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OPTIMIZATION OF SPASTICITY REDUCTION USING BOTULINUM TOXIN TYPE A INJECTIONS IN CHILDREN WITH CEREBRAL PALSY

ROLE OF MOTOR END PLATE-TARGETED INJECTIONS

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“Beter begrijpen om beter te genezen.”

Christian De Duve

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1. Background of the research project

In the early 1990's intramuscular injections with Botulinum toxin (BTX) were introduced as a method to produce a selective tone reduction of human skeletal muscles.[1] Over the past 2 decades, BTX has become a fundamental part of the treatment of children with spastic cerebral palsy (CP).[2,3] Many clinical studies have reported good results of this treatment, but also demonstrate considerable variation in outcome. The lack of congruent responses can be attributed to patient and muscle selection but also to inconsistent injection variables and problems with outcome measures.[4]
The clinical use of this product in the care for children with CP has made us realise that many factors of the injection protocol still need to be optimized to obtain good and reproducible results.

Several practical guides[5,6] provide descriptions of the BTX injection-technique. They report on dose, dilution and location of the injection for the frequently injected human skeletal muscles. A number of studies delineate the optimal dose and dilution of BTX for a limited number of muscles. Interestingly, there is almost a complete lack of information regarding the optimal location along the targeted muscles to inject BTX. Specification of this location is also not consistent in the different papers describing the injection technique. Although, in some animal studies[7,8] and in one human study[9], it has been documented that injecting the toxin at specific places in the muscle, improves the clinical effect. Finding the optimal injection sites for the frequently injected muscles in patients with CP, might improve the effectiveness of the treatment.

Not only from a clinical, but also from an economical point of view, it is important to improve the BTX injection technique. A more effective injection with BTX will give a better spasticity reduction with less of the expensive toxin.

2. Cerebral Palsy

Cerebral palsy is defined as a disorder of the development of movement and posture that is attributed to non-progressive disturbances of the developing fetal or infant brain. The motor disorders of CP are often accompanied by problems with sensation, cognition, communication, perception, behavior or by a seizure disorder. It is caused by an event that interrupts the normal brain maturation before the age of two years, resulting in a permanent brain lesion. The brain lesion itself is static, but the clinical picture of CP evolves over time.[10,11]
Cerebral palsy is the most common cause of physical disability in children, with a prevalence between 2 and 3 in 1000 live births. Its severity can range from a subtle motor impairment to involvement of the whole body. There are several classifying methods, based on anatomic distribution of the motor disorder, neuro-imaging findings, type of motor abnormalities, causation and timing of the lesion. Patients with CP have traditionally been grouped by the predominant type of motor disorder (spastic, dyskinetic or ataxic) or by the distribution of the motor impairment (diplegia, hemiplegia, quadriplegia).[10] More recently, functional grading scales such as the GMFCS (Gross Motor Function Classification System)[12] or the FMS (Functional Mobility Scale)[13] are widely used to describe ambulatory status or walking ability.
In the clinical picture of the child with CP, we discriminate primary problems, due to the brain lesion itself, from secondary problems. Primary problems include abnormal muscle tone, lack of strength,

loss of selectivity and impaired balance. The abnormal muscle tone can be either spasticity, dystonia or mixed tone. Secondary problems develop over time due to the combination of these primary problems and growth. Short skeletal muscles, fixed contractures and bony deformities such as malrotations are the most important secondary complications. These result in progressive gait disturbances in ambulatory children and hip dysplasia or hip luxation in children with more extensive motor impairment.[14,15]

Spasticity was described by Lance as being characterized by a velocity-dependent increase in tonic stretch reflexes resulting from hyperexcitability of the stretch reflex.[16] The exact pathophysiology of spasticity is still unclear, but it is generally explained as an imbalance in the central excitatory and inhibitory modulation of neuromuscular reflex loops, leading to this hyperexcitability of stretch-reflexes.

This spasticity, which occurs in 80 to 90% of children with CP[17], is a very important cause of development of the static muscle contractures and bony deformities.[15,18] Therefore, it is important to reduce spasticity to prevent the development of secondary problems and to delay or avoid the need for surgery in young children.[19]

Overall, in the early phase of development, treatment of the motor problems of children with CP is conservative (non-surgical) often including physical therapy, orthotic management, stretching casts and spasticity management. Spasticity can to some extent be addressed by oral medication when a more general spasticity reduction is wanted. A more focal spasticity reduction is obtained by BTX or phenol. The elicited neuromuscular blockade will cause a temporary reduction in reflex muscle activity. More invasive techniques for spasticity reduction are Selective Dorsal Rhizotomy (SDR) and Intrathecal Baclofen Pump (ITB). Early intervention mostly targets the primary problems with the goal to prevent secondary problems as much as possible. In particular muscle length must be maintained to prevent the need for muscle lengthening surgery, as this type of surgery adversely affects strength.[20,21] In the spasticity treatment of young children with CP, BTX has been found very effective in helping to maintain muscle length and, to some extent, to prevent the development of secondary problems.[19] As its effect is focal (limited to the injected muscle) and reversible, it is also used to guide future spasticity management when SDR or ITB are considered.

Treatment of secondary problems is mostly surgical. A limited muscle contracture can be addressed by serial casting, but more extensive treatment is needed for correction of bony malalignements. Current state of the art is a ‘single event multilevel surgery’ preferably after the age of 7 years to correct the bony lever arm dysfunction.[22] The role of the orthopaedic surgeon is to alter the biomechanics in order to improve the function of the walking or sitting child.

In view of the complexity of the motor disorder and the presence of other health issues, a multidisciplinary team must be involved in the care of children with CP.

3. Botulinum toxin type A

a. Working mechanism

BTX is produced by the bacterium Clostridium botulinum and is a potent neurotoxin that inhibits the release of neurotransmitter chemicals. Historically, it is known as the cause of botulism, a paralysis caused by the ingestion of contaminated food, wound infection or colonization of the infant gastrointestinal tract by Clostridium botulinum. After the discovery of its working mechanism, research for the use of this toxin as a therapeutic drug began. Today, there are seven different serotypes (A-G) known, with different antigenic specificity and different therapeutic profiles. Only type A and B have been developed for commercial use in routine clinical practice; other serotypes are under investigation.[23]

In clinical use the toxin is injected in the target muscle. In this muscle the toxin will cross about four to five sarcomeres (diffusion). After a few hours, it will arrive at the neuromuscular junction (NMJ), more specifically at the motor end plate (MEP). There it will block neuromuscular transmission by inhibiting the release of acetylcholine. The toxin's clinical effect is seen after about 4 days. Optimal neurotransmission blockage is seen after one month and the chemodenervation remains for three to four months.[24]

The physiology of the toxin has been comprehensively studied. Each molecule of BTX is comprised of a heavy and light chain, linked by a disulfide bond. The toxin binds to the cell surface of the cholinergic nerve terminals by means of the heavy chain and is internalized by endocytosis. The disulfide bond undergoes reduction and the heavy and light chains separate. The light chain then binds with high specificity to the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complex and cleaves specific proteins of the SNARE complex. Cleavage by BTX impedes the function or formation of this SNARE complex and therefore the docking of the acetylcholine vesicle on the inner surface of the presynaptic membrane and the release of acetylcholine are prevented.[24,25] Figure 1.

The toxin will thus cause reversible denervation atrophy without fibrosis. The end effect is weakening and relaxation of muscle overactivity in people suffering high muscle tone as in CP. This results in a biomechanical change in the muscle's function and makes it amenable to stretching and lengthening. In addition, the weakening allows an opportunity to strengthen antagonist muscles thereby providing the possibility to restore some of the balance between the two muscles.[24]

The initial phase of reinnervation occurs through sprouting. Newly formed nerve sprouts, not the parent terminal, will elicit muscle contraction with nerve stimulation after about one month. In a second and distinct phase, a return of vesicle turnover to the original terminals follows and this is accompanied by loss of exocytosis activity from the sprouts and gradual elimination of the sprouts. The return of synaptic function to the original neuromuscular junction associated with elimination of the sprouts requires approximately three months.[23-25]

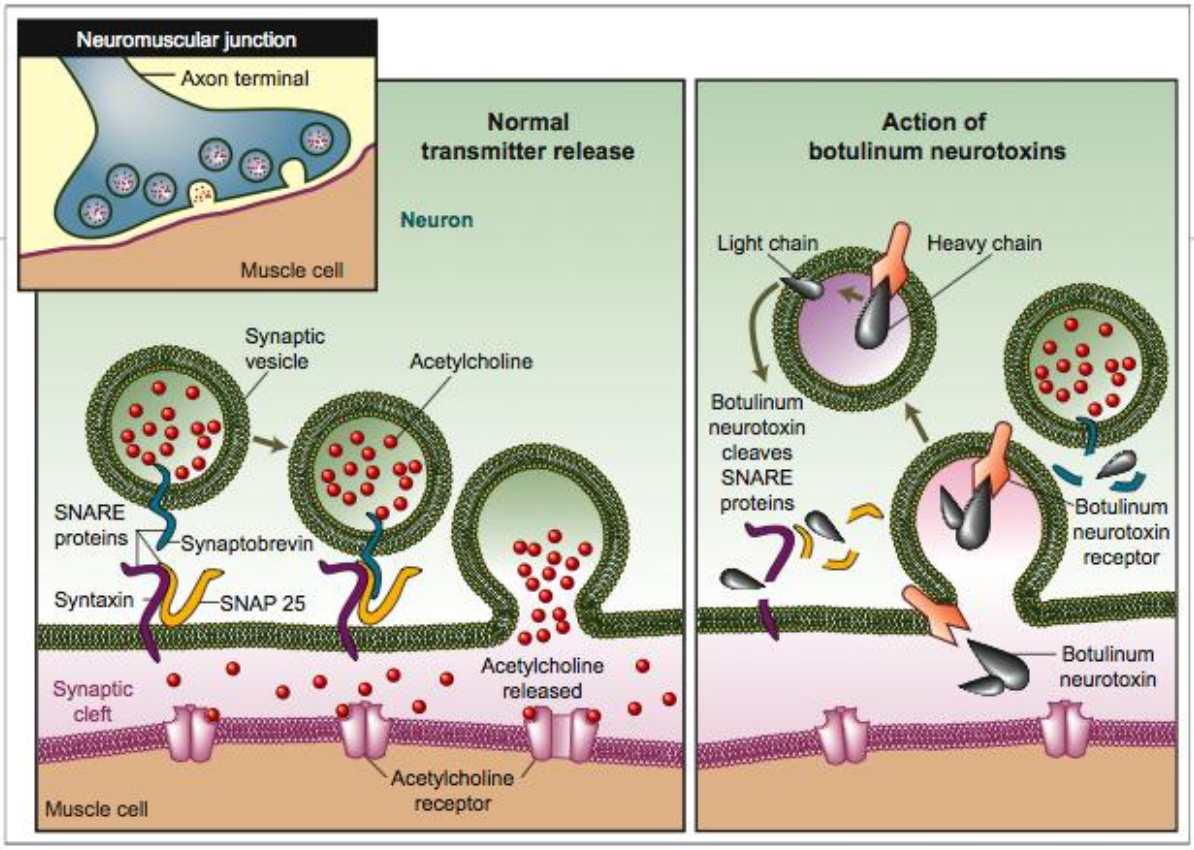

Figure 1. Working mechanism of Botulinum toxin.

b. Evidence for the use of botulinum toxin in CP

BTX is widely used for the treatment of spasticity in children and adults with CP. Since the first documentation of its use in CP in 1993 (Koman)[26], many articles have been published about its effect on spasticity in CP. When reviewing these studies -open label cohort studies, retrospective and prospective, and randomized controlled trials (RCT)- there is now convincing evidence for the -time limited- beneficial effect of BTX in decreasing muscle tone in children with upper and lower limb spasticity associated with CP.[3,27,28] There is clinical evidence establishing the role of botulinum toxin to improve function in the upper and lower limb. Decreasing muscle tone in the lower limbs translates to improved gait in CP children [28].

Numerous outcome measures have been used to describe the effect of BTX in patients with CP. When looking at these different reports, we find different measurement tools. The 'International Classification of Functioning, Disability and Health framework' classifies function into four domains: body structure and function, activity, participation and personal and environmental factors.[29] This provides a framework for classifying outcome measures in a research setting. The most immediate effect of BTX is denervation atrophy and reduction of spasticity (body structure and function). These body-level outcomes are easiest to measure objectively and are most likely to manifest changes because this domain is the most proximate to the intervention. Outcomes in the domain of activity and participation are of high interest, but represent the more secondary effects of the intervention. Most articles reporting on the effect of BTX in CP, report on the effect on body structure and

function, while only a minority reports on activity and participation. The reports on activity frequently use gait analysis parameters.
Still, the number of different outcomes measures used in all these publications is remarkable. This is most certainly partly due to the failure of traditional measures to identify the impact of BTX. High-quality outcome assessments need to be consistent and free from error (reliable) and should measure what is intended to be measured (valid). The tool also needs to be responsive to change, i.e. able to detect minimal, but clinically important differences. In many studies measures such as Ashworth score, reduction of pain or reduction of spasms are used as primary outcome measures. These however, are subjective, not very sensitive to change and do not always measure the effect of BTX itself, but either other muscle properties (visco-elastic) or secondary effects of spasticity reduction.[30-32] Recently, more direct and objective measures of spasticity have been developed; some of these in our center.[33] Also direct measurement of the denervation atrophy caused by the toxin using imaging techniques has recently been found to be a sensitive tool to measure the effect of BTX.[34]

The lack of knowledge on the long standing effect of the toxin on the injected muscles of children with CP remains a problem. In particular, the amount of atrophy of the injected muscles is a concern. By their nature, most RCTs have ethical, clinical and practical limitations including the relatively short duration of the period of control. Parents may agree to have their children randomized to standard or placebo treatment but only for short periods of time. This limits the information that can be derived from RCTs concerning longer follow-up. However, additional information exists from prospective and retrospective cohort studies. The use of BTX –integrated in a treatment regimen with other conservative measures such as physiotherapy and orthotic management- will lead to fewer secondary problems. Fewer muscles will require lengthening at a young age and fewer children will require orthopaedic surgery.[18] The children that need surgical correction of the bony malalignements can proceed to single-event-multi-level surgery at an optimal age.
Further, it has been concluded that BTX is considered safe for use in children. Adverse events in the trials are mild and/or self-limited, with the most common complaints being pain at injection sites and transient weakness.[27,28]

Finally, all reviews on the use and effect of BTX mention that future studies should focus on optimized injection schemes. In order to compare these different injection protocols sensitive outcome measures need to be used.

c. The injection procedure

Guided by the results of the static clinical examination and the dynamic evaluation of the patients gait, the clinician will differentiate the spastic muscles and will decide which muscles to inject with BTX. As most children with CP have a multilevel problem (spasticity in muscles on different anatomic levels such as plantar flexors, knee flexors and hip flexors), very often, several muscles are simultaneously injected. Due to the multiple indications and heterogeneous grouping of target muscles, it is not surprising that the evidence base for many of the injection variables concerning the most optimal use of the toxin remains limited.[27,35]

The **dose** used per muscle depends on its volume, the degree of spasticity and its involvement in the pathological gait pattern and the experience from previous BTX treatments each specific patient.

Recommended doses of BTX have been established by dose-ranging studies combined with expert opinions.[36,37] These doses per muscle are set depending on which BTX preparation is used. Several preparations of BTX are commercially determined and there are distinct differences between them. They not only have different migration characteristics, but also different conversion ratios for different muscles. It is better for the clinician to learn the effect of a specific dose of one or a limited number of the different preparations on a specific muscle than to work with a conversion factor.[3,27,28] Further, the dose-ranging studies have largely focused on the gastrocnemius muscle and there is less convincing information available to guide dose selection for most other muscles.

The **dilution** of the toxin is another important injection variable. In most RCTs dilution of BTX varies and so far, there is no high-level evidence to guide the choice of dilution. More dilute solutions appear to have a greater spread across the muscle, but this has only been demonstrated in adults.[38-41] Some studies have shown that this results in a better clinical effect, but other studies have not confirmed this.[42,43] Lack of a sensitive measure of the effect of the toxin is probably part of the problem. Furthermore, in small (especially upper-limb) muscles, working with a smaller dilution might be optimal to limit the diffusion of the toxin through the fascia.[39]

BTX has a great propensity to go to the NMJ.[24] These NMJs are situated at the MEPs of the terminating nerve branches supplying the muscle. We therefore not only need to administer the toxin accurately into the selected muscle, but also need to bring the toxin close to its site of action which is the MEP zone of the muscle.[38] In animal studies[7,8] it has been demonstrated that a MEP targeted injection increases the paralytic effect and this was confirmed in one human study in adult biceps muscles after stroke[9]. It is not clear weather this is true for all muscles and indications. The toxin also has a tendency to spread in the muscle and even to some extent, through the fascia. Animal studies have confirmed that the toxin may diffuse up to 4.5 cm along the length of the muscle[45] and the presence of fascia reduces the spread by only 23%[44]. Descriptions of the injection-technique of BTX are given in several practical guidelines[5,6] and articles[3,4,27,28,37]. However, in these guidelines, the exact localization of MEP zones is usually not taken into account. The presumed localization of the end plate zone in the middle of a muscle belly is used, because, for a lot of the frequently injected muscles, the exact anatomy of the innervation and the localization of MEP zone is unknown. Choice of the **number and exact position of the injection sites** is still based on anatomical considerations because there are no high-level clinical trials to support injection site choice.

The use of ultrasound to properly **identify the correct muscle** greatly improves injection especially for small or deep muscles. Improved clinical results using ultrasound guidance have been reported.[46] Therefore ultrasound is emerging as the preferred modality to improve the accuracy of intramuscular injections of BTX in children with CP.

Additionally, the treatment of spasticity is enhanced by a program of **physical therapy** following the BTX treatment. Physical therapy often includes intensive stretching and strengthening.[47-49] During the period in which spasticity is reduced, a more optimal enforcement of the antagonist muscles and training of a more normal gait is possible. Especially the gluteus maximus muscle should be trained as hipextension is crucial in the gait of children with CP. When the psoas muscle (hipflexor) and hamstrings (hipextensors but also kneeflexors) are weakened by BTX, strengthening of the gluteus maximus muscle is often more easily achieved. The effect of the BTX treatment will not only be augmented, but also prolonged.

Finally, a close **follow-up** is necessary. It is important to identify whether or not the treatment objectives have been met to plan further treatment. As stated earlier, spasticity management is an important part of the treatment of the young child with CP.

To conclude, there remain many unresolved issues concerning the optimal injection technique of BTX in children with CP, to some extent about the dose and dilution but even more concerning the optimal injection sites.

4. Motor end plates

The NMJ is the synapse between the nerve and the muscle cell. It will efficiently communicate the electrical impulse from the motor neuron to the skeletal muscle to signal contraction. The NMJ is comprised of portions of three cells: motor neuron, muscle fibre and Schwann cell. The nerve terminal occupies a shallow gutter in the muscle fibre and is capped by processes of Schwann cells. Active zones in the nerve terminal directly appose junctional folds in the postsynaptic muscle membrane. The motor nerve terminal is specialized for neurotransmitter release. It contains a large number of synaptic vesicles with the neurotransmitter acetylcholine. The flattened end of the motor neuron that transmits the neural impulse to the muscle is also called the *motor end plate (MEP)*. The postsynaptic muscle fibre membrane is specialized to respond rapidly and has an extremely high concentration of acetylcholine receptors. The NMJ demonstrates structural and functional variations across species, even between different muscles and between muscle-fibre types. In the typical mature mammalian muscle, it has a 'en plaque' morphology with a size of about 50 µm in diameter.[50] Figure 2.

The motor neurons that innervate vertebral skeletal muscles have their cell bodies located in the brainstem or in the ventral horn of the spinal cord. Each motor neuron has a myelinated axon that travels toward a muscle through peripheral nerves. Motor axons seldom branch en route and therefore innervate only a single muscle. However, when the axon enters the muscle, it will branch to innervate tens to hundreds of individual fibres. As each axon branch approaches its target fibre, it loses its myelin sheath and further branching or terminal branching occurs until the nerve reaches the muscle fibre forming the MEP, as part of the NMJ. The combination of a motoneuron plus all of the muscle fibres it innervates, is called a motor unit. Figure 3.

During embryonic development, motor axons already reach target muscles as the myoblasts are fusing to form myotubes. Once the motor axon contacts a newly formed myotube, synaptic transmission commences quickly. The axons contact these myotubes near their site of entry into the muscle and form synapses. The myotube will subsequently grow symmetrically at either end. Therefore, in adults, synapses are clustered in a central *end-plate bands or zones*.

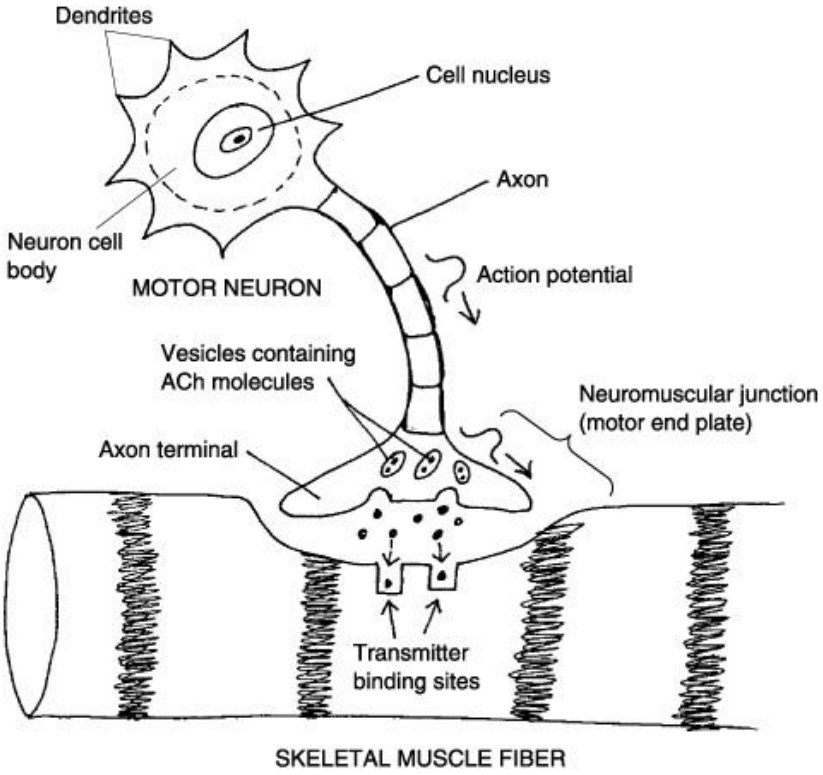

Figure 2. Schematic presentation of motor neuron, muscle fibre and neuromuscular junction.

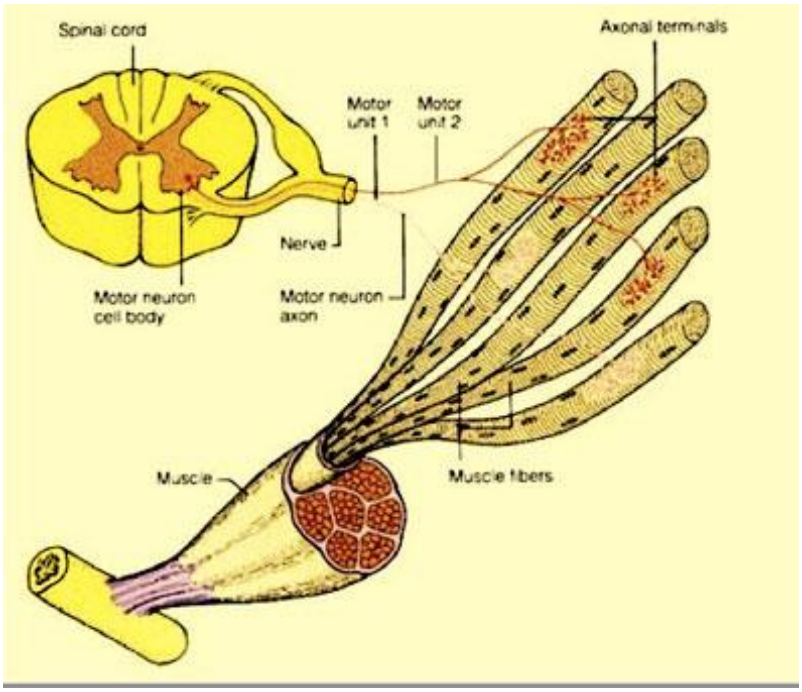

Figure 3. Cell body of the motor neuron is located in ventral horn of spinal cord. Its motor axon innervates multiple muscle fibres.

Most muscle fibres are innervated by more than one motor axon during the developmental period. All inputs but one are eliminated in the early postnatal period in a process called synapse elimination. One NMJ gradually gains territory as the others lose. Anatomically, the process occurs as a stepwise loss of branches. The time-line for this process was studied in a few animals. For example, in the rat diaphragm, about 5-15% of the fibers become singly innervated on each postnatal day until all fibers are singly innervated at two weeks of age.[51] Juvenile rats (aged 1 month) had a greater NMJ density in their muscles compared with adult rats. However, juvenile and adult rats had a similar NMJ distribution in both muscles. This study suggests that the distribution of NMJ in adult rats may be extrapolated to juvenile rats.[52] It is not clear whether distribution data from human adults can be extrapolated to juveniles. Because of very limited sources of juvenile cadavers, no anatomical studies of MEP distribution in juvenile humans have been published. Studies have been performed on adult and infant cadavers, but it is unclear whether these results can be extrapolated to juveniles.
There are some mammalian muscles that contain fibres with more than one endplate or that have polyneural innervation. For example, the human brachioradialis muscle may contain some long polyneurally innervated fibers next to short, serially linked, singularly innervated fibres.[50]

Current knowledge on the localization of the MEP zone of human skeletal muscles is based on a few older histological studies and, for some of the more frequently injected muscles, also on more recent anatomical dissection studies. The histological studies have been based on cholinesterase staining of muscles from small children. Coers[53] observed in the late 1950s in longitudinal sections of whole muscles of three infant cadavers that the endplates were always situated at the midpoint of the muscle fibre and that they were concentrated in narrow zones. Christensson (1959)[54] examined muscles from stillborn infants. She confirmed that in the unipennate muscles the MEPs formed a transverse band through the middle of the muscle, and that in the bipennate muscles there was a concave endplate band. More recently, Aquilonius (1984)[55] studied the distribution of MEPs in three adult muscles by staining longitudinal cryosections of whole muscle for cholinesterase. His findings are in agreement with the results of Coers and Christensson. In all these studies the description of MEP localization is in relation to the dissected muscle or muscle belly itself, but not with measurements in relation to external anatomical landmarks. Therefore, their practical use when injecting BTX is limited. Another problem is that not all skeletal muscles were examined and histological data on some very frequently injected muscles is missing. The more recent anatomical descriptions were done by macroscopic and in some studies by stereoscopic microscopic dissection of the nerves supplying the muscles.[56-64] In some studies the intramuscular branches and terminal arborisations are traced until it was no longer possible to follow them. Others only describe the motor points or the point where the motor branch enters the muscle belly. This allows us to determine the proximal and distal limits of the territories where most nerve endings were observed in relation to external anatomical landmarks. However, this has only been done for a limited number of human muscles and not for all of the most frequently injected muscles.

5. Aims and outline of the doctoral project

The overall goal of this thesis is to improve the effectiveness of treatment with intramuscular BTX injections in the lower limb in children with CP by optimizing the injection location. To achieve this, we first need to identify the MEP zones of the frequently injected human muscles of the lower limb. Secondly, the clinical importance of injecting these MEP zones in children with CP will be explored, through the application of innovative assessments.

These objectives will be realized through several studies, which are planned in two major research parts.

Part 1: Localization of the MEP zones of the frequently injected muscles of the human lower limb.

- All available data from literature on the innervation and MEP zones of these muscles will be examined, compared and referred to anatomical references in the lower limb.
- For clinically important muscles, for which no data on the localization of the MEP zone can be found in literature, a cadaver study will be carried out.

Part 2: MEP targeted injections of muscles of the lower limb in children with CP.

Clinical trials will compare different injection techniques for those clinically-important muscles of the lower limb where the current injection localization differs much from the MEP targeted localization. In order to measure the different outcomes, measurement tools need to be found specific to those muscles under investigation.

It is hypothesized that optimizing the location of BTX injections according to the MEP zones will improve clinical spasticity reduction and increase the effectiveness of the treatment.

Part 1

Localization of the motor end plate zones of the lower limb muscles.

1. **Introduction.**
2. **Localization of motor nerve branches of the human psoas muscle.**
3. **Localization of the motor end plate zone in human skeletal muscles of the lower limb: Anatomical guidelines for injection with botulinum toxin.**

Part 1, Chapter 1

Introduction

In this era where scientists start to decrypt human DNA, one would believe that basic anatomy such as the localization of the terminal innervation zones or MEP zones of the human skeletal muscles has its place in anatomic textbooks. Unfortunately, knowledge on muscle innervation in these textbooks is commonly limited to the motor point, this is the point where the nerve enters the muscle belly. The details of its course thereafter is not presented and for a lot of muscles even unknown.

When gathering relevant information on terminal intramuscular ramifications or MEP zones from muscles of the human lower limb in literature, there are roughly two groups of studies that provide information.
In the late 1950's two groups of researchers studied muscles from small children (stillborn infants or cadavers of young children) with cholinesterase staining. They did this for a limited number of muscles. From the lower limb, Coers and Durand[53] only studied 3 muscles (tibialis anterior, soleus and sartorius muscle); Christensson[54] examined 4 muscles (semitendinosus, gracilis, rectus femoris and extensor digitorum brevis muscle). The use of very small muscles from infants was mandatory because for these histological studies serial sections needed to be made, followed by staining and examination of the specimens under a microscope. In 1984, Aquilonius et al.[55] repeated to some extent this work using 3 adult muscles, including two from the lower limb (tibialis anterior and sartorius muscle). He worked with cryosections formed by a heavy cryostat microtome. That this was quite complicated can be indirectly demonstrated by the fact that no other reports can be found studying larger skeletal muscles using these histological methods. A limited number of studies[56,57] more recently used a Sihler's staining. This is a nerve staining technique enabling visualization of the detailed intramuscular nerve distribution. For these studies serial sections or microtome's are not necessary, but this limits these procedures to rather thin "translucent" muscles.
Only in the late 1990's, a novel interest in the localization of the MEP zones was generated. Obviously, the introduction of BTX for clinical indications a few years earlier was for some researchers the drive for their quest. For some of the frequently injected muscles anatomical descriptions were given by macroscopic and in some studies by stereoscopic microdissection of the nerves supplying the muscles.[58-64] An advantage of this research method is that it allows to determine the proximal and distal limits of the territories where most nerve endings are observed in relation to external anatomical landmarks. The previous histological studies only studied the muscle belly and not the relation to the total muscle-tendon structure or the relation to external landmarks. Unfortunately, not all of these dissection studies provide information about the terminal nerve ramifications, some only present data on motor point localization. As these studies scarcely appeared in literature, they were not yet incorporated in the textbooks on BTX injection[5,6] (often provided by the firms that commercialize the toxin) or in articles studying outcome of these injections. So, there was certainly a need for a review on this topic, ideally supported by evidence that injecting these MEP zone improves effectiveness of the BTX injection.

After gathering all available histological and anatomical studies, recalculating this to clinical relevant external landmarks and comparing this with clinical injection-practice or with the injection procedures in the BTX-textbooks[5,6], it was found that for some muscles the MEP zone can be more

precisely demarcated and for many other muscles its location is somewhat different than the currently injected areas in clinical practice. Further, there was no information on the intramuscular innervation pattern or MEP zone of the psoas muscle.

To find the MEP zone of the psoas muscle both research methods (histological and anatomic dissection) were explored. To study adult psoas muscles a large cryostat microtome was necessary, but not available. Supported by colleagues from the department of Pathology KULeuven, we studied one fetal psoas muscle. Unfortunately we were not able to collect more specimens, as the Ethical Committee advised us to seek other research methods. Finally, a cadaver study –in collaboration with the department of Anatomy University Antwerp– enabled us to identify the intramuscular terminal nerve ramifications, and thus the MEP zone, of the psoas muscle.

In the following two chapters the details of this psoas cadaver study and the review on the MEP zones of the muscles of the lower limb (already including the results of the psoas study) are presented.

Part 1, Chapter 2

Localization of motor nerve branches of the human psoas muscle.

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Abstract

Introduction End-plate targeted Botulinum toxin type A (BTX) injections achieve an optimal neuromuscular blockade. The goal of this study is to identify the motor endplate (MEP) zone in the human psoas muscle through dissection.

Method In 24 psoas muscles, the nerve branches and its intramuscular course were followed, by stereoscopic microscopic dissection, as far as their terminal ramifications.

Results From the lumbar plexus, an average of 3.7 (range 2-7) nerve branches enters the psoas muscle. The proximal and distal limits of the MEP-zone are situated at about 30% and 70% respectively of the distance between the twelfth thoracic vertebra (Th12) and the passing of the psoas under the inguinal ligament. In reference to the promontorium (P), these limits are respectively from 50% of the Th12-P distance to 20% of the P-pubis distance.

Conclusion This first study on the MEP zone of the human psoas muscle can allow the clinician to inject BTX close to its site of action.

Introduction

Spasticity is a feature of many diseases of the central nervous system. The increased tone that characterizes it, leads to disability and contractures. The psoas muscle is often involved, especially in cerebral palsy, causing hip flexion contracture and hip (sub)luxation. An optimal use of spasticity reduction with Botulinum toxin type (BTX) injections, can prevent these complications to some extent, and improve function or comfort.[1-5]

BTX-A produces a reversible, local tone reduction. It blocks neurotransmission at the neuromuscular junction (NMJ). Since the effect of the toxin depends on uptake by the presynaptic membrane of the motor end plate (MEP) of the NMJ, the injection should be given into the MEP-zone.[6,7] Data on experimental animals confirm this hypothesis. Shaari and Sanders showed in their animal model that the degree of muscle paralysis induced by BTX is proportional to the proximity of the toxin to the motor end plate.[8,9] So far, there exists only one human study to confirm this: Gracies et al. (2009) achieved a greater neuromuscular blockade with endplate targeted BTX injections versus non-targeted injections in the human spastic biceps.[10]

Local diffusion of the toxin in the muscle itself can minimise the importance of injecting at the MEP-zone. This is especially so for small muscles. Animal studies have confirmed that the toxin may diffuse up to 4.5 cm along the length of the muscle.[9,11,12] The psoas muscle however is a large muscle with considerable length in which diffusion is very unlikely to bring the toxin to the other end of the muscle. Multiple injections can bring the toxin to a larger region, but in the psoas muscle this means repeatedly injecting a deep region with its possible complications by damaging other organs.

