NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTMENT OF PHYSICS

MIGUEL MAFRA BAPTISTA

BSc in Biomedical Engineering

ELECTROMYOGRAPHY MEASUREMENTS FOR THE ASSESSMENT OF THE EFFECT OF BOTULINUM TOXIN-A IN SPASTICITY OF THE WRIST

MASTER IN BIOMEDICAL ENGINEERING

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Abstract

This study addresses the growing impact of spastic paresis resulting from conditions like stroke, with a focus on wrist spasticity assessment, and treatment using Botulinum neurotoxin A (BoNT). Spasticity, a velocity-dependent increase in muscle tone in response to passive stretching, remains a complex and insufficiently understood challenge in medical care. While the NeuroFlexor (NF) device offers a means to quantify spasticity, it is proposed that combining electromyography (EMG) with NF data could provide a more comprehensive understanding.

The study aims to determine the value of incorporating EMG alongside NF for assessing BoNT's impact on wrist spasticity. By evaluating EMG's validity and role in detecting spasticity changes pre- and post-BoNT injection, the study seeks to enhance predictive models for BoNT treatment outcomes, ultimately improving the diagnosis and therapy of wrist spasticity.

In this study, patients who had experienced a stroke or had cerebral palsy were included if they were eligible for BoNT injections at the wrist and were able to follow instructions. This study involved 18 patients and three evaluation sessions: before BoNT treatment for spasticity of the wrist joint, and at 6 and 12 weeks after treatment. EMG data was recorded from the flexor carpi radialis (FCR) and extensor carpi radialis (ECR) muscles alongside NF data (neural and non-neural contributors of wrist hyper-resistance to passive movement), and the surface EMG signals were processed and normalised. The EMG outcomes were defined as the short- (M1) and long- (M2) latency responses to passive wrist extension, and were detected using a threshold method. Clinical scales, such as the modified Ashworth scale and the Tardieu scale, and NF data provided outcome measurements, with the neural force component (NC) being a representative of spasticity.

The M1 of the FCR, but not the M2, correlated significantly with the NC of the NF. There were no significant correlations with the clinical scales. There was a significant decrease in M1-related parameters for the FCR after BoNT. EMG parameters correlated strongly with BoNT dose, with higher doses resulting in a larger decrease of EMG parameters. The accuracy, precision and sensitivity were higher for prediction models that included EMG.

The construct validity of EMG with respect to the NF was proven as was the effect of BoNT in treating wrist spasticity. The addition of EMG shows an increase in predictive power between responders and non-responders to BoNT. The extra effort in using EMG might not justify the small increase in the goodness of the model.

Keywords: Spastic paresis, Spasticity, Surface electromyography, Botulinum neurotoxin A, NeuroFlexor

Resumo

Este estudo aborda o crescente impacto da paraplegia espástica resultante de condições como o acidente vascular cerebral (AVC), com foco na avaliação da espasticidade do punho e no tratamento com a toxina botulínica A (TBo). Espasticidade, um aumento de tónus muscular dependente da velocidade, em resposta a uma distenção forçada, continua a ser um desafio médico com algumas lacunas. Embora o dispositivo NeuroFlexor (NF) ofereça um meio de quantificar a espasticidade, é proposto que a combinação de eletromiografia (EMG) com dados do NF possa fornecer uma compreensão mais abrangente. O objetivo do estudo era determinar a mais-valia da incorporação de EMG com o NF para avaliar o impacto da TBo na espasticidade do punho. Ao avaliar a validade da EMG e o seu papel na deteção de mudanças na espasticidade antes e após a injeção de TBo, esta pesquisa tenta aprimorar modelos de previsão para os resultados do tratamento com TBo, tentando melhorar o diagnóstico e a terapia da espasticidade do punho.

Neste estudo foram incluídos pacientes com AVC ou com um diagnóstico de paralisia cerebral, elegíveis para injeções com TBo no punho e capazes de seguir instruções. O estudo envolveu 18 pacientes e três sessões de avaliação: antes e após 6 e 12 semanas do tratamento com TBo para espasticidade no punho. Os dados de EMG foram adquiridos nos músculos flexor radial do carpo (FRC) e extensor radial do carpo (ERC) juntamente com dados do NF (componentes neuronal e não neuronal da hiperresistência a movimento forçado), e os sinais de EMG de superfície foram processados e normalizados. Os parâmetros da EMG foram definidos como as respostas de latência curta (M1) e longa (M2) à extensão passiva do punho e foram detetados usando um método de limiar. Escalas clínicas, como as escalas modificada de Ashworth e a escala de Tardieu, e dados do NF forneceram medidas, com a componente neuronal (CN) a representar a espasticidade.

A M1 mas não a M2 do FCR correlacionou significativamente com o CN do NF. Não foram encontradas correlações significativas com as escalas clínicas. Houve uma diminuição significativa dos parâmetros relacionados com a M1 do FRC após a TBo. Os parâmetros de EMG correlacionaram fortemente com a dose de TBo, sendo que doses mais elevadas resultaram numa maior diminuição dos parâmetros de EMG. A exatidão, precisão e sensibilidade foram maiores nos modelos de previsão que incluíram EMG.

A validade do EMG foi comprovada, assim como o efeito da TBo no tratamento da espasticidade do punho. A adição de EMG demonstrou um aumento no poder de previsão entre respondedores e não-respondedores à TBo. O esforço adicional na utilização do EMG pode não justificar o pequeno aumento da qualidade do modelo.

Palavras-chave: Paraplegia espástica, Espasticidade, Eletromiografia de superfície, Toxina botulínica A, NeuroFlexor

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Glossary

catch angle Angle at which there is a sudden increase in resistance to the passive movement [2].

clonus Involuntary and repetitive muscle contractions that can occur when a muscle is stretched passively. It is frequently observed in individuals who have upper motor neuron or spinal cord lesions [3].

co-contraction Simultaneous contraction of the agonist and antagonist muscles when the former is voluntarily contracted. If excessively present, it is considered spastic co-contraction [4].

dexterity "The ability to adequately solve any motor task... precisely, quickly, rationally and deftly" [5].

embolus Thrombus that travels through the bloodstream and lodges in a different location than its site of origin, potentially obstructing blood flow [3].

hypertonia Increase in **muscle tone** [4].

hypotonia Decrease in **muscle tone** [6].

Klippel-Feil syndrome People with this condition were found to have neurons from the motor cortex whose "axons bifurcate and make connections to homologous motor neurons on both sides of the body" [7].

muscle spindle Sensory receptor, located in the central part of skeletal muscles, able to detect absolute muscle length and changes in muscle length [7–9].

muscle tone EMG baseline level when the person is relaxed, measuring the residual muscle activity [10].

negative feature Loss of **dexterity**, **weakness**, **hypotonia** and modification of deep tendon reflexes, appearing right after a UMN lesion [11–13].

neural components	Central nervous system contribution (velocity-dependent stretch hyperreflexia (**spasticity**) and involuntary and velocity-independent background activation) to increased perceived resistance during passive motion [14].
non-neural components	Tissue changes (muscle shortening and or stiffness, elasticity, viscosity), which contribute to altered biomechanical behaviour [15, 16].
paresis	Reduction of voluntary recruitment of motor units, translating into motor weakness. Hemiparesis is relative to paresis on one side of the body [4, 8].
plegia	Complete loss or absence of the ability to move a part of the body [8].
positive feature	**Spasticity**, antagonist muscular **co-contraction**, **tendon jerking**, **clonus** and **spastic dystonia**, which usually appear later in time after a UMN lesion [11, 12, 17].
spastic dystonia	Incapability of muscle relaxation, which results in involuntary stretch-triggered muscle contraction, while the individual is at rest. The main difference from spasticity is its stretch velocity independence [4, 18].
spasticity	Lance defined spasticity as "a motor disorder characterised by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neuron syndrome" [19].
stretch reflex	The muscle stretch receptors receive the stretch impulse, which is led via motor neurons to the spinal cord or brain stem. Subsequently, this impulse is conveyed back to the previously stretched muscle. Muscle stretch reflex functions as a counteraction to the elongation of the muscle, in order to maintain its length constant [20].
tendon jerking	During a clinical exam, tapping on a muscle tendon causes a monosynaptic stretch response known as a tendon jerk [20].
thrombus	Blood clot that lodges in its site of origin [3].
weakness	"Inability to generate normal levels of force" [8].

Abbreviations

AIC	Akaike Information Criterion
AUC	area under the curve
BoNT	botulinum neurotoxin-A
CNS	central nervous system
CP	cerebral palsy
CST	corticospinal tract
EC	elastic component
ECR	extensor carpi radialis
EMG	electromyography
FCR	flexor carpi radialis
GTO	Golgi tendon organs
LMEM	linear mixed effects model
MAS	modified Ashworth scale
NC	neural component
NF	NeuroFlexor
PROM	passive range of motion
PSS	post-stroke spasticity
RF	reticular formation
RST	reticulospinal tract
sEMG	surface electromyography
TS	Tardieu scale

UMN upper motor neuron
UMNS upper motor neuron syndrome

VC viscous component
VST vestibulospinal tract

1

Introduction

The research work described in this dissertation was carried out in accordance with the norms established in the ethics code of Universidade Nova de Lisboa. The work described and the material presented in this dissertation, with the exceptions clearly indicated, constitute original work carried out by the author.

1.1 Context and motivation

With an increase in population and an improvement in medical care and knowledge, people nowadays live longer. This also means that there are more people being affected by diseases that appear at an older age, such as stroke. Stroke causes lesions to upper motor neurons (UMNs) and some patients might develop a motor disorder called spastic paresis [21]. During the chronic phase of stroke, but also sometimes in earlier phases [22], the affected individuals tend to develop spasticity [23]. A considerable number of the affected patients need to tackle this condition, due to impairments caused in their lives and due to societal burdens [13, 24, 25].

Spasticity, a condition originating in the nervous system, briefly explained as a velocity-dependent increase in muscle tone in response to passive stretching [19], has been a topic of discussion for at least the past half-century. There are still many uncertainties about its pathophysiology and, therefore, about its treatment. Botulinum neurotoxin-A (BoNT) has been used as a possible therapy and, even though it has been proven effective in reducing spasticity [17], there is still an opportunity for optimisation of treatment by predicting the outcome [26]. A correct assessment of the neural and non-neural components of hyper-resistance is needed as BoNT only affects the former [13, 27]. Failing to do this might result in other causes of hyper-resistance being treated as spasticity or vice-versa [13, 28, 29].

Spasticity can be indirectly measured at the wrist using a motor-driven device, the NeuroFlexor (NF). This device measures neural and non-neural components of hyper-resistance, the former represents spasticity [30, 31] and the latter includes the viscous and elastic components [30]. The NF imposes an isokinetic passive motion and deconstructs the measured resistance in the various components of hyper-resistance (neural, elastic and viscous components) using a biomechanical model [30, 32–34]. The idea was introduced that relying solely on the NF might not provide a complete and accurate understanding of hyper-resistance, and thus, electromyography (EMG) analysis should also be considered [26].

To date, Andringa *et al.* studied the effect of BoNT on the spasticity of the wrist using the NF. No study has been published about the assessment of BoNT effects on wrist spasticity, using the short- and long-latency responses of EMG.

This dissertation uses data provided by Aukje Andringa, the main author of "The effect of botulinum toxin-A on neural and non-neural components of wrist hyper-resistance in adults with stroke or cerebral palsy" [26], in which EMG and NF data were recorded simultaneously.

1.2 Objectives

In this dissertation, surface electromyography (sEMG) data is compared against NF data, in order to assess the construct validity of EMG with respect to the NF. The change of EMG parameters, thought to represent changes in spasticity after injection with BoNT is assessed and the relation of change with the amount of BoNT dose is investigated. Finally, the added predictive value of EMG to NF measurements of the effect of BoNT on the spasticity of the wrist is explored.

2

Theoretical background

In this chapter, some important concepts for the understanding of this dissertation will be mentioned. First, an anatomical overview of some neural circuits and their constituents is revised so then the main focus of this study, "spasticity", can be introduced. Finally, EMG and how it can be processed is also explored.

2.1 Anatomical and physiological overview

2.1.1 Structures

Muscle spindles are sensory receptors that are encapsulated and found within the central portion, or belly, of skeletal muscles, parallel to them [7, 8]. Both the detection of absolute muscle length and changes in muscle length are done by the muscle spindles [8, 9]. Within each spindle, structures such as intrafusal fibres, sensory neuron endings and gamma motor neuron endings can be found [8].

Intrafusal fibres are composed of a central non-contractile part and polar contractile zones, and they stretch alongside the muscle [7, 8]. They can be of nuclear bag or nuclear chain types [7, 8]. Moreover, bag fibres can be of static or dynamic nature, whereas chain fibres are only static [8]. The difference between them is the disposition of spherical nuclei in their centre, the former has multiple nuclei, the latter a single row [8]. Additionally, the bag type is more elastic than the chain one, stretching at a more rapid pace [8].

The central part of every intrafusal fibre is wrapped around by group Ia sensory endings, this part being more elastic and therefore more susceptible to rapid stretching [7, 8]. These fibres are very sensitive to the velocity of stretch and variations in the length of the fibre [7]. Group II fibres will primarily wrap around a region near the central part of chain fibres, which is less elastic [8]. Thus, group Ia is more responsive to dynamic muscle length and group II to static muscle length [8]. Furthermore, group II fibres are slower afferents than group I ones [3, 35].

Motor neurons

Gamma motor neurons are efferent neurons that innervate both types of intrafusal fibres at the polar regions, altering the spindles' sensitivity [20, 36]. When activated, they provoke an enhanced response of the afferent fibres since they contribute to the contraction of the polar zones, which in turn extends both extremes of the central region, where the afferent fibres are wrapped around [7, 8]. These gamma motor neurons are activated by corticospinal pathways [7].

Gamma motor neurons can be found beside alpha motor neurons. Whereas the former innervate intrafusal fibres, the latter innervate extrafusal fibres, that compose the muscle [7]. Only the contraction of the extrafusal fibres virtually contributes to the force perpetrated by the muscle [7].

Interneurons are neurons completely located within the central nervous system (CNS) [20]. These neurons can receive input from afferent fibres and descending pathways and can propagate information to efferent fibres, in both cases of inhibitory or excitatory nature [7, 37, 38].

Golgi tendon organs (GTO) can be found within tendons, close to the border with the skeletal muscle. These GTOs connect to muscle fibres, and they stretch as the muscle is contracted and vice-versa [7, 8]. The composition of these organs consists of multiple entangled collagen fibres which are encapsulated [7]. Through the holes formed by the braided fibres, pass branches of a single Ib nerve, so when the GTO is stretched, these Ib fibre endings are compressed [7]. Therefore, a GTO is a sensory receptor very sensitive to muscle tension fluctuations [7, 9].

2.1.2 Stretch reflex

One of the main causes of spasticity is the exaggeration of the stretch reflex [12]. In order to better understand this mechanism, it is important to first comprehend how the stretch reflex functions in healthy people.

If a sensory stimulus results in an involuntary response, a reflex is present [20]. From a physiological point of view, the stimulus is perceived by a receptor, which will propagate an impulse through afferent nerves until the spinal cord or the brainstem. Consequently, the response will be lead through efferent nerves to the effector organ, which can be a muscle [20]. In more complex reflexes, there are interneurons synapsing with both afferent and efferent fibres [20].

In the case of the stretch reflex, also known as myotatic reflex [9, 20], the stimuli are the rapid lengthening of the muscles, when they are passively stretched [12, 20, 37]. The muscle spindles act as receptors since they stretch with the muscle [8, 39], being able to detect the changes in its length [12, 20]. This information is propagated via the Ia afferent fibres until it reaches efferent alpha motor neurons, conveying the response which leads to the contraction of the same muscle [12, 20] and synergistic muscles [8, 9]. Interneurons controlled by descending tracts can influence the activity of the presynaptic inhibition of the Ia afferents (see section 2.1.3) [40].

If the stretch reflex results in the contraction of a muscle that was stretched, the spindles in this muscle stop being activated and the muscle can relax again. By relaxing, if the muscle surpasses the desired length, spindles are reactivated. This way, the stretch reflex functions as a negative feedback loop [7].

When there are problems with the various components of the stretch reflex, such as the afferent fibres, the motor neurons or even the muscle itself, the stretch reflex can have

a lower activity or cease to exist [7]. The CNS could also be a cause of this diminished activity since it controls the excitability of this reflex. However, if the activity is increased, the origin is always the CNS and not the other constituents of the reflex pathway [7].

Stretch reflex only appears at very high velocities for healthy people but its amplitude, in EMG, grows linearly with velocity, even for slow ones, in spastic patients [12].

2.1.3 Spinal reflex pathways

There are several reflex pathways, some of them under the scope of post-synaptic inhibitory circuits. These include reciprocal Ia inhibition, recurrent inhibition and autogenetic Ib inhibition [9, 38]. A schematic of these circuits can be found in figure 2.1.

