment effect rather than levels 1 and 2. For those that did have a statistical difference amongst leg types an alternative cause for the lack of time leg interaction is discussed.

Total Range of Motion:

Total ROM during the slow passive movement was investigated to assess the angle of the muscle length at rest, also known as the R2 parameter in the MTS (15). The expected effect of BoNT-A in regard to this measurement is an increase in the dorsiflexed ROM(6). A systematic review found an average increase in mean passive ankle dorsiflexion of 2.68 degrees for short term (2-8 week) follow-up in comparison to the placebo treated leg (6). The medium-term follow-up reported no difference between the groups. While this study showed the BoNT-A legs

had a higher estimated leg difference of +2.13 (95%CI: -5.23-9.49, p=.566) degrees from the placebo legs, without time factored in, there was an unexpected lack of significant effect *over time* amongst these legs. One probable cause is that 27% of this study's BoNT-A treated participants have had multiple BoNT-A injections. Repeated BoNT-A injections result in diminished treatment effects for ROM (24).

Peak Angular Velocity & Range of Motion *to the* Peak Angular Velocity:

Peak angular velocity was used to indicate the start of deceleration caused by muscle reactivity during the fast passive dorsiflexed movement. With increasing spasticity, the muscle reaction during testing occurs earlier in the ROM. A reduced time and or range before the muscle reaction is indicative of less time under acceleration and, therefore, reduced peak angular velocity in comparison to a less spastic muscle with a longer range before muscle reaction (14). The lack of statistical difference between leg types for peak angular velocity may establish that the spastic legs were not statistically spastic enough to differ from a non spastic leg. It is also probable that no statistical change in peak angular velocity can be assessed with low sample numbers since the non-significant means of each leg did rank in the expected order.

The ROM to the initial presence of muscle reactivity (peak velocity) was to be a similar representation of the Tardieu tests R1 parameter. This parameter indicates the angle to the catch seen in the fast passive dorsiflexed movement (25). The time and the leg overall significance did not translate to the expected time leg interactions. This may be due to a small sample size since the observed results followed expected results but were still insignificant (Table 5).

EMG:

The expected decrease in peak sEMG for the targeted muscles (GM, SOL) treated with BoNT-A over time was not statistically shown. The overall EMG results may have limited use and interpretation as they may have been influenced by other factors other than spasticity. Possible factors include inability to relax the leg during passive movement affected by the young age of the participants, atrophied muscles, quality of the EMG sensor data and EMG system availability in addition to the BoNT-A effect. Some observations were removed due to voluntary muscle activity during the passive assisted testing, increasing missing data. This is also a point of

contention in other studies with young children with CP (14). Furthermore, this study did not normalize the EMG data and therefore saw high variability in leg-to-leg comparisons. Both (GM & SOL) EMG magnitudes covered a wide range in the legs overall where BoNT-A legs (769 uv, 926 uv) varied more than the placebo (281uv, 722 uv) and the placebo more than the non-spastic non treated leg (528 uv, 381uv). While the high variability creates difficulty in assessing a physiological response change over time the practice of interpreting normalized EMG data for spastic legs is unclear as it can overexplain and underexplain trends during varying movements (26).

The spastic lower leg muscles were likely atrophied due to multiple injections and generally weak and therefore showed low signal input. This made the detection of muscle reactivity difficult as the baseline noise was moderately higher than ideal. A prerequisite for detecting a treatment effect is the presence of symptoms. The lack of difference between leg types in the GM and SOL EMGs shows no indication of increased spastic muscle activity in the treated legs compared to the non spastic non treated legs, therefore no treatment effect can be detected over time.

The GMpeaks in the straight knee position were 32uv higher ($p=.001$) than the bent knee as expected due to the preferred testing position of the GM muscle. The SOLpeak was expected to have the same response between testing positions of the knee, this was the case ($p=.935$). The EMG's ability to detect the expected change due to the differing knee positions validates its ability to detect small changes. The time effect in SOL was a false positive due to the placebo legs outliers at baseline as the model with non-treated legs as a reference saw no time effects in the post hoc analysis (table 5).