Current knowledge on the localization of the MEP-zone of human muscles is based on some older histological studies and also on more recent anatomical dissection studies. Coers and Christensen observed in the late 1950s that the endplates in longitudinal sections of whole muscles were concentrated in narrow zones.[13-16] The endplates were always situated at the midpoint of the muscle fiber. But not all skeletal muscles were examined; there are no histological data on some of the frequently injected muscles, among them the psoas muscle. The more recent anatomical descriptions of dissections on the intramuscular localization of nerve branches of some muscles allow us to determine the proximal and distal limits of the territories where most nerve endings were observed and to relate this to external anatomical references. Until now there are no such studies for the psoas muscle.

Through anatomical dissection it is possible to trace the motor points (MP), where the motor branch enters the muscle, but also to follow these motor nerve branches up to the terminal intramuscular ramifications. The zone with these terminal branches corresponds anatomically to the MEP-zone.[17-26]

The goal of this study is to identify the zone of terminal intramuscular nerve ramifications in the human psoas muscle and to relate this to reproducible surface anatomical markings. This knowledge will enable us to specifically inject the MEP-zone and thus achieve optimal spasticity reduction in the psoas muscle with minimal doses of BTX.

Material and methods

Dissection was performed on 13 embalmed human cadavers, in order to localize the nerves supplying the psoas muscle.

The study was approved by the review board of our institution. All cadavers were from persons who donated their body for research and education and were embalmed with zinc-chloride.
Each cadaver was placed supine, with the spine and the hips extended in the anatomical position. The skin, subcutaneous fat and abdominal muscles were incised in an H-shape, longitudinally along the midline and horizontally between the ribs and the pelvis, and the flaps reflected. The omentum and bowels were removed. The retroperitoneum was opened from the midline towards lateral, from cranial to caudal. The retroperitoneal part of the duodenum was not removed. The kidneys and capsula adiposa renis were mobilized so they could be reflected cranially or laterally to have good exposure of the spine and psoas muscle. The same was done for the large vessels (aorta and vena cava inferior) (figure 1).
In order to reach the nerves (rami ventrales nervi spinales and the lumbar plexus) the psoas muscle was partially stripped from the spine from anterior to posterior and this from cranial to caudal. The nerves that penetrated into the muscle belly were noted, this point was noted as the motor point (MP). We traced the intramuscular course of these nerve branches and their terminal arborisations until it was no longer possible to follow them (figure 2). Dissection was only done as far as the inguinal ligament, not to the psoas insertion on the trochanter minor. This still enabled us to follow all the nerve branches to their final ramifications.
Dissection was initially macroscopic, followed by stereoscopic microdissection with the use of a 4 times magnification lens (Keeler®).
For each cadaver the following bony landmarks were identified as reference points:

- The caudal limit of the vertebral body Th12 at the midline (Th12)
- The disk of L5S1 (promontorium) at the midline (P); in small patients the promontorium is relatively easy to palpate externally and can be used as a palpable landmark; even in normal adult patients the promontorium is palpable under anaesthesia.
- The pubis at the midline (Pu)
- The spina iliaca superior anterior (SISA)
- The point where the psoas muscle passes under the inguinal ligament (L) on a line from Pu to SISA

These reference points were chosen as they are fixed bony landmarks, except for L which was derived from the other data.

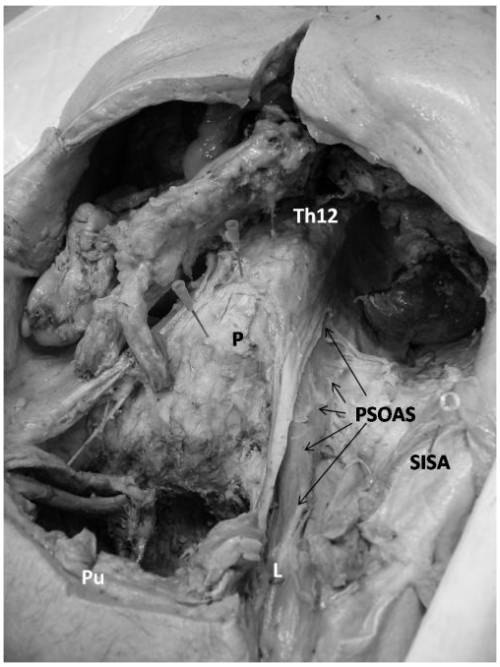

Figure 1.

Overview of dissection area.
Bowels and retroperitoneum
over psoas muscle are removed.
Pu=pubis; SISA= spina iliaca superior anterior
L= location where psoas muscle passes under inguinal ligament; P=promontorium;
Th12= twelfth thoracic vertebra

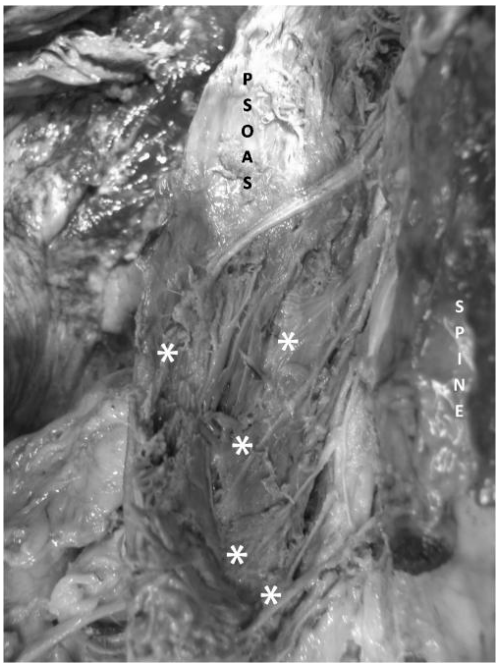

Figure 2.

Detail of psoas dissection
* intramuscular nerve

In addition to dissecting tools, a tape measure was used; each measurement was performed to the nearest millimetre. All measurements were done by the first author.
Observations, sketches and relevant data were noted in a logbook and analyzed to identify trends and variations that may exist. Photographs taken with a digital camera were used to aid analysis of the written data.
Descriptive statistics were calculated. The Wilcoxon test was used to determine the presence of symmetry between left and right sides. To assess whether the difference between number of motor branches for different ages was significant, the Spearman test was used. A similar relationship was also examined for the number of branches versus the size of the MEP zone and versus size of the psoas muscle.

Results

All 13 cadavers were of Caucasian origin; there were 7 males and 6 females; aged 50-93 years at the time of death. No evidence of abdominal or hip trauma was noted and the cause of death was not related to any pathology that could have affected the innervation pattern of the psoas muscle. Two left muscles could not be used due to other dissections, so we were able to study 11 left sided and 13 right sided muscles.

In agreement with anatomical textbooks[27], we found the psoas muscle originating at the basis of the transverse processes of all lumbar vertebrae, at the bodies of all lumbar vertebrae and sometimes part of the twelfth thoracic vertebra and at the intervertebral disks above each lumbar vertebra. The total mean length of this origin was 13.17 cm (SD 2.53 cm). The most proximal part of the origin of the psoas muscle was located at a mean distance of 1.12 cm (SD 2.13 cm) caudal to the reference point of Th12. The most distal part of the origin of the psoas muscle was located at a mean distance of 0.79 cm (SD 1.16 cm) cranial to reference point P (promontorium). Only very proximally some short tendon fibers could be observed; the rest of this broad origin consisted macroscopically immediately of muscle fibers.

The innervation of the psoas muscle comes from branches of the lumbar plexus, mainly from L2, L3 and sometimes L1 or L4, as branches from the N. Femoralis. An average of 3.7 nerve branches was observed, ranging from 2 to 7. On the left side the mean number of branches was 3.92, on the right 3.55. This was not statistically significant (p=0.2502). There was also no correlation between number of branches and the age of the person (rs= 0.08796; p=0.6827) and between number of branches and size of MEP zone (rs= 0.19376, p=0.40). The size of the psoas muscle (determined by the length of the origin and also by the length of the muscle from its origin until the L-point) was not correlated with the number of branches.
During the dissection all the other nerves originating from the plexus lumbalis were encountered. The N. femoralis and the N. genitofemoralis running through and over the psoas muscle were followed distally. On this course no branches towards the psoas muscle were noted.

In order to determine the zone of terminal intramuscular ramifications in relation to the external reference points, the location of the most proximal and most distal MP and terminal nerve branches was expressed as absolute distances and as percentages of location on reference lines.
The most proximal MP or terminal nerve ramification was located at a mean of 6.38 cm (range 3-16 cm) distal of Th12, 19.33 cm (range 15-24.5 cm) proximal of L and 8.28 cm (range 2-12.4 cm) proximal of P. In 21 of the 24 muscles, this branch ran in a distal direction; for these 21 muscles we used this MP as limit of innervation area. In 3 muscles this proximal branch had a short proximal course and the location of its terminal nerve ramifications was used as proximal limit for the MEP-zone. The proximal limit for these 3 muscles was in the same location distal of Th12 as for the other 21 muscles.
The most distal nerve branch had a distal course in all studied psoas muscles and we used its terminal nerve ramifications as distal limit of the MEP-zone. They were located at a mean of 19.08 cm (range 15-22 cm) distal of Th12, 8.34 cm (range 4-14.2 cm) proximal of L and 2.79 cm distal of P (range from 3 cm proximal to 11 cm distal).

The reference line Th12-L correlated with the course of the psoas muscle from the most proximal part of the origin to the passing of psoas under the inguinal ligament. Its mean length was 26.28 cm (SD 1.95 cm). The area of MEP-zone correlated with a zone of 30.82% to 70.25% of the Th12-L distance.

In reference to the promontorium (P) this zone was from 8.28 cm (SD 2.82 cm) proximal to P to 2.79 cm (SD 3.79 cm) distal to P. Therefore, in relation to P, the proximal limit of the MEP-zone was at 50.43% of the Th12-P distance and the distal limit was at 18.98% of the P-PU distance. So, the major part of the MEP zone is at the area of and proximal of the promontorium. Figure 3 is a schematic representation of the MEP-zone.

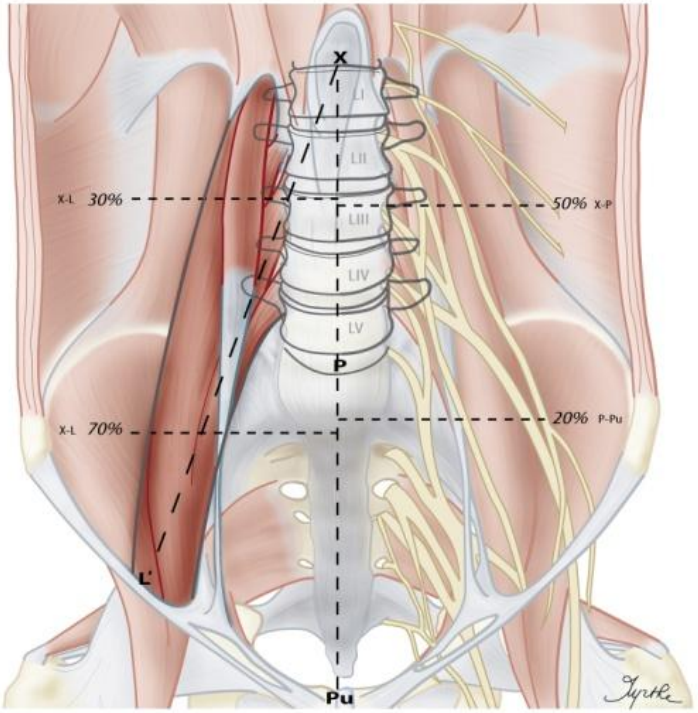

Figure 3. Schematic representation of MEP-zone of the psoas muscle
On the right side: MEP-zone in reference to Th12-L distance
On the left side: MEP-zone in reference to Th12-P and P-Pu distance
X= Th12; P=promontorium; Pu= pubis: L= location where psoas muscle passes under inguinal ligament

Variability of the location of the pubis versus the promontorium and point L (where the psoas passes under the inguinal ligament) in the frontal plane, caused by the difference in dimensions and shape of the pelvis, causes some difference between the two representations of the MEP zone if we compare the X-L versus the X-Pu reference line on a two dimensional scheme.
On the mediolateral axis, the distal limit of the MEP-zone was at a mean of 5.74 cm lateral to the midline (SD 1.45 cm). The more proximal part of the muscle lied very close to the spine; the terminal nerve endings were never more than 1 cm away from the spine. The localisation of the passing of the psoas muscle under the inguinal ligament in relation to the SISA and the pubis was somewhat closer to the SISA than to the pubis; L was at 56 % of the distance Pu-SISA.

For all these data, there were no significant differences between sex, age or left versus right sides.

Discussion

This is the first study to identify the MEP-zone of the human psoas muscle and to relate this to fixed bony landmarks. So far, no studies on innervation of the psoas muscle were done: the psoas muscle was not mentioned in the older histological studies, nor were there anatomical studies on this muscle.

The MEP is often described as being in the middle of the muscle fiber.[13-15] The psoas muscle can be described as a penniform muscle with converging fibers. With this more complex muscle morphology, the MEP-zone is not easily identifiable as the middle of a tendon-muscle unit or the middle of a muscle belly. In addition, the localization of the entry of the nerves in the muscle (motor points) will not enable us to identify the MEP-zone and especially its distal limit with enough certainty. The intramuscular path in most muscles has a distal course, but the extent is variable. Therefore, the intramuscular path of the nerve was followed until the end of each nerve branch was identified by stereoscopic dissection. This gives us a good idea of the anatomical localization of the MEP-zone.[20,22]

Similar studies to identify the MEP-zone in other muscles are described in literature. The same technique was used in a few studies, but most studies only dissect as far as the MP and do not follow the intramuscular nerve ramifications. For the muscles that were studied by Coers[14] and Christenson[13] (histological identification of NMJ by colouring techniques), the anatomic dissection findings correlate with the histological identification of the NMJ's. The knowledge of the relation of the MEP-zone to fixed bony landmarks often changes the proposed injection technique or narrows the prescribed injection zone.[17,21-23]

At present, there are several methods in clinical practice to inject BTX into the psoas muscle. A direct injection of the muscle belly through the abdominal wall in the region between the umbilicus and the SISA, as described by Molenaers et al (1999), is still routinely used by the present authors.[28] For this technique a general anaesthesia is necessary. With deep palpation, coming from lateral to push aside the intestines, the psoas is palpable. After needle placement the hip is flexed to confirm the needle's position. Some authors advice the aid of passive or active EMG guidance and ultrasonography, but this can make it more difficult to free the access to the psoas muscle with palpation.[29] An alternative technique is to inject, under analgosedation, the psoas distal to the inguinal ligament, as presented by Westhoff (2003).[30] A last alternative is injecting the psoas from dorsal, just lateral to the transverse processes of L2, L3 and L4, as presented by Ward (1999).[31]

Considering our findings, this last option very efficiently brings the toxin close to the lumbar spine in the relatively proximal area of the psoas muscle, thereby injecting the MEP zone. This dorsal approach is especially preferred in obese patients, where psoas and promontorium are difficult to palpate. In thin persons, as in children with cerebral palsy, the psoas and the promontorium are very good palpable through the abdominal wall. As general anaesthesia is often required for a multilevel BTX-injection, the proximal anterior approach is in these cases a good option. However, the infiltration must be performed very proximally. The major part of the MEP zone is next to and

proximal of the promontorium. Injecting the toxin distal to the inguinal ligament brings it in a region with no terminal nerve branches.

The importance of using end-plate targeted BTX injections is demonstrated in animal models and recently also in a clinical human study in the biceps brachii.[8,10] For other human muscles further clinical studies are necessary, but difficult because of the lack of a precise and objective measurement of spasticity for some muscles, especially for psoas muscle.[11,32]

Only adult cadavers were dissected. Our data could not be correlated to the psoas muscle in children. By describing the localization of the MEP-zone in terms of percentage distance along a reference line, we allow the measurements to be applicable to a diverse adult population, but also to paediatric and adolescent patients. However, there are no studies in humans comparing the localization of NMJ in children and adults. One animal study describes the distribution of NMJ in juvenile and adult rats as similar, although the density is greater in younger animals.[33] Further studies are necessary to determine localization in children.
Only a limited number of adult Caucasian cadavers was studied, therefore not all variations of nerve supply may have been identified. The aim of the study was not to describe individual anatomic variations in detail, but to make general observations concerning the localization of the MEP-zone. The intramuscular nerve endings are followed as far as possible, but we have not identified the motor end plates as such. Histochemical methods, such as acetylcholinesterase staining, are necessary to confirm the presence of the NMJ in the identified region or the absence of NMJ's in the more proximal and distal region. Future histological studies are needed to confirm these anatomical observations.

In summary, this study has determined the region where most nerve endings of the adult human psoas muscle are observed. We measured these data in absolute distances, but also expressed it as percentages of a reference-line. We identified an average of 3.7 nerve branches from the lumbar plexus innervating the psoas muscle. A region of intramuscular nerve endings, corresponding with the MEP zone, was identified along a reference-line from the twelfth thoracic vertebra to the passage of the psoas muscle under the inguinal ligament from about 30% until 70% of this distance. This corresponds also with a zone from 50% of the distance between the twelfth thoracic vertebra and the promontorium until about 20% of the distance between promontorium and pubis. This region of the muscle is close to the spine on the mediolateral axis.

This knowledge will enable us to give injections with BTX close to their site of action, which will improve the clinical efficacy.

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Part 1: Localizationd of MEP zones

Part 1, Chapter 3

Localization of the motor endplate zone in human skeletal muscles of the lower limb: anatomical guidelines for injection with BTX.

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Abstract

Aim Botulinum toxin gives a local tone reduction by blocking neurotransmission at the motor endplate (MEP). The importance of using MEP-targeted injections is demonstrated in animal models and in a clinical human study in the biceps brachii.

The goal of this review is to present all the available data on the localization of the MEP zone of frequently injected muscles of the lower limb zone and to compare this with current practice.

Methods Current knowledge on the localization of the MEP zone is based on some older histological studies and for some of the more frequently injected muscles also on a few more recent anatomical dissection studies.

Results We find that for some muscles the MEP zone can be more precisely demarcated and for many other muscles that its location is somewhat different than the currently injected areas in clinical practice. Optimal injection sites are presented for M. Gastrocnemius, M. Soleus, M. Tibialis posterior, M. Semitendinosus, M. Semimembranosus, M. Gracilis, M. Biceps femoris, M. Rectus femoris, M. Adductor longus, brevis and magnus and M. Psoas.

Interpretation We propose optimal injection sites in relation to external anatomical landmarks for the frequently injected muscles of the human lower limb in order to facilitate the efficiency of botulinum toxin injections.

Introduction

Spasticity is a manifestation of many diseases of the central nervous system. The increased tone leads to contractures and disability, therefore spasticity reduction is crucial. Botulinum toxin type A (BTX-A) gives a reversible, local tone reduction. It blocks neurotransmission at the neuromuscular junction (NMJ).[1-5] Outcome studies have demonstrated the effect of spasticity reduction of this drug, but there is variation in response. Many factors are responsible for this lack of a congruent result; injection technique is certainly one that must be taken into account.[6]

Since the effect of the toxin relies on uptake by the presynaptic membrane of the motor end plate (MEP) at the NMJ, the injection should be given into the MEP area.[7,8] The importance of using MEP-targeted BTX-A injections is demonstrated in animal models [9,10] and recently also in a clinical human study in the biceps brachii[11].

In several practical guides on BTX-A injection-technique descriptions are given were to inject the toxin for most of the upper and lower limb muscles.[12,13] In these guides, usually no references concerning the localization of MEP zones are given. The presumed localization of the end plate zone in the middle of a muscle belly is -were possible- taken into account, but, until recently, for a lot of frequently injected muscles the exact anatomy of innervation and localization of MEP zone was unknown.

Current knowledge on the localization of the MEP zone is based on a few older histological studies and for some of the more frequently injected muscles also on more recent anatomical dissection studies.

The histological studies have been based on cholinesterase staining of muscles from small children. Coers observed in the late 1950s in longitudinal sections of whole muscles of 3 infant cadavers that the endplates were always situated at the midpoint of the muscle fiber and concentrated in narrow zones. He described that two muscles (the sartorius and the gracilis) had endplates scattered throughout their entire length.[14] Christensson examined muscles from stillborn infants. She confirmed that in the unipennate muscles the MEPs formed a transverse band through the middle of the muscle, and that in the bipennate muscles there was a concave endplate band.[15] More recently, Aquilonius studied the distribution of motor endplates in three adult muscles by staining longitudinal cryosections of whole muscle for cholinesterase.[16] His findings are in agreement with the results of Coers and Christensson. In all these studies the description of MEP localization is in relation to the dissected muscle or muscle belly itself, but not with measurements in relation to anatomical landmarks. Therefore, its practical use when injecting BTX-A is limited. Another problem is that not all skeletal muscles were examined; there are no histological data on some very frequently injected muscles.

The more recent anatomical descriptions were done by macroscopic and in some studies by stereoscopic microscopic dissection of the nerves supplying the muscles. In some studies the intramuscular branches and terminal arborisations are traced until it was no longer possible to follow them; others only describe the motor points or the point where the motor branch enters the muscle belly. This allows us to determine the proximal and distal limits of the territories where most nerve endings were observed in relation to external anatomical landmarks.

The goal of this review is to present all the available data on the localization of the MEP zones of the muscles of the lower limb and to compare this with current practice. We will propose optimal injection sites in relation to external anatomical landmarks for the frequently injected muscles of the human lower limb in order to increase the efficiency of botulinum toxin injections.

Localization of the MEP-zone of frequently injected muscles of the lower limb.

To describe current practice, we will refer to the following two small books: "Pocket atlas. Treatment of spasticity with botulinum toxin" from Fheodoroff, Schurch and Heck (Saentis-Verlag, 2005)[12] and "Blue Book. Treatment of Cerebral Palsy with Botulinum toxin" by Steffen Berweck and Florian Heinen. (Child&Brain , 2005)[13]. For some muscles additional procedures described in literature will be mentioned.

M. Gastrocnemius

Current practice is to inject the proximal third of the leg, not too deep, in the medial and lateral head, where the muscle bellies have their largest diameter.[12,13]

Studies with cholinesterase staining on gastrocnemius muscle were done by Coers[14] on an infant muscle and by Christensson[15] on one muscle from a stillborn infant. The muscle fibers of M. Gastrocnemius are rather short, they converge towards the long insertion tendons lying in the middle of the distal part of the muscle and the MEPs form oval bands throughout the muscles. Figure 1.

Figure 1. M. Gastrocnemius (muscle belly; proximal: up, distal: down; no markings were made by Christensen concerning left-right or medial-lateral) after cholinesterase staining. The dots represent MEP: according to Christensen[15].

More recently, dissection, first macroscopic and secondly under a stereoscopic microscope on a total of 40 legs, was performed on the nerves supplying gastrocnemius (and soleus) by Parratte[17] ; the intramuscular branches and terminal arborizations were traced until it was no longer possible to follow them. These dissections have allowed us to determine the proximal and distal limits of the

territories where most nerve endings where observed. Distances were expressed as percentages of the length of the leg (from the medial malleolus up to the proximal margin of the medial tibial condyle). Limits of the area where the terminal nerve ramifications are most dense are: for the medial head proximal limit at 85.9%, distal limit at 65.3% and for the lateral head proximal limit at 87.7%, distal limit at 71%. Their proposed optimal injection sites are 3/4 of the way up the leg for the medial head of the gastrocnemius and 4/5 of the way up the leg for the lateral head of the gastrocnemius. Figure 2.

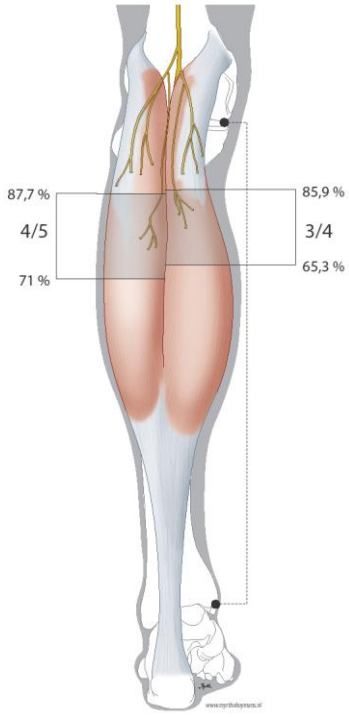


Figure 2. M. Gastrocnemius. MEP area according to Parratte[17].

Left leg, posterior view.

Kim[18] also studied the triceps surae muscles, but they described only the anatomic distribution of individual motor points (MP). 8 limbs were dissected; a MP was defined as the location where the terminal motor branch entered the muscle belly. The reference points in this study are the intercondylar and intermalleolar line and distances are expressed distal to the intercondylar line.

The MP of the medial and lateral gastrocnemius were diffusely distributed along the muscle belly with the highest and lowest MP for medial gastrocnemius at 9.6% - 37.5% and for lateral gastrocnemius at 12%-37.9% of length of lower leg. They conclude that it may be better to inject BTX into several sites than just into a middle motor point of the muscle belly.

Comparison of both studies is difficult because of the different reference points and the different definition of endpoint (terminal arborizations of intramuscular nerve branches, Parratte[17] versus motor point of terminal motor branch in the muscle, Kim[18]). We measured and compared both areas in 4 children and 4 adults. This was done by using the correct reference points and measuring the absolute dimensions of the reference lines and areas (transferred from percentages to the exact location on the limb) and marking them on both limbs of these 8 persons. For each individual child or

adult both areas were compared and we found that for all 16 limbs the region described by Parratte is smaller. For the medial head it is situated in the distal part of the somewhat larger region described by Kim and extends even a bit more distal; for the lateral head Kim's larger region extends more to proximal than distal of the region described by Parratte.

Founded upon these histological and anatomical studies, we conclude that the optimal area for injection is about the same as in current practice, only a bit smaller: at 3/4 for the medial head of the gastrocnemius and 4/5 for the lateral head of the gastrocnemius along a reference line from the medial malleolus up to the proximal margin of the medial tibial condyle. Targeting toward this area and concentrating the entire amount of the toxin in this area, might improve the effect of the toxin or enable us to reduce the dose. Figure 3.

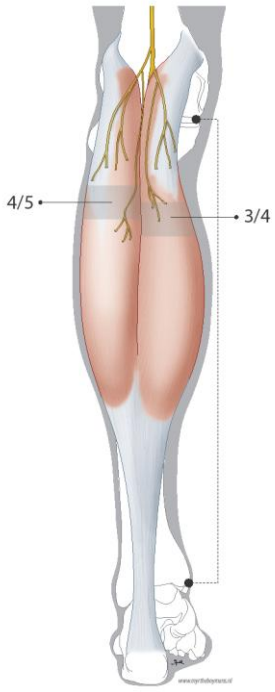


Figure 3. Optimal injection area for M. Gastrocnemius.

Left leg, posterior view.

M. Soleus

In current practice the soleus muscle is injected at the middle of the calf between the heads of gastrocnemius muscle cranial to the aponeurosis of the Achilles tendon.[12,13]

There are no descriptions of cholinesterase staining on soleus muscle in literature. In the two anatomical studies on the innervation of the gastrocnemius muscle, soleus muscle was also dissected.

Parratte[17] described the areas were the terminal nerve ramifications were densest. He found two regions: an area innervated by a posterior branch from the tibialis nerve with proximal limit at 76.5% and distal limit at 55.6% and an area innervated by the anterior nerve with proximal limit at 64.8% and distal limit at 52.3%. His proposed injections sites are at 3/5th in two sites, lateral and medial and this at several postero-anterior levels. Figure 4.

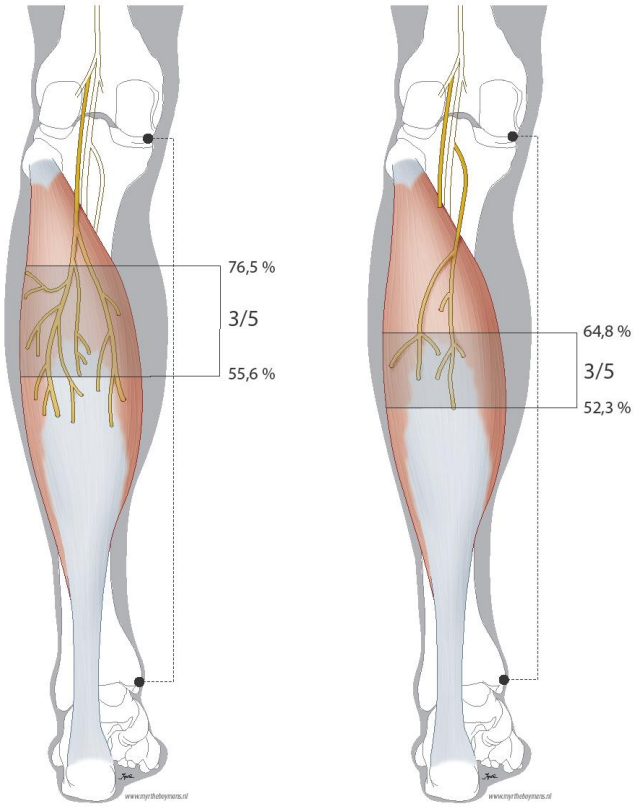
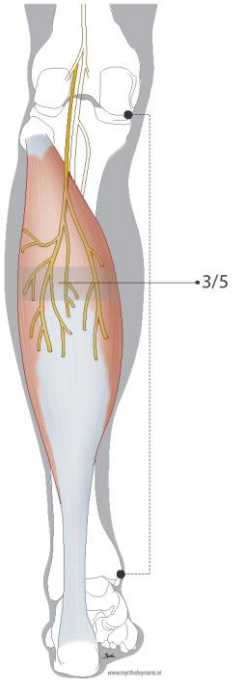
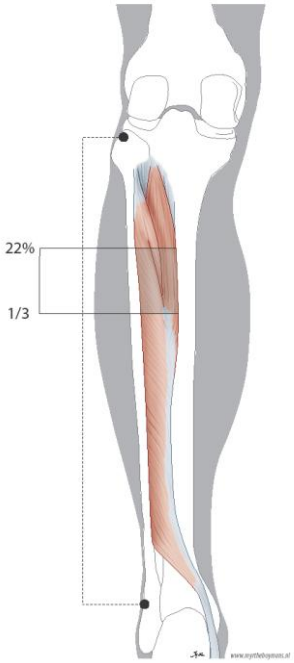


Figure 4. M. Soleus on a left leg, posterior view. MEP zone according to Parratte[17].
A. Area innervated by posterior branch from N. Tibialis
B. Area innervated by anterior branch from N. Tibialis

The MPs of the soleus muscle, as dissected by Kim[18], are diffusely distributed along the muscle belly and its highest and lowest motor points are at 20.5-46.7% of the length of the lower leg.
For this muscle, we also compared both measurements with the same method as for gastrocnemius muscle on 16 limbs and found the same result as for M. gastrocnemius muscle: Parratte describes a smaller area, more distal in the area described by Kim.

The optimal injection areas of soleus muscle are exactly the same, but a bit more precise or smaller than those currently used in clinical practice: at 3/5th in two sites, lateral and medial and this at several postero-anterior levels, along the same reference line from the medial malleolus up to the proximal margin of the medial tibial condyle. Figure 5.





Left leg, posterior view.

Figure 5. Optimal injection area for M. Soleus.

Figure 6. Optimal injection area for M. Tibialis Posterior.

M. Tibialis posterior

In current practice, we find several ways to inject the tibialis posterior muscle: a medial, anterior or posterior approach. If injected from medial, this is usually done at the middle of the leg, about halfway between medial femoral condyle and medial malleolus, behind the posterior border of the tibia. The proximal part can be reached from anterior through the tibialis anterior muscle and the interosseus membrane.[12,13] It is also possible to inject this muscle through the triceps surae from a posterior approach.

There are no histochemical staining studies on this muscle, but Oddy[19] did an anatomical dissection on 31 limbs in search for the motor points. They were found at 22.1% from the level of the head of the fibula to the intermalleolar axis. The authors state that the MP can be used as a guide to the proximal extent of the MEP-zone. They use ultrasound to identify the tibialis posterior muscle.

Injection of the tibialis posterior muscle at about the distal limit of the proximal third of the line between head of the fibula and intermalleolar axis seems optimal. This area can be reached from medial, anterior or posterior; especially for this deep muscle guidance (f.e. with ultrasound) is necessary. Figure 6.

M. Semitendinosus

Current clinical guides on BTX-A injection tell the clinician to inject the semitendinosus (ST) muscle at the middle and distal thirds on a line joining the pes anserinus and the ischial tuberosity[12] or half way to two thirds up the thigh[13]. There is disagreement between both textbooks.

Histochemical staining was done on one ST muscle from a stillborn infant by Christensson[15]. It consists of a medial part of long fibers running from the ischial tuberosity to the distal part of the insertion tendon and a shorter lateral part ending in the oblique tendon on the lateral side of the muscle. Of the two oblique end plate bands in this muscle the upper lateral one belongs to the short fibers, the lower medial one to the long fibers. Figure 7.

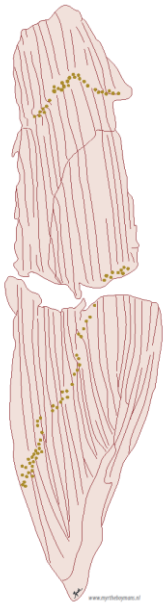


Figure 7. M. Semitendinosus (muscle belly; proximal: up, distal: down) after cholinesterase staining. The dots represent MEP, according to Christensen[15].