During the stretch reflex, the Ia afferent fibres also synapse with inhibitory interneurons that will send a response through efferent motor fibres so that the antagonist muscle relaxes to facilitate the contraction of the agonist. This sequence of events is known as (disynaptic) reciprocal Ia inhibition [7, 9, 38].

In recurrent inhibition, an alpha motor neuron will activate a Renshaw cell, which in turn will inhibit this same motor neuron from which it received the input [7, 38], and also motor neurons from the agonist and synergistic muscles [9]. This inhibitory pathway is a form of controlling motor neuron excitation, forming a negative feedback loop [7, 9]. Renshaw cells can additionally synapse with Ia inhibitory interneurons, in order to regulate the activation of antagonist motor neurons [7]. Renshaw cells are considered interneurons [7] and can be found within the spinal cord, more specifically in the ventral horn [9, 38]. These cells can additionally receive supraspinal inputs from descending pathways [7, 9].

Another spinal reflex pathway includes the autogenetic Ib inhibition, also known as non-reciprocal Ib inhibition [9, 38]. This inhibition occurs in the homonymous muscle: the activated afferent Ib fibres connected to the GTO will send an impulse to Ib inhibitory interneurons, which synapse with the motor neurons of the homonymous contracting muscle, controlling its contraction [7–9, 38], and also an excitation impulse to the antagonist's motor neurons, disynaptically via an interneuron [8]. These interneurons can also be activated from supraspinal input [9].

In presynaptic inhibition, there is a reduction of released transmitters in the synaptic cleft between the Ia afferent fibres and the motor neurons, which results in a smaller amplitude excitatory post-synaptic potential. The reduction comes after depolarisation caused by interneurons controlled by descending pathways [4, 9].

2.2 Spastic paresis and spasticity

2.2.1 Definition

Spastic paresis results from damage to the upper motor neurons, and thus is a feature of the upper motor neuron syndrome (UMNS), which can be caused by stroke and CP, among

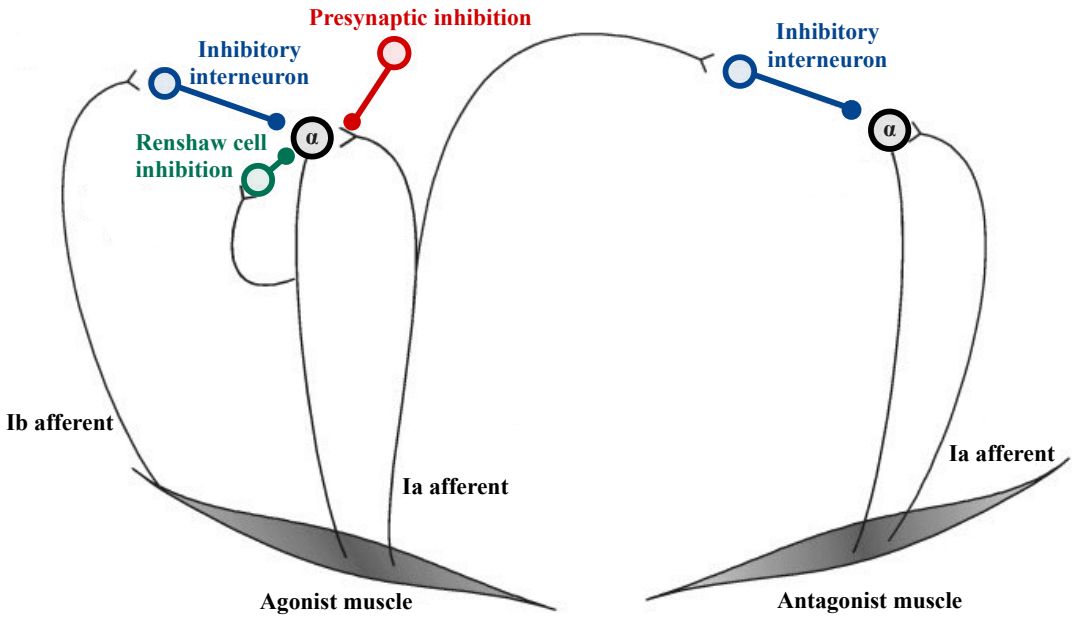

Figure 2.1: Spinal reflex pathways. Stretch reflex: the muscle spindles (not represented) in the agonist muscle activate Ia afferent fibres that synapse with α-motor neurons of the same muscle. Reciprocal Ia inhibition: the activated Ia fibres from the agonist muscle synapse with an inhibitory interneuron (blue), which synapses with the antagonist's α-motor neurons. Recurrent inhibition: a Renshaw cell (green) inhibits a motor neuron which also activates the Renshaw cell, creating a negative feedback loop. Autogenetic Ib inhibition: the Golgi tendon organs (not represented) activate Ib afferent fibres that synapse with an inhibitory interneuron (blue), which in turn synapses with α-motor neurons of the same muscle, leading to its contraction. Presynaptic inhibition (red): presynaptic inhibitory interneurons limit the transmitter release from the Ia afferent to the α-motor neuron. Adapted from [41].

others [42]. Even though spastic paresis and spasticity are often used interchangeably, spastic paresis entails multiple clinical features, one of which is spasticity [21]. Other features are spastic dystonia and alterations of muscle properties [21, 42].

Spasticity, as defined by Lance in [19], consists of "a motor disorder characterised by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyper-excitability of the stretch reflex, as one component of the upper motor neuron syndrome" [19]. This definition is possibly the most cited one, however it entails some problems since multiple other conditions fall under this same definition but have different pathophysiologies [38].

Spasticity is one form of hypertonia, which is of a neural nature [43] and is velocity-dependent [8, 44]. Not only is spasticity velocity-dependent but it is also dependent on the length of the muscle, with a higher level of spasticity being present when the flexor muscles of the upper limbs are longer [12]. The flexor muscles of the forearm are a prime area where spasticity is more commonly found [12]. The non-velocity-dependent component of hypertonia is caused by biomechanical changes in the muscle, such as the shortening

of muscle fibres when the muscle in contraction is immobilised, for a long period, due to weakness [4, 12, 15, 45].

2.2.2 Causes

Stroke can cause cortical lesions that lead to UMNS [21]. In recent years, the American Heart Association and the American Stroke Association published an updated definition of stroke, which is defined as an episode of neurological dysfunction characterised by symptoms that last more than 24 hours or result in death and are caused by a suppression of blood flow to a specific part of the brain, retina, or spinal cord, depriving said area of oxygen [3, 46, 47]. This suppression can be a consequence of vascular occlusion (ischaemic stroke), either thrombotic or embolic, or a consequence of a haemorrhage (haemorrhagic stroke) [3].

UMNS is caused by damage to the sensory pyramidal and parapyramidal networks [13, 48, 49]. The first effects are obvious immediately after the damage, and these are the negative features of the UMNS: loss of dexterity and some reflexes, weakness, hypotonia, among others [11–13]. Positive features can emerge later in time, and these encompass spasticity, spastic dystonia, co-contraction, tendon jerking and clonus [11, 12, 17]. Multiple of these signs can be present at the same time, sometimes contributing to the exacerbation of some conditions.

A descending tract, made up of upper motor neurons (UMNs), conveys impulses through the CNS [48]. There are three descending tracts that are known to participate in the altered stretch reflex activity, these being the reticulospinal (dorsal and medial), and the vestibulospinal [9, 12, 50]. The origin of the dorsal reticulospinal tract (RST) is the medullary reticular formation (RF) and for the medial RST is the pontine RF. The frontal cortex, specifically the motor area, sends information to control muscle tone via the medullary RF, therefore only the dorsal RST is influenced by this cortex [9, 51]. Changes in the spinal pathways can come from supraspinal alterations or from modifications in the spinal cord at a level lower than the lesion [38].

2.3 Electromyography

The contraction of a muscle, either actively generated or passively induced, involves the activation of the motor units producing a measurable potential that is recorded as the electrical activity of said muscle, using a technique called electromyography (EMG) [8]. The acquisition of EMG can be invasive, with needles in direct contact with the muscle fibres, or using electrodes that measure the signals at the skin surface [52]. This option is called surface electromyography (sEMG) and has a myriad of advantages compared to the needle EMG, it is more accessible due to the lack of invasiveness, and it uses more affordable electrodes. However, it also has some disadvantages, being more prone to measuring noise [52].

Normally, the analysis of an EMG signal is preceded by the treatment of the raw signal since these signals have a lot of noise and artefacts. The overall noise has multiple sources, either intrinsic or extrinsic [53]. The former consists of noise related to the contact between the skin and the electrodes, also known as electrochemical noise [53]. The electrical circuit responsible for the amplification can also cause a type of intrinsic noise, specifically thermal noise [53]. Regarding extrinsic noise, this has a power line component (50 Hz in Europe [52]) and another component derived from the movement of the cables [53]. During measurements, possible movement by the person or motion of the cables will cause artefacts in the signal. While the person is moving, the impedance of the interface between the skin and the electrode will fluctuate, resulting in artefacts that can go up to 20 Hz. The movement of the cables can lead to artefacts of 50 Hz or less [52].

The two types of intrinsic noise make up the baseline noise, which is detectable as the electrode contacts with the skin and the muscle is not contracted [52, 53]. However, there are other forms of noise detected while the muscle is at rest, which in addition to baseline noise, form the background noise. Interference originated by physiological activity, such as an electrocardiogram or cross talk from other muscles can partake in this background noise [52].

sEMG signals typically comprise amplitudes up to 2 mV [54] and frequencies from 10 to 500 Hz, above that value being considered noise [52]. The white Gaussian noise shares the same characteristics as this noise, so a low-pass filter with a cutoff frequency close to 500 Hz is recommended to eliminate it [52, 55]. This cutoff value can be as low as 350 Hz since higher frequencies do not have a high amplitude in an sEMG power spectrum [52, 54].

Regarding the other sources of noise, a high-pass filter with a cutoff value of at least 20 Hz is recommended [55]. Depending on the type of acquisition, this value could change [52, 53]. A frequency of 20 Hz is the recommended value for analysis of isometric contractions or normal movements and should be increased if the signals are measured in people performing high-velocity movements or with motor disorders, such as spasticity [53]. In summary, sEMG signals should be band-pass filtered, with the lowest value being at least 20 Hz and the highest value between 350 to 500 Hz.

To be able to retrieve the envelope of a signal, a full wave rectification should be performed [55]. This procedure transforms all the negative values into their respective absolute value.

3

Literature review

This chapter gathers state-of-the-art information about various topics surrounding spasticity: pathophysiology, prevalence, common measuring techniques and treatments. These matters are still surrounded by some uncertainty, and have been, and will continue to be a target of change and adaption over the years.

3.1 Pathophysiology of spasticity

In chapter 2.2.2, it was briefly mentioned that there are specific tracts that partake in the source of spasticity. The corticospinal tract (CST), one of the pyramidal tracts [15], is not known to be related to spasticity but rather to other problems such as hypotonia and weakness [9, 12, 50, 51]. This tract descends from the cerebral cortex, whereas the RST and the vestibulospinal tract (VST) descend from the brainstem [51]. The RST has an influence on alpha and gamma motor neurons, affecting reflexes, muscle tone and movement of voluntary nature [20]. Whereas the dorsal RST is stimulated by the motor cortex through corticoreticular projections [25] and has an inhibitory effect, the medial one is not and has an excitatory effect [7, 9, 12, 15, 50, 51]. The VST also has an excitatory effect, specifically on extensor motor neurons to control muscle tone and maintain posture [9, 12, 20, 50, 51]. The excitatory effect originating from the VST is not as important as the one from the medial RST [9].

These tracts work together to maintain a balanced muscle tone activity [9, 15, 51]. When there is a lesion at the cortical level, the facilitatory input to the medullary RF can diminish and with it the inhibiting information carried by the dorsal RST [25]. Only the excitatory remains, through the medial RST [9, 43, 51]. The lesion can occur directly in the brain stem where the medullary RF is located in [13]. In case the lesion is at the spinal level, if it is incomplete then the dorsal RST loses its effect, happening the same as with the cortical lesion. In both situations, there is high excitability of the circuits involving the stretch reflex, which ultimately results in spasticity [9, 15, 43]. In humans, impairments to the RST appear to be more related to spasticity than the VST [51]. The exact contribution of each descending tract to the spasticity of the wrist is not well known, and the RST is not believed to be of importance since there is no anatomical projection of this tract in the distal part of the upper limb. However, Li in [25] mentions a finding of RST importance in distal muscles of the limbs, complementing its importance in proximal muscles.

The first signs of spasticity, which result from the aforementioned unbalance, usually happen one to six weeks after the onset of the lesion [25, 51]. The fact that they do not

appear right after the lesion is an argument for neuronal plastic rearrangements [15]. Damaged descending fibres can leave gaps that are occupied by new interneuron endings that synapse with motor neurons [4]. This sprouting occurs at the segmental spinal levels [4, 25]. Additionally, plastic changes can include sprouting of undamaged descending tracts [4]. There is also the possibility of unaffected fibres from the CST branching out [4]. Overall, new excitatory synapses are created, which previously were inhibitory, by the sprouting of afferent fibres [15].

It has been proved that hyper-activity of the muscle spindles is not a cause of the exaggerated stretch reflex, as it was once thought to be [9, 12, 13, 38, 45].

A reduction in the reciprocal Ia inhibition, due to damaged descending fibres that control the interneurons, will see an increase in the stretch reflex and is, therefore, one cause of spasticity [9, 38]. Altered recurrent inhibition is not thought to be related to spasticity [38, 45]. Regarding Ib afferents, there could also be a relationship between their excitation and inhibition and spasticity [38, 45]. Non-reciprocal Ib inhibition was not demonstrated in hemiplegic patients in comparison to healthy ones, some of the former even showing some facilitation. This could be a sign of causality of spasticity [9]. Depression in post-synaptic activation is thought to be related to spasticity during rest and also to its degree [45]. Patients with hemiplegia demonstrated reduced presynaptic inhibition in the upper limb, specifically at the flexor carpi radialis (FCR) muscle [12, 56].

It is possible to conclude that the origin of spasticity depends on the origin of the lesion, which will vary from patient to patient. It is believed that spasticity is caused by the interaction of multiple of these paths rather than by just one [9].

3.2 Spasticity epidemiology

Data on post-stroke spasticity (PSS) varies from study to study, probably due to the different types of considered populations. Spasticity as a cause of stroke is more prevalent in the chronic phase (up to 42.6%) than in the acute phase (up to 27%) [23]. Since stroke is such a common disease, the amount of people with PSS is tremendous. In patients with spinal cord injury, at the time of discharge from the hospital, Holtz *et al.* found that 65% of patients presented some kind of spastic characteristics, but only 35% were problematic. When analysed one, two and five years after discharge, interference by spasticity was positive in 27%, 24% and 20% of patients, respectively [24]. Patients with cerebral palsy (CP) are born with CNS lesions [8]. The prevalence of CP has been declining and a recent 2022 study by McIntyre *et al.* reports a new number of 1.5 for every 1000 live births [57]. When the lesions happen in the cortex and the CST, which accounts for up to 60% of CP cases, they have spastic CP [8]. The number of spastic CP patients is not as large as PSS patients, but it still shows that many spastic patients' origin is CP.

3.3 Spasticity measurement

Spasticity has to be correctly measured in order to provide the best-tailored treatment or therapy [28, 29]. A biomechanical assessment is the most frequently used way to measure spasticity, either with a motorised velocity and torque-controlled quantification of resistance, a manual quantification or the use of clinical scales [58]. Whereas the previous methods attempted an indirect evaluation of spasticity, other studies followed neurophysiological methods, using EMG, which lie on the direct measurement [58]. Indirect methods are flawed and a simultaneous measurement of muscle activity should be employed for the quantification of spasticity [2, 17, 58].

The use of robots to evaluate spasticity is uncommon, although it might be a useful tool [29]. These instruments, with the help of sensors, are able to measure individual feedback, as they apply perturbations and forces [11, 29]. They function in association with neuromechanical models, which contribute by differentiating the neural and non-neural components [31]. Most previous investigations that employed robotic equipment did so for the first time, addressing their validity and reliability; thus, there is still a lack of support for their diagnostic usage [29].

Trials of static nature, in which there is no stretching action and spasticity is evaluated by ultrasonography or EMG with electrical stimulation, are also uncommon. These options, even if clinically valid, lack specificity and repeatability [2].

Outcome-wise, most studies retrieve force/torque data, followed by EMG data and the angle at which there is a sudden increase in resistance to the passive movement (catch angle) [2]. Studies that exerted a controlled extension used kinematic measurements in conjunction with robotics [2]. In opposition, manual extension relied predominantly on muscle activity measurements, since there is no direct provision of torque or force [2]. A vast number of studies have demonstrated that the use of torque, force or work, along the stretching movement, is a good option, with favourable psychometric properties [2].