TApeak was not expected to have a large change in physiological response since it was not injected. The response here was to determine its ability to counteract a spastic response and hypertonia from the GM and SOL. Spastic responses convert to high levels of coactivation between the GM/TA and the SOL/TA (14, 26). One study found the antagonistic muscle had even higher activation than in the targeted muscle (14). Another study testing this coactivation during gait saw high levels of both pairs during the terminal phase in the normalized and absolute sEMG amplitudes. The stretch seen in the MTS is comparable to the transition between terminal phase and pre-swing. Likely more TA activation is necessary for adjustment of

increased muscle tone with spastic GM and or SOL. In figure 11 the range of peak amplitudes during P1 in the BoNT-A legs appears to be of the smallest, indicating the muscle has little tone correction to perform.

4.2 Agreement Level Among Methods

The comparison between methods, the MTS and the kinematic and neuromuscular measures was expected to have a strong level of agreement when assessing the presence of a 'catch'. One study found about 26-51% non-agreement, and 49-74% agreement between the methods(14). This study had 56% non-agreement and 43% agreement based on catch determination on a categorical yes/no basis. This represents no meaningful consistent agreement other than that of which could happen by random chance (50/50). The two studies based the EMG spasticity recognition characteristics differently. This study defined the EMG catch and no catch distinction solely via neuromuscular measures where the presentation of a significant peak among at least one of the targeted muscles (GM or SOL) was defined as a 'catch'. In addition to the neuromuscular component this study covered, the former study also noted kinematic measures (peak velocity, and ROM to peak velocity) to represent the degree of resistance to passive stretch as indicators of the severity of the resistance to passive stretch. The expanded definition of the former study could explain higher agreement. Future analysis could review if the definition expansion would recreate the previously observed agreement levels.

4.3 Strengths and Limitations

This study was an explorative post hoc sub study based off of a double blinded RCT's partial participant data. A common observance among explorative post hoc studies is the lack of control over the available data set. In this case a strength of this study was that two of the locations remained randomized, however the third location encountered limitations due to incomplete/ missing data and was unable to retain randomization. With an unrandomized dataset the baseline between each leg type was unable to be assessed as one. In a limited manner the participants available may not have been the ideal participant groups for testing. The data shows the participants were not as spastic as they had reported prior to baseline assessment. Therefore, finding treatment-based spasticity changes among spastic groups that were already presenting minimal spasticity levels leaves minimal to no change as the possible outcome, as seen in the

results. Furthermore, the number of participants was only 26, divided into subgroups after this left a small number for analysis. This limited the format of the LMM. Adding all the levels available in the model could result in a smaller count per level for the model to run on, and an effect on significance and power that could lead to misleading interpretations of the dataset.

4.4 Future Implications/ Further Research

Had this study's agreement between methods been higher, more than can be said by chance, and significant BoNT-A effects been seen, another aim was of initial interest for further evaluation: To assess which of the two methods would be better at predicting the physiological effects of BoNT-A. Future research would benefit from including age groups categorized between 2 to 6 and 6 to 12 years based on the CP: Musculoskeletal Management Algorithm (8) which demonstrates the younger group to have a higher indication for BoNT-A and the older group for surgical measures. It would be valuable to include a signal pattern recognition for the EMG that not only would show the peak amplitudes per muscle group but would characterize the spastic muscle reaction seen, such as clonus, in future studies.

5. Conclusion

This study evaluated the effect of BoNT-A injections over time in spastic lower legs in relationship to spastic placebo injected legs and non spastic non treated legs for children with spastic CP, through kinematic and neuromuscular measurement methods. Finding no significant change from baseline at any timepoint, a future adjustment in the inclusion criteria for more spastic participants and an increased sample size is suggested to verify BoNT-A effectivity on an electromechanical level. Further studies with this relatively new measurement method are needed before its use for clinical treatment prediction and for stronger comparison to the clinical assessment. The spasticity decision of the MTS and the neuromuscular testing methods resulting in a lack of consensus in the categorization between ‘catch’ and ‘no catch’ presentation also supports a larger study and more spastic participants in the inclusion requirements. Alternatively, redefining and or expanding the criteria required for distinguishing between the ‘catch’ vs ‘no catch’ categories within the kinematic and neuromuscular testing method may affect the consensus between methods.

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