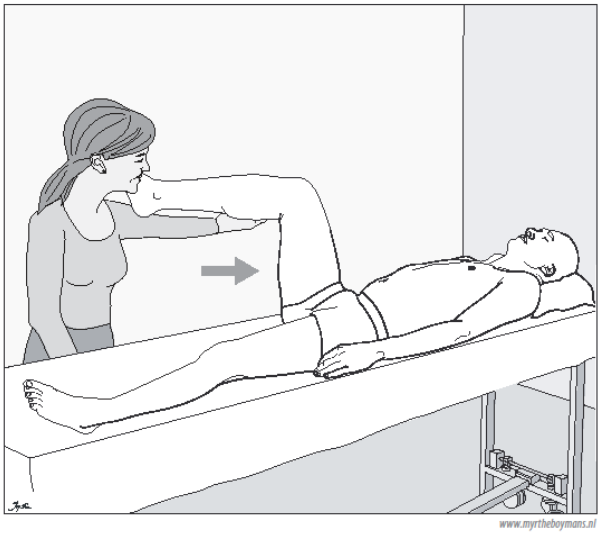
So far, no studies are published with a detailed anatomic dissection of the intramuscular course of the terminal nerve branches. The following two articles only describe the motor branches and motor point localization. Motor point (MP) is defined as the point where a motor branch (MB) enters the muscle belly. Seidel et al[20] dissected 30 adult cadaver limbs to find 2 MB in 77% and only 1 MB in 23 % of the ST muscles. 93% has 2 motor points (at 23 and 51% of femur length) along a line from the ischial tuberosity to the lateral femoral condyle.

Woodley and Mercer[21] also found two distinct partitions arranged in series that were divided by a tendinous inscription; a singular muscle nerve or a primary nerve branch innervated each partition. They expressed distances in centimetres distal from ischial tuberosity and studied 6 embalmed human lower limbs. Terminal branches of the nerve supplying the superior fascicles entered the muscle relatively proximally (between 4.2 and 12.2 cm from the ischial tuberosity). Branches of the nerve innervating the inferior fascicles entered the muscle distal to the tendinous inscription, between 7.5 and 19.0 cm from the ischial tuberosity.

The macroscopic dissection studies confirm the older findings with histochemical staining. There are two zones of concentration of MEPs, confirmed by two partitions in the muscle with two motor

points. No studies on the intramuscular course of terminal branches have been done; only the motor points (where the nerve enters the muscle belly) were described. If the comparison of the results from Parratte[17] versus Kim[18] for M. Gastrocnemius follow through, we can assume that a zone more distal than the MP zone is the zone of the terminal nerve ramifications.

The optimal injection area for the semitendinosus muscle is a bit more distal than 21 and 50% of femur length along a line from the ischial tuberosity to the lateral femoral condyle. This is at least for one injection point more proximal than the current practice. Figure 8B.



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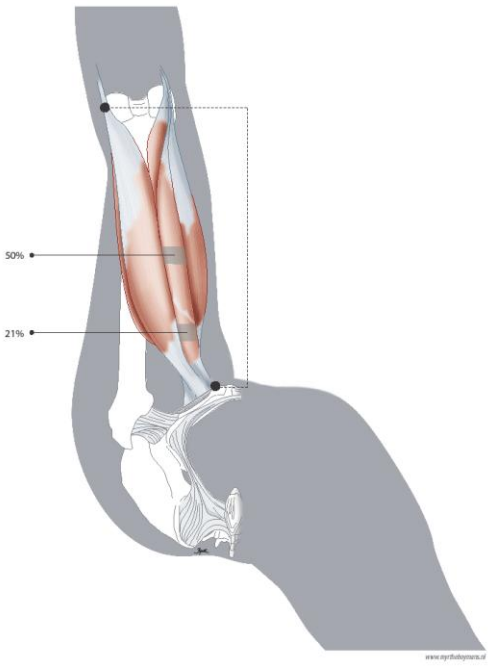

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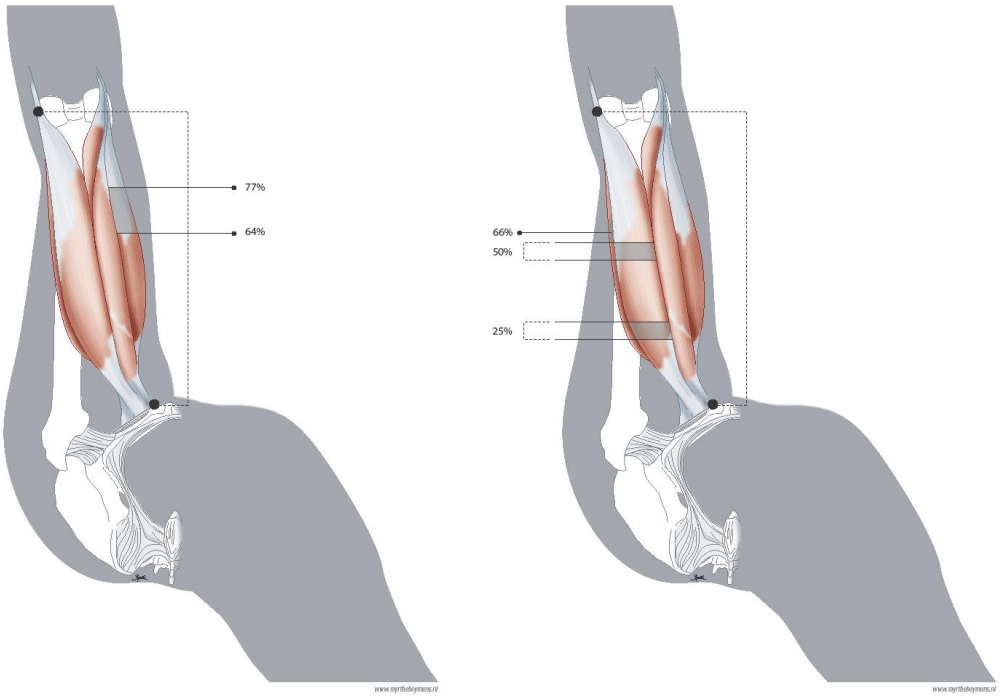


C **D**

Figure 8. Infiltration of the knee flexors.

A. Position of the patient: supine with hip flexed 90° and right knee in as maximal extension as possible (as for measuring popliteal angle).
B. Optimal injection areas for M. Semitendinosus
C. Optimal injection areas for M. Semimembranosus
D. Optimal injection areas for M. Biceps femoris, long head en short head

M. Semimembranosus

In the textbooks semimembranosus (SM) muscle is injected medial and lateral of the semitendinosus muscle at the limit of middle and distal third of the thigh[12] or halfway to two thirds up the thigh[13]. Again we find no consensus.

Studies with cholinesterase staining are not available for the SM. The same studies as for ST describe the innervation of SM. Seidel[20] found 1 MB and generally 5 or 6 MP, widely dispersed within the SM muscle belly. Within a 5 cm span (64 to 77% of femur length, measured from superior medial aspect of ischial tuberosity to medial femoral condyle) 50% of the MP from the SM can be located. Woodley and Mercer[21] describe three regions on basis of the muscle architecture; a primary nerve branch innervates only two regions, the third receiving a secondary branch. The point at which the nerve entered the muscle was 14.6 – 21.2 cm distal to the ischial tuberosity. The most distal branches entered the muscle 34.5 cm distal to the tuberosity.

Motor points seem to be widely dispersed, but highest concentration is relatively distal (more distal than in M. Semitendinosus) and this is more distal than in current practice (for some textbooks).

With the available data, the optimal injection site for the SM muscle seems to be between 64% and 77% of the femur length along a reference line from the ischial tuberosity to the lateral femoral condyle . Figure 8C.

M. Biceps Femoris

Current practice is to inject the long head of the biceps femoris muscle (BF) just distal of proximal third on the line joining the lateral border of the popliteal fossa and the ischial tuberosity. The short head is injected lateral to the long head in the middle and distal third of the thigh.[12]

No studies with cholinesterase staining are available for the biceps femoris muscle. The same studies as for ST and SM are describing the innervation of BF. Seidel[20] found along the long head in 33% one MB and in 66% 2MB; 2 or 3 MP were usually found at 9.2cm (24%), 17.9cm (46%) and 19.7cm (49%). Woodley and Mercer[21] describe that the BF long head is supplied by one muscle nerve with multiple terminal branches. The short head has one nerve that enters the muscle 20 cm distal to ischial tuberosity and another nerve enters it between 22.2 and 34.2 cm distal to ischial tuberosity; both with few terminal branches.

Optimal injection for BF long head is one injection at 25% of the length of the thigh (measured from superior medial aspect of ischial tuberosity to medial femoral condyle) and one injection around 50%. For the BF short head the optimal injection site is at the proximal limit of the distal third of the femur. Figure 8D.

M. Gracilis

Again for the gracilis muscle there is no uniform injection technique in current practice. One textbook tells to inject in the middle third of the thigh into a line joining the pes anserinus and the symphysis[12], the other advices to inject proximal[13].

The gracilis muscle consists of parallel fibers running through the whole muscle. Christensson[15] demonstrated with cholinesterase staining that two MEP bands exist: one oblique band between the upper and the middle part of the muscle and another band between the middle and the lower third of the fibers. Figure 9. This and other observations (interruption by fine collagen fibers between the muscle fibers, no double innervation) suggest that at least part of the muscle fibers in the gracilis muscle have been developed from two myoblasts, each with its own end plate. Kumar[22] demonstrated on 6 human gracilis muscles the intramuscular neural pattern with Sihler's staining technique and identified the motor endplates with the method of Koelle and Friedenwald for localizing cholinesterase activity. The gracilis was found to have numerous bands of motor end-plates scattered throughout its entire length. However, two zones of concentration were observed: one at the proximal third and another at the distal third of the muscle. Unfortunately, in both studies this was not referred to external anatomical landmarks, nor was the length of the tendon taken into account.

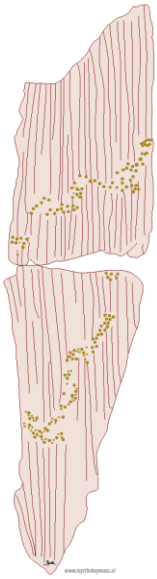
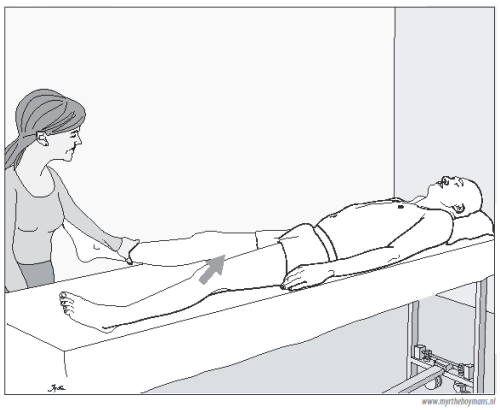


Figure 9. M. Gracilis (muscle belly; proximal: up, distal: down) after cholinesterase staining. The dots represent MEP, according to Christensen[15].

An anatomical description of the localization of the MP was given by Crystal et al[23] after dissecting 20 legs in 15 cadavers. They found the MP of the gracilis muscle at 44% of the reference line (symphysis pubis to medial joint line at the distal point of the medial femoral condyle). There was no further dissection of the intramuscular course of the terminal nerve branches in this special muscle.
The studies from Christensson[15] and Kumar[22] give a good description of the MEP zones: one at the limit of the proximal and middle third and one at the limit of the middle and distal third of the muscle. As the proximal part of the muscle has almost no tendon –it starts almost as a muscle belly from the bone-, we only have to consider the length of the distal tendon.

The optimal places to inject the toxin in the gracilis muscle are at the limit of the proximal and middle third and one at the limit of the middle and distal third of the muscle belly and not as is mentioned in the textbooks in the middle third or very proximal in the muscle. Figure 10.



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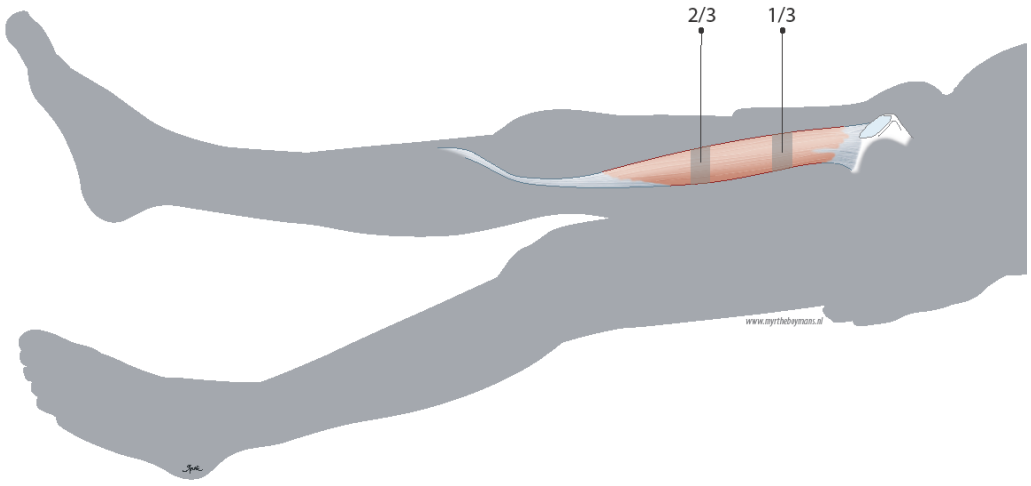


B

Figure 10. Infiltration of M. Gracilis.
A. Position of the patient: supine, with hip in extension and maximal abduction
B. Optimal injection areas for M. Gracilis.

M. Rectus Femoris

Current practice is to inject the rectus femoris muscle (RF) at the middle third[12] or the upper third[13] of the thigh along a line joining the superior border of the patella and halving the inguinal ligament between the symphysis and the anterior superior iliac spine.

Histochemical staining by Christensson[15] showed an oval shaped end plate band in this bipennate divergent muscle. Figure 11.

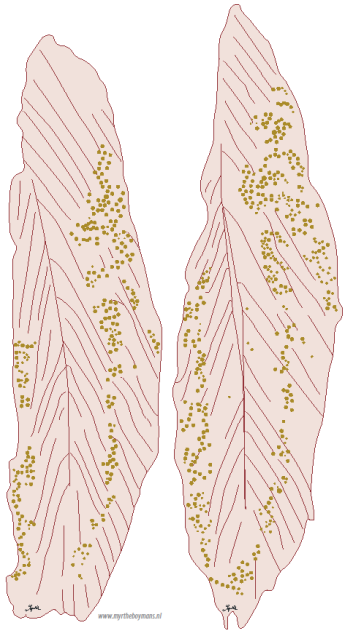


Figure 11. M. Rectus Femoris (2 different muscle bellies; proximal: up, distal: down) after cholinesterase staining. The dots represent MEP, according to Christensen[15].

Sung[24] studied the localization of the motor branch of rectus femoris in 22 cadavers, in order to find an ideal target point for selective motor branch block. There are 2 subbranches of the branch from N. Femoralis for RF; 1 penetrates at about 1/3 (on a line from anterior superior iliac spine to medial femoral condyle), 1 goes more distally, the exact localization of the entrance in the muscle is not determined in this study. The oval shaped end plate band gives a widespread distribution of MEPs in the rectus femoris muscle. The presence of two sub-branches with divergent distribution confirms this.

Injection in the middle third of the thigh, possibly in 2 or 3 sites, is very likely to block a substantial part of the NMJ. Injection can easily be done with the patient supine, hip extended and knee flexed. The RF is then the most stretched muscle and easily palpable. After insertion of the needle, the needle will move with a knee-flexion motion and with a hip-flexion motion, as the RF arises proximal from the hip joint in contrast with the vastus muscles, which are easily avoided using this technique. This palpation-mobilisation technique can be combined with ultrasound very easily. Figure 12.

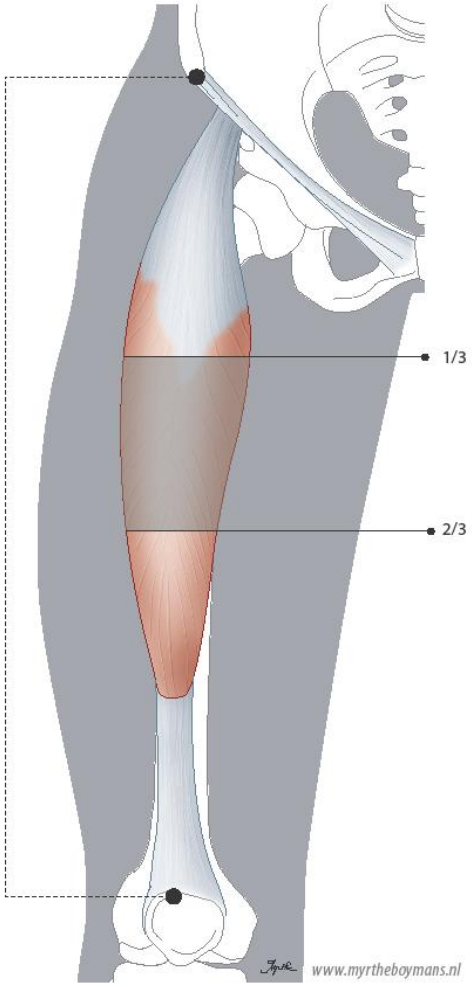


Figure 12. Optimal injection area for M. Rectus Femoris.

Mm. Adductor longus, brevis and magnus

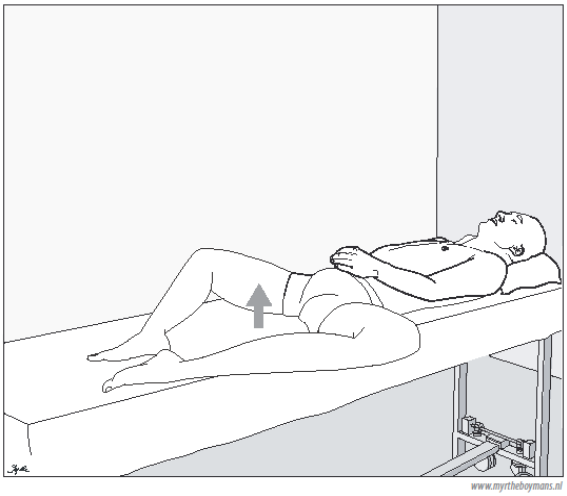
The current description of the injection points of the adductor longus (AL) and adductor brevis (AB) muscles is at the proximal third of the thigh. Injection in adductor magnus (AM) muscle is described in side-lying position anterior and posterior of the gracilis muscle at the middle third of the thigh.[12,13]

There are no studies on the adductors with histochemical staining. Crystal[23] dissected 20 legs in 15 cadavers and described the motor points of the adductor muscle group; there is no information on the intramuscular course of the terminal branches or the exact localization of the end plate zone. The localization of the MP is referred to a line from the symphysis pubis to the medial knee joint line. The MP of AL is at 31%, MP of AB at 22.4% and for AM at 38.1% of the reference line. Although, we know from other studies that the MEP zone is usually more distal than the motor point.

With the current knowledge (derived from the localization of the MP), optimal injection point for AL would be distal from 31% of the reference line, and not in the most proximal part of the muscle. For AB the optimal injection probably is around 25% of the reference line. Injection of AM is best done distal of 38 % (at 40%) of the reference line and this is in the middle third of the thigh as described in the current textbooks. Figure 13.

Figure 13. Injection of the adductors.

- **A.** Position of the patient: supine with both hips flexed and abducted, both knees flexed.
- **B.** Optimal injection area for M. Adductor magnus
- **C.** Optimal injection area for M. Adductor longus
- **D.** Optimal injection area for M. Adductor brevis



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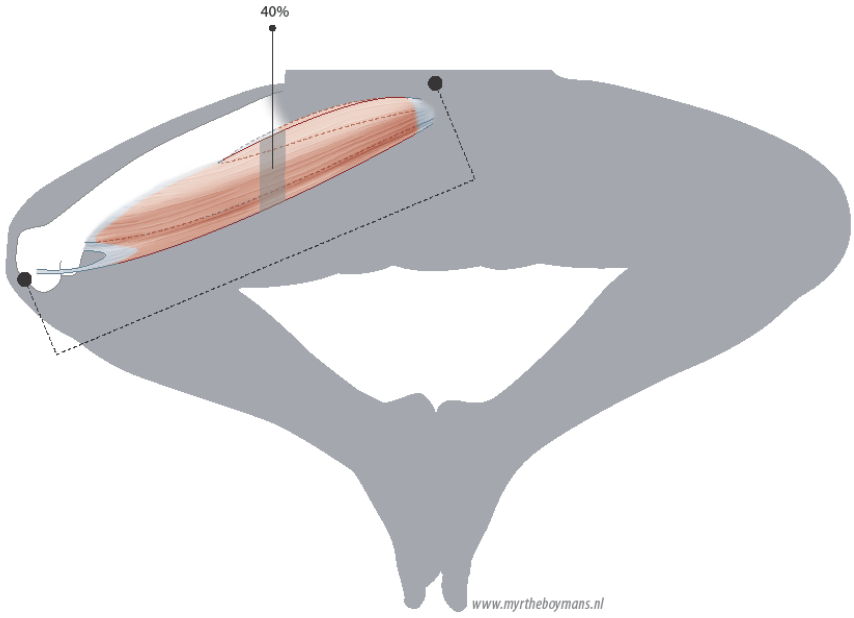
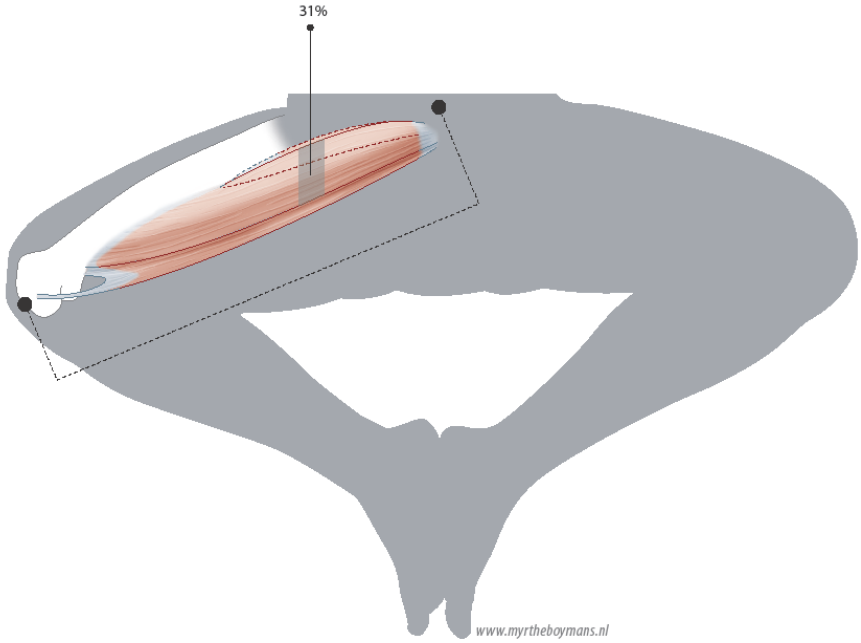


B

Part 1: Localizationd of MEP zones



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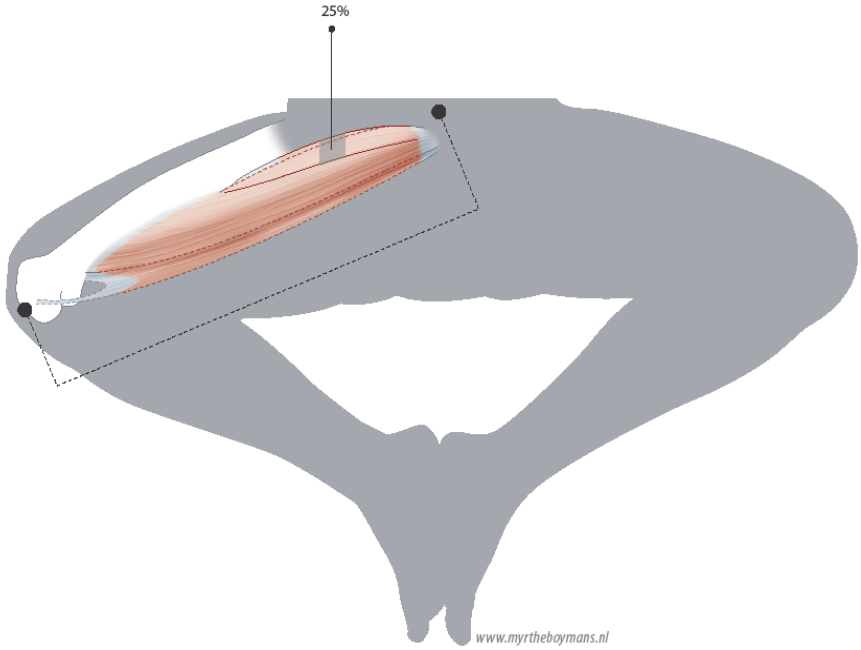

D

M. Psoas

There are several methods described in literature to inject BTX-A into the psoas muscle.

A direct injection of the muscle belly through the abdominal wall in the region between the umbilicus and the anterior superior iliac spine, as described by Molenaers[25], is still routinely used by the present authors. With deep palpation, coming from lateral to push aside the intestines, the psoas is palpable. After needle placement the hip is flexed to confirm the needle's position. Some authors[26] advise the aid of passive or active EMG guidance and ultrasonography, but this can make it more difficult to freely access the psoas muscle with palpation. For this technique however a general anaesthesia is necessary. An alternative technique is to inject, under analgosedation, the psoas distal to the inguinal ligament, as presented by Westhoff[27]. A last alternative is injecting the psoas from dorsal, just lateral to the transverse processes of L2, L3 and L4, as presented by Ward[28].

No studies with cholinesterase staining were done so far. Knowledge of the localization of the MEP-zone of the psoas muscle in relation to external anatomical landmarks has recently been acquired.[29] In 24 muscles the intramuscular course of the nerve branches from the lumbar plexus to the psoas muscle and their terminal arborisations was dissected until it was no longer possible to follow them. The proximal and distal limit of the MEP-zone were situated at about 30% and 70% respectively of the distance between the twelfth thoracic vertebra (Th12) and the passing of the psoas under the inguinal ligament (L). In reference to the sacral promontory (P), these limits are respectively from 50% of the Th12-P distance to 20% of the P-pubis distance. Figure 14. During this study, it was also noted that the nerve branches had a distal course after entering (MP) the muscle; the proximal MP was at about the region of the proximal limit of the MEP-zone, the distal limit of the MEP zone was more distal the most distal MP.

Considering these recent findings, injecting the psoas from dorsal most efficiently brings the toxin close to the lumbar spine in the relatively proximal (30 to 70 % of Th12-L distance) area of the psoas muscle. In thin persons, as in children with cerebral palsy, the psoas and the sacral promontory are very good palpable through the abdominal wall. As general anaesthesia is often required for a multilevel BTX-injection, the proximal anterior approach remains another option. In that case, however, the infiltration must be performed more proximally than is currently done. Injecting the toxin distal to the inguinal ligament brings it in a region without terminal nerve branches.

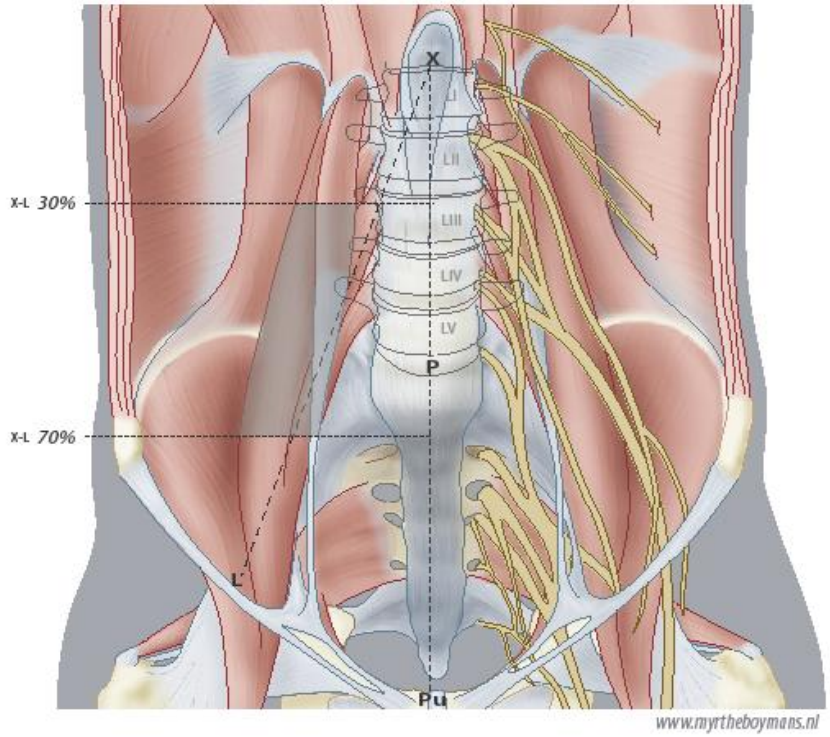

Figure 14. Optimal injection area for M. Psoas, according to Van Campenhout[29].

Discussion

This review collects all scientific knowledge on the localization of the MEP-zones from frequently injected muscles of the lower limb. Some of the recent literature refers this information on the localization of the MEP-zones to external anatomical landmarks.[17-24,29] With these guidelines the injection of BTX-A can be directed to the areas with the largest concentration of MEPs. As the effect of the toxin relies on its uptake at the presynaptic membrane of the motor end plate, the injection should be given into the MEP area.

Shaari and Sanders[10] showed in their animal model that the degree of muscle paralysis induced by BTX-A is proportional to the proximity of the toxin to the motor end plate. Gracies[11] achieved a greater neuromuscular blockade with endplate targeted BTX-A injections versus non-targeted injections in the human spastic biceps. These data on experimental animals and a human study demonstrate that an optimal injection technique is crucial to the success of treatment with BTX-A.[2,6,30] Current practice of BTX-A injections is often guided by the knowledge that the MEP zone is situated in the middle of a muscle fiber, and therefore it is assumed to be in the middle of the muscle belly.[8] This is so for unipennate muscles, but more complex muscles have a different dissipation of their NMJ's.

After gathering all available histological and anatomical studies and comparing this with clinical practice, we find that for some muscles the MEP zone can be more precisely demarcated and for

many other muscles its location is somewhat different than the currently injected areas in clinical practice. For M. Gastrocnemius and M. Soleus the MEP zone is slightly smaller than the areas described in the clinical injection books. When we inject this more limited area, we assume that we can either limit our dose or achieve a better neuromuscular blockade with the same dose.[15,17,18] The MEP-zone of M. Tibialis posterior is at the distal limit of the proximal third of the line between head of the fibula and intermalleolar axis.[19] From motor point localisation, we calculate for M. Semitendinous two optimal injection areas which are located slightly more distal than 21 and 50% on a line from the ischial tuberosity to the lateral femoral condyle. For M. Semimembranosus this is around 70% on a line from ischial tuberosity to medial femoral condyle and for the long head of M. Biceps femoris at 25% and just proximal of 50% of this line.[15,20,21] The current injection of M. Gracilis at the proximal or middle part of the muscle can best be replaced by two injections: one at the limit of the proximal and middle third and one at the limit of the middle and distal third of the muscle.[15,23] M. Rectus femoris has a widespread distribution of MEPs, therefore multiple injections are advised.[15,24] For the MEP-zone of the adductors we also have to rely on the localization of the motor points. This locates the optimal areas just distal of 31% for M. Adductor longus, 22% for M. Adductor brevis and 38% for M. Adductor magnus along a line from symphysis pubis to the medial joint line and this is more distal than current practice for many clinicians.[23] Especially for M. Psoas the knowledge of the localisation of the MEP-zone can help the clinician to improve spasticity reduction and clinical outcome of the toxin injections. Its MEP zone is situated at the level of the promontorium and one injection just proximal of the promontorium and close to the spine and one injection just distal of the promontorium in the more easy palpable part of the muscle will bring the toxin optimal in the MEP-zone.[29]

Local diffusion of the toxin in the muscle itself can minimise the importance of injecting at the MEP-zone. This is especially so for small muscles. Animal studies have confirmed that the toxin may diffuse up to 4.5 cm along the length of the muscle.[31] However, most muscles in the lower limb are large muscles with considerable length in which diffusion is very unlikely to bring the toxin to the other end of the muscle. Multiple injections can bring the toxin to a larger region, but this means repeated injections. In doing this, you will also disperse the toxin over areas which have none or only a limited number of MEPs. This toxin -if not bound to the Acetylcholine receptor of the NMJ- can diffuse and reach other muscles or blood vessels, with its possible complications. In the psoas muscle this also means repeatedly injecting a deep region with its possible complications of damaging other organs. Botulinum toxin easily passes through muscle fascia even at subclinical doses. The presence of fascia reduces the spread by 23%.[32] This again suggests that the spread of botulinum toxin can be prevented only by delivering small doses to the MEP-zone of a target muscle. In CP, BTX-A is often used at multiple levels, leading to the use of high total doses.[33] In these cases it is even more important to use this powerful drug with an optimal injection technique.

A lot of questions still remain. For many muscles there is no information on the exact location of the MEP zone and we have to deduct the localisation of the MEP-zone from the localization of the motor points. We know that the motor endplate is not located where the motor branch enters the muscle belly. Comparison of some studies (Parratte[17] versus Kim[18]) and the results from the psoas dissection study[29] teach us that the motor points are usually more proximal than the motor end plates. For some muscles we can let the MP guide us to the MEP-zone. Still, it would be better to have good anatomical studies on the localization of the MEP-zone in reference to external anatomical markers on all these frequently injected muscles; this ideally would imply studies in which the intramuscular

path of the nerve is followed with stereoscopic dissection until the end of each terminal nerve ramification.