Incorporating both diagnosis and therapy capabilities in the same device has recently attracted curiosity. In this way, a single machine would measure the spasticity level prior to treatment and enforce a procedure tailored to that level, resulting in cost savings [11].

It is worth pointing out that, even though the manually-assessed spasticity systems have several limitations and disadvantages, they are also less costly and time-consuming, making them easier to employ clinically [2]. These advantages, however, do not seem sufficient to favour their sole continued use.

Overall, an instrumented assessment of spasticity seems to be more advantageous than a clinical measurement [11, 29]. Instrumentation allows for standardisation on speed and applied pre-load [29].

3.3.1 Clinical scales for spasticity

In a clinical setting, spasticity is seen as any form of hyper-resistance against passive motion. There are scales used to measure spasticity, two of the most commonly used ones are the modified Ashworth scale (MAS) and the Tardieu scale (TS) [17, 28, 59, 60]. In table 3.1, a score overview of these two clinical scales can be observed.

3.3.1.1 Ashworth and modified Ashworth scales

The MAS is a subjective assessment based on the clinician's expertise and perception [11, 29, 61]. The MAS is an ordinal scale [62], with values ranging from 0 to 4, with 0 reflecting normal muscle tone and 4 signifying limb stiffness when passively moving the limb (table 3.1) [63]. With the transition from the Ashworth scale to the MAS, level 1+ was introduced between levels 1 and 2 [63].

Table 3.1: Modified Ashworth scale (MAS) and Tardieu scale (TS) [17, 64].

Score	MAS	TS
0	No increase in muscle tone	No muscle reaction
1	Small increase in tone, with a catch and release at the end of the ROM	Weak resistance
1+	Small increase in tone, with persistent weakness during the rest of the ROM after the catch	N/A
2	Considerable increase in tone during most of the ROM, still easily movable	Clear catch followed by release
3	Considerable increase in tone, difficultly movable	Fatiguing clonus (<10 s)
4	Rigidity while flexing or extending	Non-fatiguing clonus (>10 s)

ROM, range of motion.

To be valid, the MAS must properly measure the construct it is intended to test, which is spasticity [63]. This validity is difficult to achieve because the increase in resistance should only be associated with an over-activity of the stretch reflex [62], but it is known that other signs of the UMNS can contribute to the clinician's perception of resistance increase [62]. The clinician alone cannot distinguish between the various possible causes of this hyper-resistance [13]. It also has low inter-rater reliability and does not account for the dependency on velocity [38, 60]. Therefore, the MAS is not a tool capable of distinguishing between spasticity and other sources of resistance to passive motion and should be carefully used [13, 65].

3.3.1.2 Tardieu scale

Measuring spasticity with the TS requires the muscle to be fully relaxed when the passive lengthening is applied [64]. This scale accounts for two aspects 1) quality of muscle reaction and 2) angle of muscle reaction at different velocities of stretch [17]. The first aspect consists of a five-level ordinal scale, from 0 to 4, where 0 means there is no muscle

reaction and 4 represents a persistent clonus for more than 10 seconds (table 3.1) [17, 64]. The second aspect consists of moving the limb of interest at three different velocities: a very slow speed (V1), a velocity at which the limb would fall under gravitational force (V2) and a very fast speed (V3). At each of these velocities, an angle is taken when a reaction from the muscle is felt [28, 60]. Some studies opt to leave out the measurement of V2 and use only V1 and V3 [28].

For V1, since the velocity is too slow to elicit any abnormal stretch reflex response, the muscle reaction is considered the final angle of the passive range of motion (PROM) [28]. During V3, the quality of muscle reaction is measured and the angle is taken when resistance is felt [64]. A measure of spasticity is assumed as the difference in angle between V3 and V1 [64].

This scale becomes more advantageous in relation to the MAS because it considers the velocity-dependency of spasticity [17] and it is able to separate spasticity from other sources of hyper-resistance, being a more valid alternative [28].

3.3.2 The NeuroFlexor

The NeuroFlexor (NF) (Aggero MedTechAB, Solna, Sweden) is a motor-driven device that imposes an isokinetic passive motion, at the wrist joint. It has an incorporated force transducer and it is able to extract the neural, elastic and viscous components of the perceived resistance, with the help of a biomechanical model [26, 31, 33, 66]. The NF operates unidirectionally in the sagittal plane at a constant, but modifiable velocity [33, 34, 66]. A more detailed explanation of the use of the NF is done in chapters 4.2 and 4.5.2.

Both the machine and the model have been validated and used in several studies regarding spasticity measurement [30–34, 66, 67]. The NF measurements are valid [30, 31, 33], standardised [32], reliable [31, 33, 66, 67] and change-sensitive [34, 66], allowing clinical practicality [33, 34, 66].

Early studies on NF presented this machine as an advantageous alternative to other spasticity assessments, in part due to it being able to separate neural and non-neural components and without the use of EMG data [30, 33].

3.3.3 EMG latency responses

Surface and regular EMG have also been used to measure the neural component of hyper-resistance [58, 68]. A sudden rapid stretch of a muscle elicits a stretch reflex, of monosynaptic nature [7, 8, 69–71], which causes a response in an EMG signal. The resulting peak, commonly denominated as M1 or short latency response, is normally followed by another peak, the latter being called M2 or medium/long latency response, depending on the author [8, 35, 69, 72]. The M1 response is known to depend on the velocity of the stretch [70]. Whereas there is an overall agreement for the monosynaptic stretch reflex to be the origin of the M1 [71, 73], the M2's origin is still uncertain.

The M2 response can be elicited by different pathways, depending on the muscle, or even have differences within a single muscle, in case the stimulation is not the same [69]. For distal muscles of the upper limb, which is the case for finger and wrist muscles, the motor cortex is believed to participate in the pathway that mediates the M2 response [7, 36, 70, 71, 74]. Even though the slower group II afferents are not believed to contribute to the stretch duration dependency of the M2, they are thought to participate in its elicitation [70]. The M2 response could also be task-related, with a higher activity associated with a deliberate effort to resist movement [71, 75]. Multiple authors have proposed some hypotheses [35, 76], and the activation of group II afferent fibres seems to be frequently suggested [35, 69, 72, 77], since their transmission speeds match the appearance of the M2 for upper limb muscles [36]. However, there are inconsistencies in the results from these studies [37]. Some authors argue that Ia afferents may have a role in the origin of the M2 and that the group II afferents do not act alone in this process [70, 71, 78]. Schuurmans *et al.* even suggest that the group II afferents are not the main contributor [71], as other authors had done. Group II afferent fibres might not be activated in situations when the stretch is of small amplitude, failing to elicit the M2 response in some cases [69]. Moreover, the modulation of the M2 response is probably independent of the M1's one [71].

After analysing the timing of both M1 and M2 responses for a distal and a proximal muscle of the upper limb, the trans-cortical hypothesis with the participation of the motor cortex seems to be more reasonable than the signals transversing a similar pathway of the M1 response, but through slower afferents (group II) [36]. The presence of cortical structures for the involvement in eliciting of the M2 response is also supported by studies with patients suffering from Klippel-Feil syndrome [36].

M2 response eliciting cannot be attributed to a single circuit of spinal or supraspinal nature. Multiple factors already mentioned, and some not discussed here, contribute to the presence of the M2 [36, 37, 70, 75]. It is still not completely clear the contribution of each factor [36].

3.3.3.1 Alterations with spasticity

In a spastic patient, some characteristics of the M1 and M2 waves might exhibit alterations. For instance, M1's amplitude tends to be exaggerated, whereas the one from M2 can decrease to a point where it disappears [35, 69]. One possible cause of the increase in the M1 response is the depression of presynaptic inhibition [79]. Regarding the M2, Cody *et al.* present motor neuron refractoriness as a contributing factor for the decrease in its amplitude [35].

Taking this into consideration, EMG can be used as a technique able to retrieve outcomes representative of spasticity, thus probably a better assessment of the efficacy of BoNT treatment [58]. The M1 response taken as a representation of the stretch reflex, can validly represent spasticity (increase stretch reflex) by having a larger amplitude than normal [80].

3.4 Treatment of spasticity

The decision to the treatment of spasticity relies on the necessity perceived by the affected person and the physicians that accompany their situation [17]. In some cases, spasticity might not hamper any voluntary movement or could even help in the locomotion of some patients [38, 45]. If spasticity does not have a high contribution to a patient's disability, then treatment could be discouraged [45].

Nevertheless, spasticity is a limiting condition for most people who have it. It is mostly present in the upper limb [81], which can affect the precise coordination needed by some motor activities, interfering with daily tasks and hindering the work capacity of some people [43, 50]. Furthermore, it can be an inconvenience to more people as some patients need the help of caregivers [50]. Spasticity coupled with weakness can exacerbate the overall condition of the patient, creating additional problems [43, 58, 68, 82].

Some medication for the treatment of spasticity also exists, such as baclofen, tizanidine, dantrolene and benzodiazepine [13, 17, 70, 71]. Some of these drugs can have negative side effects in patients affected by stroke and their efficacy might depend on the location of the damage [13, 17]. The adherence to this option is not as high as with other options, since there are not many reliable studies reporting on their efficacy [17]. Nevertheless, these medications may be a more affordable choice [17]. Additionally, these drugs are a general treatment for spasticity while other options treat spasticity locally [13].

Some types of surgery could be a treatment option. If hyper-resistance is of neural cause (spastic hypertonia), then neurosurgery is more advised. A non-neural cause, where the aforementioned drugs have barely any effect, could be treated with orthopaedic surgery [13, 27].

3.4.1 Botulinum neurotoxin A

When spasticity can be treated locally, the generally utilised method is the injection of botulinum neurotoxin-A (BoNT) [13, 48, 83, 84]. This method has been shown to be effective in reducing pain, involuntary movement and the perceived resistance at the wrist, therefore improving the PROM [16, 27]. BoNT is a biological toxin from the pathogen *Clostridium botulinum*, whose name comes from the Latin word for sausage, *botulus*, since the bacteria are normally associated with infected sausages [85]. It was initially used to treat strabismus and various forms of spasms and, more recently, motor disorders [85].

In a neuromuscular synapse, BoNT acts as a presynaptic inhibitor of the release of acetylcholine [13, 34, 65, 85, 86], possibly resulting in diminished hypertonia, temporary paralysis and reduction of pain [13, 17, 83, 85–87]. Stretching of the affected muscle(s) becomes easier. These effects normally last 12 weeks, before a new injection should be given [83, 86].

Even though some authors report an improvement in hand function after the application of BoNT [51], a recent meta-study conducted by Andringa *et al.* in [27] suggests that

arm-hand capacity sees no improvement with BoNT injections.

The American Academy of Neurology considered the onaBoNT-A and the incoBoNT-A to be of level A of effectiveness, the highest level, for the treatment of upper limb spasticity, in the 2016 guidelines [85]. Not only is muscle tone reduced but there is also an increase in the PROM [17] and a decrease of the MAS scores of the wrist [27]. The effectiveness of the use of any type of BoNT also depends, of course, on the injected muscles and specific injection site, and the amount of dose given [17, 85]. BoNT has been used for several decades, however some questions about its application still persist, such as what the initial amount injected should be and what the best technique for the application is [85].

A wrong diagnosis of spasticity as the cause of hyper-resistance can lead to a person being treated with BoNT when they should have undergone a different treatment. In this case, since the cause of hyper-resistance was non-neural, BoNT will not have any effect and the person will be considered non-responsive to the toxin [13]. A non-response to this toxin can also happen if the injected person has already undergone previous injections, which could be a reason for non-responsiveness [85].

Some limitations of the use of BoNT encompass its high cost, the discomfort felt during the injection and the relatively short actuation period [85]. Additionally, since it only works locally, it cannot be used generally [13].

4

Methodology

In this chapter, the population of this study is presented, as well as the measurement procedure for both NF and EMG data. The model behind the NF is briefly explained and the analysis of EMG data is presented. Finally, every step employed in the statistical analysis is discussed. Hereinafter, the term EMG concerns surface EMG.

4.1 Subjects

Inclusion criteria for this study were: 1) stroke at least 3 months prior to screening or diagnosis of cerebral palsy (CP), 2) eligibility for BoNT treatment in the wrist or finger flexor muscles, or both, evaluated by a rehabilitation physician, 3) 18 years or older and 4) ability to follow test instructions. Exclusion criteria were: 1) inability to passively extend the wrist, while extending the fingers, more than 0^{o} and 2) other disorders that could influence hyper-resistance at the wrist.

The study was conducted within the framework of standard medical care, resulting in the exemption of medical ethical certification by the Medical Ethics Committee of the Vrije Universiteit medical center (VUmc) in Amsterdam, The Netherlands (2017.440). Additionally, every participant provided written consent, as required by the Declaration of Helsinki.

Patients with stroke or CP eligible for the study underwent BoNT treatments between January 2018 and June 2019. Treatments were done at the outpatient rehabilitation department of a hospital, the Spaarne Gasthuis, in Hoofddorp, The Netherlands.

This study follows a previous prospective cohort study in patients before and after BoNT treatment, assessed by both the NF and EMG [26].

Patients were evaluated three times in this prospective clinical cohort study: before the intervention (just prior to BoNT treatment for spasticity of the wrist joint), and at 6 and 12 weeks after the intervention. All measurements were carried out by trained physiotherapists or occupational therapists who were blinded for BoNT treatment dosage. Except for one patient, who received incobotulinum toxin-A (Xeomin, Merz Pharmaceuticals GmbH, Frankfurt, Germany), the patients received intramuscular injections of onabotulinum toxin-A (Botox, Allergan, Irvine, CA, USA). Only one doctor (W.P.) managed the injections, with the help of ultrasound guidance.

An individualised assessment of dosage, chosen muscles and exact local for injection was performed by the treating rehabilitation physician, so to achieve the best treatment

results. The treating physician was blinded to the outcomes of previous sessions, including clinical and NF measurements.

4.2 Experimental protocol

Prior to any trials, the PROM of each patient was measured, using a goniometer. EMG signals were acquired in parallel with NF data. The starting position of the NF was -20º, in flexion, and the motion ended at 30º, in extension. The patient was seated comfortably with the NF by their side. The elbow was flexed by 90º and the shoulder was abducted by 45º, with no flexion. Additionally, the forearm was pronated and in direct contact with the NF base. The patient's arm was positioned in the platform in order to have the wrist and the rotation axis of the NF aligned. Finally, the patient was strapped to the machine by the fingers, using velcro straps (figure 4.1). The patient was asked to remain relaxed during the whole procedure and to not create any resistance to the active motion of the NF.

Each measuring session started with a recording of approximately 15 seconds of rest-activity, followed by 5 slow trials (5º/s) and 10 fast trials (236º/s). The slow and fast trials were also separated by 15 seconds.

Figure 4.1: The NeuroFlexor with forearm and hand positioning [32].

4.3 EMG acquisition

EMG signals were acquired by a Mobi (Twente Medical Systems International B.V., Oldenzaal, The Netherlands), an electrophysiological signal acquisition device with bipolar inputs that can be connected to electrode leads applied to the patient. This device can have up to six channels, with a maximum sampling frequency of 2048 Hz. The leads were positioned at the forearm, one on the flexor side and the other on the extensor side, to measure the activity of the flexor carpi radialis (FCR) and the extensor carpi radialis (ECR), respectively. A ground electrode was placed at the mastoid process on the unaffected side.

4.4 EMG processing

Figure 4.2 shows six panels with an overview of the processing steps of the EMG signals for both muscles of one of the patients. Using MATLAB version R2021a (The MathWorks, Inc., Natick, Massachusetts, United States), EMG signals were 3rd-order band-pass filtered with cutoff frequencies of 30 and 450 Hz. After that, the signals were normalised against baseline background activity, using a time period before the start of any contraction in any signal, from the 10^{th} to the 17^{th} second. Signals were full-wave rectified and low pass filtered by a 3rd-order Butterworth filter with a cutoff frequency of 10 Hz to get the linear envelope. Finally, signals were normalised to the maximum value of all signals of each person (both muscles and every session).

EMG data for both FCR and ECR were acquired simultaneously with data from the NF triggers and Mobi triggers. The goal was to use the NF triggers in order to know where each movement's onset took place and then retrieve the M1 and M2 waves. Ideally, the triggers would give us the exact moment when the contraction is initiated. This was verified by visual inspection in most of the measuring sessions, however, in some cases, the trigger appeared moments after the start of the contraction, making the usage of the triggers as onset points unviable. Nevertheless, the triggers still provided useful information since we knew, once again by visual inspection, that the onset of the contraction would be found within a one-second interval before and after the NF trigger point. This way, for each session, a window was defined for every trigger. In each window, a threshold method was applied to identify the onset of the M1 wave. The threshold, T, was defined following the expression

$$T = \mu + h \cdot \sigma$$

where μ is the mean of the activity at rest, h is the threshold level and σ is the standard deviation of the activity at rest. The value for h was defined empirically and based on some studies which applied the same method to EMG signals [88, 89]. In the present study, h was equal to 5. For some files, where there were problems in triggers acquisition, the M1 onset was detected visually. In the end, the waves were averaged to obtain the mean M1 and M2 waves.

4.5 Outcome data

4.5.1 Clinical data

A therapist measured the resistance to passive movement, in wrist flexor muscles and finger flexor muscles, using the MAS. The TS was also used, where the quality of muscle response at a fast speed was measured as TS_Q. The $TS_{R2\text{-}R1}$, corresponding to the difference between the range of motion at a very slow velocity (R2) and the catch angle for a very fast velocity (R1), was also taken.

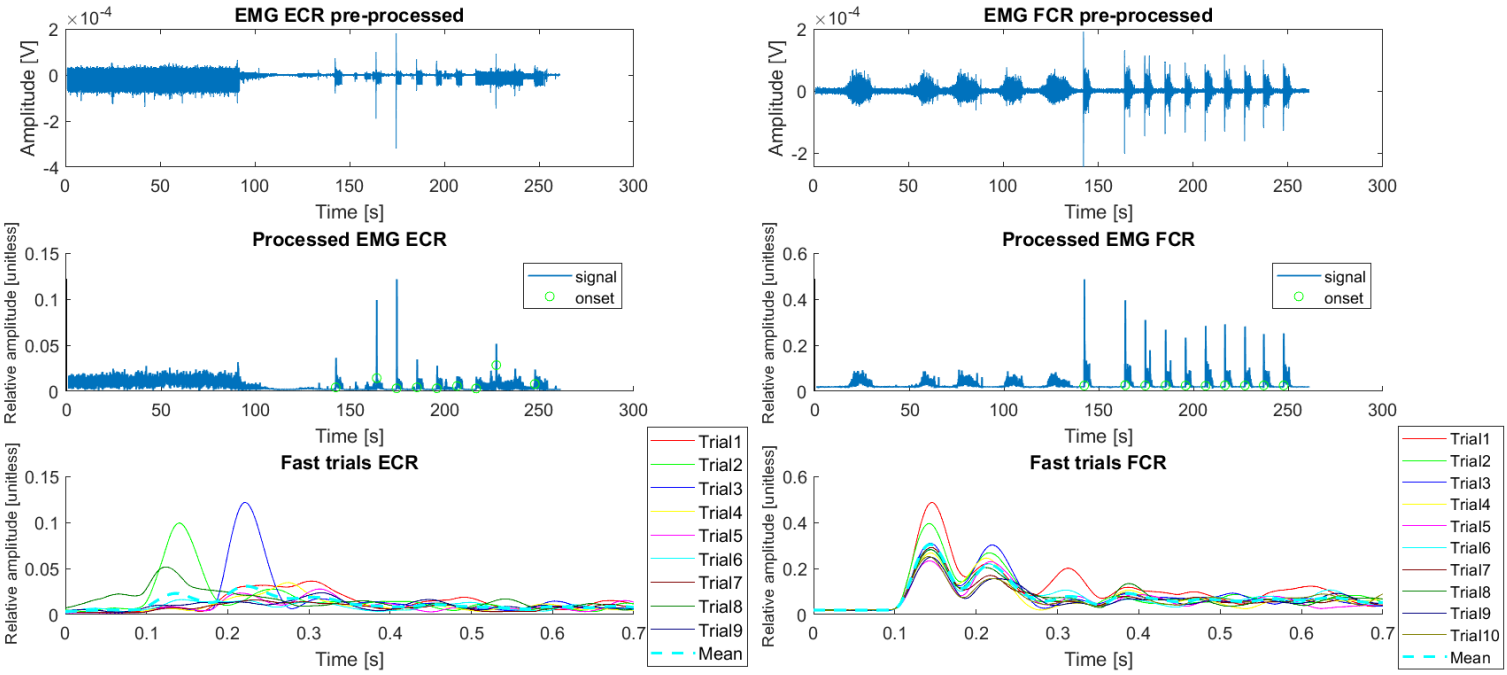

Figure 4.2: Example of pre-processed and processed EMG signals.

4.5.2 NeuroFlexor data

Data from the NF is retrieved by the use of a biomechanical model, which is able to decompose the resistance measured by the device in its multiple components. In Appendix A, a series of images that provide a visual guide to using this model can be found. As soon as the device finishes the fast movement, the resisting force is measured and the elastic and viscous components are removed, ultimately resulting in the neural component (NC), in newtons (N), which represents spasticity. The elastic component (EC) is measured one second after the slow movement ends. The viscous component (VC) is 20% of the difference between the total force measured approximately 15 ms after the fast movement onset (F_{total}) and the inertia component. This results in the following expression

$$VC = 0.2 \cdot (F_{total} - m \cdot a)$$

where m is the mass of the hand and a its acceleration (21 m/s^2). The mass of the hand is defined as being 0.6% of the total weight of the person [26, 30, 32–34, 66, 67].

4.5.3 EMG data

Figure 4.3 shows an example of the retrieving of outcomes for one session of one of the patients. From the processed EMG signals, the area under the curve (AUC) and the peaks of both M1 and M2 waves (AUC M1, AUC M2, peak M1, peak M2) were used as outcome parameters. All signals were averaged to find the average abscissa of the peak of each

wave. Then, for each signal, local maxima and minima and their respective abscissas were found, using the in-built MATLAB functions `islocalmin` and `islocalmax`, respectively. The abscissa of the peak for both waves of each signal corresponded to the abscissa of the local maximum closer to the average value previously found. These steps allowed for the peak of each wave to be found and their abscissa was then used to determine the onset and offset of each wave. The onset and offset of the M1 wave corresponded to the abscissa of the local minimum right before and after the abscissa of the M1 peak, respectively. For the M2 wave, the onset was the same as the offset of the M1 wave and the offset was the abscissa of the local minimum right after the abscissa of the M2 peak.

After this was done for every signal, a visual inspection was performed and, for a few cases, different points were chosen. Finally, the AUC for each wave was calculated using trapezoidal numerical integration, employing the in-built function `trapz`.

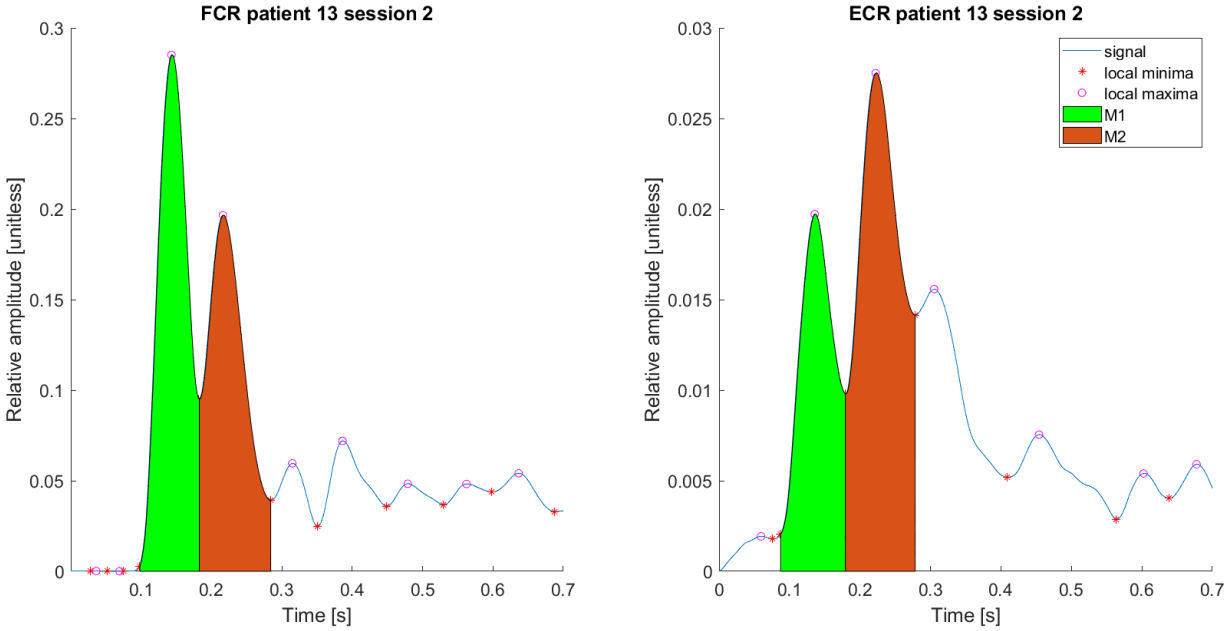

Figure 4.3: Example of detection of the M waves.

4.6 Statistical analysis

The normality of every EMG parameter was investigated, by Shapiro-Wilk tests and by histograms and Q-Q plots.

4.6.1 EMG validity

EMG validity was first studied, comparing the outcomes from EMG with outcomes from the NF and the clinical scales. The correlation between the changes in values of each EMG-related parameter and the NF parameters, and between EMG parameters and the clinical scales was performed. These values were calculated for both the numerical change

within sessions 1 and 0, and 2 and 1. Spearman's correlation coefficients were selected since most of the data were not normally distributed.

4.6.2 Effect of BoNT

Face validity of EMG measurements was assessed by plotting a graph showing the trend of change, between sessions, for every patient and for the median value, for each parameter of both ECR and FCR. Spearman's correlation coefficients were computed between the decrease of EMG-related parameters of the FCR, between sessions 1 and 0, and the dose of BoNT, using a 1-tailed test of significance since a higher dose of BoNT was expected to decrease the EMG-related parameters measured at the two sessions. For the ECR, a 2-tailed test of significance was preferred. The effect of BoNT is most prominent right after being injected into the muscles, so for this part of the analysis, only the decrease of the parameters between sessions 1 and 0 was considered.

This part of the study was meant to investigate whether the use of BoNT reduced any of the EMG-related parameters. To analyse the effect of BoNT, parameter change over time of both M1 and M2 waves was assessed by a linear mixed effects model (LMEM). This model was chosen instead of other options because the data was non-normally distributed and there were missing data. For every parameter, the model was run four times with the variable "session" as a fixed effect, every time with a different repeated covariance type: auto-regressive, compound symmetry, unstructured and toeplitz. The Akaike Information Criterion (AIC) was used to evaluate model performance across different covariance types in each iteration, and the choice of the model was made based on the one with the lowest AIC. The unstructured covariance type was the chosen one. Post-hoc analysis was done in order to see the differences between each session. The LMEM assumes a normal distribution of the residuals, which was proved following the statistical analysis.

4.6.3 Added value of EMG

The aim was to determine whether incorporating EMG measurements, in addition to using the NF and clinical scales, could enhance the ability to classify patients as responders or non-responders to BoNT treatment.

Taking the various clinical scales used in this study, the study population was divided into two groups: responders and non-responders to BoNT. Only scales which allowed for an almost equal division of groups were considered. For this part of the analysis, only the FCR was taken into account.

Using the MAS, a responder was defined as a patient whose MAS value in the wrist had a decline more negative than -0.5, between sessions 1 and 0. If the value was also -0.5 for both the MAS of the wrist and of the fingers, then the patient would also be considered a responder. Using the TS, if the change of scores between sessions 1 and 0 was negative, then the patient was considered a responder. For the PROM of the wrist, if the variation

of the values between sessions 1 and 0 was larger than 10^{o}, the patient was regarded as a responder.

Binary logistic regressions were performed for the three aforementioned situations, with the combination of predictors that can be seen in section 5.3.1. The model was evaluated based on parameters such as -2 Log-likelihood, accuracy, precision and sensitivity. For the -2 Log-likelihood, the smaller the value, the better the model. For the other evaluation parameters, higher values translate into a better model. If a model that included the EMG parameters resulted in a better outcome than the models which did not include them, then it would be possible to conclude that the inclusion of EMG was beneficial to determine if a patient was or not a responder to BoNT.

A significance value of 0.05 was taken for every analysis. Correlation values below 0.400 are considered weak. Higher than 0.400 and lower than 0.599 are seen as moderate. From 0.600 up to 0.799 are strong and above 0.800 correlation coefficients are very strong. Statistical testing was done using SPSS Statistics for Windows, version 29.0.0.0 (IBM Corp., Armonk, NY, USA).

4.6.4 Hypotheses

Based on previous studies [30, 31, 90, 91], the construct validity of EMG with respect to the NF is expected to be achieved. The EMG-related parameters of the FCR are predicted to have a good to excellent correlation with the NC of the NF, and a low correlation with the other components of the NF, due to representing different constructs.

It is unlikely that the construct validity of EMG with respect to the MAS will be achieved, as it has been proven that the MAS does not provide a good outcome for spasticity. The correlation coefficients between EMG-related parameters and these scales are not expected to be significant. However, a decrease in EMG-related parameters should also be accompanied by a decrease in MAS scores.

The effect of BoNT is the reduction of spasticity. Therefore, EMG-related parameters are expected to decrease at least in the FCR as a result of BoNT, since the injections of BoNT were administered in this muscle or surrounding ones. The effect of the BoNT in spasticity should be stronger in the first weeks after its application, so a bigger change in the EMG parameters' values is expected between session 1 and session 0, and probably a lesser decrease between the last two sessions, as the effect of BoNT is less pronounced. It is likely that a higher dose of BoNT would result in a bigger difference between sessions of the EMG-related parameters, at least for the M1 response. Thus, a strong correlation (with a correlation coefficient higher than 0.6) is predicted between the EMG-related parameters of the FCR and the dose of BoNT. Regarding the parameters of the ECR, no significant decreases after BoNT injections are anticipated.

Because EMG can directly quantify spasticity, the -2 log-likelihood is expected to be smaller and the accuracy, precision and sensitivity higher in prediction models of responsiveness to BoNT in which EMG parameters are included.

5

Results

In this chapter, the results retrieved from the statistical analysis mentioned in the previous chapter are reported. Sessions 0, 1 and 2 correspond to baseline, 6-week follow-up and 12-week follow-up, respectively.

5.1 Baseline characteristics of the patients

Figure B.1 contains a flowchart explaining every step of patient recruitment and data acquisition. Out of the 213 patients scheduled for a BoNT treatment, only 28 were deemed eligible. From these, 9 patients decided not to get the injections and another patient failed one inclusion criterion after the screening procedure. In the end, a total of 18 participants, 15 with chronic stroke and 3 with CP, were included in this study. At baseline, the EMG outcomes were not available in 3 patients for the FCR, and in 2 patients for the ECR. There was a loss to follow-up after week 6, with 2 patients not being included in the last measuring session. Outcome data from session 1 (6-week follow-up) was not retrieved from 3 of the patients. After session 2 (12-week follow-up), EMG parameters were only available for 13 of the patients.

Table 5.1 summarises the characteristics of the study population at baseline. Table B.1 contains information about the muscles injected and the respective median number of units. Table B.2 shows individual data for each patient. The mean age of the population was approximately 58 years old, and the number of men was 1.5 times larger than that of women. On average, stroke patients had a time post-onset of approximately seven years. With the exception of two patients, the majority of individuals had previously received BoNT injections at some point. Overall, stroke patients were injected with a higher dosage of BoNT in comparison with CP patients (442 ± 151 units vs 158 ± 52 units in general and 323 ± 101 units vs 108 ± 14 units in finger and/or wrist muscles). Eleven patients were injected in flexor muscles of the forearm and almost every patient received injections at finger flexor muscles.

5.2 EMG validity

Spearman's correlation coefficients and their significance are represented in table 5.2. Strong correlations were found between the change within sessions 1 and 0 of the peak of M1 and the NC ($r_s = 0.709$, $p = 0.007$) and between the AUC of M1 and the NC ($r_s = 0.747$, $p = 0.003$). These relationships can be seen in figures B.2 to B.3. Strong to very

Table 5.1: Patients' characteristics before BoNT injections (adapted from [26]).

Characteristic	N	Value
Age (years)	18	57.8 ± 13.3
by sex		
Male	11	57.2 ± 15.9
Female	7	58.7 ± 8.9
by diagnosis		
CP	3	59.7 ± 26.7
Stroke	15	57.4 ± 10.6
iCVA	12	60.2 ± 9.9
hCVA	3	46.3 ± 4.5
Time post-stroke (years)	15	7.2 ± 5.4
NIHSS score (points)	15	4.6 ± 3.0
Affected side		
Left		7
Right		11
Number of previous BoNT treatments		
0		2
1 to 5		1
6 to 10		8
More than 10		7
BoNT total dose (units)		394 ± 176
CP	3	158 ± 52
stroke	15	442 ± 151
BoNT dose in finger/wrist joints (units)		288 ± 123
CP	3	108 ± 14
Stroke	15	323 ± 101
MAS score (points)		
Wrist flexors		1+ [1-2]
Finger flexors		1+ [1-2]
TS_Q		
Wrist flexors		2 [1-2]
Finger flexors		2 [1-2]
$TS_{R2\text{-}R1}$ (º)		
Wrist flexors		50.7 ± 42.3
Finger flexors		43.0 ± 42.5

The values reported are means and standard deviation or median [25th–75th percentile]. Abbreviations: N, number of patients over which values were calculated; BoNT, botulinum neurotoxin-A; CP, cerebral palsy; hCVA, hemorrhagic stroke; iCVA, ischaemic stroke; MAS, modified Ashworth scale [range 0–4]; NIHSS, National Institutes of Health Stroke Scale [range: 0–42]; TS_Q, Tardieu scale, quality score; $TS_{R2\text{-}R1}$, Tardieu scale, range of motion at a slow speed (R2) minus catch angle for a fast speed (R1).

strong correlations were found between the change of EMG-related parameters for the FCR and the VC, within sessions 2 and 1. No significant correlations were found between the change of the FCR-related EMG parameters and the other components of the NF or the clinical scores.