The histological studies reported in literature have been based on cholinesterase staining of muscles from small children[14,15]. Only Aquilonius[16] has studied the distribution of motor endplates from adult muscles by staining longitudinal cryosections of whole biceps brachii, tibialis anterior and sartorius muscles for cholinesterase. Their findings are in agreement with the results of Coers and Christensson. So far, no other skeletal muscles from adults were examined with histochemical staining or immunolabeling methods. This is technically very demanding and has only been done in the very small laryngeal and facial muscles.[34-39] Histochemical studies have been performed on adult[16] and infant[15,16] cadavers; their findings are in agreement. It is unclear whether these results can be extrapolated to juveniles. Jianjun et al[40] studied the distribution of NMJ in juvenile and adult rats and stated that the distribution on NMJ in adult rats can be extrapolated to juvenile rats, but it is not clear whether distribution data from human adults can be extrapolated to juveniles.

Currently, there is only one human study to confirm the importance of injecting the toxin close to its site of action. Gracies described this for M. Biceps brachii.[11] Some older studies could not find a difference. Childers[41] sought to test the hypothesis that injections of BTX-A at the mid belly of the gastrocnemius muscle in spastic hemiplegic adults produce superior clinical results to proximal injections directed toward the muscular origin. One of the problems of this study is that the wrong assumption concerning the localization of the MEP-zone was made. Further clinical studies on other human skeletal muscles are necessary to demonstrate the importance of injecting the toxin close to the MEP-zone.

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Part 2

MEP targeted injections of muscles of the lower limb in children with CP.

1. **Introduction.**
2. **MEP targeted injections of the medial hamstrings.**
3. **MEP targeted injections of the psoas muscle.**

Part 2, Chapter 1

Introduction

The review of the MEP zones of the frequently injected muscles of the lower limb (part 1-chapter 3) taught us that especially for the medial hamstrings (semitendinosus, semimembranosus and gracilis muscle) and for the psoas muscle the current popular injection techniques are different from the proposed optimal injection sites according to MEP localization. As these muscles are very often involved and injected in children with CP, there is a high clinical challenge to optimize these injection techniques.

Therefore, the different injection techniques need to be compared in prospective clinical trials.
To measure the effect of BTX, most intervention studies use clinical spasticity grading scales and/or more invasive assessments of spasticity reduction. These clinical spasticity scales, such as the Modified Ashworth Scale or Tardieu Scale, assess spasticity by grading the resistance as felt during an externally applied fast stretch. They have been found to be not objective, sensitive or valid enough.[30-32] The perceived resistance to the performed passive movement during these tests may be a result of reflex muscle activity, but also of non-neural mechanical properties such as changes in visco-elastic properties of joint structures and soft tissues. Clinical scales fail to decompose both components in order to measure spasticity.[65]
More objective and qualitative measures can be categorized as biomechanical and/or electrophysiological. Biomechanical methods measure the behavior of muscles by capturing joint position, angular velocity and torque during well-defined motions. In some studies isokinetic devices are used, but these are often bulky and difficult to apply in children. Neurophysiological methods measure the muscle's activity in reaction to specific motions, active or passive. Surface electromyography has been used to identify different spasticity patterns, but to be able to provide information about reactive-resistance a combination with some biomechanical approach is mandatory. So far, hardly any measurement that fully integrates multidimensional signals is clinically feasible and few have been assessed for reliability.[33,66,67]

Therefore, a project was set up in the center of movement analysis of University Hospital Leuven, in collaboration with the engineering department KULeuven (and supported by a grant from the Flemish Agency for Innovation by Science and Technology, IWT-TBM). Goal was to create an instrumented clinical spasticity measurement and to determine a set of quantitative spasticity-sensitive parameters based on integrated biomechanical and electrophysiological signals. For this both a passive single joint measurement and a functional dynamic assessment during gait with 3D gait analysis (multiple joint) were developed.

- A portable, non-invasive tone assessment device was developed to measure three groups of signals during standardised passive isolated movements in different lower limb joints, which are (1) stretch characteristics (joint angle parameters), (2) reactive resistance and (3) muscle activity (EMG). Details of the procedure (data collection and data analysis), validity and repeatability of this instrumented spasticity assessment have been published[33,68]. This procedure is best suited to measure spasticity in the gastrocnemius, adductors en medial hamstrings (semitendinosus, semimembranosus and gracilis).

- A multiple joint evaluation during gait is a standard evaluation in ambulatory children with CP and in our CP center also mandatory before and after every BTX treatment. An evaluation of kinematic, kinetic and electromyographic activity of the muscles is done at a self selected speed. As spasticity is a velocity dependent feature, it has been suggested that increasing the walking velocity will highlight signs of spasticity.[69,70] Therefore the impact of increased walking velocity on kinematic, kinetic and EMG parameters and on muscle length and muscle lengthening velocity is studied.

In part 2-chapter 2 the single joint measurement of spasticity in the medial hamstrings is documented in two articles explaining the procedure, its sensitivity and validity; a third article finally compares two injection protocols of the gracilis muscle. In a fourth article the multiple joint procedure is presented with an emphasis on gastrocnemius and hamstrings related parameters of spasticity.

An objective evaluation of the spasticity of the psoas muscle remains a challenge. Both single and multiple joint measurements are not very suited to measure its tone. For the single joint measurement, a surface EMG is needed and this is not possible for the psoas muscle. Using fine wire EMG for this muscle is not an option in children and due to its direct retroperitoneal position even dangerous in adults. For the multiple joint assessment during gait, we are confronted with the fact that the psoas muscle does not only cross the hip joint. It originates on the moving vertebras of the lower thoracic and lumbar spine. This makes it difficult to model its muscle length. Further, several other hip flexors (f.e. the iliacus muscle) play a role in kinematics and kinetics and again, no EMG signal can be obtained during gait.
Therefore, an alternative tool to measure the effect of different injection protocols was selected. As BTX selectively blocks neurotransmission, it will cause a temporary chemical denervation and muscle atrophy. Schroeder et al.[34] already used volume assessment to demonstrate the effect of BTX in two healthy adults. They used magnetic resonance imaging, measuring the cross-sections of the injected muscle. As the psoas muscle is a multipennate muscle and we want to compare injections at different locations, we need a 3D reconstruction of the complete muscle volume to be able to compare the different injection protocols. The comparison of two injection procedures for the psoas muscle using volume assessment by digital MRI segmentation is presented in part 2-chapter 3.

Part 2, Chapter 2

MEP targeted injections of the medial hamstrings.

1. **Instrumented assessment of the effect of BTX in the medial hamstrings in children with CP.**
2. **Is an instrumented spasticity assessment an improvement over clinical spasticity scales?**
3. **Motor end plate targeted BTX injections of the gracilis muscle in children with CP.**
4. **Can we unmask features of spasticity during gait in children with CP by increasing their walking velocity?**

Part 2: MEP targeted injections

Part 2, 2.1

Instrumented assessment of the effect of BTX in the medial hamstrings in children with CP.

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Abstract

This study examined the sensitivity of an instrumented spasticity assessment of the medial hamstrings (MEH) in children with cerebral palsy (CP). Nineteen children received Botulinum Toxin type A (BTX) injections in the MEH. Biomechanical (position and torque) and electrophysiological (surface electromyography, EMG) signals were integrated during manually-performed passive stretches of the MEH at low, medium and high velocity. Signals were examined at each velocity and between stretch velocities, and compared pre and post BTX (43±16 days). Average change between pre and post BTX was interpreted in view of the minimal detectable change (MDC) calculated from previously published reliability results. Improvements greater than the MDC were found for nearly all EMG-parameters and for torque parameters at high velocity and at high versus low velocity (p<0.03), however large inter-subject variability was noted. Moderate correlations were found between the improvement in EMG and in torque (r=0.52, p<0.05). Biomechanical and electrophysiological parameters proved to be adequately sensitive to assess the response to treatment with BTX. Furthermore, studying both parameters at different velocities improves our understanding of spasticity and of the physiological effect of selective tone-reduction. This not only provides a clinical validation of the instrumented assessment, but also opens new avenues for further spasticity research.

Introduction

Spasticity is characterized by a velocity-dependent increase in tonic stretch reflex[1] with an accompanying increase in muscle resistance when a muscle is passively stretched.[2] This definition, as well as the methods for spasticity assessment, has been under much debate in the last decade. Nonetheless, neuromuscular tone reduction remains an important treatment modality in children with cerebral palsy (CP).[3] For example, Botulinum Toxin type-A (BTX), injected intramuscularly, causes a temporary reduction in reflex muscle activity by selectively blocking the release of acetylcholine at the cholinergic nerve terminal. Whilst this has been found effective to decrease spasticity in children with CP, there remains a large variability in treatment response.[4] A comprehensive assessment of the effect of BTX on spasticity could increase our knowledge of the pathology and improve our understanding of this reported variability.

In children with CP, the effect of BTX is most commonly assessed with clinical scales (Modified Ashworth-MAS[5], or Modified Tardieu Scale-MTS[6]). These scales assess spasticity by subjectively interpreting the resistance felt during passive stretch. Nonetheless, the perceived resistance may be a result of reflex muscle activity as well as of changes in visco-elastic properties of the joint and muscle. The available clinical scales fail to distinguish between both components and are thus not deemed sensitive or valid to quantitatively assess the effect of BTX on the stretch reflex. Moreover, they have also been criticized for their low reproducibility and poor accuracy[7,8]. As such, clinical scales have a limited ability to differentiate between patients or to explain the response variability after treatment. Instrumented methods could provide a more comprehensive assessment.

Electromyography (EMG) has been used in adults to quantify the effect of BTX on the pathological response during passive muscle stretch.[9,10] Simultaneously assessing muscular resistance using torque sensors provides an integrated (EMG and torque) instrumented measurement method.[11] However, in children with CP, clinically-applicable integrated approaches to assess the effect of BTX have only been applied to the upper limb[12], whereas lower limb muscles are most commonly treated. We therefore used an instrumented method, that integrates EMG and torque, as described by Bar-On et al.[13]. The repeatability and discriminate validity to measure spasticity in the medial hamstrings (MEH) in children with CP has previously been shown.[13,14] However, it is yet to be determined whether this instrumented assessment is sensitive to detect treatment efficacy and if it can help to understand variability in treatment outcome.

Therefore, the aim of this study was to quantify and understand the effects of BTX injection in treating MEH spasticity in children with CP, using an integrated assessment based on EMG and torque.

Method

Children aged 3-18 years and scheduled for BTX in the MEH (Mm. Semitendinosus and Semimembranosus) were recruited from the multidisciplinary clinic for patients with CP (University Hospital Leuven). The exclusion criteria were: presence of ataxia or dystonia; severe muscle weakness (<2+ on the Manual Muscle Test[15]); poor selectivity[6]; bony deformities or contractures hindering neutral alignment; cognitive problems that could impede the measurements; previous lower limb orthopedic surgery (soft tissue or bony procedures); intrathecal Baclofen pump or

selective dorsal rhizotomy. Minimal strength production and good selectivity were required because a voluntary contraction was used as an individual reference to evaluate surface EMG (sEMG) signals in previous studies with the same subject group.[13,14] In the current study however, voluntary contractions were expected to be influenced by the BTX injections and the normalized sEMG was thus not analyzed. The University Hospitals' ethical committee approved the experimental protocol and all children's parents signed an informed consent.

As part of a regular multilevel BTX treatment, muscles to inject and dosages were selected based on standard multidisciplinary evaluation. Injection with BTX *(Botox®, Allergan Ltd, UK)* was done under a short anesthesia and ultrasound was used to confirm needle position. All children underwent casting for a period of 10 days (lower-leg with optional removable upper-part used as a knee-extension device), intensive physical rehabilitation as well as orthotic management (day and night) following the BTX injections.

Data acquisition

The set-up of the instrumented assessment for the MEH is presented in Figure 1. In children with unilateral CP, only the affected side was tested. In children with bilateral involvement, the most involved side was tested. This was defined as the side with the highest MEH MAS-score or, in case of symmetrical MAS-scores, the most severe MTS-score. All assessments were performed prior to injection and 14-70 days after injection, by the same trained assessor. For more details regarding the measurement method, the reader is referred to Bar-on et al.[13]

Four repetitions of passive MEH muscle stretches over the full range of motion (ROM) were carried out at three velocities. Firstly, the knee joint was moved at low velocity (*LV*) during 5 seconds, followed by a movement at intermediate, medium velocity (*MV*) during 1 second, and finally at high velocity (*HV*), which was performed as fast as possible. The interval between repetitions was 7 seconds in order to avoid post-activation depression of the electrophysiological response.

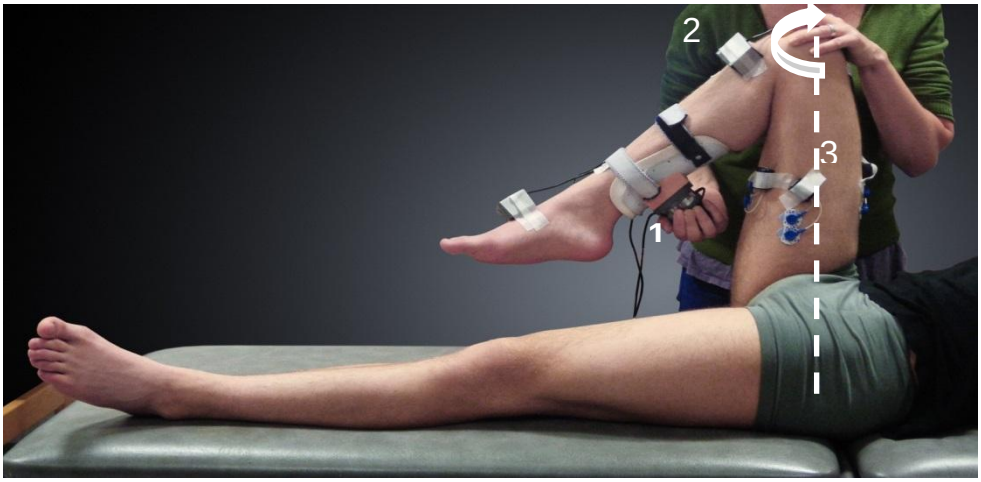


Figure 1. Instrumented spasticity assessment of the medial hamstrings muscle: test starting position, direction of stretch (white arrow) and instrumentation. (1) a six DoF force-sensor attached to a shank orthosis on the posterior aspect of the lower leg (torque measurement); (2) two inertial measurement units (joint angle measurement); and (3) surface electromyography (sEMG) of the medial hamstrings and rectus femoris (muscle activity measurement). sEMG data from the rectus femoris were utilized to ensure no active assistance of the patient during the passive stretches.

Data analysis

A 6th order zero-phase Butterworth bandpass filter ranging from 20-500Hz was applied to filter the raw sEMG signal. The root mean square envelope of the sEMG (RMS-EMG) signal was computed using a low-pass 30Hz 6th order zero-phase Butterworth filter on the squared raw signal. EMG onset, ROM, maximum angular velocity (V_{MAX}), and the net internal joint torque were computed as previously described.[13]

Repetitions were excluded when passive stretches were performed out of plane, at inconsistent velocities, in case of poor quality sEMG signal (loss of signal, low signal-to-noise ratio or obvious artifacts), or when there was indication of antagonist activation (rectus femoris sEMG activity). All data analyses were carried out with MATLAB® Software 7.6.0 R2010a.

Outcome parameters

ROM was determined during *LV*; V_{MAX} during all velocities. All other parameters were calculated at each velocity and were extracted from the RMS-EMG and the computed torque signals. Average RMS-EMG, expressed in mV, was calculated as the area underneath the RMS-EMG time curve, divided by the duration of the time interval considered. The time interval started 200ms prior to the time corresponding to V_{MAX} and ended at the time corresponding to 90% of the full ROM. From the computed torque signal, four instrumented spasticity parameters were developed. Firstly, the amount of work required to stretch the muscle was calculated as the integral of torque with respect to position starting at V_{MAX} up to 90% of the ROM (referred to as 'work' and expressed in J). Torque was additionally analyzed at 70° knee flexion, an angle that corresponded to the overall mid-ROM of all children ('torque', expressed in Nm). The angle of catch (AOC) was defined as the angle that corresponded to the time of minimum power after maximum power and was expressed as a percentage of the ROM.[14] Finally, the value of the power at the AOC was used to quantify catch severity[14] ('AOC power', expressed in W). The AOC and AOC power were calculated from the first *HV* stretch following the procedure described in Bar-On et al.[14] All other parameters were calculated by taking the average of 2-4 repetitions per velocity. To provide a measure of the severity of spasticity, the absolute change between *MV* and *LV* (*MV-LV*) and between *HV* and *LV* (*HV-LV*) was also calculated for every parameter (except ROM, AOC, and AOC power).

Statistical analysis

All parameters were checked for normal distribution using the Kolmogorov-Smirnov test with $p>0.1$ indicating a normal distribution. To ensure that the velocity of passive stretches was performed consistently between measurement sessions, V_{MAX} at each velocity was first compared between sessions using a paired samples t-test, or in case of non-normal distributions, a Wilcoxon Matched Pairs Test (WMPT). Next, to evaluate the sensitivity of the parameters to treatment with BTX, the average change between pre- and post-treatment sessions was calculated. It was hypothesized that ROM, AOC, and AOC power would increase and that RMS-EMG, torque, and work parameters would decrease post-treatment. Average change between pre- and post-treatment sessions was interpreted in view of the minimal detectable change (MDC). MDC values were calculated from the standard error of measurement (SEM) values reported by Bar-On et al.[13,14] ($MDC=SEM*1.645*\sqrt{2}$) [16](Supplementary Material 1). Those parameters whose average change exceeded the MDC were compared between sessions using a paired samples t-test, or a WMPT, as appropriate.

Finally, to explore the relationships between different outcome parameters, Pearson product-moment correlation coefficients (or Spearman rank correlation coefficients, as appropriate) were

computed between all parameters. Correlations <0.20 were considered poor; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 good; and 0.81–1.00, very good.[17] It was hypothesized that the pre-post change in torque parameters would have the highest positive correlations to pre-post change in EMG parameters at *HV* and at *HV-LV*. Significance was set at $p<0.05$. All statistical analyses were performed using Statistica 10 (*StatSoft*).

Results

Nineteen children with CP participated in the study (Table 1). The mean dose for the MEH was 3.02 U/kg (SD 0.75 U/kg; range: 1-4 U/kg).

Children's characteristics (n=19)	
Mean age (SD) (years)	7.20 (3.09)
Male/female (n)	male: 10, female: 9
Diagnosis (n)	
- Unilateral involvement	3 right hemiplegia, 3 left hemiplegia
- Bilateral involvement	11 diplegia, 2 quadriplegia
GMFCS (I-IV) (n)	I: 6, II: 9, III: 3, IV: 1
MAS MEH (0-4) (number of muscles)	0: 1, 1: 1, 1+: 4, 2: 11, 3: 2
Average MTS MEH (SD)	-70.47˚ (26.84˚)

GMFCS: Gross Motor Function Classification Score; MAS MEH: Modified Ashworth Score of the medial hamstrings from the pre-treatment session; MTS MEH: average Modified Tardieu Score of the medial hamstrings from the pre-treatment session.

Table 1. Children's characteristics

Assessments were performed on average 9±15 days before and 43±16 days after BTX injection (see Figure 2 for a representative example of EMG, torque, position, and power signals pre and post BTX-A). All change pre-post parameters, except RMS-EMG at *LV*, had a normal distribution. Mean values of all parameters at both sessions can be found in Table 2. Comparison of V_{MAX} between sessions indicated that at *HV*, muscles were stretched significantly faster during the post-treatment session (increase of 31.5°/sec). ROM increased around 10° post BTX, which was significant. At *HV* stretches, all muscles had an EMG onset during both pre and post BTX assessments. RMS-EMG parameters significantly decreased post BTX at all stretch velocities. Torque and work reduced significantly at *HV-LV* (decrease of 2.79Nm and 1.18J, respectively), as well as torque at *HV* (decrease of 3.82Nm). AOC appeared 12.73% further in the ROM and AOC power increased by 3.50W post BTX. Both improvements were significant.

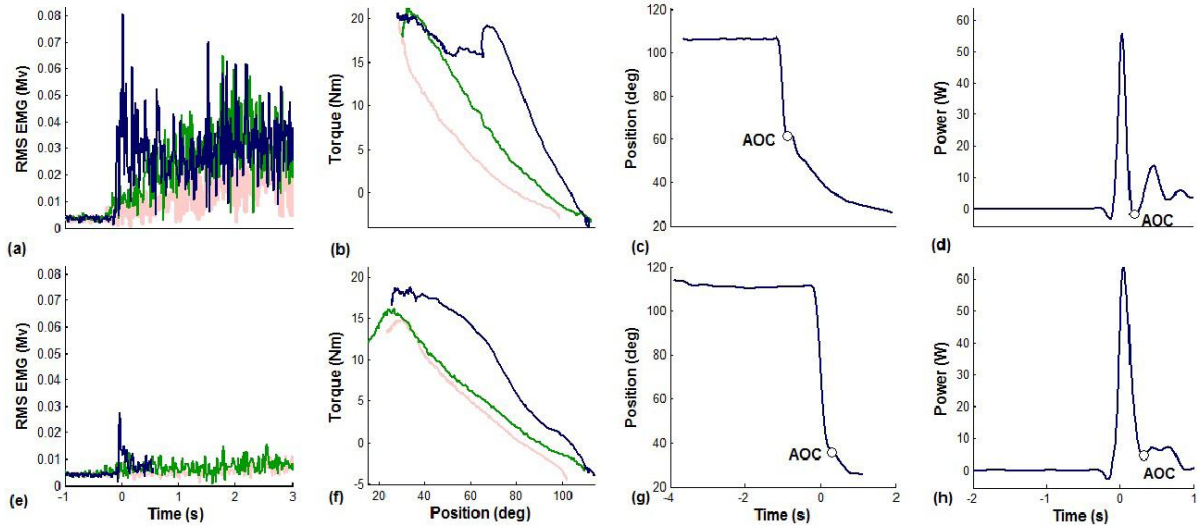


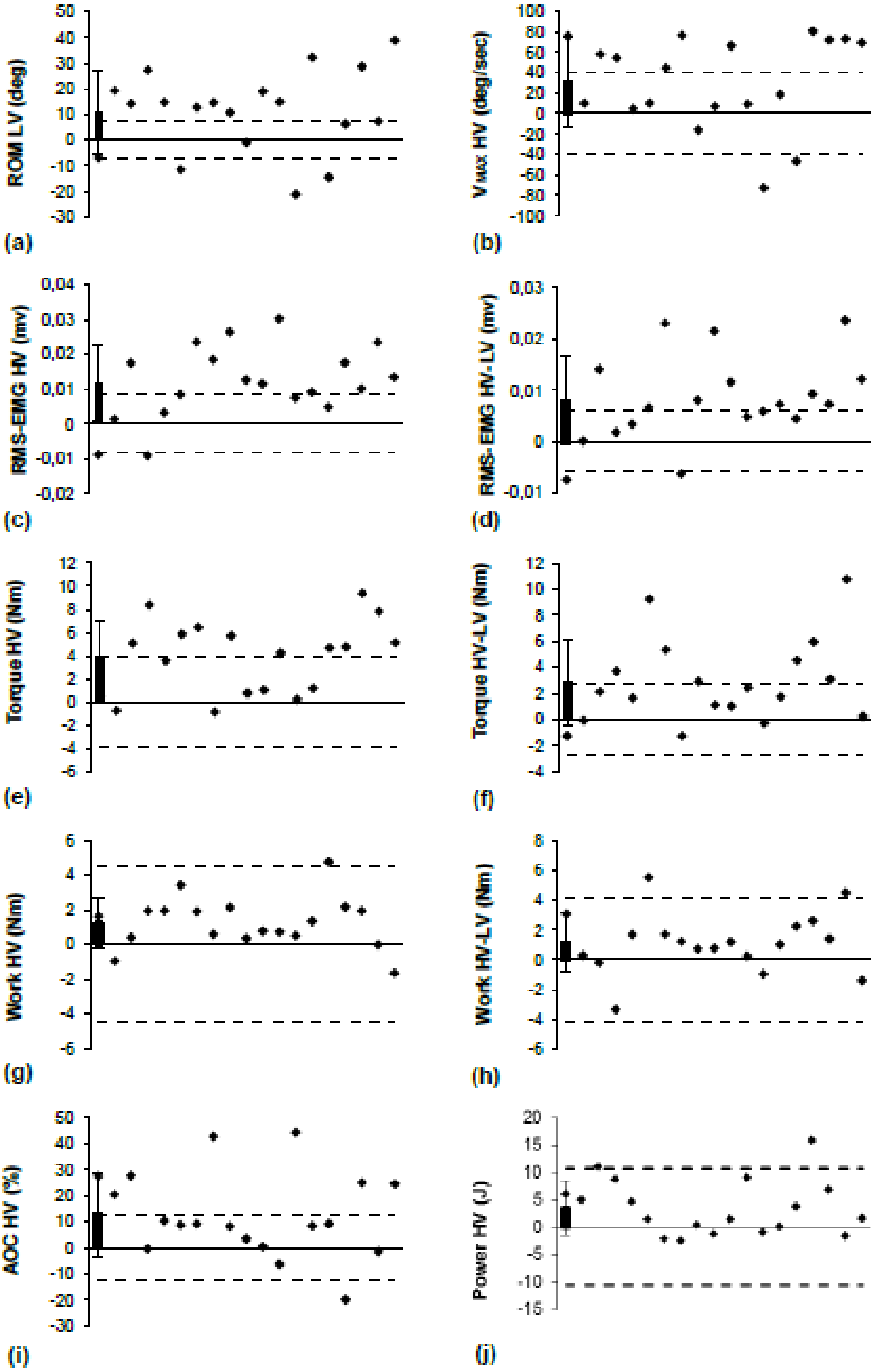
Figure 2. Pre (top row) and post (bottom row) BTX measurement of a child with spastic CP: RMS EMG-time (a, e), torque-position (b, f), position-time (c, g), and power-time graphs (d, h) during low (pink/ light gray), medium (green/gray), and high (blue/black) velocity stretches. The position of the angle of catch (AOC) is indicated on the position- and power-time graphs.

The individual and average change pre-post BTX for all outcome parameters and their corresponding MDC values can be found in Figure 3.The average change values of ROM at *LV* (10.90±16.16°), RMS-EMG at *HV* (0.012±0.011mv), RMS-EMG at *HV-LV* (0.008±0.009mv), torque at *HV-LV* (2.79±3.27Nm), and AOC (12.73±16.31%) were larger than their corresponding MDC values (Supplementary Material 1).

At *HV-LV*, good correlations were found between RMS-EMG change and torque change pre-post BTX (r=0.52), between AOC change and torque change pre-post BTX (r=0.58), and a moderate correlation between ROM change and work change pre-post BTX (r=0.45). At *LV*, a moderate correlation was found between ROM change and work change pre-post BTX (r=0.45).

Figure 3. Change between pre and post BTX measurements in: **a)** range of motion (ROM) at low velocity (*LV*); **b)** maximum angular velocity (V_{MAX}) at high velocity (*HV*); **c)** root mean square electromyography (RMS-EMG) at *HV*; **d)** change in RMS-EMG between *HV* and *LV* (*HV-LV*); **e)** torque at 70˚ knee flexion at *HV*; **f)** torque at 70˚ knee flexion at *HV-LV*; **g)** work at *HV*; **h)** work at *HV-LV*; **i)** relative position of the angle of catch (AOC) at *HV*; and **j)** the power value at the AOC at *HV*. Each diamond represents the individual value of a MEH (per child). The bar represents the mean value for all muscles. The dashed horizontal lines represent the minimal detectable change values.

Part 2: MEP targeted injections



Discussion

This study provides a clinically-applicable, instrumented method to quantify the response to BTX in spastic MEH in children with CP. Selected parameters, extracted from EMG and torque, were shown to be sensitive to measure effect post BTX.
Clinical spasticity assessments, such as the MAS and MTS, have been criticized for their poor reliability[7,8] and questionable sensitivity in identifying the response to treatment.[18] Moreover, the limited range of the ordinal scoring of the MAS results in patients being clustered into broad severity groups.[19] Although the MTS has a smaller gradation, Fosang et al. reported intra-assessor measurement errors up to 9° in the MEH.[20] This results in an MDC value of 21°, which is higher than the average reported change post BTX ranging from 2-12°.[21,22] In this study, on the other hand, parameters from the instrumented assessment provided not only sensitive continuous data, but also captured higher variable levels of response to BTX treatment. Understanding this variability could enhance treatment delineation and ensure more targeted and individualized anti-spasticity care.

Clinical tests in isolation cannot discern the relative contributions of neural and non-neural components of muscle tone. By integrating electrophysiological and biomechanical parameters, a more comprehensive assessment was achieved. Parameters investigating the change between velocities are able to capture velocity-dependent spasticity as defined by Lance.[1] These proved most sensitive to treatment with BTX, with an average of 53% reduction in velocity-dependent RMS-EMG and a 47% reduction in torque. The moderate correlation between the change in RMS-EMG% and in torque post BTX confirms that the decrease in torque is partially influenced by velocity-dependent neurogenic factors.

All injected muscles showed an increased electrophysiological response to passive *HV* stretches, indicating that spasticity was correctly diagnosed in all children. In 14 of the 19 muscles that were tested, we also found an EMG onset during *LV* stretches. This may imply the presence of position- or muscle length-dependent spasticity, as also reported in stroke[23] and spinal cord injured[24] patients. It has been suggested that with increasing muscle length, group II afferent neurons activate baseline muscle spindle activity, which in turn lowers the threshold, but not the amplitude of the stretch reflex.[25] In the current study, the torque parameters at *LV* did not change post BTX and the decrease in EMG at *LV* was clinically not relevant (below the MDC). Furthermore, at *LV*, the change in torque was not correlated to the change in EMG. This suggests that the low intensity muscle activity present during *LV* stretches does not contribute towards the simultaneously measured torque and that neither parameter is affected by BTX treatment. Torque parameters at *LV* are thus believed to represent intrinsic stiffness (due to secondary changes of the spastic muscle) rather than the neural components of tone.[26] On the other hand, the change pre-post BTX in ROM at *LV* did significantly exceed the MDC. Since little significant effect of BTX on intrinsic stiffness has been reported [27], and casting is known to alter the extensibility of muscle and joint, this increased ROM is thought to reflect the effect of post-injection casting. Alhusaini et al.[27] have also reported increased ROM and unchanged intrinsic stiffness due to BTX during low-velocity stretches of the gastrocnemius in children with CP. We conclude that it is important to distinguish those patients with increased intrinsic stiffness from those with increased reflex-related torque. This will already help determine the optimal treatment modality for the individual child.

The AOC and its power value increased post BTX, suggesting an increase in the velocity-threshold and a reduction in the catch severity after treatment. However, it should be noted that Wu et al.[28] have warned that the AOC position is positively correlated to the velocity of stretch, with later catches occurring the higher the velocity. Since in the current study, the MEH muscles were stretched at a significantly faster velocity post BTX-A, this could have affected the results. We believe that considering the intensity with which the catch occurs, together with the position, improves the interpretation of the spastic catch. However the repeatability of this parameter should be enhanced.

In the current study, 12 children showed an improvement in RMS-EMG at *HV-LV* that exceeded MDC, 6 were within the positive and negative MDC values, and 1 showed increased RMS-EMG activity (i.e. worsening of spasticity) that was smaller than the negative MDC value (Figure 3). In comparison, Pandyan et al.[18] measured RMS-EMG during fast passive stretches of the biceps muscle in stroke patients before and after BTX-A injections. In accordance to our findings, they reported large response variability, whereby 9 of their 14 subjects had decreased and 4 had increased RMS-EMG post BTX. They did not however quantify the MDC of the RMS-EMG parameter, which may have led to an overestimation of responders and non-responders. In fact, to the best of our knowledge, no study has used information on the measurement error of instrumented spasticity assessments to interpret the effect of BTX, making comparisons difficult.

Despite the sensitivity of the instrumented measurement method, some methodological limitations need to be considered. A first limitation is the significantly higher stretch velocity during post-treatment sessions. However, despite faster stretching post BTX, there was still an overall reduction in the spasticity parameters. Furthermore, as has been reported by Chen et al.[12], BTX can increase the velocity threshold of the spastic muscle, which could have accounted for the increased V_{MAX} post BTX. The V_{MAX} post BTX was also closer to the velocity at which the MEH of typically developing children is stretched.[13] A second possible limitation is the lack of EMG normalization. This may have accounted for some of the response variability seen among children. However, since BTX is known to affect strength, normalization to e.g. maximum voluntary contraction is not suitable and will only increase variability.[29] To ensure reliability of the calculated parameter and minimize variability, a thoroughly standardized electrode application was applied. This standardized procedure without normalization resulted in reliable RMS-EMG parameters (Supplementary Material 1). Finally, while this study investigated the effect of BTX on passive spasticity, it is also recommended to capture the effect of BTX on functional activities (e.g. walking). More specifically, as only weak to moderate correlations between clinical spasticity scores and gait parameters have been reported[30], it would be useful to explore correlations between parameters from instrumented tests and gait analysis.

In conclusion, the current study proposes an instrumented method to quantify the effect of BTX on MEH spasticity in children with CP. Spasticity parameters that were sensitive to treatment and larger than the MDC were identified. These could potentially be used to categorize subjects according to the level of response and thus assist in treatment planning. This consolidates the clinical validity of the proposed method and opens up possibilities of exploring the effects in other muscles or of other tone-reducing treatments such as selective dorsal rhizotomy and intrathecal baclofen. Furthermore, combining multiple, integrated parameters was found superior over interpreting a single parameter obtained from an isolated signal to assess treatment efficacy. Increased torque at *LV*, representing intrinsic rather than reflex-related stiffness, did not change post BTX. However, the large response variability among children requires further studies using objective, instrumented measurements that

assess the effect of BTX better understanding of factors that determine treatment outcome will allow individualized treatment planning and increase the functional potential of children with CP.

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Supplementary material 1

Results from intra-rater reliability study of instrumented spasticity parameters for the medial hamstrings (MEH) in 12 children with spastic CP (9.86±3.1 years) tested on two occasion on average 13±9 days apart by the same assessor (see Bar-On et al., 2012 for more details on study and subject characteristics [1]).

		Test mean (SD)	Retest mean (SD)	ICC	SEM	MDC
ROM (˚) *LV		88.12 (18.06)	92.19 (17.77)	0.90	4.05	9.43
V_{MAX} (˚/sec)	*LV	17.37 (7.28)	17.69 (6.04)	0.86	3.424	8.00
	*MV	108.55 (35.67)	103.53 (26.07)	0.47	26.71	62.15
	*HV	270.61 (41.50)	269.60 (44.54)	0.91	17.52	40.76
RMS-EMG (mV)	*LV	0.005 (0.006)	0.005 (0.005)	0.96	0.002	0.004
	*MV	0.011 (0.009)	0.012 (0.009)	0.96	0.003	0.006
	*HV	0.020 (0.015)	0.021 (0.015)	0.97	0.004	0.008
	*MV-LV	0.007 (0.004)	0.007 (0.005)	0.91	0.002	0.004
	*HV-LV	0.015 (0.012)	0.016 (0.011)	0.98	0.003	0.006
Torque (Nm)	*LV	4.65 (4.21)	3.96 (2.75)	0.84	1.91	4.44
	*MV	8.87 (7.81)	7.14 (5.22)	0.92	2.39	5.56
	*HV	12.45 (7.79)	13.32 (7.00)	0.97	1.68	3.91
	*MV-LV	4.22 (3.82)	3.18 (2.75)	0.92	1.12	2.61
	*HV-LV	6.46 (3.39)	6.09 (2.97)	0.93	1.19	2.76
Work (J)	*LV	2.28 (1.46)	2.41 (1.17)	-0.07	1.37	1.19
	*MV	5.45 (3.53)	4.84 (2.44)	0.71	2.07	4.82
	HV	8.20 (4.25)	8.74 (4.17)	0.89	1.80	4.19
	*MV-LV	3.17 (2.39)	2.43 (1.43)	0.84	0.96	2.23
	*HV-LV	6.62 (3.79)	7.33 (3.16)	0.84	1.80	4.17
AOC (%)	HV	88.60 (11.24)	87.64 (10.27)	0.87	5.40	12.52
AOC power (J)	HV	- 7.29 (5.05)	-4.84 (2.61)	0.50	4.56	10.61

ICC: Intraclass correlation coefficient; SEM: standard error of measurement; MDC: minimal detectable change [2]; *LV*: low velocity stretch; *MV*: medium velocity stretch; *HV*: high velocity stretch; *MV-LV*: medium velocity stretch minus low velocity stretch; *HV-LV*: high velocity stretch minus low velocity stretch; ROM: range of motion; V_{MAX}: maximum angular velocity; Torque: torque at 70˚ knee flexion; AOC: angle of catch defined as the angle corresponding to the time of the first minimum power value after the time of the maximum power, expressed as a percentage of the full range of motion; AOC power: the power value at AOC. *These results were additionally calculated from the subject group previously described [1].

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Part 2, 2.2

Is an instrumented spasticity assessment an improvement over clinical spasticity scales?

An exploration of the responsiveness and predictive ability of instrumented and clinical spasticity assessments to BTX treatment in the medial hamstrings of children with cerebral palsy.

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Abstract

Objectives To compare (a) responsiveness and (b) predictive ability of clinical and instrumented spasticity assessments after treatment with Botulinum Toxin A (BTX) in the medial hamstrings (MEH) in children with spastic cerebral palsy (CP).

Design Prospective cohort study

Setting University Hospital

Participants 31 children (40 MEH muscles) with CP, consecutive sample, requiring BTX injections.

Interventions Instrumented and clinical spasticity assessments before and 53±14 days after BTX.

Main outcome measures Clinical spasticity scales included the Modified Ashworth Scale (MAS) and the Modified Tardieu Scale (MTS). The instrumented spasticity assessment integrated biomechanical (position and torque) and electrophysiological (surface electromyography-sEMG) signals during manually-performed low- and high-velocity passive stretches of the MEH. Signals were compared between both stretch velocities and examined pre and post-BTX. Responsiveness of clinical and instrumented assessments was compared by percentage exact agreement (PEA). Prediction ability was assessed with a logistic regression and the area under the Receiver Operating Characteristic (ROC) curves of the baseline parameters of the responders versus the non-responders.

Results Both clinical and instrumented parameters improved post-BTX ($p \leq 0.005$), though showed a low PEA. Baseline MTS was the only clinical scale predictive for response (area under ROC curve=0.7). For the instrumented assessment, baseline values of root mean square (RMS) EMG and of torque were better predictors for a positive response (area under ROC curve=0.82). RMS-EMG remained an important predictor in the logistic regression.

Conclusions The instrumented spasticity assessment showed higher responsiveness than the clinical scales. The amount of RMS-EMG is considered a promising parameter to predict treatment response.

Introduction

Cerebral palsy (CP) is the most common cause of physical disability in children. Spasticity, occurring in 80-90% of these children, is characterized by a velocity-dependent increase in tonic stretch reflexes resulting from hyperexcitability of the stretch reflex.[1] It is considered the main cause of secondary muscle contractures and bone deformities.[2] Consequently, spasticity management in children with CP aims to prevent these secondary problems and to delay or avoid the need for surgery.[3]

Intramuscular injected Botulinum Toxin type-A (BTX) is effective in temporarily decreasing spasticity,[4] though a large variability in response has been reported in children with CP (37-80%, depending on the outcome measure used).[5,6] Common outcome measures include the Modified Ashworth Scale (MAS)[7] and the Modified Tardieu Scale (MTS).[8] However, the intrinsic subjective character of these classical clinical scales restricts their reliability.[9-12] Additionally, it remains unclear whether their predictive ability is sufficient for clinical decision making.[13-16] The value of clinical scales may be questioned as they cannot differentiate between neural and non-neural components of increased resistance.[10,17-21] This may be essential information to support treatment planning and help understand response.

Instrumented tests that integrate biomechanical and electrophysiological measures of spasticity collect quantitative data.[20,21] Recently, such instrumented spasticity assessment usable in a clinical setting has proved repeatable and valid in measuring spasticity in the medial hamstrings (MEH) in children with CP.[22,23] However, it has yet to be assessed if parameters obtained from instrumented assessments are more sensitive than clinical scales in detecting treatment response and if these could provide further insights that help explain response variability.

In this study we used instrumented and clinical spasticity assessments to define the effect of BTX in the MEH of children with CP. For both assessments, we first analyzed their responsiveness to change and secondly, their ability to predict it. We hypothesized that an instrumented assessment was more responsive and could better predict the effect of BTX on spasticity in the MEH of children with CP.

Methods

Participants

In this prospective cohort study, participants were recruited from the multidisciplinary clinic for patients with CP of the University Hospital Leuven. Children aged 3-18 years and scheduled for BTX of the MEH (Mm. Semitendinosus and Semimembranosus) were screened for inclusion. Exclusion criteria were: presence of ataxia or dystonia; severe muscle weakness (<2+ on the Manual Muscle Test[24]); poor selectivity[8]; bone deformities or contractures compromising the performance of purely sagital plane passive knee flexion/extension movements; cognitive problems that could impede the measurements; previous lower limb orthopedic surgery; intrathecal Baclofen pump or selective dorsal rhizotomy. Children's parents signed an informed consent for participation. The experimental protocol was approved by the university hospital's ethical committee.

BTX dosage *(Botox®, Allergan Ltd, UK)* was selected based on findings of a clinical examination (MAS[7], MTS[8], range of motion-ROM, strength[24], selectivity[8]) and 3D-gait analysis. Injection was done under a short anesthesia and ultrasound was used for visual identification of muscles and depth control of needle placement.[25] Post-BTX, all patients received casting for a period of 10 days (lower leg with optional removable, upper-leg night splint used as a knee-extension device), intensive physical rehabilitation and orthotic management (day and night), as previously described.[26]

Data acquisition

Spasticity assessments were performed before injection and between 14-90 days after. Clinical (MAS[7], MTS[8]) and instrumented spasticity assessments[22] were performed consecutively by two independent assessors on the same day. MTS measurements were only performed in cases of MAS≥1+ by applying a quick passive stretch and recording the angle at which a catch was felt (R1-value).[8] In children with unilateral CP, only the affected side was tested. In children with bilateral involvement, both sides were tested.

The set-up of the instrumented assessment is presented in Figure 1. All evaluations were conducted as previously outlined.[22] Surface electromyography (sEMG) electrodes were placed according to standardized procedure on the MEH and rectus femoris.[27] Data from the rectus femoris were utilized to ensure the absence of active assistance of the patient during passive stretches. Four repetitions of passive muscle stretches of the MEH, at three velocities, over the full ROM were carried out. The knee joint was first moved at low-velocity during 5sec, followed by an intermediate-velocity (not included in the current data analysis) and finally at high-velocity, performed as fast as possible. The interval between repetitions was 7sec.

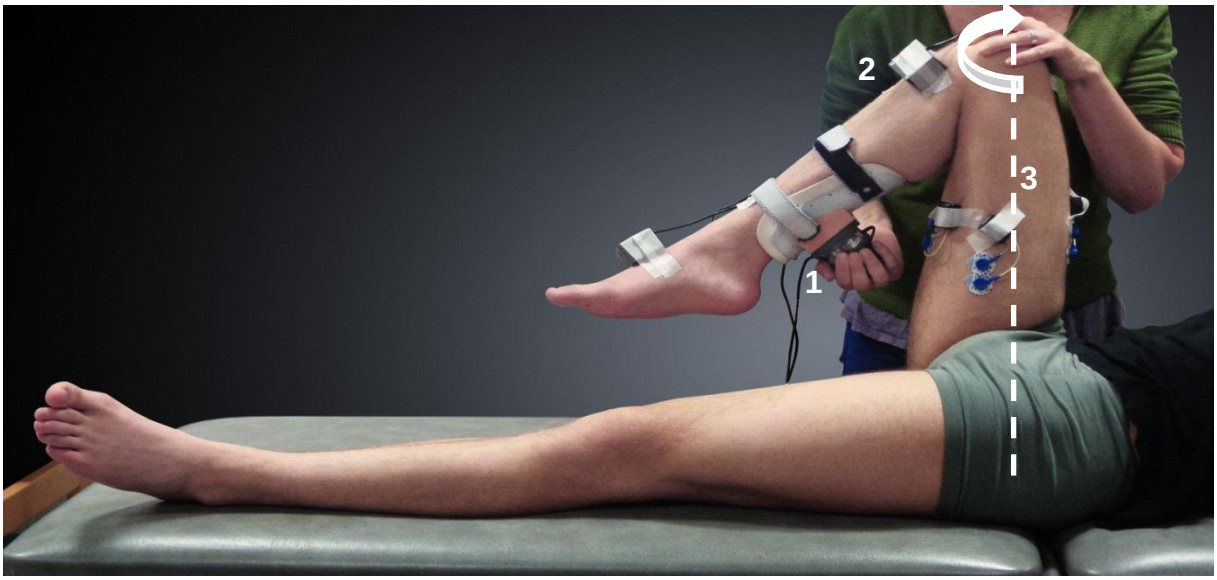


Figure 1. Test starting position, direction of stretch (white arrow) and instrumentation for the instrumented spasticity assessment of the medial hamstrings muscle. Overview of the test instrumentation: (1) a six degrees of freedom force-sensor attached to a shank orthosis on the posterior aspect of the lower leg was used to measure torque; (2) two inertial measurement units measured joint angle characteristics; and (3) surface electromyography measured muscle activity of the agonistic and antagonistic muscle groups.

Data analysis

A 6th-order zero-phase Butterworth bandpass filter ranging from 20-500Hz was applied to filter the raw sEMG-signal. The root mean square envelope of the sEMG (RMS-EMG) was computed using a low-pass 30Hz 6th-order zero-phase Butterworth filter on the squared raw signal. ROM, maximum angular velocity (V_{MAX}), and the net internal joint torque were obtained as previously described.[22]

Data with a missing post-test were excluded. Additionally, repetitions were excluded when passive stretches were performed out-of-plane (see Suppl. 1 in[22]), at non-similar velocities (difference >20˚/sec within a velocity trial), in case of poor quality sEMG (low signal-to-noise ratio or obvious artifacts), and in case of antagonist activation. Data analyses were carried out using MATLAB® Software 7.6.0 R2008a.

Outcome parameters

MAS and MTS-scores constituted the clinical spasticity parameters. From the instrumented test, nine spasticity parameters were extracted from joint angles, RMS-EMG and torque signals. ROM was analyzed from low-velocity stretches, V_{MAX} from high-velocity stretches. Non-normalized, average RMS-EMG (V) was calculated as the area underneath the RMS-EMG time curve, divided by the duration of the time interval considered. This time interval started 200ms prior to the time corresponding to V_{MAX} and ended at the time corresponding to 90% of the full ROM. The normalized EMG-parameter was calculated conform Bar-On et al. (2012)[22], i.e. as the ratio between the average RMS-EMG signal during the passive stretch and the peak of the RMS-EMG signal during a maximum voluntary contraction (RMS-EMG%). However, BTX also affects strength and thus the stability of the maximum voluntary contraction. The RMS-EMG% of the post-tests was therefore calculated using the maximum voluntary contraction from the pre-test.[28]

From the computed torque, five additional spasticity parameters were assessed. Torque was first computed at the time at which V_{MAX} occurred, and secondly at 70° knee flexion, an angle that corresponded to the overall mid-ROM of all subjects. Thirdly, work was calculated as the integral of torque with respect to the position starting at V_{MAX} to 90% of the ROM. Power, defined as the product of angular velocity and torque, has recently been found to be a sensitive and valid parameter to detect the position and intensity of the angle of catch (AOC) in children with CP.[23] Therefore, the AOC was defined as the angle corresponding to the time of minimum power after maximum power in the first high-velocity stretch, expressed as a percentage of ROM.[23] Finally, this minimum power value was used to report the intensity of the catch.[23] All parameters (except AOC and AOC power) were calculated by taking the average of 2-4 repetitions per velocity. Additionally, all parameters (except ROM, V_{MAX}, AOC and AOC power) were calculated as the absolute change between low- and high-velocity stretches.

Statistical analyses

Responsiveness

To evaluate group response, clinical and instrumented parameters were compared pre and post-BTX using Wilcoxon Signed Rank Test. While the clinical scales have been found responsive to BTX[8], no such evidence exists for instrumented parameters. To identify the most responsive instrumented parameters, the group average change between pre and post-BTX was evaluated in view of the minimal detectable change (MDC).[29] MDC-values were calculated from standard error of

measurement values previously reported[23] (MDC=standard error of measurement*1.645*√2)[29] (Appendix 1). Only those parameters with average change values above the MDC were regarded responsive and retained for further analyses. To evaluate response per muscle, a distinction was made between *clinical response* and *instrumented response*.

CLINICAL RESPONSE

Per muscle, the change in clinical scores between pre and post-BTX was calculated. A positive response was defined as a change to a lower MAS-score[30] or a change to a less severe catch angle (change >8.45°, i.e. the standard error of measurement of the MTS for the MEH[12]). A muscle that responded in either clinical scale was categorized as a *clinical responder* (scored as 1). A muscle showing no improvement in either clinical scale was categorized as a *clinical non-responder* (scored as 0).

INSTRUMENTED RESPONSE

Per muscle, the change between pre and post-BTX values of every responsive instrumented parameter was calculated, and expressed as a percentage of its corresponding MDC-value. Next, these change-values were averaged to produce one value per muscle. A muscle with a value >100% was considered an *instrumented responder* (scored as 1). A muscle with a value ≤100% was considered an *instrumented non-responder* (scored as 0).

Percentage exact agreement values between the *clinical* and *instrumented responders* were calculated. The significance of the differences in percentage exact agreement was determined using Fisher's exact *p*-value of the chi-square statistic. The difference between the ability to detect a responder was determined using the McNemar test. To account for agreement due to chance, Kappa (*K*) values were calculated. These were interpreted as poor when <0.2, fair when 0.2-0.4, moderate when 0.4-0.6, good when 0.6-0.8 and very good when >0.8.[31]

Prediction

We explored whether baseline values of the clinical scales and the instrumented parameters could predict a *clinical and* an *instrumented responder,* respectively. Baseline MAS, MTS and instrumented parameters were therefore compared between responders and non-responders, using either Fisher-exact (dichotomized parameters), Freeman-Halton (categorical parameters) or Man Whitney U tests (continuous parameters). To investigate whether patient and treatment characteristics affected response, following parameters were also compared between responders and non-responders (tests as appropriate): age, Gross Motor Function Classification Score[32], diagnosis (right/left hemiplegia, diplegia, triplegia, quadriplegia), casting (with/without upper-leg casts), BTX dosage, and time between injections and post-evaluation.

Significance was set at $p<0.05$. When more than one parameter differed significantly between responders and non-responders, a multivariate logistic regression was performed. Parameters were examined for co-linearity using Spearman rank correlation coefficients. Only those parameters with poor to moderate inter-correlations[33] were retained and submitted to the multivariate logistic regression. Areas under the Receiver Operating Characteristic (ROC) curves were calculated per predictive parameter and for the combination of parameters. Statistical analyses were performed using SPSS (IBM Statistics 20).

Results

Thirty-one children (Table 1) with CP were included, of whom 19 were injected bilaterally. Data could not be collected bilaterally in 11 participants, due to time restrictions or poor quality EMG. In these cases, data was collected from the most affected side (highest baseline MAS-score or the more severe MTS in case of symmetrical MAS). Clinical scores of the excluded muscles did not differ significantly from the included muscles at baseline or post-BTX [(pre median (IQR): MAS 2 (0.5), MTS -82.5º (10º); post median (IQR): MAS 1.5 (0.5), MTS -75º (10º)]. Therefore, a total of 40 MEH muscles were studied. The mean dose for the MEH was 3±0.6U/kg *Botox®* (range: 2-4U/kg). Assessments were executed on average 22±16 days (0-46 days) before and 53±14 days (17-90 days) after injection.

Table 1. Participant and treatment characteristics

Participant characteristics	**participants (n=31), muscles (n=40)**
Mean age (SD) (years)	8.77 (2.48)
Male/female (n)	male: 18, female: 13
Diagnosis (n)	
- Unilateral involvement	5 left hemiplegia, 6 right hemiplegia
- Bilateral involvement	17 diplegia, 1 triplegia, 2 quadriplegia
GMFCS (I-V) (n)	I: 12, II: 12, III: 6, IV: 1
Treatment characteristics	
Amount of BTX injected in the MEH	2.0: 6, 2.67: 13, 3.0: 2,
(units/Kg) (number of muscles)	3.33: 14, 4.0: 4, 4.67: 1
Casting post BTX (number of muscles)	Knee-extensor casts: 33
	No knee-extensor casts: 7

GMFCS: Gross Motor Function Classification Score

Responsiveness

All spasticity parameters significantly improved post-BTX (Table 2). The average change in RMS-EMG (9.27±9.02V), RMS-EMG% (7.61±7.91%), torque at 70º knee flexion (2.82±2.60Nm) and AOC (13.83±8.94%) was larger than each corresponding MDC-value (Appendix 1).

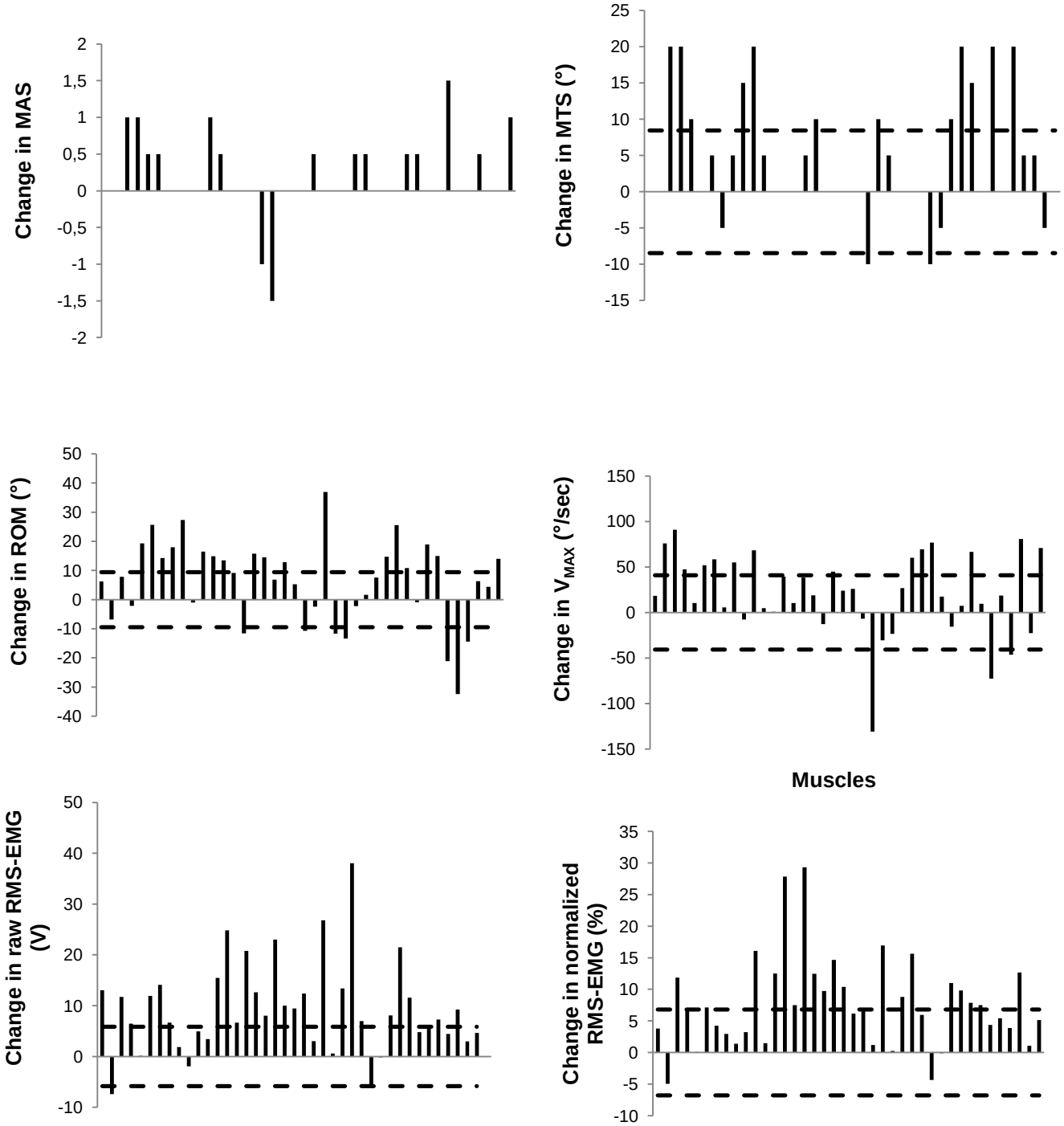
Table 2. Median and interquartile range (IQR) values of spasticity parameters pre- and post-BTX.

	Pre-BTX	**Post-BTX**	***p***
MAS	2.00 (0.50)	1.50 (0.50)	*0.036
MTS (º)	-80.00 (15.00)	-75.00 (10.00)	*0.002
RMS-EMG (V)	15.53 (9.69)	5.93 (6.14)	*<0.001
RMS-EMG (%)	11.89 (7.92)	3.91 (4.84)	*<0.001
Torque at 70˚ (Nm)	7.09 (5.11)	4.02 (3.21)	*<0.001
Torque at V_{MAX} (Nm)	5.39 (4.64)	4.36 (3.57)	*0.009
Work (J)	4.53 (3.03)	3.18 (3.15)	*<0.001
ROM (°)	75.21 (16.00)	79.61 (15.27)	*0.024
AOC (%)	76.84 (22.23)	92.03 (13.11)	*<0.001
AOC power (W)	-5.31 (8.10)	-1.01 (8.08)	*<0.001

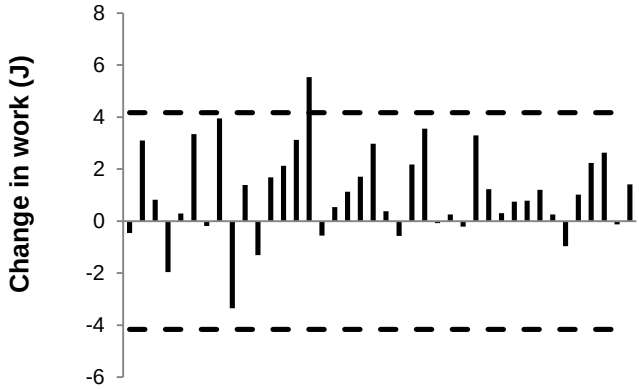
RMS-EMG (%):the ratio between the average RMS-EMG signal during the passive stretch and the peak of the RMS-EMG signal during a maximum voluntary contraction; Torque at 70˚: torque at 70˚ knee flexion; Torque at V_{MAX}: torque at maximum angular velocity; AOC (%): angle of catch expressed as a percentage of the full range of motion; AOC power: the power value at the angle of catch. *p<0.05

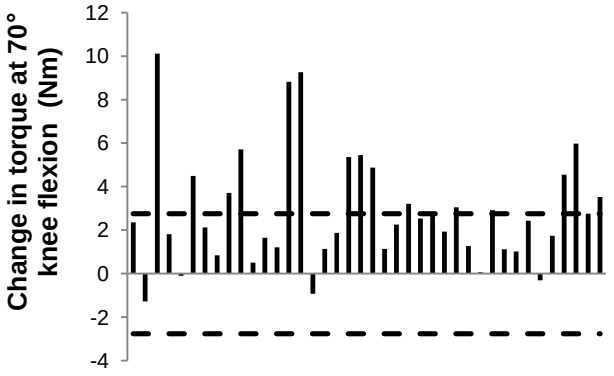
Individual change values of clinical and instrumented parameters and their corresponding MDC-values are shown in Figure 2. Six muscles at baseline and 8 muscles post-BTX received a MAS-score ≤1 and thus not assessed with MTS. The MAS identified 14 responders, the MTS 12. Combined, there were 19 *clinical responders*. Responsive instrumented parameters together identified 25 *instrumented responders*. Despite low percentage exact agreements between clinical and instrumented assessments, detection ability was only statistically different between MAS and *instrumented responders* (Table 3).

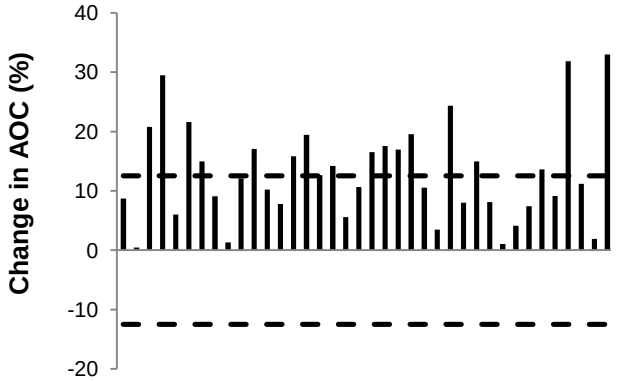
Figure 2. Individual change between pre- and post-treatment sessions in: (A) Modified Ashworth scores (MAS); (B) Modified Tardieu scores (MTS); (C) range of motion (ROM); (D) maximum angular velocity (V_{MAX}); (E) root mean square electromyography (RMS-EMG); (F) RMS-EMG expressed as percentage of maximum voluntary contraction; (G) Work (H) torque at 70˚ knee flexion; (I) relative position of the angle of catch (AOC); (J) AOC power. Each bar represents one muscle. A positive value indicates a decrease in that parameter or an improvement post-BTX. The dashed horizontal lines represent the minimal detectable change values, or in case of the MTS, the standard error of measurement value.



Part 2: MEP targeted injections







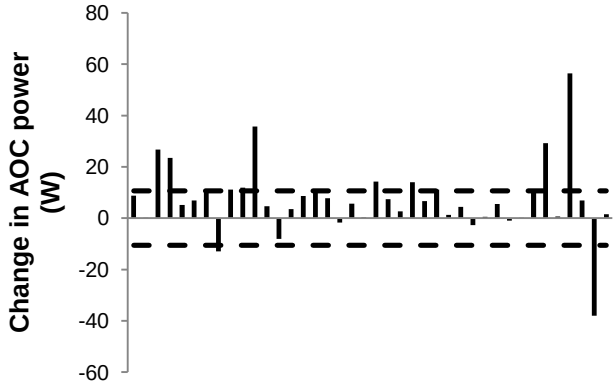


Table 3. Responsiveness to BTX: Percentage Exact Agreement (PEA) between the clinical scales and the instrumented assessment.

		MAS			PEA	K	Fisher's exact	McNemar test
		Non-responders	Responders	Total			*p*-value	*p*-value
Instrumented	Non-responders	9	6	15	No response=23%			
	Responders	17	8	25	Response=20%			
	Total	26	14	40	Total 43%	-0.07	0.736	0.035
		MTS						
		Non-responders	Responders	Total				
Instrumented	Non-responders	8	6	14	No effect=25%			
	Responders	12	6	18	Effect=19%			
	Total	20	12	32	Total 44%	-0.09	0.718	0.238
		Clinical						
		Non-responders	Responders	Total				
Instrumented	Non-responders	6	9	15	No effect=15%			
	Responders	15	10	25	Effect=25%			
	Total	21	19	40	Total 40%	-0.19	0.328	0.307

K: Kappa values

Prediction

Table 4 shows baseline parameters of responders versus non-responders. Patient and treatment characteristics did not differ between responders and non-responders. MTS was significantly different between *clinical responders* and *non-responders*. The area under the ROC curve for predicting a *clinical responder* using the MTS was 0.7, p=0.04 (Table 4). Values of RMS-EMG, RMS-EMG% and torque at 70° were significantly different between *instrumented responders* and *non-responders*. As RMS-EMG and RMS-EMG% were highly correlated (r=0.8, p<0.05), only RMS-EMG was retained for multivariate logistic regression. Combination RMS-EMG and torque at 70° knee flexion

Table 4. Median and inter-quartile range values of baseline parameters of clinical and instrumented responders and non-responders. Univariate analyses (Man Whitney U, Fisher-exact or Freeman-Halton, as defined in the methods section) between baseline and outcome parameters.

Outcome parameters	Clinical scales			Instrumented assessment		
Clinical baseline parameters	Non-responders (n=21)	Responders (n=19)	p	Non-responders (n=15)	Responders (n=25)	p
Pre MAS (grades 1-3)	0:2 ;1:3; 1+:9; 2:5; 3:2	1+:5; 2:10; 3:4	0.08	1+:5; 2:8; 3:2	0:2; 1:3; 1+:9; 2:7; 3:4	0.43
Pre MTS (°)	75 (14)	85 (15)	*0.04	80 (15)	80 (19)	0.73
Instrumented baseline parameters						
Pre RMS-EMG (V)	17.49 (13.50)	12.43 (6.50)	0.05	17.23 (11.90)	10.62 (10.90)	*0.01
Pre normalized RMS-EMG (%)	13.03 (10.04)	11.39 (6.45)	0.35	6.93 (10.90)	13.40 (6.21)	*0.01
Pre Work (J)	5.56 (3.90)	3.62 (3.07)	0.49	3.50 (3.52)	5.19 (4.35)	0.21
Pre torque at VMAX (Nm)	5.49 (4.66)	6.17 (5.54)	0.86	5.31 (3.25)	6.17 (5.58)	0.08
Pre torque at 70˚ knee flexion (Nm)	8.30 (4.52)	6.23 (5.37)	0.88	5.37 (4.97)	7.85 (5.96)	*0.04
Pre ROM (°)	76.24 (14.63)	74.55 (17.60)	0.78	77.36 (18.36)	73.77 (18.64)	0.05
Pre AOC (%)	79.0 (21.24)	74.09 (24.81)	0.22	86.13 (40.27)	74.92 (18.49)	0.08
Pre AOC power (J)	-4.56 (9.88)	-9.89 (15.49)	0.12	-4.25 (7.75)	-9.25 (10.73)	0.19
Pre VMAX at high velocity (°/s)	284.72 (47.28)	278.32 (66.72)	0.10	306.4 (43.33)	283.1 (59.09)	0.26

Patient characteristics						
Age (years)	8.75 (2.87)	8.62 (5.23)	0.89	8.70 (4.36)	8.62 (3.30)	0.86
GMFCS (I-IV)	I:10; II:8; III:3	I:3; II:11; III:4; IV:1	0.21	I:4; II:7; III:3; IV:1	I:9; II:12; III:4	0.83
Paralysis injury (RH; LH; Di; Tri; Quad)	RH:3; LH:5; Di:13	RH:3; LH:1; Di:11; Tri:2; Quad:2	0.18	RH:2; LH:1; Di:10; Tri:1; Quad: 1	RH:4; LH:5; Di:14; Tri:1; Quad:1	0.81
Treatment characteristics						
Timing of post-measurement (days)	56.00 (12)	53.0 (10)	0.20	56.0 (11)	52.0 (8)	0.12
Knee extension casts (yes/no)	Yes=17; No=4	Yes=16; No=3	0.79	Yes=12; No=3	Yes=21; No=4	0.75

Pre normalized RMS-EMG (%): the ration between the RMS-EMG signal during a passive stretch and the peak of the RMS-EMG signal during a maximum voluntary contraction; Torque at VMAX: torque at maximum angular velocity; ROM: range of motion; AOC (%): angle of catch expressed as a percentage of the full range of motion; AOC power: the power value at the angle of catch; VMAX: maximum angular velocity during a high velocity stretch; GMFCS: Gross Motor Function Classification Score; RH: right hemiplegia; LH: left hemiplegia; Di: diplegia; Tri: triplegia; Quad: quadriplegia.

resulted in an area under the ROC curve of 0.82 (p<0.01), although only RMS-EMG (p=0.02) remained significant in the logistic regression (Table 5).

Table 5. Multiple logistic regression results for predicting the clinical and instrumented outcome scores

Predictor variable	B	S.E	Wald chi square	*p*-value	Estimated odds ratio	Area under ROC curve	ROC curve *p*-value
Clinical responder							
Baseline MTS	0.08	0.04	3.95	0.05	1.08	0.70	0.04
Constant	-5.95	3.08	3.73	0.05	0.003		
Model				0.03		0.70	0.04
Instrumented responder							
Baseline RMS-EMG (V)	0.163	0.069	5.54	0.02	1.18	0.77	<0.01
Baseline Torque at 70° knee flexion (Nm)	0.22	0.12	3.41	0.07	1.25	0.69	0.01
Constant	-3.53	1.47	5.75	0.02	0.03		
Model				<0.01		0.82	<0.01

B: coefficient for predicting dependent variable; S.E: standard errors associated with the coefficients

Discussion

This study compared the responsiveness of clinical and instrumented spasticity assessments following BTX and investigated the value of clinical and instrumented baseline parameters to predict treatment response.