In table 5.3, the correlation coefficients for interactions between EMG-related parameters of the ECR and NF and clinical scales outcomes can be seen. Every coefficient was non-significant.

Table 5.2: Correlation coefficients between changes in values of EMG parameters of FCR within sessions 1 and 0, and 2 and 1, and various factors associated with hyper-resistance in the wrist and fingers.

FCR	$\Delta_1$peak M1		$\Delta_1$peak M2		Δ_1AUC M1		Δ_1AUC M2	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Δ_1NC	**0.709**	**0.007**	0.418	0.156	**0.747**	**0.003**	0.280	0.354
Δ_1EC	0.022	0.943	0.060	0.845	0.005	0.986	0.187	0.541
Δ_1VC	-0.404	0.171	-0.165	0.590	-0.303	0.315	-0.052	0.865
WF muscles								
Δ_1MAS	0.343	0.251	0.309	0.305	0.252	0.407	0.246	0.418
Δ_1TS_Q	-0.109	0.724	0.068	0.826	-0.172	0.571	0.009	0.977
$\Delta_1TS_{R2\text{-}R1}$	0.500	0.667	-0.500	0.667	0.500	0.667	0.500	0.667
FF muscles								
Δ_1MAS	0.425	0.147	0.383	0.196	0.185	0.546	0.322	0.284
Δ_1TS_Q	-0.134	0.661	-0.071	0.819	-0.289	0.338	0.074	0.810
$\Delta_1TS_{R2\text{-}R1}$	0.800	0.200	-0.400	0.600	0.800	0.200	-0.400	0.600
	$\Delta_2$peak M1		$\Delta_2$peak M2		Δ_2AUC M1		Δ_2AUC M2	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Δ_2NC	0.098	0.762	-0.014	0.966	-0.021	0.948	-0.070	0.829
Δ_2EC	0.007	0.983	0.021	0.948	-0.063	0.846	-0.070	0.829
Δ_2VC	**0.692**	**0.013**	**0.797**	**0.002**	**0.720**	**0.008**	**0.846**	**<0.001**
WF muscles								
Δ_2MAS	-0.146	0.651	-0.246	0.441	-0.238	0.427	-0.321	0.309
Δ_2TS_Q	-0.118	0.715	-0.267	0.401	-0.244	0.445	-0.330	0.294
$\Delta_2TS_{R2\text{-}R1}$	-0.500	0.667	-0.500	0.667	-0.500	0.667	-0.500	0.667
FF muscles								
Δ_2MAS	-0.341	0.278	-0.326	0.301	-0.378	0.225	-0.363	0.245
Δ_2TS_Q	-0.552	0.063	-0.552	0.063	-0.626	0.029	-0.552	0.063
$\Delta_2TS_{R2\text{-}R1}$	-0.486	0.329	-0.657	0.156	-0.200	0.704	-0.543	0.266

Significant values are highlighted with bold font. r_s is the Spearman's correlation coefficient. Δ_1 and Δ_2 represent the change in values between the measuring sessions 1 and 0, and 2 and 1, respectively. Abbreviations: FCR, flexor carpi radialis; ECR, extensor carpi radialis; AUC, area under the curve; M1, short latency response; M2, medium latency response; NC, neural component of the NeuroFlexor; ECR, elastic component of the NeuroFlexor; VC, viscous component of the NF; MAS, modified Ashworth scale; BoNT, botulinum neurotoxin-A; WF, wrist flexor; FF, finger flexor; TS_Q, Tardieu scale, quality score; $TS_{R2\text{-}R1}$, Tardieu scale, range of motion at a slow speed (R2) minus catch angle for a fast speed (R1).

Table 5.3: Correlation coefficients between changes in values of EMG parameters for the ECR within sessions 1 and 0, and 2 and 1, and various factors associated with hyper-resistance in the wrist and fingers.

ECR	$\Delta_1$peak M1		$\Delta_1$peak M2		Δ_1AUC M1		Δ_1AUC M2	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Δ_1NC	0.390	0.188	-0.066	0.831	0.368	0.216	-0.099	0.748
Δ_1EC	0.473	0.103	-0.374	0.209	0.451	0.122	-0.363	0.223
Δ_1VC	-0.264	0.383	0.270	0.373	-0.256	0.399	0.325	0.279
WE muscles								
Δ_1MAS	0.060	0.846	0.031	0.919	0.031	0.919	0.082	0.791
Δ_1TS_Q	-0.047	0.879	0.135	0.660	-0.091	0.767	0.188	0.538
	$\Delta_2$peak M1		$\Delta_2$peak M2		Δ_2AUC M1		Δ_2AUC M2	
	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
Δ_2NC	-0.119	0.713	0.042	0.897	-0.154	0.633	0.126	0.697
Δ_2EC	0.385	0.217	0.727	0.007	0.448	0.145	0.622	0.031
Δ_2VC	0.105	0.746	0.049	0.880	0.154	0.633	-0.049	0.880
WE muscles								
Δ_2MAS	-0.136	0.674	0.535	0.073	-0.209	0.515	0.197	0.539
Δ_2TS_Q	-0.209	0.515	0.197	0.539	-0.205	0.523	0.138	0.669

Significant values are highlighted with bold font. r_s is the Spearman's correlation coefficient. Δ_1 and Δ_2 represent the change in values between the measuring sessions 1 and 0, and 2 and 1, respectively. Abbreviations: FCR, flexor carpi radialis; ECR, extensor carpi radialis; AUC, area under the curve; M1, short latency response; M2, medium latency response; NC, neural component of the NeuroFlexor; ECR, elastic component of the NeuroFlexor; VC, viscous component of the NF; MAS, modified Ashworth scale; BoNT, botulinum neurotoxin-A; WE, wrist extensor; TS_Q, Tardieu scale, quality score; $TS_{R2\text{-}R1}$, Tardieu scale, range of motion at a slow speed (R2) minus catch angle for a fast speed (R1).

5.3 Effect of BoNT

Table 5.4 displays the median value and respective 25^{th}-75^{th} percentile, for each parameter for every session. The sub-figures of figure B.4 show a graphical overview of the EMG-related parameters for the FCR, where a decrease can be observed between baseline and 6 weeks after and a smaller decrease between the last two measuring sessions, for every parameter. In the sub-figures of figure B.5, no big decreases can be seen for the EMG outcomes relative to the ECR.

In table 5.5, the correlation coefficients for the correlation of the change within the first two sessions for every EMG-related parameter and the dose of BoNT can be found. For both muscles, strong to very strong correlations were found between the change of the M1 peak value, within sessions 1 and 0, and the injected dose of BoNT (FCR: r_s = -0.864, $p < 0.001$; ECR: r_s = -0.623, p = 0.023) and also between the change of the value of the AUC of the M1 wave and the dose of BoNT (FCR: r_s = -0.864, $p < 0.001$; ECR: r_s = -0.628, p = 0.022). The correlation between the change of the peak of the M2 wave and the dose of BoNT was strong (r_s = -0.613, p = 0.013), for the FCR. Furthermore, the correlation between the change of the AUC of the M2 wave and the injected BoNT was also strong (r_s = -0.528, p =

0.032), for the FCR. The relationships between every parameter of the FCR and the dose of BoNT can be seen in the sub-figures of figure B.6.

Table 5.4: Median and 25th-75th percentile for the EMG outcomes and modified Ashworth scale values at the three measuring sessions (adapted from [26]).

Parameter	Baseline	6 weeks	12 weeks
FCR	N = 15	N = 15	N = 13
peak M1	0.294 [0.087; 0.683]	0.146 [0.059; 0.203]	0.117 [0.040; 0.293]
peak M2	0.169 [0.036; 0.276]	0.086 [0.013; 0.171]	0.061 [0.029; 0.239]
AUC M1	0.020 [0.006; 0.033]	0.008 [0.003; 0.010]	0.007 [0.003; 0.017]
AUC M2	0.012 [0.002; 0.020]	0.007 [0.001; 0.011]	0.004 [0.002; 0.018]
ECR	N = 16	N = 15	N = 13
peak M1	0.072 [0.042; 0.179]	0.092 [0.027; 0.128]	0.075 [0.024; 0.150]
peak M2	0.045 [0.021; 0.085]	0.037 [0.010; 0.059]	0.036 [0.022; 0.060]
AUC M1	0.005 [0.003; 0.010]	0.007 [0.002; 0.009]	0.005 [0.001; 0.008]
AUC M2	0.003 [0.001; 0.006]	0.002 [0.001; 0.004]	0.002 [0.002; 0.006]
WF muscles (N = 18)			
MAS	1+ [1-2]	1 [0-1+]	1+ [1-2]
TS_Q	2 [1-2]	1 [0-2]	2 [1-2]
TS_{R2-R1}	54 [0-96]	0 [0-66]	4 [0-60]
FF muscles (N = 18)			
MAS	1+ [1-2]	1 [0-1+]	2 [1-2]
TS_Q	2 [1-2]	2 [0-2]	2 [1-2]
TS_{R2-R1}	42 [0-85]	10 [0-69]	50 [0-79]

The values presented for the peaks and AUC are unitless since they were normalised. Abbreviations: N, number of patients over which values were calculated; FCR, flexor carpi radialis; ECR, extensor carpi radialis; AUC, area under the curve; M1, short latency response; M2, medium latency response; MAS, modified Ashworth scale [range 0–4]. TS_Q, Tardieu scale, quality score; TS_{R2-R1}, Tardieu scale, range of motion at a slow speed (R2) minus catch angle for a fast speed (R1); WF, wrist flexor; FF, finger flexor.

Table 5.5: Correlation coefficients between changes in EMG parameters within session 0 and session 1 and the dose of BoNT in the wrist and fingers.

BoNT dose	Δpeak M1		Δpeak M2		ΔAUC M1		ΔAUC M2	
wrist/fingers	r_s	P-value	r_s	P-value	r_s	P-value	r_s	P-value
FCR	**-0.864**	**<0.001**	**-0.613**	**0.013**	**-0.864**	**<0.001**	**-0.528**	**0.032**
ECR	**-0.623**	**0.023**	0.149	0.628	**-0.628**	**0.022**	0.163	0.596

Significant values are highlighted with bold font. r_s is the Spearman's correlation coefficient. Δ represents the change in values between the measuring sessions 1 and 0. Abbreviations: FCR, flexor carpi radialis; ECR, extensor carpi radialis; AUC, area under the curve; M1, short latency response; M2, medium latency response; BoNT, botulinum neurotoxin-A.

Table 5.6 presents the results of the LMEM statistical analysis. Significant values are highlighted with bold font. For the peak of the M1 wave relative to the FCR, there is a significant difference between at least two of the sessions (p = 0.037). The decreases between session 0 and session 1 (difference = 0.233, p = 0.015) and again between session 0 and session 2 (difference = 0.197, p = 0.017) are significant. There is a small increase

between the last two sessions, but this is not significant (difference = -0.036, p = 0.547). Analysis of the AUC of M1's wave shows similar significant differences, a decrease between session 0 and the other two sessions (difference = 0.012, p = 0.010 for session 0 vs. session 1; difference = 0.010, p = 0.037 for session 0 vs. session 2). For other FCR-related outcomes and all ECR-related outcomes, no statistically significant differences were found.

5.3.1 Added value of EMG

In table 5.7, there is an overview of the division between responders and non-responders using the MAS, the TS and the PROM of the wrist. Tables 5.8, 5.9 and 5.10 show the outcomes (-2 log-likelihood, accuracy, precision and sensitivity) for multiple models with different predictors, using different scales for the initial grouping of responders and non-responders. In general, the more the number of predictors, the better the outcomes from the model. Comparing the model that only had the change of the NC of the NF as a predictor with the models that also included the EMG-related parameters, it is possible to observe lower values in the -2 log-likelihood outcome (20.9 vs 13.7 for MAS responders; 23.1 vs 10.4 for TS responders; 23.3 vs 17.2 for PROM responders) and higher accuracy (47.1% vs 76.9% for MAS responders; 47.1% vs 84.6% for TS responders; 47.1% vs 53.8% for PROM responders), higher precision (44.4% vs 66.7% for MAS responders; 25.0% vs 83.3%) and higher sensitivity (50.0% vs 80.0% for MAS responders; 40.0% vs 83.3%), except for PROM responders that showed a decrease in precision and an unchanged sensitivity. This is more noticeable between the models that were built around the decision of responders and non-responders using the TS (table 5.9). The best prediction model seems to be the one that includes the NC of the NF, the EMG parameters and one clinical scale. It is also true that, if we compare a model with the NC of the NF and clinical scales as predictors with a model with the NC and EMG parameters, the former presents slightly better outcomes for MAS responders and for PROM responders.

In the prediction models, M2-related parameters were not included since for almost every, or even every model their inclusion did not change significantly the metrics used.

Table 5.6: Results of the linear mixed effects model of the EMG outcome parameters, with "session" as the fixed effect.

Variable	Parameter/comparison	Estimate	P-value	95% CI
		FCR		
peak M1	Session		**0.037**	
	0vs1	0.233	**0.015**	[0.055; 0.410]
	0vs2	0.197	**0.017**	[0.042; 0.351]
	1vs2	-0.036	0.547	[-0.161; 0.089]
peak M2	Session		0.179	
	0vs1	0.090	0.078	[-0.012; 0.192]
	0vs2	0.054	0.198	[-0.033; 0.142]
	1vs2	-0.036	0.448	[-0.134; 0.062]
AUC M1	Session		**0.030**	
	0vs1	0.012	**0.010**	[0.003; 0.020]
	0vs2	0.010	**0.037**	[0.001; 0.019]
	1vs2	-0.002	0.613	[-0.010; 0.006]
AUC M2	Session		0.335	
	0vs1	0.004	0.195	[-0.002; 0.010]
	0vs2	0.002	0.330	[-0.003; 0.007]
	1vs2	-0.002	0.632	[-0.008; 0.005]
		ECR		
peak M1	Session		0.473	
	0vs1	0.033	0.368	[-0.042; 0.107]
	0vs2	-0.019	0.783	[-0.167; 0.128]
	1vs2	-0.052	0.404	[-0.182; 0.079]
peak M2	Session		0.863	
	0vs1	0.006	0.743	[-0.034; 0.046]
	0vs2	-0.005	0.848	[-0.068; 0.057]
	1vs2	-0.012	0.634	[-0.064; 0.040]
AUC M1	Session		0.509	
	0vs1	0.002	0.382	[-0.002; 0.005]
	0vs2	-0.001	0.795	[-0.009; 0.007]
	1vs2	-0.003	0.445	[-0.009; 0.004]
AUC M2	Session		0.562	
	0vs1	0.0005	0.723	[-0.002; 0.003]
	0vs2	-0.002	0.463	[-0.008; 0.004]
	1vs2	-0.003	0.326	[-0.008; 0.003]

Significant values are highlighted with bold font. A p-value less than 0.005 means that there is a significant difference between the values of the two sessions. The estimate represents the difference between the sessions. 0, 1 and 2 are respective to each session. Abbreviations: CI, confidence interval; FCR, flexor carpi radialis; ECR, extensor carpi radialis; AUC, area under the curve; M1, short latency response; M2, medium latency response; vs, versus.

Table 5.7: Type of response to BoNT based on three different scales, for every patient.

Patient	ΔMASw	ΔMASf	Response	ΔTSw	Response	ΔPROMw	Response
1	-1.5	-2	1	-2	1	52	1
2	0	0	0	0	0	24	1
3	0	0	0	0	0	-14	0
4	-0.5	-0.5	1	-2	1	45	1
5	0	0	0	0	0	2	0
6	0	-1	0	0	0	30	1
7	0	1	0	-1	1	-3	0
8	-0.5	0.5	0	-1	1	-32	0
9	-0.5	-0.5	1	0	0	10	1
10	1.5	0.5	0	2	0	-11	0
11	-1	0	1	0	0	18	1
12	-1	-1	1	0	0	25	1
13	-1.5	-3	1	-2	1	48	1
15	-1	-1	1	-2	1	-15	0
16	-0.5	0	0	0	0	10	1
17	-1	-1	1	-1	1	5	0
18	-1	-0.5	1	-1	1	5	0

Δ represents the change in values between sessions 1 and 0. The values 0 and 1 represent non-responders and responders, respectively. The 1+ value in the modified Ashworth scale is transformed into 1.5 for calculations, hence the .5 values. Abbreviations: MASw, modified Ashworth scale for the wrist; MASf, modified Ashworth scale for the fingers; TSw, Tardieu scale for the wrist; PROMw, passive range of motion for the wrist. PROMw values are in degrees, °.