Responsiveness

Despite the lower thresholds of responsiveness for the MAS and MTS compared to the instrumented parameters, both scales were less sensitive than the instrumented assessment to detect change in spasticity post-BTX. Although the average MAS-score reduced significantly post-BTX, such reduction was measured in only 14 of 40 muscles. The MAS tends to cluster muscles into broad severity categories, thereby limiting its ability to detect response. Conversely, the instrumented assessment offers continuous parameters and thus a wider range of possible outcome changes.

The average change in MTS did not exceed the measurement error, which was used as the cut-off to determine response.[12] Consequently, a low number of *clinical responders* were detected. However,

goniometry, as used in the MTS, has been repeatedly shown to be an imprecise method to measure the catch angle due to joint repositioning errors.[10,34] This indicates that the reliability of the MTS must be considered when assessing treatment response. Unlike the clinical parameters, several average change values from instrumented parameters did exceed their corresponding MDC-values. This further emphasizes their superiority over the clinical scales in detecting response, and highlights the possibility of these parameters to refine treatment strategies such as muscle selection, injection technique and dosage.

The MAS or the MTS do not discern the relative contributions of neural and non-neural components of tone. Integrating electrophysiological and biomechanical parameters provided a more comprehensive assessment. On average, velocity-dependent EMG during passive stretch reduced 57% post-BTX. According to Lance's definition[1], this indicates spasticity reduction. Nevertheless, large response variability was seen. Only 25 of 40 muscles showed a change in RMS-EMG above the MDC, and 4 muscles had increased RMS-EMG (spasticity increase). However, the instrumented parameters were specifically developed to highlight velocity-dependent spasticity, whereas spasticity might also be position dependent. This is defined as an augmentation of muscle activity already present during low-velocity stretches.[35,36] By only investigating the change between the two velocity conditions, we may have underestimated the effect of BTX on this type of spasticity.

There was an average reduction of 38% in torque post-BTX. Two of four MEH muscles with increased muscle activity also showed increased torque post-BTX, confirming that torque is partially influenced by neurogenic factors. The AOC appeared at a higher percentage of the ROM in half of the MEH muscles post-BTX, suggesting a possible increase in the velocity threshold and thus a reduction of spasticity severity. Unfortunately, no comparable studies are available in children with CP. In stroke patients, similar large response variability post-BTX has been demonstrated, even with instrumented assessments.[18,37,38] However, the lack of reported measurement errors hinders proper result interpretation.

Prediction

Despite the smaller standard deviations of most instrumented parameters post-BTX (Table 2), large response variability among muscles was also present. Possible explanations were sought in the baseline spasticity measurements. In general, muscles with higher pathological EMG activation at baseline tended to be good responders and vice versa. As BTX targets reflex muscle activity by selectively blocking the release of acetylcholine at the cholinergic nerve terminal, it was not surprising that baseline RMS-EMG showed highest sensitivity to identify responders. Conversely, the MAS and the MTS had no predictive ability. The amount of torque at 70° knee flexion did not survive the multivariate logistic regression. Although the parameter captures the effect of increasing stretch velocity, torque was not decomposed into its neural and non-neural components, e.g. viscosity and stiffness. Nevertheless, the instrumented spasticity assessment captured the variability due to treatment, thereby confirming its clinical validity. This consolidates its value to optimize BTX treatment and opens new pathways for other tone-reducing treatments, e.g. selective dorsal rhizotomy and intrathecal baclofen.

In contrast to previous literature, age[26], diagnosis[13,14] and functional level[39] could not distinguish *instrumented responders* from *non-responders*. Casting, which is also known to affect outcome,[26] was not found to be predictive. However, these dissimilarities may have been due to the sample size

and/or lack of variability in the post-BTX treatment protocol. The fact that time was not a predictive factor was unexpected, given the ample evidence of the decreasing effect of BTX with time.[40] Larger, longitudinal studies are warranted to analyze the time course of the effect of BTX.

Study limitations

A first limitation is the dependence of the normalized RMS-EMG parameter on a representative maximum voluntary contraction. BTX induces temporary weakness.[41] Conform literature, we therefore used the maximum contraction at baseline to quantify spasticity post-BTX.[28] The non-normalized parameter was also reported and care was taken to minimize the disadvantage of using non-normalized EMG through standardization of the sEMG-electrode placement. Importantly, conclusions were similar for the normalized and non-normalized parameters.

The current study was lacking a control group. However, previous studies[22,23] which assessed the repeatability of the presented method may serve as a reference for the stability of parameters in case of no treatment. The post-BTX physical therapy was not considered a predictive factor of treatment response. The impact of this factor was considered negligible as we previously showed little variability in the frequency and duration of post-BTX physical therapy.[26]

Finally, the sample size limited the number of predictive variables that could be entered in the multivariate logistic regression. Although instrumented parameters were more responsive and had improved ability to predict treatment response than the clinical scales, this study remains explorative and should be confirmed in larger groups.

Conclusions

This study provides an objective, integrated method to quantify lower limb spasticity and its response to BTX in children with CP. Several instrumented parameters indicated reduced spasticity in most MEH muscles post-BTX. However, the degree of response was still variable. Adequate identification of those muscles that benefit most from BTX is thus essential. Baseline spasticity parameters from an instrumented assessment assist in the prediction of treatment response and may thus be superior to the commonly used clinical scales.

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Part 2: MEP targeted injections

Part 2, 2.3

Motor end plate targeted Botulinum toxin injections of the gracilis muscle in children with CP.

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Abstract

Introduction Intramuscular Botulinum toxin type A (BTX) injections reduce spasticity in children with cerebral palsy (CP) by blocking neurotransmission at the motor end plates (MEP). Injection of BTX close to the MEP zone seems crucial, but current injection techniques do not always aim for this region.

Methods Thirty four gracilis muscles in 27 children with CP (8.5±2.5y) were injected with Botox® (fixed dosage and dilution): 17 muscles by proximal (at 25% of the length of the upper leg) and 17 muscles by MEP targeted (half the dosage at 30 and half at 60% of the upper leg) injections. Clinical (modified Ashworth scale MAS) and instrumented spasticity assessments using surface electromyography (EMG) during passive motion at different velocities were performed before and after the injections. The difference of the averaged root mean square (RMS) EMG at low versus high velocity was calculated and normalized to the pre-injection EMG at maximal voluntary isometric contraction.

Results MEP targeted injections showed a significantly better decline in pathological EMG signal compared to the conventional proximal injections, demonstrated by a higher reduction of the normalized RMS-EMG parameter. This difference could not be demonstrated using the MAS.

Discussion For long muscles, such as the gracilis, the injection site is an important variable in the BTX injection procedure. Using instrumented spasticity assessment, different injection protocols can be compared.

Conclusion BTX injection in the gracilis muscle at the sites with a high concentration of MEPs resulted in improved spasticity reduction. This may eventually lead to reduced BTX-dosages.

This study was registered under the EudraCT number 2010-023631-41.

Introduction

Intramuscular Botulinum toxin type A (BTX) injections reduce spasticity in patients with cerebral palsy (CP). Clinical studies have documented this, but also mentioned variation in outcome.[1] Many factors, including injection technique, are responsible for this inconsistency.[2] BTX blocks neurotransmission at the neuromuscular junction by inhibiting the release of Acetylcholine at the motor endplate (MEP).[3] We therefore not only need to administer the toxin into the preferred muscle, but also need to bring it close to the MEP zone. This was first demonstrated in animal studies[4,5] and more recently in humans[6,7]. The importance of injecting the toxin close to the MEPs in children with CP has recently been demonstrated by increased muscle atrophy after MEP targeted injections in the psoas muscle.[7] However, there is no study that confirms an improved decrease in spasticity after MEP targeted injections.

Spasticity of the gracilis muscle is a common clinical sign in children with CP. The gracilis muscle is one of the muscles involved in hip(sub)luxation and deteriorating gait in CP, as it works as a hip adductor and knee flexor.[8-10] Therefore, this muscle is often treated with BTX. As the gracilis muscle is a long muscle, running from the symphysis pubis till the proximal tibia, it is important to localize the optimal injections site(s). The localization of the MEP zones of the gracilis muscle can be derived from several histological and dissection studies. Christensson[11] and Kumar et al[12] observed two dense bands of MEP zones using cholinesterase staining: one oblique band between the upper and middle thirds and another between the middle and lower thirds along the length of the muscle. This and other observations (interruption by fine collagen fibers between the muscle fibers and the absence of double innervation[11-13]) suggest that at least part of the muscle fibers in the gracilis muscle have been developed from two myoblasts, each with its own motor end plate. Won et al.[13] demonstrated the intramuscular branching pattern, with reference to surface landmarks of the thigh, using Sihler staining. The branches of the obturator nerve spread out from 17.8% to 86.0% of the distance from the pubic tubercle to the medial epicondyle of the femur, with an area of even denser distribution of nerve branches between 25 and 35%. However, in most textbooks describing injection techniques for the toxin, this information is not taken into account. Instead, it is advised to inject the gracilis muscle in the middle third of the thigh along a line joining the pes anserinus and the symphysis pubis[14] or to inject proximally[15].

In order to find the most efficient injection technique for the gracilis muscle, two different injection localizations were compared: MEP targeted versus a popular proximal injection[14]. Outcome was quantified by means of a quantitative spasticity assessment using surface electromyography (sEMG) measured during passive muscle stretch at different velocities.[16] It was hypothesized that MEP targeted BTX injections would result in a more effective tone reduction compared to proximal BTX injections in the gracilis.

Methods

Participants

Participants were recruited from the multidisciplinary clinic for patients with CP of the University Hospital of Leuven. Children with CP, aged between 3 and 18 years, with the indication for BTX injection of the gracilis muscle were reviewed for participation. This injection was part of a multilevel BTX treatment. For all children, at least the semitendinosus and semimembranosus muscles were also injected. The exclusion criteria were: presence of ataxia or dystonia; severe muscle weakness (<2+ on the Manual Muscle Test[17);] previous lower limb orthopedic surgery (soft tissue or bony procedures); intrathecal Baclofen pump or selective dorsal rhizotomy. Minimal strength production was required because a representative voluntary contraction was used as an individual reference to evaluate sEMG signals during the spasticity assessment. Parents of the children signed an informed consent for participation. The experimental protocol was approved by the university hospitals' ethical committee and registered internationally.

BTX procedure

The BTX (Botox®, Allergan Ltd, UK) injection was done under a short anesthesia and ultrasound was used to confirm needle position. A fixed dosage of 2 U/kg body weight (with a dilution of 5ml per 100U) was used for the gracilis muscle. All patients underwent casting for a period of ten days (lower leg cast with removable, upper-leg night splint used as a knee-extension device), intensive physical rehabilitation as well as orthotic management (day and night) following BTX treatment.
For each patient the injection procedure was chosen at random (Randomisation generator, SPSS). The injection was either given MEP oriented – this is at two sites: half of the dosage at 30% and half of the dosage at 60% of the total distance from the pubic tubercle to the medial epicondyle of the femur (Figure 1A) - or only proximal which is at 20% to 25% of the reference line (Figure 1B). In the latter procedure, the total dose was given at this site when this was less than 50U. If more than 50U needed to be injected this was distributed at two sites with three centimeters distance. All other muscles that needed BTX were injected MEP targeted[18]with standard dosages[14] in a dilution of 5 ml per 100U.

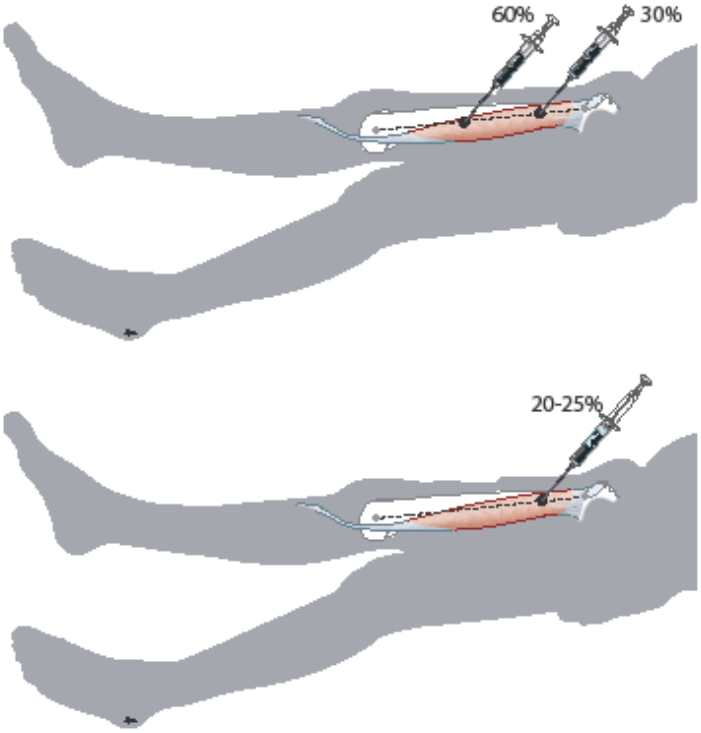
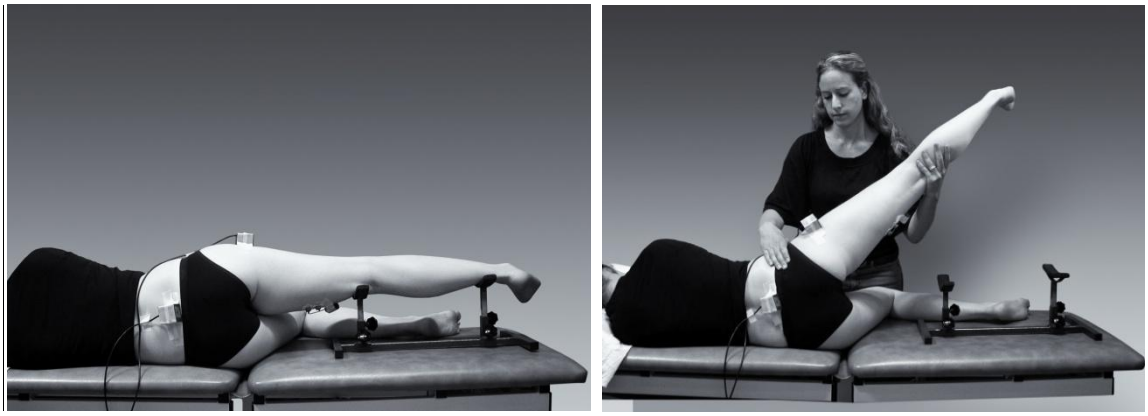


Figure 1. Injection procedures of M. Gracilis.
A. MEP oriented injection: half of the dose at 30% and half of the dose at 60% of reference line
B. Proximal injection: at 20% to 25% of the reference line
Reference line: from the pubic tubercle to the medial epicondyle of the femur

Instrumented spasticity assessments

Assessments were performed before and 3 to 12 weeks after the injection. As part of a standard clinical spasticity assessment, the modified Ashworth scale (MAS)[19] of the medial hamstrings and hip adductors was measured as the gracilis crosses the knee joint as a hamstring and also works as an adductor.
An instrumented assessment to measure spasticity[16] of the gracilis muscle was performed in a side lying position as presented in Figure 2. Data acquisition was carried out using Labview 8.6 (National Instruments, Austin, TX, USA). The evaluation included collection of sEMG using Zerowire (Wave Wireless EMG, Milan, IT) at a sample rate of 2000 Hz. The gracilis muscle was palpated by moving the leg in full abduction and applying knee extension. sEMG-electrodes were then placed on the gracilis, at one third of the length of the upper leg down from the groin area. sEMG data was collected during the entire instrumented measurement procedure, thus also during the rest periods in between the passive muscle stretches to ensure the subject was relaxed. Two inertial measurement units (IMU: Analog Devices, ADIS16354) were used to track the movement of the upper leg with respect to the pelvis. One IMU was placed in line with the sacrum half way between the posterior superior iliac spines and the other was placed on the upper leg. To compute the anatomical joint angles from the IMU measurements, a calibration trial in side lying was performed. During the calibration trial, the

pelvis was vertically aligned with the table ensuring that there was no pelvic rotation. The lower leg was supported and the hip was in 0° abduction (Figure 2A).
Three repetitions of maximal voluntary isometric contractions (MVIC) were carried out. Then, four repetitions of passive muscle stretch of the gracilis at two velocities over the full range of motion were performed (Figure 2B). Firstly, the leg –with the knee extended- was moved into abduction at low velocity during 5 seconds repeated four times. Secondly, the same movement was performed four times at high velocity, this is as fast as possible. The interval between all repetitions was 7 seconds. All evaluations were conducted by the same trained assessor.



A **B**

Figure 2. Measurement procedure.
A. Test starting position with instrumentation for the instrumented spasticity assessment of the gracilis muscle. Overview of the test instrumentation: (1) two inertial measurement units measured joint angle characteristics; and (2) surface electromyography measured muscle activity of the gracilis muscle.
B. End position after bringing the leg in abduction with the knee extended.

Data analysis

Data was processed and visualized offline using custom-made software in MATLAB 7.6.0 R2010 (Mathworks). A 6th order zero-phase Butterworth bandpass filter ranging from 20-500Hz was applied to filter the raw sEMG signal. The root mean square envelope of the sEMG (RMS-EMG) signal was computed using a low-pass 30Hz 6th order zero-phase Butterworth filter on the squared raw signal. To estimate joint position, angular velocity and acceleration, a Kalman smoother[20] was applied on the IMU-data. Stretch repetitions were excluded from analysis when passive stretches were performed out-of-plane, at non-similar velocities (difference >20˚/sec within a particular velocity trial), or in case of poor quality sEMG data (low signal-to-noise ratio or obvious artifacts).

Outcome parameters

Quantitative parameters extracted from sEMG signals, explored around maximum velocity (V_{MAX}) and compared between the two velocity conditions, were used to measure spasticity. Average RMS-EMG was calculated as the area under the RMS-EMG time curve divided by an interval starting 200ms prior to the time corresponding to V_{MAX} and ending at the time corresponding to 90% of the full range of motion. In accordance with the measurement protocol previously described by Bar-On et

al[16], average RMS-EMG was additionally expressed as a percentage of the peak RMS-EMG from the three MVIC repetitions collected during the pre-BTX measurement. Thus, both a normalized RMS-EMG parameter (in %) as well as a non-normalized RMS-EMG parameter (in μV) were evaluated.
An average of the RMS-EMG parameters of the repetitions at low velocity was subtracted from the average of the RMS-EMG parameters of the repetitions at high velocity, resulting in two major outcome parameters: non-normalized '*RMS-EMG high-low*' (in μV) and normalized to pre-injection MVIC '*RMS-EMG high-low normalized*' (in %). These measures have been reported to be reliable and valid measures for spasticity.[16] V_{MAX} from the high velocity stretches was collected at each test condition in order to compare consistency of the measurement procedures. Finally, also the MAS of hamstrings and was included in the final set of outcome parameters.

Statistical analysis

Pre-injection subject characteristics, RMS-EMG parameters, timing of pre- and post-injection measurements, MVIC, V_{MAX}, as well as BTX dosages were compared between both groups using Freeman-Halton (categorical parameters) or Mann Whitney U tests (continuous parameters). The change between pre and post injection condition for the RMS-EMG parameters, V_{MAX} and the MAS of the hamstrings were compared between groups using Mann Whitney U tests. Significance was set to $p<0.05$. All statistical analyses were performed using SPSS (IBM Statistics 20).

Results

Twenty-nine children participated in the study, but the data of two children, one from each injection group, were excluded due to technical problems during the processing phase. The characteristics of the excluded patients and their MAS were within the range of the included patients, giving confidence that exclusion did not bias the results. All children had there post injection evaluation between 40 and 90 days after the injections, except for two children –one from each group- who had their post injection evaluation already after 17 and 23 days.
Fifteen children with diplegia, three with triplegia and nine with hemiplegia were included, with the following distribution of gross motor function classification scale (GMFCS): I 6, II 14, III 5, IV 2. The mean age was 8.5±2.5 years (range: 4.3-14.7y). From the 15 children with spastic diplegia, bilateral measurements were performed in seven patients. In the other eight bilaterally involved children, a bilateral measurement procedure was not possible due to the young age (and thus limited time of appropriate cooperation) or weak mental condition. In those cases, data were collected from the side with the highest average baseline MAS-score for the hamstrings and adductors. In case of symmetrical MAS-scores, the side was randomly chosen. A total of 34 muscles were measured before and after injection. For half of the muscles (N=17) the MEP targeted approach was applied, while the other half of the gracilis muscles (N=17) were injected proximally. An average dosage of 40.44U (range: 20-100) per gracilis muscle was injected.

There was no significant difference in the following pre-injection characteristics between both groups: GMFCS, anatomic distribution of the motor impairment, age and MAS of adductors and hamstrings. Furthermore, absolute dosage of BTX injected in the gracilis muscle, total dosage of BTX for the medial hamstrings, timing of the post-injection measurements and V_{MAX} during the high

velocity stretches (indicating that the measurement was performed consistently) were also not found to be different. Finally, also the pre-injection MVIC values and the baseline spasticity levels (RMS-EMG parameters) did not differ between groups. (Table 1)

MAS of the hamstrings did not significantly change after the BTX injections in both groups. (Table 2). The median change values of the RMS-EMG parameters (pre-post BTX) for both groups are expressed in Table 2. A statistically significant difference (p=0.04)was found for RMS-EMG *high-low normalized*, with a larger reduction in the RMS-EMG in the MEP injected gracilis muscles (median 6.38%, IQR 3.77%) than in the proximally injected gracilis muscles (median 1.26%, IQR 8.13%). The same trend could be observed for the non-normalized values, however this did not reach statistical significance with a median reduction of 4.78 µV (IQR 5.37 µV) for MEP injected muscles and of 1.35 µV (IQR 9.14 µV) for the proximally injected muscles (p=0.14).

Table 1. Baseline patient, measurement and treatment characteristics.

	MEP oriented injection (n=15 subjects, 17 muscles)	**Proximal injection (n=16 subjects, 17 muscles)**	***p***
Motor impairment (unilateral/bilateral)	Uni: 4; Bilat: 11	Uni: 4; Bilat: 12	0.92
GMFCS (I-IV)	I:3; II:7; III: 4; IV:1	I:3; II:9; III: 3; IV:1	0.94
MAS hamstrings (0-5)	1:3; 1+:5; 2:6;3:3	1:1; 1+:11; 2:3;3:2	0.25
MAS adductors (0-5)	0:2; 1:2; 1+:6; 2:4; 3:2; 4:1	0:5; 1:4; 1+:7; 2:1; 3:0; 4:0	0.26
	Median (IQR)		
Age (years)	7.85 (4.80)	8.68 (3.38)	0.53
BTX dosage in gracilis (Units)	40 (10)	40 (20)	0.74
BTX dosage in hamstrings (Units/Kg)	5 (1.0)	5 (0.5)	0.49
Timing of pre-treatment measurement (days)	0 (5)	0 (2)	0.92
Timing of post-treatment measurement (days)	52 (13)	51 (10)	0.27
Pre-treatment peak MVIC (µV)	115.75 (54.72)	76.36 (58.52)	0.13
Pre-treatment V_{MAX} (°/sec)	90.45 (45.37)	114.45 (74.98)	0.21
Pre-treatment RMS-EMG *high-low* (µV)	9.23 (8.58)	8.20 (7.50)	0.32
Pre-treatment RMS-EMG *high-low normalized* (%)	11.88 (9.48)	8.02 (11.41)	0.27

GMFCS: gross motor functional classification scale; MAS: modified Ashworth Scale; BTX: Botulinum toxin Type-A; MVIC: maximum voluntary isometric contraction; V_{MAX}: maximum angular velocity during high velocity trials; RMS-EMG: root mean square electromyography; high-low: average during high velocity trials minus average during low velocity trials; p: significance level for Mann whitney u test; IQR: interquartile range

Table 2. Median and interquartile range values of change (pre-post) in the outcome parameters

	MEP oriented injection	**Proximal injection**	***p***
RMS-EMG *high-low* (μV)	4.78 (5.31)	1.35 (9.14)	0.14
RMS-EMG *high-low normalized* (%)	6.38 (3.77)	1.26 (8.13)	0.04
Post-treatment V_{MAX} (°/sec)	99.50 (42.35)	110.2 (33.02)	0.27
MAS hamstrings	0 (0.5)	0 (0)	0.85

RMS-EMG: root mean square electromyography; high-low: average during high velocity trials minus average during low velocity trials; V_{MAX}: maximum angular velocity during high velocity trials; MAS: modified Ashworth scale

Discussion

Injection of BTX in the gracilis muscle at the sites of the muscle where the MEP zones are most concentrated, results in a larger reduction in pathological electrophysiological muscle activity compared to an injection with the same dosage and dilution in the proximal part of the muscle. This was documented using an instrumented spasticity assessment that integrates biomechanical and electrophysiological measures. This assessment was developed for use in a clinical setting and has proven to be repeatable and valid in measuring spasticity in children with CP.[16] In clinical practice, outcome measures for spasticity reduction include scales such as the MAS[19] and the Modified Tardieu Scale[21]. However, the intrinsic subjective character of these clinical tests restricts their reliability.[21,22] Further, these tests cannot differentiate between neural and non-neural components of increased resistance.[23-25] MAS was also evaluated in this study group, but did not show statistically significant differences after the injections or between the injection protocols.

When comparing different injection techniques with respect to the localization of the injection, Gracies et al.[6] used mean rectified voltage during maximal isometric muscle action for the biceps brachii muscle in adults with spasticity. Van Campenhout et al.[7] measured the reduction of muscle volume using MRI segmentation of the psoas muscle of children with CP as a primary outcome. Both studies used measures of muscle activity, but not true measures of spasticity. In this study a reliable and valid measure of spasticity reduction could confirm the superiority of MEP targeted BTX injections, therefore confirming the hypothesis of the study.

A study limitation is the dependence of the normalized RMS-EMG parameter on a representative MVIC. BTX induces temporary weakness.[26] Conform literature, we therefore used the maximum contraction at baseline to quantify spasticity post-BTX.[27] The non-normalized parameter was also reported and care was taken to minimize the risks of using non-normalized EMG, by strict standardization of the sEMG-electrode placement. Importantly, for both normalized and non-normalized EMG data, the conclusion was similar, however statistical significance was only reached for the normalized data.

A limited number of patients with a large age range and different functional levels (GMFCS levels) was studied. Despite the heterogeneity of the group, a difference between the injection techniques could still be noted.

We only tested the gracilis muscle. The dependence on sEMG in the outcome assessment of our patient group (children with CP) limits the measurement method to investigate surface muscles from

which activity can be easily differentiated from that of neighboring muscles. In selected patient groups, however, fine wire EMG may broaden the indications.

As treatment often indicates multi-level BTX injections, additional muscles need to be tested for their optimal injection site. A review on the localization of the MEP zones[18] is available for the frequently injected muscles of the lower limb. Additionally, for many upper limb muscles, anatomical studies on terminal nerve ramifications have been published.[28-31]

Especially for longer muscles there is a high clinical relevance to use MEP targeted injections. For small muscles local diffusion of the toxin in the muscle itself can minimise the importance of injecting at the MEP-zone. Animal studies have confirmed that the toxin may diffuse up to 4.5 cm along the length of the muscle.[32] However, most muscles in the lower limb are large muscles with considerable length in which diffusion is very unlikely to bring the toxin to the other end of the muscle. Multiple injections can bring the toxin to a larger region, but this means repeated injections. In doing this, the toxin will also disperse over areas which have no, or only a limited number of MEPs. This toxin –if not bound to the acetylcholine receptor of the neuromuscular junction- can diffuse and reach other muscles or blood vessels, with possible complications. In CP, BTX is often used at multiple levels, leading to the use of high total dosages.[33] In these cases, it is even more important to use this powerful drug with an optimal injection technique.

Using MEP targeted injections has proven to give a better spasticity reduction. In the future, the goal is to study different dosages and dilutions using the instrumented spasticity assessment as outcome measure, in order to further improve the injection technique. This will even more increase safety of this treatment and will eventually lead to reduced dosages and thus reduced treatment costs.

Using the instrumented spasticity assessment, we were able to document improved reduction in pathological electrophysiological muscle activity indicating a better spasticity reduction. This spasticity reduction is most likely to manifest changes because this domain is the most proximate to the intervention[34]. Future studies also need to delineate the effect on gait, function and participation.

Conclusion

Using MEP targeted injections improved the efficacy of BTX injections of the gracilis muscle in children with CP. Different injection protocols could be compared sensitively and objectively using an instrumented spasticity assessment that integrates biomechanical and electrophysiological measures.

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Part 2: MEP targeted injections

Part 2, 2.4

Can we unmask features of spasticity during gait in children with cerebral palsy by increasing their walking velocity?

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Abstract

Background and aim Spasticity is a velocity dependent feature present in most patients with cerebral palsy (CP) . It is commonly measured in a passive condition. The aim of this study was to highlight markers of spasticity of gastrocnemius and hamstring muscles during gait by comparing the effect of increased walking velocity of CP and typical developing (TD) children.

Methods 53 children with spastic CP and 17 TD children were instructed to walk at self-selected speed, faster and as fast as possible without running. Kinematics, kinetics and electromyography (EMG) were collected and muscle length and muscle lengthening velocity (MLV) were calculated. To compare the data of both groups, a linear regression model was created which resulted in two non-dimensional gait velocities. A difference score (DS) was calculated between the high and low velocity values for both groups.

Results 75 gait parameters were analyzed of which 16 had a statistically significant DS between TD and CP groups. The spastic gastrocnemius muscle presented at high velocity with a higher ankle angular velocity, plantar flexion moment, power absorption and increased EMG signal during loading response. The spastic hamstrings demonstrated at high velocity a delayed maximum knee extension moment at mid-stance and increasing hip extension moment and hip power generation. The hamstrings also presented with a lower MLV during swing phase.

Conclusions A limited number of gait parameters differ between CP and TD children when increasing walking velocity, giving indirect insight on the effect of spasticity on gait.

Introduction

Children with cerebral palsy (CP) present with varying motor deficits, including neuromotor impairments and pathological gait patterns. Spasticity is one of the most present and disabling neuromotor problems. Lance[1] defined spasticity as "a velocity dependent increase in tonic stretch reflexes or muscle tone". Spasticity is commonly evaluated in a passive condition, either by clinical scales such as the modified Ashworth Scale[2] (MAS) or the Tardieu scale[3], or in laboratory settings using biomechanical and electrophysiological methods[4-7]. The latter provide more objective measures, but still lack functional aspects. In particular, passive measurement may not reflect the effect of spasticity on gait. More-over, CP patients have a multifaceted disorder and different studies highlight the complex relationship between spasticity and gait leaving the interactions largely uncovered.[8-10]

In line of the original definition of spasticity which stresses the velocity dependency, it has been suggested that signs of spasticity during gait may be highlighted by increasing the walking velocity.[11,12] Walking faster will change the gait pattern both in CP and in typically developing (TD) children.[11-15] Indirect insight into the effect of spasticity on gait can be achieved by studying the differences in effect of increased walking velocity on gait in CP and TD children.

A number of previous studies (Schwartz et al. 2008, van der Linden et al. 2002, Stansfield et al, 2001) indicated that in TD children, several aspects of gait appear to be sensitive to walking speed.[13-15] However, the effect of walking speed on gait of CP children has only been studied for a few gait parameters. For example, Van der Krogt et al. demonstrated the effect of walking speed on muscle-tendon length and lengthening velocity of spastic plantar flexors and hamstrings.[11,12]

The aim of this study was to highlight markers of spasticity of gastrocnemius and hamstring muscles in gait analysis data by comparing the effect of increased walking velocity in CP and TD children. We studied the impact of increased walking velocity on kinematic, kinetic and EMG parameters and on muscle length (ML) and muscle lengthening velocity (MLV).

Methods

Subjects

Fifty-three patients diagnosed with spastic CP (28 boys, 25 girls; mean age 9.8 y ± 3.0 y) and 17 TD children (11 boys, 6 girls, mean age 10.46 y ± 2.36 y) volunteered for this study. All children's parents signed an informed consent form, approved by the local Ethics Committee of the hospital.

Children with CP were selected from the list of planned gait analyses of the Clinical Motion Analysis Laboratory of the University Hospital of Leuven, based on the following inclusion criteria: (1) spastic CP, (2) ambulatory status, (3) aged between 4 and 17 years old and (4) spasticity ($MAS^2 \geq 1,5$) in gastrocnemius and hamstrings in the same lower limb. Children presenting with the following criteria were excluded: dystonia and/or athetosis, severe cognitive impairment, previous orthopaedic surgery, Intrathecal Baclofen pump or botulinum toxin-A treatment less than 6 month prior to evaluation. The TD children were children of colleagues of the research team of the Clinical Motion Analysis Laboratory or siblings of patients and had no medical history of cardio-vascular,

neurological or musculoskeletal disorders.

In children with CP, the most affected side was selected based on the MAS scores. For 10 patients the MAS scores were symmetrical; their side was selected according to Tardieu score. In case of symmetrical MAS and Tardieu, the right side was selected. For the TD group, the right side was selected unless no kinetics were available or in case of bad quality electromyographic (EMG) signal, in which case the left was selected.

Study design

The evaluation involved a clinical examination and a three-dimensional (3D) gait analysis. The clinical examination included evaluation of range of motion (ROM), bony alignment, MAS[2], Tardieu scale[3], strength and selectivity[16] of both lower limbs.

The gait data were collected using an 8 camera VICON system, operating at 100 Hz, with 15 reflective markers located at specific anatomical landmarks of the lower limbs, according to the lower limb Vicon PluginGait marker configuration (VICON, Oxford Metrics, Oxford, UK). The ground reaction forces were recorded using two AMTI force plates (Advanced Mechanical Technology, Inc., Watertown, Massachusetts) integrated in the walkway. Surface EMG was collected for 8 lower extremity muscle groups bilaterally, however, this study only reported on the results of gastrocnemius and medial hamstrings. The surface EMG (Zerowire, Cometa, Milan, Italy) was recorded at a sample rate of 1500 Hz and filtered through a bandpass filter (20-500Hz) (Nexus, Vicon, Oxford Metrics Group, UK).

All children walked barefoot along a 10m walkway. First, they were instructed to walk at a self-selected walking speed, secondly, to walk faster, and finally to walk at the fastest speed they could achieve without running. At least 3 successful trials were collected at each speed condition. A trial was considered successful when there was good marker visibility and an overall artifact-free EMG signal.

Data analysis

Three dimensional kinematic data were calculated for the pelvis, hip, knee, ankle and foot bilaterally, decomposed in the sagital, coronal and transverse plane. Kinetic analysis included the net internal moments and joint power for hip, knee and ankle. All kinematic and kinetic analyses were based on the lower limb PlugInGait model (VICON, Oxford Metrics, Oxford, UK). Two gait cycles per trial were determined using kinematic and kinetic data. An additional visual quality control of the all EMG, kinetic and kinematic signals was carried out in custom-made MATLAB software (Mathworks®, Natick, MA, USA), prior to further data analysis. In the same software, the root mean square (rms) was calculated from the raw surface EMG signals. Average rms-EMG values were calculated by dividing the rms-EMG signal by a given time phase during the GC including: 1) 0-100% GC, 2) 0-20% GC, 3) stance phase, 4) swing phase and 5) 80-100% GC. These values were then normalized to the averaged maximum rms-EMG value of the gait cycles collected at self-selected walking speed, to enable comparison between subjects and between different velocities.

Muscle lengths were estimated using the musculoskeletal model introduced by Delp[17], using the lower limb segment and joint kinematics as input data. The algorithms of muscle length estimations were created through a custom-made bodybuilder program (VICON). All muscle lengths were expressed as a percentage of the corresponding muscle length in the anatomical position. The derivative of the muscle length was then calculated to obtain the muscle lengthening velocity (MLV).

From the continuous waveforms organized in gait cycles for kinematics, kinetics and muscle lengths a set of discrete parameters were selected based on clinical relevance. These included maxima, minima and mean values, ROM and the timing of maxima and minima within the GC and/or for sub-phases (stance and swing) and several specific parameters (including kinematic and kinetic values at specific events such as initial contact and toe off and ROM values for specific sub-phases such as loading response and push-off). For EMG the mean square roots of EMG of the gastrocnemius and hamstrings for the full gait cycle as well as for specific sub-phases (stance, swing, and first and last 20% of the gait cycle) were calculated. Finally, EMG and muscle length parameters were combined into EMG ratios, which are the averaged rms EMG values in swing divided by the maximum muscle lengthening velocity in swing, calculated for gastrocnemius and hamstrings.[18] All these parameters were automatically extracted from the waveforms using custom-made MATLAB software (Mathworks®, Natick, MA, USA). Gait parameters in TD children were also expected to change with increasing walking velocity and this effect was important to consider when studying pathological gait.[13] To allow focusing on comparable walking speed between CP and TD children, the gait velocities were first rendered to dimensionless using the scheme proposed by Hof[19], $\breve{v} = v/\sqrt{gl}$leg , in which v is the walking speed, g the acceleration of gravity and $l$leg the leg length (Figure 1). Per subject, the value of each gait parameter for each of the nine trials (three per velocity condition: self selected speed, faster and fastest; two gait cycles per trial), were plotted against dimensionless velocity. Subsequently, a linear regression model was fitted through all data points to enable comparison of each individual parameter with increasing non-dimensional velocity. From these linear regression lines, two non-dimensional gait velocity values of 0.3 (low velocity) and 0.6 (high velocity) were selected which were identical for all subjects. These two velocities were selected as they were the closest values common to both CP and TD groups. All parameters were therefore estimated at these two non-dimensional gait velocities using the individual linear regression equations (Figure 1).

Additionally, per parameter, a difference score (DS) was calculated between the high and low velocity values, representing the change in the parameter due to increased velocity. We were therefore able to study the difference in gait pattern between both study groups (CP and TD) at each non dimensional velocity (low and high) and also to measure the impact of speed on both groups through the DS.

Statistical analysis

To verify how well the data points fitted the regression line, the R^2 values were calculated per subject per parameter. Parameters of subjects in which the R^2 values were <0.1 were directly excluded from the analysis. Subsequently, of the remaining values, parameters with a mean R^2 values of <0.4 were removed for all subjects. All parameters were checked for normal distribution using the Kolmogorov-Smirnov test with $p>0.1$ indicating a normal distribution. Estimated parameters at low and high non-dimensional velocities, as well as DS were compared between the CP and TD groups using Mann-Whitney U test. Significance was set to $p<0.05$. All statistical tests were carried out using Statistica version 11 (Statsoft Inc. 2012)

Results

The characteristics of the CP (n=53) and TD (n=17) groups are detailed in Table 1. Kinetic data were not available for 21 children. Additionally, the EMG data from one subject was not used due to bad quality.

Characteristic	CP	TD
Subjects (N)	53	17
Gender (F:M)	25:28	6:11
Average age (years)	9.8 (3.0)	10.46 (2.36)
MAS gastrocnemius (N)	1,5 (17); 2 (26); 3 (10)	0
MAS medial hamstrings (N)	1,5 (26), 2 (25), 3 (2)	0
GMFCS (N)	I:26, II:24;III:3	I:17
Selected left side (N)	29	6
Selected right side (N)	24	11
Kinetics measured (N)	33	17
Walking aid (N)	4	0

Table 1 Subjects characteristics**.**
Age is displayed as mean (±SD).
MAS: Modified Ashworth Scale, GMFCS: Gross Motor Function Classification Score, N: number of subjects.

1830 of the 7210 values had an R^2 of <0.1 and were therefore removed from the analysis. After removal of these values, 28 of the 103 parameters evaluated still had an average R^2 of less than 0.4 and were therefore also removed. Therefore, 75 parameters were analyzed of which 16 had a statistically significant DS between CP and TD groups. Table 2 gives the R^2, median scores and interquartile ranges of the low and high velocities and DS for the TD and CP groups, with their corresponding *p*-values for these 16 parameters. These 16 parameters are also described below.

Table 2. Median and interquartile range (IQR) values of parameters whose difference scores (DS) were significantly different between CP and TD groups

Time and distance parameters	CP group	TD group	*p*-value
Cadence (number of steps/s)			
low velocity Median (IQR)	1,80 (0,30)	1,74 (0,20)	0,065
high velocity Median (IQR)	2,83 (0,60)	2,44 (0,30)	0,0001
DS Median (IQR)	0,96 (0,43)	0,68 (0,31)	0,001
Average R^2	0,89	0,92	
n=	53	17	
Single support (s)			
low velocity Median (IQR)	0,37 (0,06)	0,41 (0,04)	0,024
high velocity Median (IQR)	0,30(0,07)	0,34 (0,05)	0,00003
DS Median (IQR)	-0,09 (0,05)	-0,06 (0,04)	0,002
Average R^2	0,65	0,63	
n=	48	16	
Double support (s)			
low velocity Median (IQR)	0,26 (0,07)	0,25 (0,06)	0,610
high velocity Median (IQR)	0,11 (0,06)	0,14 (0,02)	0,001
DS Median (IQR)	-0,14 (0,10)	-0,11 (0,04)	0,025
Average R^2	0,76	0,85	
n=	51	17	
Kinematic parameters			
Maximum ankle angular velocity during mid-stance (deg/s)			
low velocity Median (IQR)	90,45 (54,29)	80,83 (40,30)	0,560
high velocity Median (IQR)	179,19 (107,42)	148,69 (38,26)	0,004
DS Median (IQR)	81,36 (58,65)	46,80 (29,32)	0,001
Average R^2	0,5	0,5	
n=	45	15	
Kinetic parameters			
Maximum hip moment during stance (Nm/kg)			
low velocity Median (IQR)	0,16 (0,48)	0,27 (0,50)	0,630
high velocity Median (IQR)	0,66 (0,37)	0,40 (0,08)	0,005
DS Median (IQR)	0,37 (0,36)	0,23 (0,49)	0,012
Average R^2	0,51	0,48	
n=	26	11	
Maximum hip power generation during stance (W/kg)			
low velocity Median (IQR)	-0,10 (0,83)	-0,22 (0,44)	0,528
high velocity Median (IQR)	1,44 (1,04)	0,42 (0,50)	0,00002
DS Median (IQR)	1,40 (1,35)	0,70 (0,65)	0,003
Average R^2	0,64	0,43	
n=	28	13	

Percentage of the GC at which the maximum knee moment occurs during mid-stance (%)			
low velocity Median (IQR)	8,27 (4,86)	13,79 (3,33)	0,0004
high velocity Median (IQR)	10,10 (2,46)	12,06 (1,05)	0,0004
DS Median (IQR)	1,82 (4,47)	-1,85 (3,39)	0,012
Average R^2	0,52	0,44	
n=	26	17	
Ankle moment at loading response (Nm/kg)			
low velocity Median (IQR)	0,63 (0,84)	0,28 (0,53)	0,439
high velocity Median (IQR)	1,15 (0,93)	0,48 (0,59)	0,001
DS Median (IQR)	0,50 (0,56)	-0,04 (0,54)	0,009
Average R^2	0,56	0,46	
n=	26	9	
Minimum ankle power during loading response (W/kg)			
low velocity Median (IQR)	0,60 (0,70)	-0,54 (0,53)	0,372
high velocity Median (IQR)	-1,61 (1,65)	-0,92 (0,78)	0,031
DS Median (IQR)	-1,20 (1,38)	-0,43 (0,47)	0,036
Average R^2	0,6	0,49	
n=	29	9	
Muscle lengths			
Maximum muslce lengthening velocity of the medial hamstrings during swing (%ML/s)			
low velocity Median (IQR)	47,32 (18,48)	57,76 (6,65)	0,003
high velocity Median (IQR)	75,08 (24,04)	92,96 (9,22)	0,0002
DS Median (IQR)	26,53 (15,39)	34,76 (5,49)	0,006
Average R^2	0,58	0,88	
n=	50	16	
Percentage of the GC at which maximum muscle length of gastrocnemius occurs during stance (%)			
low velocity Median (IQR)	46,15 (8,33)	47,18 (2,09)	0,370
high velocity Median (IQR)	29,65 (18,58)	40,20 (2,44)	0,004
DS Median (IQR)	12,42 (17,6)	7,04 (2,50)	0,009
Average R^2	0,44	0,67	
n=	42	16	

Maximum muscle lengthening velocity of the gastrocnemius during swing (%ML/s)			
low velocity Median (IQR)	15,58 (11,91)	27,90 (9,82)	0,0002
high velocity Median (IQR)	32,39 (15,57)	36,19 (8,66)	0,204
DS Median (IQR)	14,81 (14,31)	10,65 (6,63)	0,009
Average R^2	0,42	0,41	
n=	39	16	
Muscle lengthening velocity of the gastrocnemius at initial contact (%ML/s)			
low velocity Median (IQR)	10,58 (21,75)	-12,43 (4,15)	0,001
high velocity Median (IQR)	23,14 (41,70)	-22,48 (13,62)	0,001
DS Median (IQR)	11,21 (30,39)	-14,75 (7,60)	0,003
Average R^2	0,4	0,4	
n=	34	9	
EMG			
Average RMS-EMG in the gastrocnemius during 0-20%GC (%)			
low velocity Median (IQR)	0,97 (0,72)	0,32 (0,30)	0,0001
high velocity Median (IQR)	2,13 (1,07)	0,80 (0,27)	0,000001
DS Median (IQR)	1,22 (0,91)	0,48 (0,56)	0,0002
Average R^2	0,49	0,55	
n=	48	13	
Average RMS-EMG in the gastrocnemius during stance (%)			
low velocity Median (IQR)	0,90 (0,58)	0,22 (0,38)	0,000001
high velocity Median (IQR)	2,07 (0,75)	2,37 (0,96)	0,357
DS Median (IQR)	1,21 (0,88)	2,17 (1,35)	0,0003
Average R^2	0,63	0,75	
n=	50	16	
Average RMS-EMG in the gastrocnemius during 80-1000%GC (%)			
low velocity Median (IQR)	0,65 (0,50)	0,15 (0,36)	0,00003
high velocity Median (IQR)	1,53 (0,64)	2,00 (0,81)	0,005
DS Median (IQR)	0,81 (0,83)	1,85 (1,03)	0,00003
Average R^2	0,6	0,73	
n=	48	16	

DS: difference score; GC: gait cycle; ML: muscle length;
RMS-EMG: root mean square electromyography normalized to maximum EMG at self-selected speed

With increasing walking velocity, cadence increased significantly more in CP than in TD children. Single support was shorter in CP children and, with increasing walking velocity, diminished more than in TD children. Double support time diminished slightly more in CP versus TD children with increasing walking velocity.

The description of the results of the remaining parameters are organized in two groups - gastrocnemius related parameters and hamstrings related parameters- and this according the phase of the GC.

1.Gastrocnemius related parameters

During loading response (LR), the ankle of TD children showed a plantarflexion motion, while the majority of CP children presented with a dorsiflexion motion. As a result, the gastrocnemius was shortening in TD children and lengthening in CP children and in both groups this pattern increased with velocity. Maximum ankle angular velocity from initial contact (IC) till midstance was higher in the CP group compared to TD, at low walking velocity and increased even more at high velocity. Ankle moment during LR increased more in CP children with increasing walking velocity. Accordingly, the power absorption in the ankle at LR was similar for both groups at low speed, but with increasing velocity the CP group showed a more pronounced power absorption. Additionally, during the first 20% of the gait cycle, gastrocnemius muscle activity was higher at both velocities and the increase between velocities was more pronounced in CP than in TD children.
With increasing walking velocity, the maximum length of the gastrocnemius occurred earlier in stance in both groups; but this premature timing was significantly more pronounced in CP than in TD children. For the whole stance phase, the CP children showed more pronounced gastrocnemius muscle activity at low velocity. In both groups, this muscle activity increased with increasing velocity, but to a larger degree in the TD group.

In swing phase, the maximum MLV was higher for the TD group at both velocities, but in CP children this MLV increased more from low to high walking velocity. When focusing on the values of the averaged EMG activity for the end of swing phase (80-100% of the GC) there was an influence of speed that was different between CP and TD, expressed by a larger increase of muscle activity at high walking velocity in TD children.

2. Medial hamstrings related parameters

The timing of maximum knee extension moment during mid-stance was delayed with increasing speed in the CP group while it was slightly premature in the TD group. Maximum hip extension moment in sagittal plane during stance phase increased more with increasing walking velocity in CP than in TD children. Additionally, maximum hip power generation in stance increased much more in CP patients in comparison to the increase in TD children.
MLV for the medial hamstrings muscles during swing phase was lower in CP children at both walking velocities and this difference became more pronounced when walking velocity increased.

Discussion

Gait parameters of TD and CP children were compared at similar non-dimensional velocities, estimated by a linear regression model. Many parameters showed differences between the CP and TD group at the lowest, at the highest or at both velocities. However, for many parameters, similar

strategies to increase walking velocity were used by both groups, as indicated by the non-significantly different DS between CP and TD. Still, a number of gastrocnemius and hamstrings related parameters can be considered as functional markers for spasticity, due to significantly different DS between CP and TD.

The spastic gastrocnemius muscle presented at high velocity with a higher ankle angular velocity, plantar flexion moment and power absorption during LR. Additionally, this muscle demonstrated increased EMG signal during early stance phase. Crenna[20] previously also observed gastrocnemius lengthening early in stance in children with CP, while TD children presented with gastrocnemius lengthening during the second ankle rocker. Pathological muscle length patterns during stance and lower MLV during swing have been reported by van der Krogt et al[12] for the gastrocnemius muscle, in a study comparing ML and MLV at different walking speeds in 17 CP and 11 TD children.

The increased walking velocity affected the spastic hamstrings at the level of the hip and knee joints at mid-stance, reflected by a delayed maximum knee extension moment and by increasing the hip extension moment and hip power generation. The hamstrings also presented with a lower MLV during swing phase, as was also previously reported.[11,19]

Throughout the gait cycle, a larger number of markers of spasticity in the gait analysis were linked to the gastrocnemius muscle compared to the number of spasticity markers for the hamstrings. The crucial phases in the gait cycle characterized by large lengthening velocity in the hamstrings are frequently linked to specific biomechanical demands and/or require similar EMG activity for both CP as well as TD children (such as eccentric activity at terminal swing to decelerate knee extension), reducing the discriminating capacity between both subject groups. On the other hand, the gastrocnemius showed clear lengthening velocities in passive conditions (such as gastrocnemius lengthening in swing) which were found to more clearly distinguish between groups, and which may explain the higher number of markers for spasticity in gait data.

A limitation of this study is the loss of some kinetic data in the CP group, mainly due to inclusion of children with GMFCS level III. The advantage of recruiting these children is the inclusion of more involved children, often with higher levels of spasticity. Furthermore, we only differentiated between non-spastic TD and spastic CP muscles and not between different degrees of spasticity. Future studies, exploring spasticity in different subgroups (according to MAS or according to more objectively biomechanically and electrophysiological measured spasticity) and including additional muscles can further improve our knowledge on the effect of spasticity during gait. While the use of the regression procedure to study spasticity markers at similar velocities is found to be successful when comparing heterogeneous groups, a limitation of this procedure is that parameter values do not reflect real values, but estimated ones.

In general, the spastic gastrocnemius muscle presented with kinematic, kinetic and EMG changes during stance and swing phase when increasing walking velocity. The spastic hamstrings demonstrated lower MLV during swing at higher walking velocity. CP children used a different strategy than the TD children to increase their walking velocity: they increased their hip extension moment during stance phase and their cadence to a larger extent than the TD group.

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Part 2: MEP targeted injections

Part 2, Chapter 3

MEP targeted injections of the psoas muscle.

Botulinum toxin type A injections in the psoas muscle of children with cerebral palsy: muscle atrophy after motor end plate-targeted injections.

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Abstract

MEP targeting during BTX injections has been demonstrated to improve outcome. Two injection techniques of the psoas muscle –proximal MEP targeting versus a widely used more distal injection technique- are compared using muscle volume assessment by digital MRI segmentation as outcome measure.

Method 7 spastic diplegic children received injections in both psoas muscles: two different injection techniques randomly in 5 patients, in 2 patients bilateral MEP targeting. MRI images of the psoas were taken before, after 2 months and in 3 patients after 6 months.

Results Average post injection volume (in relation to pre-injection volume) for the MEP targeted muscles (9) is 79,5% versus 107.8% in the 5 distal injected psoas muscles (p=0.0033). In all 5 asymmetric injected patients the MEP targeted psoas had a larger volume reduction than the more distal injected psoas muscle. This atrophy remains even 6 months after the injection.

This is the first study were a longitudinal follow-up by MRI demonstrates muscle atrophy after BTX in children with CP. Injections in the MEP zone of the muscle, which is the more proximal part of the psoas muscle, cause atrophy in contrary to more distal injections were this atrophy is not observed.

Introduction

Intramuscular Botulinum toxin type A (BTX) injections are known to reduce spasticity in children with cerebral palsy (CP). Clinical studies have documented good response, but demonstrate considerable variation in outcome. Many factors are responsible for this lack of congruent results (Graham et al., 2000). Injection technique may be one of the main causes (Lim & Seet, 2008). BTX blocks neurotransmission at the neuromuscular junction by connecting to the presynaptic membrane of the motor endplate (MEP) (Melling, Hambleton & Shone, 1988). We therefore not only need to administer the toxin accurately into the preferred muscle, we also need to inject the toxin close to its site of action, this is in the MEP zone of this muscle. In animal studies (Childers et al., 1998; Shaari & Sanders, 1993) it has been demonstrated that a MEP targeted injection increases the paralytic effect. This hypothesis was confirmed in one human study (Gracies et al., 2009) where the biceps brachii was injected at the region of the MEP zone resulting in superior efficacy compared to more diffuse injection.

A review of the localization of the MEP zones of the commonly injected lower limb muscles was recently published (Van Campenhout & Molenaers, 2011). Herein, anatomical guidelines for intramuscular BTX injections were proposed based on histologic and anatomical dissection studies. For some muscles, this proposed injection location was somewhat different than the currently injected areas routinely used in clinical practice. Particularly for the psoas muscle the difference between the frequently applied distal injection technique and the proposed proximal injection sites was intriguing. Indeed, in a cadaver study on the innervation of the psoas muscle and the localization of its terminal nerve arborisations (Van Campenhout, Hubens, Fagard & Molenaers, 2010) it was highlighted that the MEP zone is proximal in the muscle belly: at the level of and proximal to the promontorium ossis sacri. Figure 1.

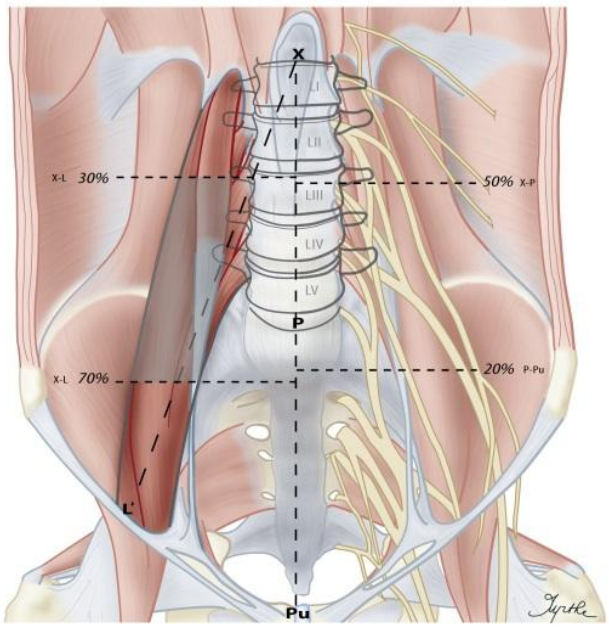

Figure 1. Innervation of the psoas muscle.On the right side: MEP zone in reference to Th12-L distance, on the left side: MEP zone in reference to Th12-P and P-Pu distance. From Van Campenhout et al[8].
X= thoracic vertebra 12 (Th12); P=promontorium; Pu= pubis: L= location where psoas muscle passes under inguinal ligament

Currently in clinical practice, several methods exist to inject BTX into the psoas muscle. Injection of the psoas muscle using a distal anterior approach from the groin with ultrasound guidance was presented by Westhoff et al. (Westhoff, Seller, Wild, Jaeger & Krauspe, 2003). After insertion of the needle distal of the inguinal ligament, the long needle is directed proximally towards the muscle belly. Another technique is to use a direct injection of the muscle belly through the abdominal wall in the region between the umbilicus and the anterior superior iliac spine, as described by Molenaers et al. (Molenaers, Eyssen, Desloovere, Jonkers & De Cock, 1999). This gives access to the more proximal part of the muscle belly. For this technique a good relaxation of the abdominal wall, preferably using general anaesthesia is necessary. A last alternative is injecting the psoas from dorsal, just lateral of the transverse processes of the second to fourth lumbar vertebra, as presented by Ward (Ward, 1999).
The temporary chemical denervation caused by BTX injections leads to muscle atrophy (Tsai, Hsieh & Chou, 2010). Magnetic resonance imaging (MRI) sensitively identifies these changes in muscle volume (Kamath, Venkatanarasimha , Walsh & Hughes, 2010; Kolzenburg & Bendszus, 2008). In this study, we compared two injection techniques of the psoas muscle (MEP targeting versus the distal injection technique) in order to find the most optimal procedure and to confirm the role of MEP targeting. We used muscle volume assessment by digital MRI segmentation to compare the effect of the different BTX injection techniques.

Method

Participants
Children with diagnosis of a symmetric spastic diplegia caused by cerebral palsy with a clinical need for BTX injection of the psoas muscle were included. Patients were excluded if there was a major fixed psoas contracture, dystonia or athetosis, previous surgery of the psoas muscle, previous injection with BTX in the last 6 months, previous neurosurgery and severe cognitive impairment interfering with the ability to cooperate in the study. Children were also screened for contraindications for performing the MRI. Finally their ability to cooperate with the MRI protocol (lying silently for about 15 minutes) on at least two occasions (before and 3 months after the injections) determined whether we could use their data. Initially 14 children entered the study, due to moving or fear during the (sometimes second) MRI, seven children were excluded. The age of the remaining 7 children with spastic diplegia (all female) ranged between 7y and 16y5m (mean:12y).

Study design
All patients received BTX injections in both psoas muscles as part of a multilevel treatment. All clinically involved muscles of the lower limbs were injected. This was done under anesthesia in a day-clinic setting. In 5 children these BTX injections were administered using different injection techniques for the left and right psoas muscle, randomly chosen (Randomisation generator, SPSS). In two patients the psoas muscle was injected bilaterally with the MEP-targeted injection technique. We always used 2 U Botox® (Allergan) /kg body weight per psoas muscle with a dilution of 5ml per 100U (20 U Botox®/ml).

For the distal technique a long needle was placed in the psoas muscle just distal of the inguinal ligament using ultrasound guidance (Westhoff, Seller, Wild, Jaeger & Krauspe, 2003). The toxin was injected in two sites, one as proximal as possible and one after retracting the needle about 2 cm in order to spread the toxin to avoid accumulation of the toxin. Figure 2A.

For the MEP targeted injection a direct injection of the muscle belly through the abdominal wall was performed (Molenaers, Eyssen, Desloovere, Jonkers & De Cock, 1999). With deep palpation from lateral to hold the intestines medially, the psoas can be manually localized. After needle placement in front of the palpating hand the hip is flexed to confirm the needle's position: when the tip of the

needle is in the psoas muscle the needle will move when the hip is flexed. The psoas is localized by a combination of palpation of the muscle and mobilisation of the adjacent joint. We do not use ultrasound for this technique as with one hand pushing on the abdomen and the needle in place the ultrasound probe is difficult to position. In order to inject the toxin at the MEP zone, two sites were injected: one just proximal of the promontorium and close to the spine and one just distal of the promontorium in the more easy palpable part of the muscle belly. Figure 2B.

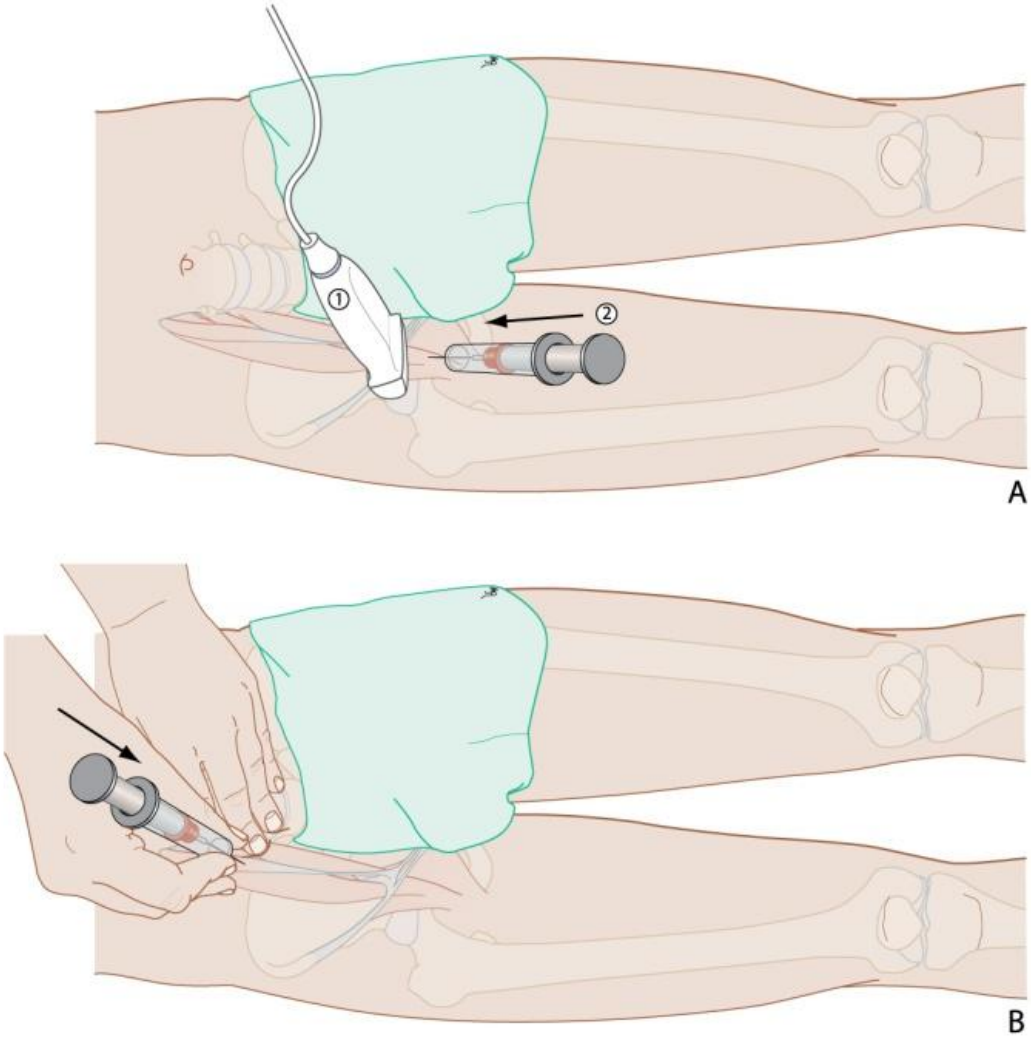

Figure 2. Injection techniques of the psoas muscle.
2A: Injection distal of the inguinal ligament; 1. Ultrasound probe, 2. Needle directed proximally
2B: Injection close to MEP zone around promontorium using an anterior abdominal approach. Palpating hand holding intestines medially, injection in front of fingers of palpating hand.

To reduce variability and maximize protocol blindedness, all injections were performed by one of the investigators (AV, unblinded) and all assessments were done by the first author (AVC) blinded to the injection technique.

Base line MRI scanning was performed on the morning of the injection or maximum 3 weeks before the injection. Post-injection MRI was done 2 months after the injections. Three patients agreed to have an extra MRI 6 months after the injections.

Outcome parameters

A 3.0 Tesla MR (Philips Ingenia, Philips Medical, Netherlands) was used to acquire bilateral transverse and sagital images from the tenth thoracic vertebral body to trochanter minor. Subjects were positioned supine with hips and knees in slight flexion using a pillow under the knees. Patients were scanned using an anterior Torso coil and posterior built-in coil. Following MRI sequences were taken:

- T2-Haste weighted-imaging sequence (WI) in a transverse plane (TR: 1158 ms, TE: 80 ms, 6 mm slice thickness, field of view (FOV) 375 x 303 mm, 376 x 302 scan matrix , total scan duration of 1:09 minutes (min))
- mDixon-sequence in a transverse plane (TR: 3,5 ms, TE1: 1,21 ms, TE2: 2,3 ms, 2,5mm slice thickness, FOV 375 x 303 mm with a scan matrix of 252 x 169 , total scan duration 18,2 seconds)
- T1 WI in transverse plane (TR: 537 ms, TE: 20 ms, slice thickness 8 mm, field of view 200 x 232 mm, 272 x 280 scan matrix, total scan duration of 5:09 min)
- STIR-W in a transverse plan (TR: 6663 ms, TE: 60 ms, inversion time (TI):200 ms, 8mm slice thickness, FOV 300 x 218 mm with a 308 x 163 scan matrix and total scan duration 3:33 min)

After data collection, the images were transferred to MeVisLab (Mevis Medical Solutions, Germany) for digital reconstruction. The slice areas of the psoas muscle were manually traced on the T1 sequences along the length of the muscle using a digitization tablet (Cintaq 24 HD, Wacom Europe). With this software muscle volume is calculated via linear interpolation and presented in mm^3. These tracings and measurements were repeated three times (on different days) by the same assessor (AVC) to obtain an intra-observer reliability and measure of variability.

Furthermore, acutely denervated skeletal muscles have been show to present with prolongation of the T2 relaxation time and increased signal intensities in short tau inversion recovery (STIR) due to muscle oedema (Kamath, Venkatanarasimha , Walsh & Hughes, 2010; Kolzenburg & Bendszus, 2008; Fanucci et al, 2001; Schroeder et al, 2009). These sequences were studied for signal intensity changes after the toxin injection.

Statistics

The intra-observer reliability of the volume measurement was assessed on the pre-injection measurements which were repeated three times by the same rater. The intraclass correlation (ICC) was calculated using a 2-way mixed model, considering each leg (N=14) as a separate unit. The within-subjects coefficient of variation (WSCV) is given, which is the within-subjects standard deviation divided by the mean of the volume measurements. 95% confidence intervals (CI) are reported for the ICC and WSCV. The change between the muscle volumes at the different time-points is compared using Wilcoxon signed rank test. The relative change with respect to the pre-injection measurement is compared between MEP and distal injected muscles, using Mann-Whitney U (MWU) and Wilcoxon signed rank tests. This has been done using the average of the three repeated volume measurements at each time point.

Ethical approval

This research using different within muscle localizations for injection with BTX has been approved by the research ethics committee of the University Hospital (blinded for review). The committee's recommendations have been adhered to and written informed consent for participation and publication has been obtained from the parents.

Results

Injection details

All patients had multilevel BTX injections, always including the psoas muscle as one of the targeted muscles. Depending on the clinical picture BTX was also injected in certain patients in the following muscles: m. adductor longus, m. adductor brevis, m. gracilis, m. semitendinosus, m. semimembranosus and m. gastrocnemius. An average total dose of 17 U Botox® /kg BW (range: 11-22) was used. The maximum dose per muscle never exceeded 4 U/kg BW. For the psoas muscle the fixed dose of 2 U Botox® /kg BW was applied, leading to an average amount of 70 U Botox® (range: 40 – 100) per psoas muscle.

All patients have had BTX injections previously as part of their medical history, however at least 12 months before start of the study (range: 12 m – 64 months ago). Most patients even had more than one injection of the psoas muscle in the past (mean: 3.8 ; range: 1 – 7 times); these injections were done proximal in the muscle.