Table 5.8: Outcomes for different prediction models using the MAS responders.

MAS responders	-2 log-likelihood	Accuracy	Precision	Sensitivity
ΔNC	20.9	47.1%	44.4%	50.0%
ΔNC + ΔM1 peak	14.7	61.5%	50.0%	60.0%
ΔNC + ΔM1 peak + ΔM1 AUC	13.7	76.9%	66.7%	80.0%
ΔM1 peak + ΔM1 AUC	15.9	61.5%	50.0%	60.0%
ΔPROMw	19.7	64.7%	66.7%	66.7%
ΔTSw	17.6	70.6%	66.7%	75.0%
ΔTSw + ΔPROMw	13.9	70.6%	77.8%	70.0%
ΔNC + ΔPROMw	16.7	64.7%	66.7%	66.7%
ΔNC + ΔTSw	15.3	82.4%	77.8%	87.5%
ΔNC + ΔTSw + ΔPROMw	10.0	88.2%	88.9%	88.9%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔPROMw	9.7	84.6%	83.3%	83.3%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔTSw	11.6	92.3%	83.3%	100.0%

Δ represents the change in values between sessions 1 and 0. Abbreviations: TSw, Tardieu scale for the wrist; MASw, modified Ashworth scale; NC, neural component of the NeuroFlexor; M1, short latency response; AUC, area under the curve; PROMw, passive range of motion for the wrist.

Table 5.9: Outcomes for different prediction models using the TS responders.

TS responders	-2 log-likelihood	Accuracy	Precision	Sensitivity
ΔNC	23.1	47.1%	25.0%	40.0%
ΔNC + ΔM1 peak	10.9	84.6%	83.3%	83.3%
ΔNC + ΔM1 peak + ΔM1 AUC	10.4	84.6%	83.3%	83.3%
ΔM1 peak + ΔM1 AUC	17.3	46.2%	33.3%	40.0%
ΔMASw	18.1	70.6%	62.5%	71.4%
ΔPROMw	23.5	70.6%	37.5%	100.0%
ΔMASw + PROMw	17.4	76.5%	75.0%	75.0%
ΔNC + ΔMASw	17.8	70.6%	62.5%	71.4%
ΔNC + ΔPROMw	23.0	52.9%	25.0%	50.0%
ΔNC + ΔMASw + ΔPROMw	16.7	76.5%	75.0%	75.0%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔMASw	8.6	92.3%	83.3%	100.0%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔPROMw	10.3	84.6%	83.3%	83.3%

Δ represents the change in values between sessions 1 and 0. Abbreviations: MAS, modified Ashworth scale for the wrist; NC, neural component of the NeuroFlexor; M1, short latency response; AUC, area under the curve; TS, Tardieu scale; PROMw, passive range of motion for the wrist.

Table 5.10: Outcomes for different prediction models using the PROM responders.

PROM responders	-2 log-likelihood	Accuracy	Precision	Sensitivity
ΔNC	23.3	47.1%	77.8%	50.0%
ΔNC + ΔM1 peak	17.6	53.8%	50.0%	50.0%
ΔNC + ΔM1 peak + ΔM1 AUC	17.2	53.8%	50.0%	50.0%
ΔM1 peak + ΔM1 AUC	17.3	53.8%	50.0%	50.0%
ΔMASw	21.5	64.7%	77.8%	63.6%
ΔTSw	23.4	58.8%	100.0%	56.3%
ΔMASw + ΔTSw	20.1	70.6%	66.7%	75.0%
ΔNC + ΔMASw	19.5	76.5%	77.8%	77.8%
ΔNC + ΔTSw	23.1	64.7%	77.8%	63.6%
ΔNC + ΔMASw + ΔTSw	16.5	76.5%	77.8%	77.8%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔMASw	13.3	84.6%	83.3%	83.3%
ΔNC + ΔM1 peak + ΔM1 AUC + ΔTSw	16.4	61.5%	50.0%	60.0%

Δ represents the change in values between sessions 1 and 0. Abbreviations: PROM, passive range of motion; MASw, modified Ashworth scale for the wrist; NC, neural component of the NeuroFlexor; M1, short latency response; AUC, area under the curve; TSw, Tardieu scale for the wrist.

6

Discussion

In this longitudinal study, 18 stroke or CP patients were injected with BoNT with the intent of treating spasticity at the wrist. Measurements were done using the NF and EMG to address the added value of the latter. Strong correlations were found between M1-related parameters of the FCR and the NC of the NF. These were mainly the only significant correlations found between EMG-related parameters and NF outcomes. No correlations were found between EMG and clinical scales. Almost every change in EMG parameters between sessions correlated significantly with the dose of BoNT. Significant decreases were only found for the M1 of the FCR, between the first two sessions. The inclusion of EMG parameters showed improved predictive outcomes for responsiveness to BoNT.

The strong correlations between the parameters of the M1 wave from the FCR and the NC of the NF show the construct validity of the EMG with respect to the NF. The NF retrieves its NC from the flexor muscles and not from the extensor muscles, due to this muscle being the one resisting the enforced movement of the NF. Therefore, it is logical that the parameters of the ECR do not correlate with the NC of the NF. A different study by Pennati *et al.*, showed a significant correlation between EMG outcomes and NC of the NF, after electrical stimulation [90]. Some of the authors from the aforementioned study also conducted another research with EMG and NF measurements, this time in leg muscles. They found strong correlations between the EMG outcomes and the NC of the NF [91]. Significant correlations between EMG and the NF were also found in [30, 31]. Even though there are some differences between the aforementioned studies and the current one, such as EMG measured on different muscles and different EMG outcomes, these results back ours. For the M2-related parameters, the correlation was not significant possibly because the M2 wave represents a different construct than the ones of the NF.

The correlation between any parameter of the EMG and the VC and EC of the NF was low probably due to the EMG measuring neural components of hyper-resistance and the EC and VC being non-neural components. There was one exception, the correlation between the change within the last two sessions of EMG-related parameters and the VC of the NF, which could be coincidental or could reflect an inability to detect small BoNT effects.

For the correlations between every EMG parameter and clinical scales, the fact that there were not any significant values could be explained by the lack of validation of the commonly used clinical scales for the quantification of spasticity and rather measuring hyper-resistance as a whole, as previously mentioned [13, 38, 60, 65]. Pandyan *et al.* demonstrated a lack of correlation between EMG parameters of the elbow flexor muscles

and the MAS scores after BoNT injections [86]. The TS, which is thought to be a more valid way of measuring spasticity [28], had insufficient data so the correlations that involved the TS do not provide very useful information.

Overall, most of the results were in line with the hypotheses and one can conclude that the EMG is a valid way of quantifying the effect of BoNT in spasticity, as it was already concluded in multiple studies [65, 86, 92]. Even though EMG measurements had already been validated, the EMG parameters used in these analyses were different to the ones used here. Most studies use the H- and M-waves [65], but we used the short (M1) and long latency (M2) responses. Nevertheless, it appears that the M1 response effectively represents the stretch reflex, while the M2 response does not demonstrate validity as an EMG outcome for spasticity.

The moderate to very strong correlations between the change of EMG-related parameters of the FCR, within the first two sessions, and the dose of BoNT injected at the muscles around the wrist indicate that the higher the dose, the larger the decrease of the EMG parameters. The effect of the dose of BoNT is undoubtedly seen for the M1 wave. The pathways involved in the increase of the stretch reflex are inhibited by the toxin, leading to the reduction of the parameters. There are studies that proved that the injection of BoNT in a muscle resulted in increased presynaptic inhibition on motor neurons of the injected muscle [16]. Therefore, the decrease of the M1 wave after the injection of BoNT, which translates into a decrease in stretch reflex, could be explained by the increase in presynaptic inhibition. In a recent study, it was reported that BoNT has the power to constrain Renshaw cells which can lead to a restoration of reciprocal Ia inhibition [93]. Pandyan *et al.* also found a decrease in spasticity after the BoNT treatment and argued that it could be related to a smaller number of activated motor units [86]. Miscio *et al.* likewise saw a reduction in stretch reflex following injections [80]. These results further corroborate ours, even though the muscles are different and the EMG parameters they retrieved were not the same as ours.

For the M2, the correlations are still significant, but not as strong, which could be related to the different pathways responsible for the elicitation of the M2 not being as affected by BoNT. As seen in chapter 3.3.3, there are multiple pathways that can contribute to the origin of the M2. Therefore, it is possible that BoNT interacts with the components of some of these pathways, which would explain the decrease between sessions, however not a significant decrease that could justify a very high correlation with the amount of BoNT injected. Currently, no studies can be found about the effect of BoNT on the M2 response for distal upper limb muscles. The effect of BoNT on the M1 response is clear but one can only speculate about the results regarding the M2. BoNT affects the neuromuscular synapses of the injected muscles, so the ones part of the M2 pathway are also affected. Thus, the correlation between the decrease of M2 parameters and the injection of BoNT is not a peculiar finding. The increase/decrease of inhibitory/excitatory circuits responsible for the M2 wave, caused by BoNT, needs further research. One big problem is the lack of certainty surrounding these circuits.

The significant differences between baseline and the other sessions, for the parameters of the M1 wave of the FCR, were in line with the suppositions. The non-significant difference between session 1 and session 2 could indicate a loss of effectiveness of the BoNT as time passes, possibly due to the appearance of immunoresistant activity from antibodies [94]. Once again, the results are different for the parameters related to the M2 wave, since this response might not be the most representative of muscle tone.

Our results showed a decrease, even if not significant, in the M2 following the injection of BoNT. Deuschl and Lücking reported an amplitude decrease of the M2 wave in patients with spasticity, specifically in hand muscles, compared with healthy individuals [69]. This would mean that, as BoNT is injected and spasticity decreases, the amplitude of the M2 augments, and therefore would go against our results. However, the muscles analysed in this study were wrist muscles, and not hand muscles. Additionally, the responses in this study were elicited mechanically, contrarily to electrical eliciting in [69]. These factors could explain the differences between the results.

Performing binary logistic regressions using responder and non-responder data, defined by the TS scale, seems to have resulted in prediction models with the best outcomes. The overall agreement appears to be that, the more predictors that are included in the model, the better said model is. EMG-related parameters should be included as predictors, because not only are they valid but they also improve up to three times some outcomes of the model if we compare with one with only the NC of the NF.

Bar-On *et al.* studied the prediction of the response to BoNT treatment of spasticity in lower limb muscles of children with CP. They concluded that, from multiple outcome parameters, the one related to EMG had the best predictive power compared to models with outcomes from clinical scales or torque values [92]. Even though the study population and the target muscles differed from the ones in the present study, their results could support our findings to add EMG in spasticity measurements.

7

Conclusion

Compared to the study by Andringa *et al.*, where only the NF data was assessed, their overall results are very similar to the results of this study. The correlation between the M1 response of the FCR and the NC of the NF was strong, which seems to confirm that both the NF and EMG are able to provide a good measurement of spasticity. Validity of the M1 response with respect to the NC of the NF was achieved and EMG is sensitive to changes in spasticity after BoNT injections.

Even though a larger number of predictors might indicate a better prediction model for the responsiveness to BoNT, it is preferable to find a balance between the number of predictors and the goodness of the model. Therefore, one can decide whether to use a model with the NC of the NF and M1-related parameters as predictors or the same model but also with a clinical scale.

EMG is easily accessible, due to its affordability, and it is readily implemented, but it cannot be used alone since it needs a biomechanical device to enforce the isokinetic passive motion. Thus, the option is to either use the NF or to use the NF and the EMG simultaneously. If the desired parameters from EMG are not the short and long latency responses, then there might be no need for the NF.

Based on the obtained results, even though EMG measurements improve the prediction models, other models without EMG parameters also show satisfactory outcomes, with values approximately from 50% up to 89%. Therefore, considering the extra effort involved with EMG, its inclusion might not be advocated.

The final conclusion is that the objectives of this study were accomplished, EMG is able to detect changes in spasticity resultant from BoNT injections. There is an added value in including EMG for spasticity measurements based on the outcomes of the prediction models, however the complexity of the study will increase significantly. Nevertheless, EMG should be included due to the significantly higher prediction outcomes and due to being a more direct and valid representation of the neural component of hyper-resistance.

7.1 Limitations

This study had some limitations that could have influenced the results. Firstly, every limitation presented by Andringa *et al.* in [26] is shared with the present study since these limitations are related to data acquisition with the clinical scales and the NF, also used in our analyses: 1) small sample study population; 2) lack of blinding which could have

affected the outcomes of clinical scales; 3) previous BoNT injections; 4) the use of the NF requires the wrist to be able to passively extend to 40º.

There were some problems with data acquisition and processing. The system used for EMG acquisition failed in some sessions for some of the patients. There were two missing files and for some instances, the signals were corrupted, thus the detection of the M-waves was not possible.

It is also known that the M2 wave is higher if people try to resist the movement. Even though patients were asked to relax for every trial, this is something that might be difficult to achieve for some people. If some patients failed to relax in a few trials, this could have had an unwanted contribution to the M2-related parameters.

Another limitation was the fact that the triggers from the Mobi device and the NF were not reliable, so they could not be used as the onset of the contraction. In some cases, when visually analysed, the trigger clearly happened after the muscle contraction had started so, even though it seemed to work for most of the trials, the triggers could not be reliably used as onset. Due to this, a threshold-based algorithm was written in MATLAB, which is not a perfect option unfortunately. This algorithm was built to correctly detect the start of every contraction but, even after visually checking every trial, the algorithm could have picked an incorrect start for the contraction. This then leads to an incorrect detection of the M1 and M2 waves.

The second algorithm, built to find the onset of each response, could have also detected parts of the signal that were not representative of the M-waves. The probability of this happening is not very high, since the signal amplitude was low and every signal was checked afterwards. However, there were a few cases where some doubts existed.

7.2 Future work

Some of the limitations could be tackled in future studies. It would probably be helpful to develop advanced algorithms for the extraction of EMG parameters that more accurately represent spasticity. This can be done by exploring machine-learning techniques. A cost-effectiveness analysis could also be performed in order to assess if the added value of EMG justifies the additional resources required. It would also be interesting to include between-patient differences, by taking into account characteristics such as age, origin of spasticity, and previous BoNT injections, among others. Ideally, this would be done with a larger study population.

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A

NeuroFlexor software

This appendix has the intent to briefly explain the functioning of software that includes the model used alongside the NF. Figure A.1 shows the launch window of the NF software, where the settings for each trial can be defined. The characteristics of the slow and fast modes can be customised, with velocities from 5 to 280 º/s. The starting and final angles can also be changed.

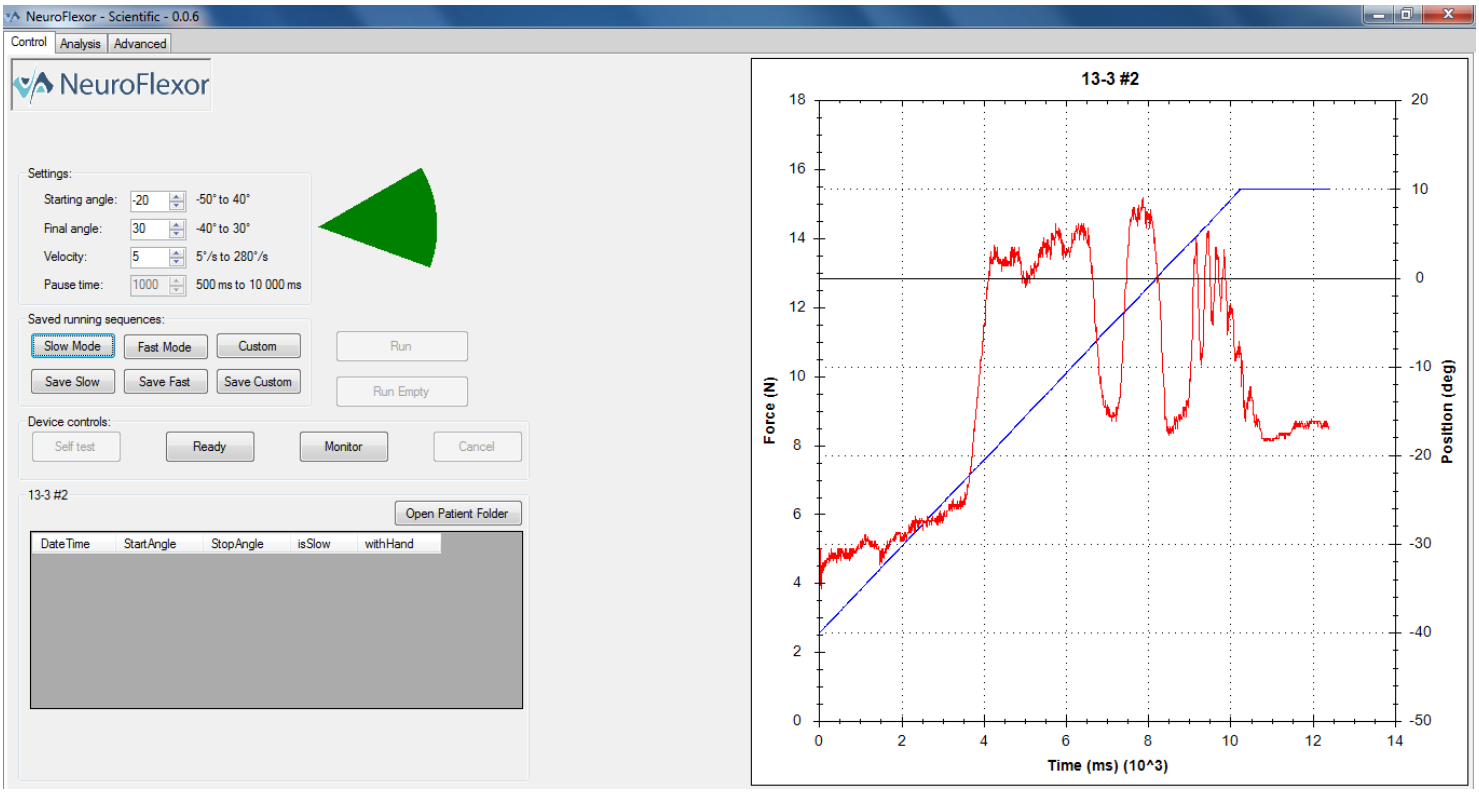
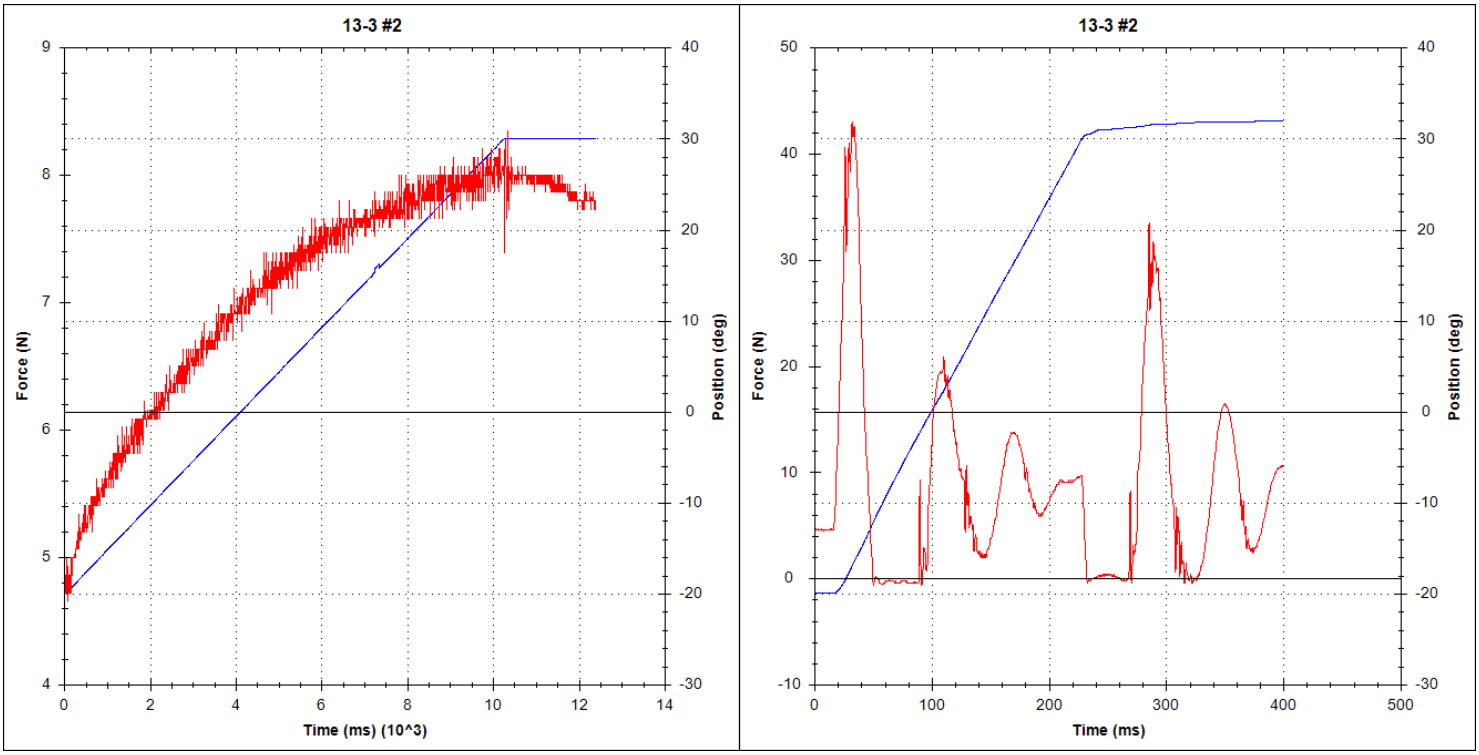


Figure A.1: Control of the NeuroFlexor software. The top left corner shows the customisation boxes; the box in the bottom left corner will show the characteristics of the trial, such as the starting time of the recording, the starting and final angles, if the trial was a slow or a fast one and if it was done with the hand on the device; the right side shows the plot of the measured force (red), in Newtons, and wrist angle (blue), in degrees against time, in milliseconds.

Figure A.2 shows two examples of trials, a slow one (A.2a) and a fast one (A.2b), both with the hand of the person on top of the platform.

Finally, figure A.3 shows the software window where the analysis takes place. In this window, the multiple fast and slow trails are superimposed and shown in two different



(a) Slow trial.

(b) Fast trial.

Figure A.2: Examples of experimental trials, with the hand on the NF platform. In each graph, the plot of the measured force (red), in Newtons, and wrist angle (blue), in degrees against time, in milliseconds, are shown.

plots. In this window, some individual characteristics can be adjusted, such as body weight, hand size and sex. From the mean fast trial, the software retrieves the resting force (P0), the initial force peak (P1) and the late force peak (P2). The force magnitude (P3) is retrieved from the slow trial at the end of the extension movement. With these components, the neural, viscous and elastic components of the measured resistance can be calculated.

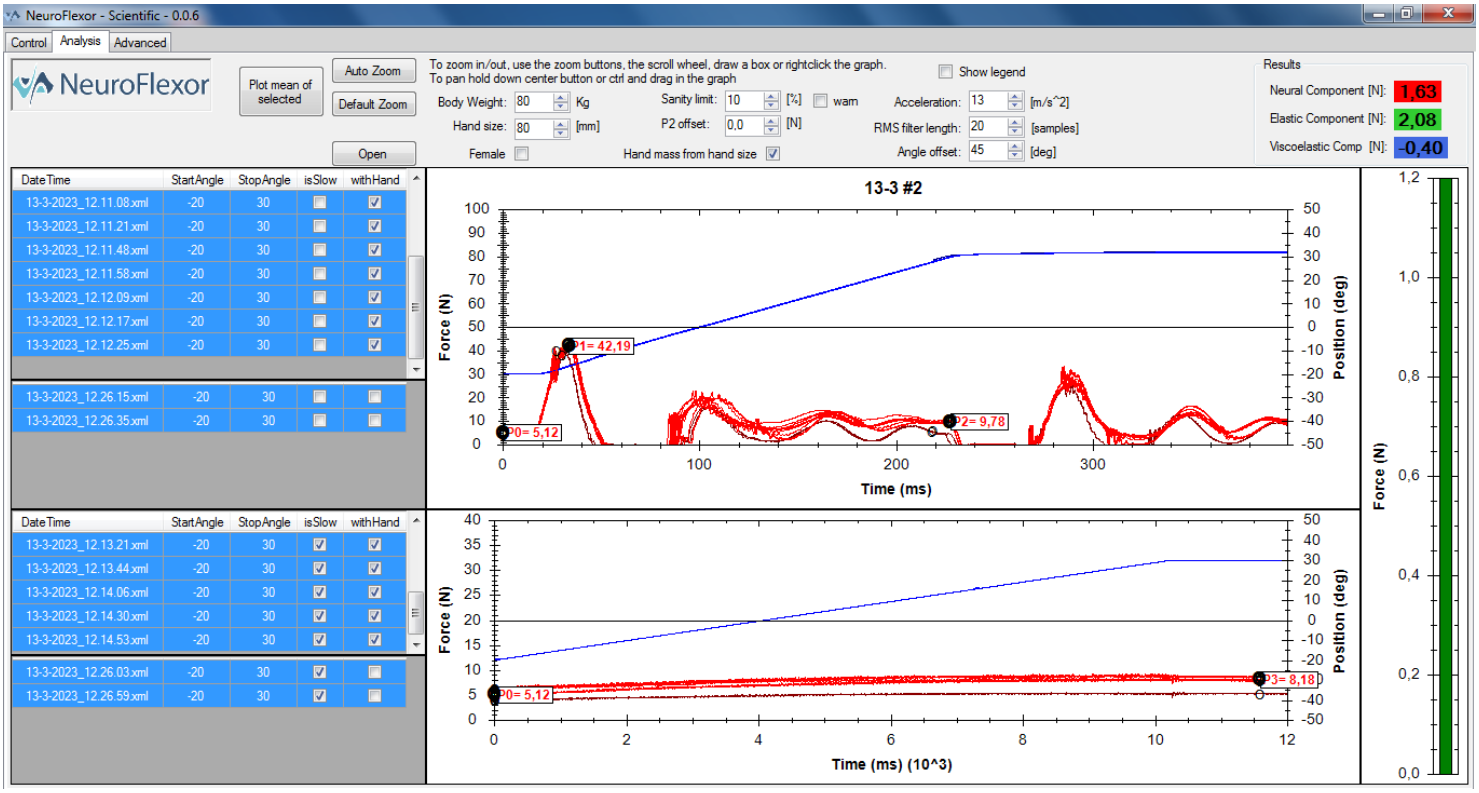


Figure A.3: Data analysis of the NeuroFlexor. At the window's upper section, certain parameters that are involved in the calculations can be modified. On the top right corner, the values of the neural, viscous and elastic components are reported, in Newtons. Most of the window is occupied by the superimposed plots of the multiple fast trials (on the top) and multiple slow trials (on the bottom). In each graph, the plot of the measured force (red), in Newtons, and wrist angle (blue), in degrees against time, in milliseconds, are shown. The left side of the window shows the multiple trials and the respective characteristics and allows for the inclusion or exclusion of specific trials.

B

Extra results

Table B.1: Median dose of injected BoNT by muscle (from [26]).

Muscle	Patients injected (n)	Dose, unit (median)
m. flexor digitorum superficialis	16	75
m. flexor digitorum profundus	16	75
m. flexor carpi radialis	10	75
m. flexor carpi ulnaris	8	50
m. lumbricalis	10	50
m. palmaris longus	6	25
m. flexor pollicis longus	9	50
m. flexor pollicis brevis	7	25
m. opponens pollicis	7	25
m. extensor carpi radialis	1	25
m. extensor carpi ulnaris	1	40
m. extensor digitorum	1	50
m. extensor pollicis brevis	1	25
m. abductor digiti minimi	1	25
m. pronator teres	8	50
m. pronator quadratus	1	25
m. brachialis	9	50
m. brachioradialis	3	50
m. biceps brachii	6	75
m. triceps brachii	2	37.5
m. pectoralis	3	100

Muscles with a grey background participate in wrist hyper-resistance.

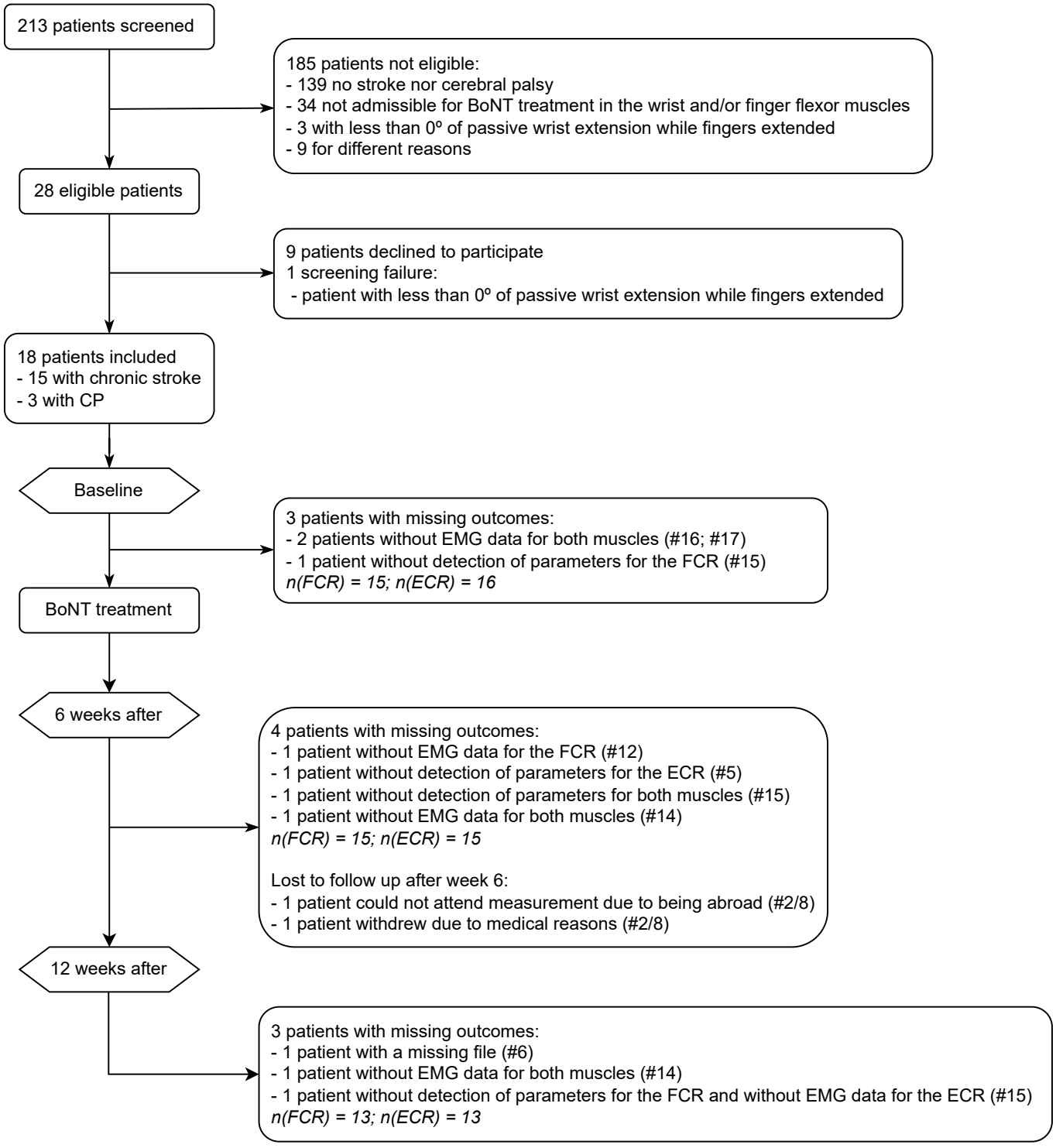


Figure B.1: Flowchart (adapted from [26]).

Table B.2: Individual data of BoNT dose (adapted from [26]).

ID	Age	Sex	Disease	BoNT total [U]	BoNT wrist-finger [U]	Injected muscles and BoNT affecting wrist and/or finger joints
1	69	F	Stroke	375	325	FDS-75, FDP-75, FCR-50, PL-25, FPL-50, FPB-25, OP-25
2	51	F	Stroke	475	175	FDS-75, FDP-75, ECR-25
3	67	M	Stroke	450	450	FDS-75, FDP-100, FCR-75, FCU-50, L-50, FPL-50, FPB-25, OP-25
4	60	F	Stroke	225	225	FDS-75, FDP-50, FPL-50, FPB-25, OP-25
5	53	F	Stroke	600	375	FDS-75, FDP-75, FCR-75, L-50, PL-25, FPL-50, FPB-12.5, OP-12.5
6	72	F	CP	200	100	FCR-50, FCU-50
7	29	M	CP	175	125	FDS-50, FDP-25, ED-50
8	78	M	CP	100	100	FCU-35, ECU-40, ADM-25
9	49	F	Stroke	400	250	FDS-75, FDP-75, L-50, FPL-50
10	56	M	Stroke	350	350	FDS-100, FDP-100, L-50, FPL-50, FPB-25, OP-25
11	57	F	Stroke	700	375	FDS-75, FDP-75, FCR-75, FCU-75, L-25, PL-50
12	46	M	Stroke	600	500	FDS-100, FDP-100, FCR-100, FCU-50, L-50, PL-25, FPL-50, FPB-12.5, OP-12.5
13	71	M	Stroke	275	275	FDS-100, FDP-100, L-75
14	72	M	Stroke	600	425	FDS-100, FDP-100, FCR-100, FCU-75, PL-50
15	45	M	Stroke	250	150	FDS-50, FDP-50, L-25, EPB-25
16	50	M	Stroke	350	275	FDS-75, FDP-100, FCR-75, FPL-25
17	42	M	Stroke	625	400	FDS-75, FDP-75, FCR-75, FCU-50, L-50, FPL-25, FPB-25, OP-25
18	73	M	Stroke	350	300	FDS-75, FDP-75, FCR-50, FCU-25, L-50, PL-25

Abbreviations: BoNT, botulinum neurotoxin A; U, unit; M, male; F, female; CP, cerebral palsy; FDS, m. flexor digitorum superficialis; FDP, m. flexor digitorum profundus; FCR, m. flexor carpi radialis; FCU, m. flexor carpi ulnaris; L, m. lumbricalis; PL, m. palmaris longus; FPL, m. flexor pollicis longus; FPB, m. flexor pollicis brevis; OP, m. opponens pollicis; ECR, m. extensor carpi radialis; ECU, m. extensor carpi ulnaris; ED, m. extensor digitorum; EPB, m. extensor pollicis brevis; ADM, m. abductor digiti minimi.

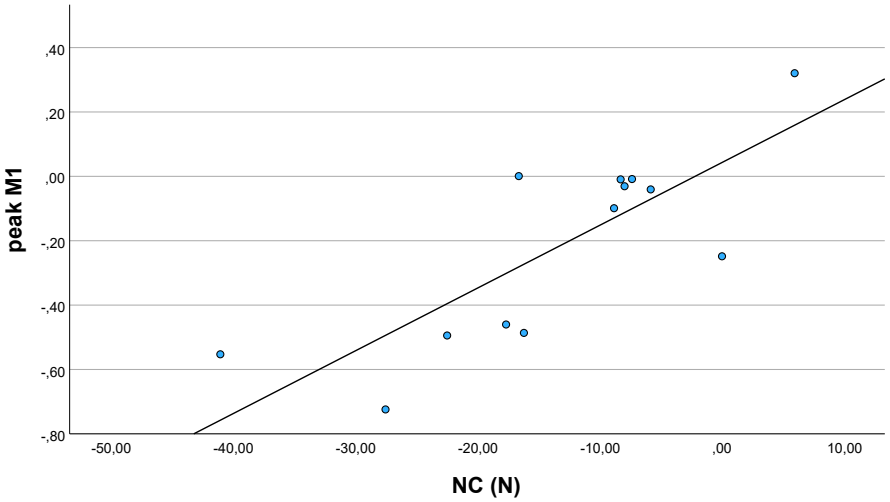


Figure B.2: Scatterplot of change in values of the amplitude of the peak of the M1 wave and the change in the neural component of the NF, for the FCR. Every dot represents a patient.

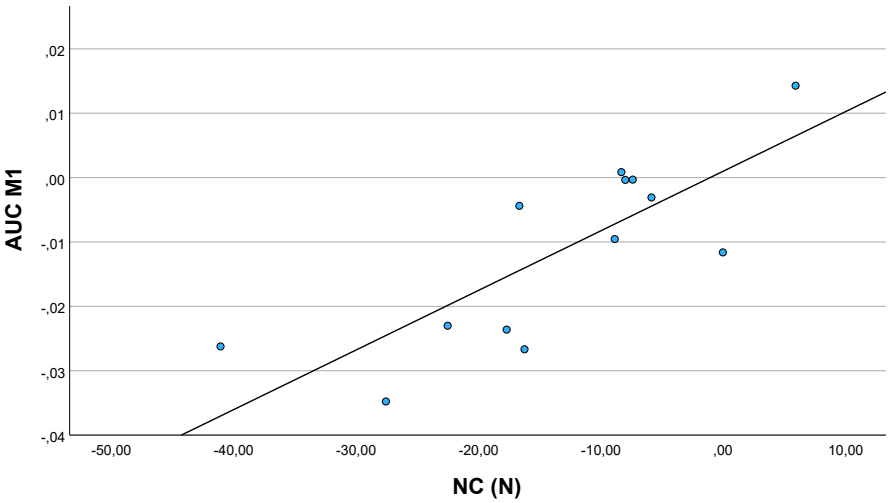
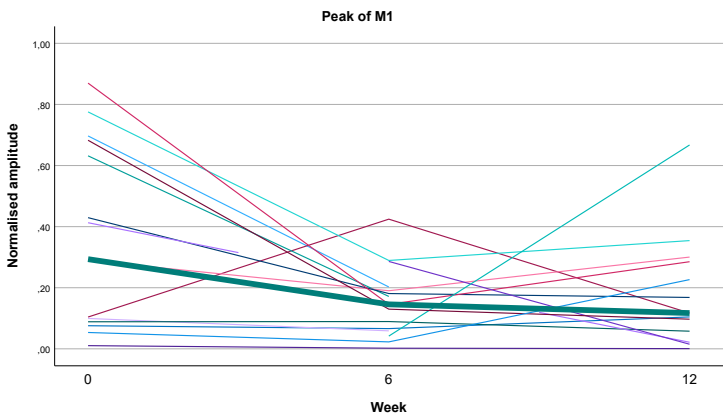
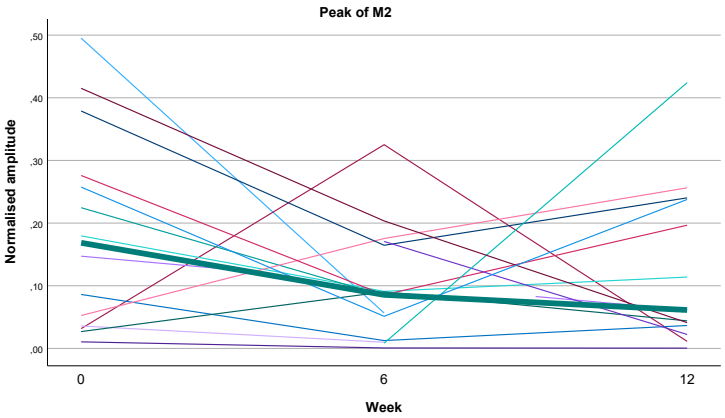


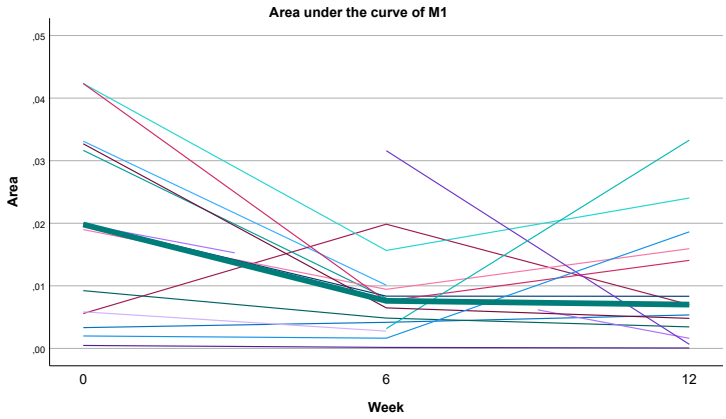
Figure B.3: Scatterplot of change in values of the AUC the M1 wave and the change in the neural component of the NF, for the FCR. Every dot represents a patient.



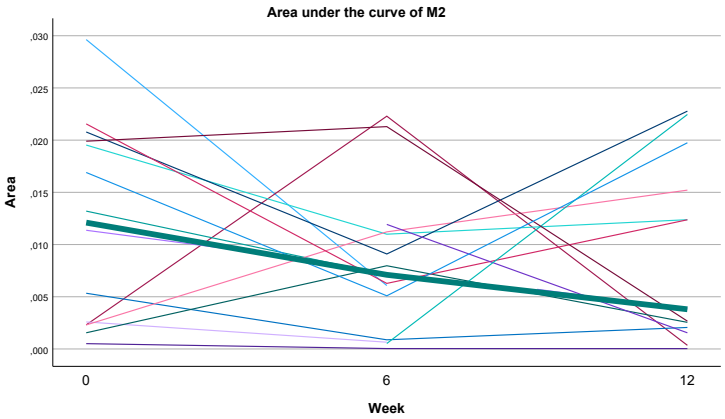
a) Peak of M1



b) Peak of M2

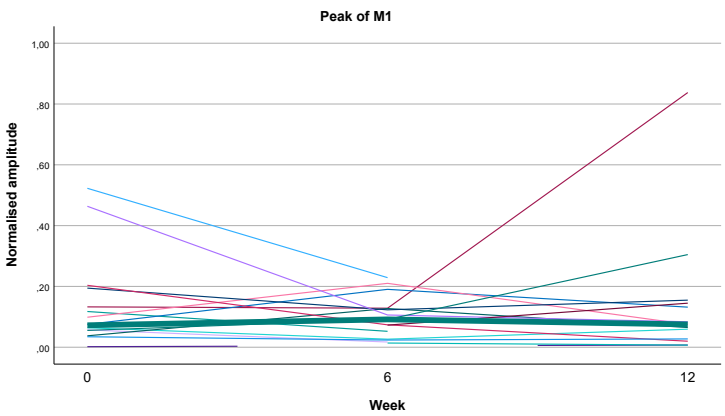


c) Area under the curve of M1

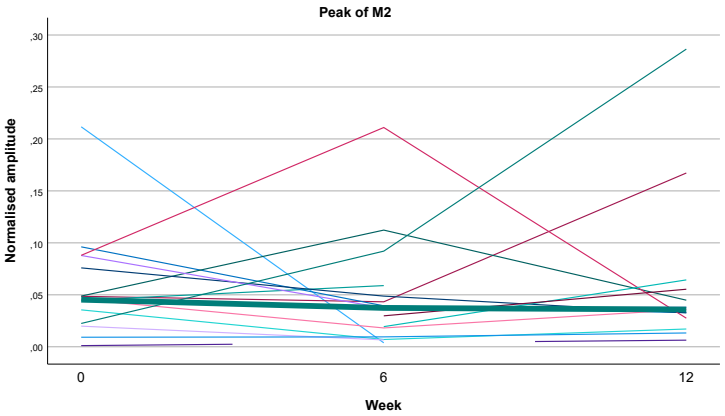


d) Area under the curve of M2

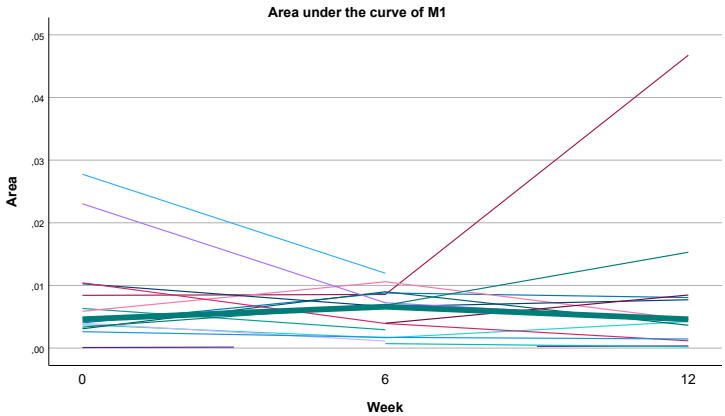
Figure B.4: Change of the EMG-related parameters of the FCR, between baseline, 6 and 12 weeks after. Each line represents a patient and the thick line represents the median.



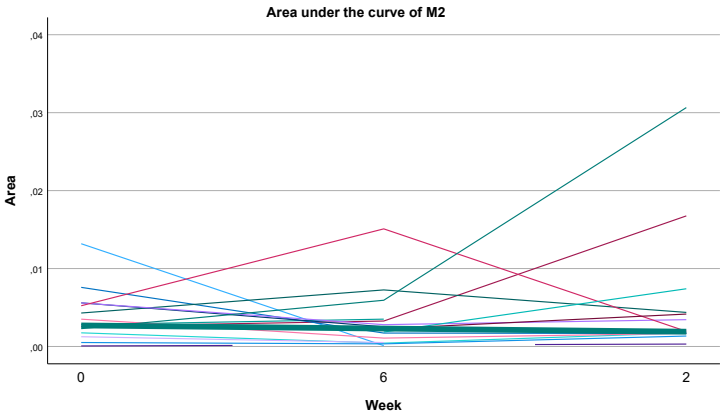
a) Peak of M1



b) Peak of M2

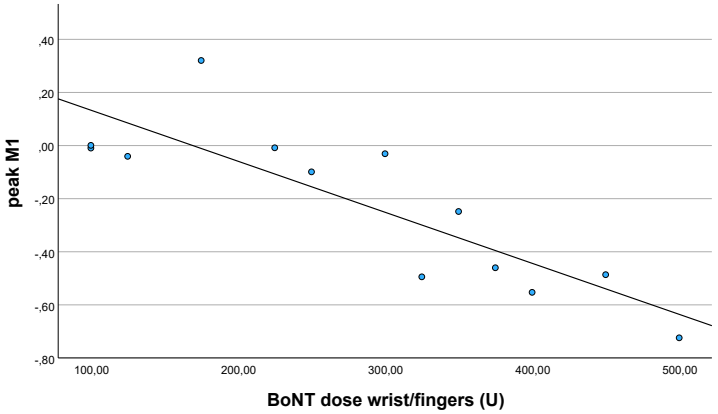


c) Area under the curve of M1

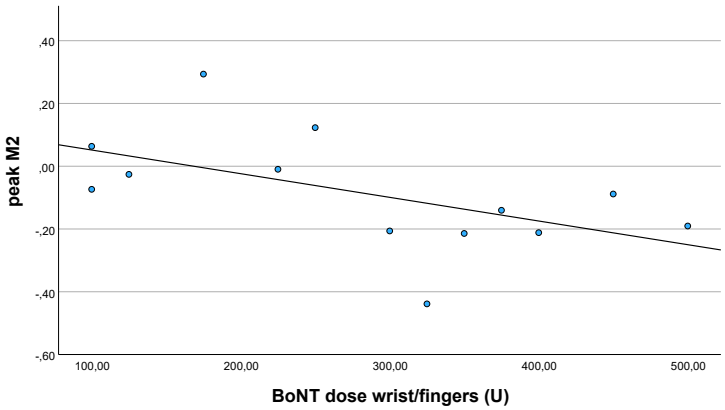


d) Area under the curve of M2

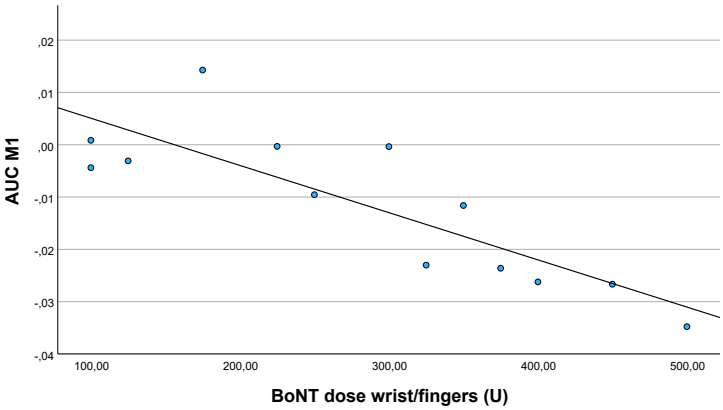
Figure B.5: Change of the EMG-related parameters of the ECR, between baseline, 6 and 12 weeks after. Each line represents a patient and the thick line represents the median.



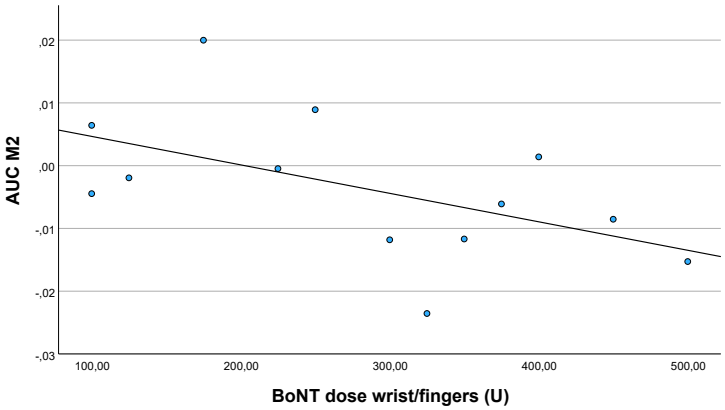
a) Peak of M1



b) Peak of M2



c) Area under the curve of M1



d) Area under the curve of M2

Figure B.6: Scatterplot of change in values of EMG-related parameters, between sessions 1 and 0, for the FCR, and dose of injected BoNT, for the FCR. Every dot represents a patient.