Volume assessment

Pre-injection MRI was taken at an average of 11 days (range: 0 - 23) before the injections. The first post-injection MRI was taken at an average of 2 months and 4 days (range: 57-72 days); in 3 patients a third MRI was taken after 6 months.

The within-subjects standard deviation for the volume measurement of the psoas muscle equaled 2071 mm^3, yielding a WSCV of 3.5% (CI:2.4%-4.6%) The ICC equaled 0.988 (CI: 0.971-0.996).

Starting volumes of all psoas muscles was between 35072 and 91374 mm^3 (mean: 61052 mm^3); all children under the age of 10 years had a volume below 50000 mm^3, the older children had higher volumes.

Five psoas muscles (in 5 patients) were injected using the distal injection technique and 9 psoas muscles (in 7 patients of which 2 patients bilaterally) were injected with the MEP oriented injection technique. In figure 3 the results of the volumes per time-point were presented. In all the MEP injected psoas muscles (dashed lines) there was a decline in volume after 2 months. In the distal injected psoas muscles (solid lines) the volume was stable or slightly increased. The volume at 6 months after the injection was measured in only 6 muscles (3 distal and 3 MEP injected psoas muscles in 3 spastic diplegic patients asymmetrically injected) and did not change with respect to the volume after 2 months. The difference between the initial volume and the volume 2 months after the MEP oriented injection was statistically different (p=0.004); there was no statistical significance for the differences in volume after 6 months (only 3 muscles) or for the muscles that were injected distally.

Part 2: MEP targeted injections

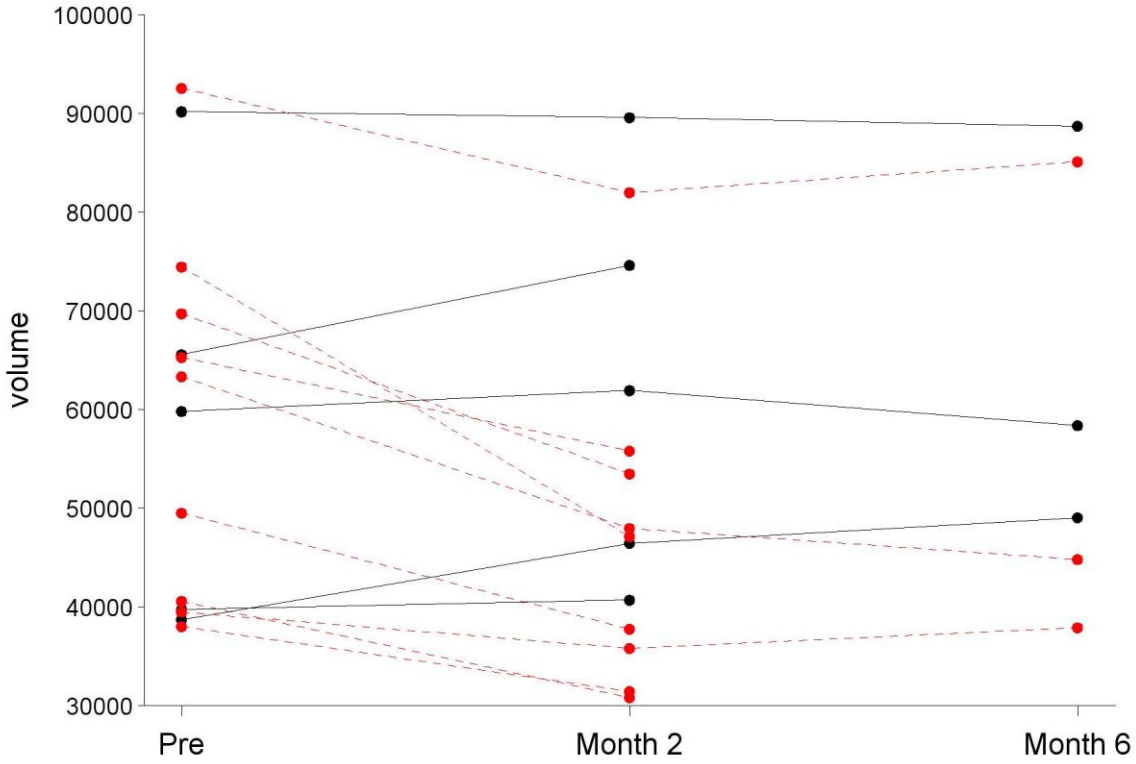

Figure 3. Volume (mm^3) of the psoas muscle pre-injection, 2 months and 6 months after injection. Dashed lines: MEP oriented injection technique; solid lines: distal injection technique.

Figure 4 represents these data as ratios of the post-injection volume in relation to the pre-injection volume for the different muscles (dashed lines: MEP; solid lines: distal injection). The mean of these ratios after 2 months was 0.795 (range: 0.633-0.907, SD:0.084) for the 9 MEP targeted muscles versus 1.078 (range: 0.993-1.200; SD:0.087) for the 5 distal injected psoas muscles. This difference is statistically significant (p=0.0033). Ratio of the volume after 6 months is 0.862 (range: 0.707-0.960; SD:0.136) for the 3 MEP injected psoas muscles versus 1.076 (range:0.976-1.267; SD:0.166) for the distal injected psoas muscles(p=0.08). Note that with only 3 observations in each group, there is no power to detect differences after 6 months.

In all 5 asymmetric injected spastic diplegic children the MEP targeted psoas had a larger volume reduction 2 months after injection than the more distal injected psoas muscle. The average difference of the pre-post ratio of muscle volume in both muscles was 0.27 (range: 0.09-0.37; SD: 0.12, p=0.063).

We could not observe a denervation zone with high-signal intensity pattern on the STIR sequences of the post-injection images. We have to mention although that the STIR sequences were often from an inferior quality due to (respiratory) movement of the patient; these sequences take more time and make a different and loud noise from which the children with CP very often get upset.

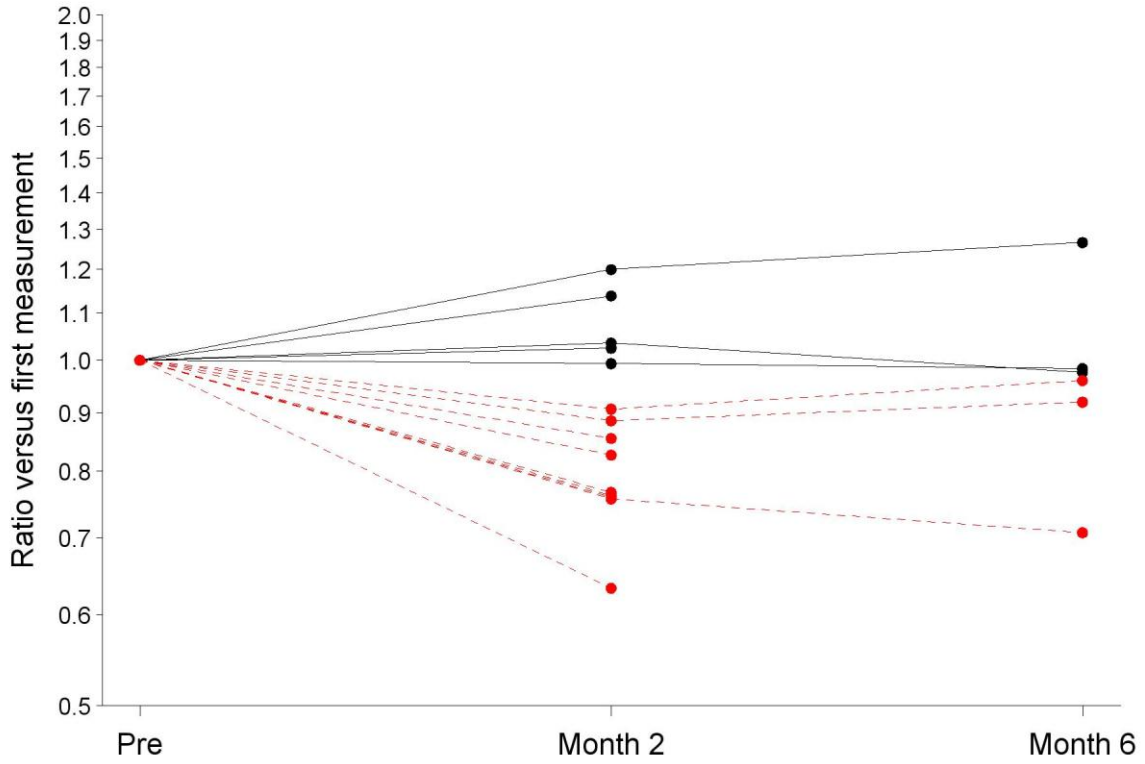

Figure 4. Ratio of the post-injection volume of the psoas muscle in relation to the pre-injection volume at 2 months and 6 months after injection. Dashed lines: MEP oriented injection technique; solid lines: distal injection technique.

Discussion

This is the first study that demonstrates muscle atrophy after BTX injection with a longitudinal follow-up by MRI in children with CP. This method was applied to find the most optimal injection technique for the psoas muscle. Indeed, when injecting the psoas muscle with BoNT-A in children with CP, a MEP oriented injection leads to a volume reduction of the psoas muscle of about 20%. Instead, a more distal injection of the toxin does not result in this muscle atrophy.

There is a large variability in outcome measures between studies evaluating the effect of BTX injections in CP including those that compare different injection techniques. In our study we did not select any assessment of function, activity or participation as outcome measure because we believe that changing the injection technique of one of the injected muscles would not give a measurable difference for those global measures. We specifically focused on body function and structure as described by the ICF-model (WHO, 2001). In this respect measures of spasticity are often reported as primary outcome measure. Clinical manual measures (for example modified Ashworth scale, Tardieu scale), instrumented analogs measuring torque increase as an answer to passive muscle stretch or electromyographic parameters measured during passive muscle stretch at different velocities can be used. For the psoas muscle however electromyography (EMG) is not an option as surface EMG is not possible in this retro-peritoneal deep-seated muscle, neither is repeated needle EMG in children with CP. Clinical and instrumented spasticity measures are either not sensitive enough to rate small differences in spasticity (Bohannon & Smith, 1987) or they are not designed to test hip flexor spasticity. As pelvis motion and hip extension is influenced by many factors including both psoas

muscles, we opted not to use 3-dimensional gait analysis to search for a difference between both injection techniques. Sätila et al. (2005) and Childers (2004) both tried to demonstrate the importance of MEP targeted localization of the injection in the gastrocnemius muscle using some of the before mentioned outcome measures and partly failed due to lack of sensibility of the outcome measures. Gracies et al (2009) used mean rectified voltage of the injected muscle measured by surface EMG during maximal active flexion to demonstrate the difference between endplate targeting injection versus a more dilute four-quadrant injection technique of the biceps brachii. They were also not able to demonstrate the difference between injection techniques with more clinical assessments of spasticity and range of motion. On the contrary, MRI can be used to identify even small changes in muscle volume. High sensibility and ability to accurately measure individual muscles using MRI has been demonstrated before (Koltzenburg & Yousry, 2007). This was confirmed by our good intra-class correlation (0.988) and by a within-subject coefficient of variation of 3.506% for MRI parameters. The averaged difference of 27% in volume difference pre-post between the MEP injected and distal injected psoas muscle largely exceeded the measurement error.

Using MRI, it has been demonstrated that the acutely and subacutely denervated muscle shows volume reduction. Already in 2001 Fanucci et al. (2001) used MRI results in 9 patients to demonstrate the effect of piriformis infiltration with BTX, administered to treat piriformis syndrome. They found atrophy in all cases and signal intensity changes on STIR sequences in 7 of the 9 cases defined by MRI 3 months after infiltration. More recently Schroeder et al. (2009) reported on the BTX injection in the lateral gastrocnemius muscle of two healthy adults. MRI at 3, 6, 9 and 12 months showed a high-signal-intensity pattern in STIR sequences and a reduction of cross-sectional area to 62 to 88% of the initial volume. We found a decreased volume to about 80 % of the initial volume in the MEP oriented injected psoas after the BTX injection. We preferred to use 3-dimensional muscle volume -measured by MRI segmentation- above 2-dimensional cross-sectional area. The use of volume takes the full muscle into account and may therefore better reflect change. As the aim was to compare injection at different sites we preferred to evaluate the effect on the total muscle instead of comparing cross-sections at a limited number of sites. We could however not document the denervation on the STIR sequences. It is not clear weather this is due to the previous history of BTX injections before the initial MRI or to the inferior quality of the images.

The atrophy of the 9 MEP targeted psoas muscles after 2 months (ratio 0.795 or a reduction to 79.5% of the initial volume) persisted after 6 months (ratio 0.862 for the 3 muscles that were measured after 6 months). This longstanding atrophy confirms the data from Schroeder et al (2009) from the two healthy lateral gastrocnemius muscles, highlighting a reduction in cross sectional area to 81 and 86% of initial after 3 months, 73% in both muscles after 6 months and even still an atrophy of 88 and 78% of the initial area after 12 months.

As all these children had BTX injections before their first study (pre-injection) MRI, it is not clear what their normal psoas volume would have been without a previous BoNT-A treatment. There might have been atrophy caused by the previous injections. However we found the same amount of atrophy in the patients with more recent injections (12 months, in 3 patients) compared to patients who had their previous injections a longer time ago (62 and 64 months). The current results also highlighted that the number of previous injection sessions did not change the amount of atrophy.

The 5 distal injected psoas muscles show no atrophy on MRI after 2 months, but a stable or slightly increased volume (range: 0.993-1.200; mean: 1.078).

There are several limitations to this study. Ideally CP patients without a previous history of BTX injections in the psoas should be studied. However, due to our treatment policy, all children whom are old enough to cooperate for the MRI without anesthesia have had injections already at a younger age. Including very young patients that have their first psoas injection would imply the need for

sedation for the MRI study, which should be avoided. Further, we do not have measurements at one year after the injection.
We did not include other outcome measures. We can therefore not be sure that the absence of muscle atrophy in the distal injected psoas muscles correlates with a total absence of clinical effect of spasticity reduction. We also didn't correlate the atrophy of the muscle to change in power.
Despite the small sample size, the difference in muscle volume after 2 months was significant. It should be noted that the MWU test considers the result at each leg as independent information. As such, this might have resulted in overestimation of the information for symmetric injected patients. On the other hand, the increase in power from the paired observations (asymmetric injected patients) is not used by the MWU test. The sample size is not large enough to use appropriate statistical techniques (e.g. a linear mixed model) to deal with the simultaneous presence of paired and unpaired comparisons.

Conclusion

This study has made several important contributions to the current understanding of the effect of BTX injections in the psoas muscle of children with CP. Injections given in the MEP zone, which is the more proximal part of the muscle cause muscle atrophy, which can be documented by MRI. On the other hand, injections of the distal part of the psoas muscle don't produce this muscle atrophy.

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General discussion

The scope of this doctoral project was to improve the intramuscular Botulinum toxin type A (BTX) treatment in children with cerebral palsy (CP). Literature and daily clinical practice have shown that this treatment is characterized by a large variability in outcome.[3,27,35] This is partly due to injection variables.[4] Animal studies already had shown that injecting the toxin near the motor end plate (MEP) zone increases its paralytic effect.[7,8] This was, so far, only confirmed in one human study for the adult biceps brachii muscle.[9] Besides this lack of strong clinical evidence of the importance of MEP targeted injections in children with CP, the clinician was confronted with the very limited information on the localization of the MEP-zones in the lower limb muscles. Therefore, the first part of this doctoral project was a search for the localization of these MEP zones in relation to external anatomic landmarks. In a second part, the clinical importance of injecting at the MEP zone was controlled in prospective randomized studies. For two muscles -for which a substantial difference in current injection technique versus proposed MEP targeted injection localization was found- both injection techniques were compared using novel sensitive outcome measures. The development of these evaluation methods was necessary because current evaluations are either too subjective and do not register small changes[30-32] or are too invasive and difficult to apply in children[33,66,67].
This general discussion first gives a summary of the main findings of this doctoral thesis. This summary is followed by an overall interpretation and discussion of these results, some critical considerations and recommendations for future research.

Summary

Until recently, routine practice of BTX injections was guided by the knowledge that the MEP zone is situated in the middle of a muscle fiber[53] and, therefore, it was assumed to be in the middle of the muscle belly. While this is true for unipennate muscles, more complex muscles have a different dissipation of their MEPs.[54] A thorough literature search provided information on the exact localization of the MEP zone or the terminal nerve ramifications of the frequently injected muscles of the lower limb. After studying all available histological and anatomical results and comparing these with clinical practice, it became clear that for some muscles the MEP zone could be more precisely demarcated and for many other muscles its location was somewhat different than the currently injected areas in clinical practice. **(part 1, chapter 3)**. More-over, no information was found for the psoas muscle. To overcome this lack of information, a cadaver study was performed on 24 adult psoas muscles. The nerve branches and their intramuscular course were followed, by macroscopic and stereoscopic microscopic dissection, as far as their terminal ramifications. An average of 3.7 nerve branches from the lumbar plexus innervating the psoas muscle was found. A region of intramuscular nerve endings, corresponding with the MEP zone, was identified along a reference-line from the twelfth thoracic vertebra to the passage of the psoas muscle under the inguinal ligament from about 30% until 70% of this reference. This corresponded also with a zone from 50% of the distance between the twelfth thoracic vertebra and the promontorium until about 20% of the distance between promontorium and pubis. This region of the muscle was close to the spine on the mediolateral axis. **(part 1, chapter 2)**. In the aforementioned review **(part 1, chapter 3)** optimal injection sites were presented for M. Gastrocnemius, M. Soleus, M. Tibialis posterior, M. Semitendinosus, M. Semimembranosus, M. Gracilis, M. Biceps femoris, M. Rectus femoris, M. Adductor longus, brevis and magnus and M. Psoas in relation to external anatomical landmarks. Interestingly, the optimal injections zones for many lower limb muscles were different than the sites

defined by the guidelines of popular injection techniques. Most specifically for the medial hamstrings (semitendinosus, semimembranosus and gracilis muscle) and the psoas muscle, there was enough evidence to conclude that current popular injection techniques were not injecting the toxin at a site close to the MEP zone. The medial hamstrings and the psoas muscle are very often involved in patients with spastic CP. Therefore, both injection techniques ('current' versus MEP targeted) were compared for these muscle groups. This required a set of methodological studies and prospective randomized clinical trials.

To compare both injection techniques for the medial hamstrings, and more specifically for the gracilis muscle, a quantitative evaluation of spasticity reduction using an instrumented assessment[33,38] was used.

First, the sensitivity of this instrumented spasticity assessment for the medial hamstrings in children with CP was studied. The measurement -using biomechanical (position and torque) and electrophysiological signals when applying passive stretches to the medial hamstrings at different velocities- was performed on nineteen children before and after BTX injections. Improvements that exceeded the minimal detectable change (calculated from previously published reliability results[33]) were found for nearly all electromyography (EMG)-parameters and for torque parameters at high velocity and at high versus low velocity. The biomechanical and electrophysiological parameters proved to be adequately sensitive to assess the response to treatment with BTX with an average of 53% reduction in velocity-dependent root mean square (RMS)-EMG and a 47% reduction in torque. **(part 2, chapter 2.1)**.

A second methodological study was set up to assess whether parameters obtained from the instrumented spasticity assessment were more sensitive than clinical scales in detecting treatment response. In addition it was studied if these instrumented spasticity assessments could provide further insights that help to explain response variability. Thirty-one children with CP (40 medial hamstring muscle groups) had clinical (modified Ashworth scale MAS and modified Tardieu scale MTS) and instrumented spasticity assessments of the medial hamstrings before and after BTX injection. Responsiveness of clinical and instrumented assessments was compared by percentage exact agreement (PEA). Prediction ability was assessed with a logistic regression and the area under the Receiver Operating Characteristic curves of the baseline parameters of responders versus the non-responders. Both clinical and instrumented parameters improved post-BTX, though showed a low PEA. Baseline MTS was the only clinical scale predictive for response. For the instrumented assessment, baseline values of RMS-EMG and of torque were good predictors for a positive response. RMS-EMG also remained an important predictor in the logistic regression. It was concluded that the instrumented spasticity assessment showed higher responsiveness than the clinical scales. The amount of RMS-EMG was considered a promising parameter to predict treatment response. **(part 2, chapter 2.2)**.

Following these methodological studies, a prospective randomized trial was set up, including 34 gracilis muscles which were injected with BTX (using a fixed dosage and dilution) in 27 children with CP (8.5±2.5y). Seventeen muscles were treated by proximal injections (at 25% of the length of the upper leg) and 17 muscles by MEP targeted injections (half the dosage at 30 and half at 60% of the upper leg). Clinical (MAS) and instrumented spasticity assessments using surface EMG (sEMG) during passive motion at different velocities were performed before and after the injections. The difference of the averaged RMS-EMG at low versus high velocity was calculated and normalized to the pre-injection EMG at maximal voluntary isometric contraction. The MEP targeted injections showed a

significantly better decline in pathological EMG signal compared to the conventional proximal injections, demonstrated by a higher reduction of the normalized RMS-EMG parameter. This difference could not be demonstrated using the MAS. We concluded that BTX injection in the gracilis muscle at the sites with a high concentration of MEPs resulted in improved spasticity reduction in children with CP. Further, we demonstrated that different injection protocols could be compared sensitively and objectively using the instrumented spasticity assessment that integrates biomechanical and electrophysiological measures. **(part 2, chapter 2.3)**.

The ultimate goal is to optimize motor function and thus to understand the influence of spasticity and tone reduction treatment on functional activities, such as gait. Therefore, a study was set up to search for functional markers of spasticity of the gastrocnemius and hamstring muscles during gait. Because spasticity is a velocity dependent feature[16], it has been suggested that signs of spasticity during gait may be highlighted by increasing the walking velocity. Walking faster will change the gait pattern both in CP and in typically developing (TD) children and indirect insight into the effect of spasticity on gait can be achieved by studying the differences in effect of increased walking velocity on gait in CP and TD children. We studied the impact of increased walking velocity on kinematic, kinetic and EMG parameters and on muscle length (ML) and muscle lengthening velocity (MLV) in 53 patients diagnosed with spastic CP (9.8 y ± 3.0y) and 17 TD children (10.46 y ± 2.36y). Gait parameters of the TD and CP children were collected during three dimensional (3D) gait analysis at different walking velocities (normal, fast and as fast as possible without running) and compared at similar non-dimensional velocities, estimated by a linear regression model. Many parameters showed significant difference between the CP and TD group at the lowest, at the highest or at both velocities. But, for many parameters, similar strategies to increase walking velocity were used by both groups, as indicated by the non-significantly different difference score (DS) between CP and TD. Still, a number of gastrocnemius and hamstrings related parameters could be considered as functional markers for spasticity, due to significantly different DS between CP and TD. The spastic gastrocnemius muscle while walking at high velocity was characterized by a higher ankle angular velocity, plantar flexion moment and power absorption during loading response. Additionally, this muscle demonstrated an increased EMG signal during early stance phase. The increased walking velocity affected the spastic hamstrings at the level of the hip and knee joints at mid-stance by a delayed maximum knee extension moment and by an increased hip extension moment and power generation. The hamstrings also presented with a lower MLV during swing phase. **(part 2, chapter 2.4)**.

For the psoas muscle, the instrumented spasticity assessment could not be used to test hip flexor spasticity, mainly due to the dependence on sEMG. This is not possible in this retro-peritoneal deep-seated muscle. More-over, we opted not to use 3D gait analysis to search for a difference between both injection techniques, as pelvis motion and hip extension is influenced by many other factors than spasticity of both psoas muscles. Therefore, a quantitative evaluation using muscle volume assessment by digital magnetic resonance imagination (MRI) segmentation was done to compare both injection techniques. The temporary chemical denervation caused by BTX injections leads to muscle atrophy.[72] MRI sensitively identifies these changes in muscle volume.[73] This was confirmed by our good intra-class correlation (0.988) and by a within-subject coefficient of variation of 3.506% for the MRI parameters. The MEP targeting versus a widely used more distal injection technique were compared in seven spastic diplegic children. Five patients received two different injection techniques randomly applied to both psoas muscles and in two patients a bilateral MEP targeting technique was

used. MRI images of the psoas were taken before, two months and -in three patients- six months after the injections. The average post injection volume after two months (in relation to pre-injection volume) for the nine MEP targeted muscles was 79,5% versus 107.8% for the five distal injected psoas muscles. This difference was statistically significant. In all five asymmetric injected patients, the MEP targeted psoas had a larger volume reduction than the more distal injected psoas muscle. This atrophy remained even six months after the injection. We therefore concluded that injections in the MEP zone of the muscle, which is the more proximal part of the psoas muscle, caused muscle atrophy, in contrary to more distal injections were this atrophy was not observed. The effect of the toxin in the psoas muscle could only be demonstrated when the injection was done in the region where the MEPs are concentrated. This was the first study to prove the importance of MEP targeting in children with CP. Further-more, the study was also original in providing a longitudinal follow-up of muscle atrophy demonstrated by MRI, after BTX injections in children with CP. **(part 2, chapter 3)**

Interpretation of the findings and critical considerations

In the first part of this doctoral research project we found that, even after collecting all available studies on innervation of human skeletal muscles, it was necessary for a limited number of lower limb muscles to deduct the localization of the MEP zone from the localization of the motor points (MP). Because of a lack of histological studies or detailed anatomical studies, this was required for the tibialis posterior, semimembranosus, biceps femoris and adductor muscles. It should be noted that the MEP is not located at the MP or where the motor branch enters the muscle belly, but comparison of some studies (Parratte[58] versus Kim[59]) and the results from our psoas dissection study, learned us that the MPs are usually more proximal than the MEPs. So, we can, to some extent, let the MPs guide us to the MEP zone. Still, it would be better to have good anatomical studies on the localization of the MEP zone in reference to external anatomical markers for all of these muscles. This ideally would imply studies in which the intramuscular path of the nerve is followed with stereoscopic dissection until the end of each terminal nerve ramification. Fortunately, this has recently - after the completion of the MEP review- been done for the semimembranosus and biceps femoris muscles by An et al.[71] using dissection of terminal nerve endings and for the adductor longus muscle by Won et al.[57] using Sihler staining. For these three muscles, the assumptions made from the localization of the MPs concerning the localization of the MEP zone were found to be correct, based on the comparison to the findings of these recent studies. We can thus conclude that the proposed MEP locations for the few remaining muscles can be used, until new studies emerge.

The histochemical studies on the neuromuscular junctions (NMJ) were carried out on infant[53,54] and adult[55] cadavers. The findings in the two groups were in agreement. It is unclear whether these results can be extrapolated to juveniles. Ma et al[52] studied the distribution of NMJs in juvenile and adult rats and stated that the distribution of NMJ in adult rats can be extrapolated to juvenile rats, but it is not clear whether distribution data from human adults can be extrapolated to juveniles. All anatomical dissections studies were also done on adult cadavers.[56-64,712] Also for the psoas muscle, our data could not be compared to data of the psoas muscle of children. Juvenile cadavers are -fortunately- only seldom available. But by scaling the localization of the MEP-zone in terms of percentage distance along a reference line, we allow the measurements not only to be applicable to a diverse adult population, but also to pediatric and adolescent patients.

Recently, the use of electrophysiological mapping to precisely localize MEP zones of superficial muscles has been demonstrated. Lapatkia et al.[74] presented a high-density surface EMG for localizing the main endplate zone of the extensor digitorum brevis muscle. Multi-electrode grids (8x15 electrodes, each 1.5 mm in diameter and spaced 4 mm apart) were used to record the compound muscle action potential (CMAP). These CMAP amplitudes have been shown to be sensitive and specific to MEP location. This technique potentially solves the problem of very precisely localizing the MEP zones of superficial muscles for which this localization is unknown and allows to study inter-subject variability of MEP localizations for a specific muscle. Lapatkia et al.[74] also demonstrated that even a small misplacement of BTX injections resulted in a significantly lower BTX efficacy. This method, however, is tailored to those muscles that have MEPs clustered in single or several well-defined areas. Further, this localization technique requires surface EMG, which means that it is not applicable for deep musculature. Finally, it is time-consuming. The authors state that approximately 15 minutes are needed for this procedure. This makes it difficult to use in case of multilevel BTX treatments, where several muscles need injection. For this indication, the clinician will still rely on descriptions of the average localization of the MEP zones to find the most optimal injection sites.

In the study by Lapatkia et al.[74] healthy volunteers were injected. Until now, there was only one human study in patients to confirm the importance of injecting the toxin close to its site of action. Gracies et al.[9] described this for the biceps brachii muscle of adults with hemiparesis due to stroke or traumatic brain injury. In a previous study, this research team used histologic staining to identify the MEP zone in five biceps brachii muscles.[75] Subsequently, the effect of BTX in seven diffuse injected muscles was compared to the effect of the toxin in seven MEP targeted injected muscles. Mean rectified voltage (MRV) from the elbow flexor during maximal isometric flexion, measured with sEMG, was used as primary outcome measure. Secondary outcome measures were maximal voluntary power, Tardieu scale and active range of motion of the elbow. Superior efficacy of the MEP targeted injection was demonstrated by a greater neuromuscular blockade -i.e. lower MRV (although this did not reach statistical significance)-, greater reduction of Tardieu scale and improvement of active range of elbow extension (both statistical significant better).
It should be noted that older studies could not find a difference in effect between injection techniques applying different injection sites. Childers[38] (2004) sought to test the hypothesis that BTX injections at the mid belly of the gastrocnemius muscle in spastic hemiplegic adults produce superior clinical results compared to proximal injections. One of the problems of this study is that the wrong assumption concerning the localization of the MEP zone was made. Further, Childers[38] used outcome measures that have been proven (later on) to be not very sensitive to change, such as Ashworth scale. The same problem was encountered by Sätilä et al.[76] (2005) when comparing two injection localizations of the gastrocnemius muscle.
In our gracilis study (part 2, chapter 2.3), MAS was also evaluated, but did not show a statistically significant difference. In both clinical trials (on gracilis and psoas muscle; part 2, chapter 2.3 and 3), the results of the different injection techniques were determined using newly developed assessment tools.

The development of the valid assessments of the effect of BTX made it possible to objectively and sensitively compare injection protocols. Both the instrumented passive spasticity assessment (part 2, chapter 2.1-2.3) and the 3D MRI muscle volume assessment (part 2, chapter 3) are measures of body function and structure. Changes in this domain are the most straight forward to be measured objectively and most likely to manifest changes, because this domain is the most proximate to the

intervention. Outcomes in the domain of activity and participation (for example gait) are of high interest, but represent the more secondary effects of the intervention. The identification of dynamic markers of spasticity (part 2, chapter 2.4) will make it possible to study the effect of different injection techniques also during more complex actions such as gait. In the future, a correlation between the dynamic markers of spasticity and the spasticity parameters from the instrumented passive single joint assessment (part 2, chapter 2.2 and 2.3) need to be made by combining both measures in one patient group. Studying the change of the dynamic markers of spasticity after BTX injections will further contribute to the validation of these markers. However, both studies are beyond the scope of this doctoral project, and are part of future (and already ongoing) research.

In the psoas study (part 2, chapter 3), change in muscle size was used as outcome measure. A reduction in muscle volume of the psoas muscle was documented after BTX infiltration at the MEP zone. This neurogenic atrophy was also reported by Fanucci et al.[77] after BTX injections of the piriformis muscles in adult patients with piriformis syndrome and by Schroeder et al.[34] in two healthy adults after BTX injections of the lateral gastrocnemius muscle. This is also in agreement with results from animal research and reports from the cosmetic industry.[78,79] A recent study by Williams et al.[80] confirms our findings of muscle atrophy after BTX injections in children with CP, but this in the gastrocnemius and medial hamstrings. It is well recognized that muscle size and structure are associated with muscle strength. Further, children with CP have smaller and weaker muscles. In this patient group, it is important that a treatment that potentially leads to further muscle atrophy and weakening is well understood.

The duration of the muscle atrophy and its reversibility is not completely clear. Fanucci et al.[77] only measured muscle volume after 3 months, Williams et al.[80] only after 5 weeks. A persistent reduction of the cross-sectional area of the muscle was documented until 12 months after the injection by Schroeder et al.[34] in the healthy lateral gastrocnemius muscle. We also observed a decreased muscle volume of the psoas muscle in children with CP until 1 year after the BTX injections. Longer follow-up has not been reported yet.

A treatment with BTX in children with CP is usually performed when local spasticity impedes function or leads to contractions. In some muscle groups the observed muscle atrophy and thus weakening of the muscle can be considered as a desirable effect of the toxin. Longstanding weakening of the spastic muscle will enable the child to increase work and load of synergistic muscles and to improve function of antagonistic muscles, thus improving overall function. The study by Williams et al.[80], studied the muscles volumes of both gastrocnemius and soleus muscles after injection of the gastrocnemius muscle. They uncovered the possibility of this compensatory hypertrophy in synergist muscle as the muscle volume of the (non-injected) soleus muscle slightly increased in contrary to the mild muscle atrophy of the injected gastrocnemius muscle. It was hypothesized that the increased physiological demand on the synergistic soleus muscle would lead to work-induced hypertrophy. Further, no strength deficits of the group of ankle plantar flexors were seen following a single site injection. This possibly can explain the good clinical and functional response -often longer than the duration of the chemical denervation- in children with CP after BTX injections. This muscle atrophy is more concerning when it involves muscles essential for gait such as the soleus muscle. Just as it is a bad idea to lengthen surgically and thus weaken the soleus muscle in a child with spastic diplegia, injection of the soleus muscle might also lead to a weaker muscle and might induce evolution to crough gait. Future research is necessary to understand better the correlation between BTX injections, muscle atrophy and weakening and functional outcome.

Parameters from the instrumented spasticity assessment provided not only sensitive continuous data, but also captured higher variable levels of response to BTX treatment. Understanding this variability could enhance treatment delineation and ensure more targeted and individualized anti-spasticity care. Baseline spasticity parameters from the instrumented assessment can assist in the prediction of treatment response. Muscles with higher pathological EMG activation at baseline tended to be good responders and this knowledge needs to be implemented in clinical decision making. Further, by integrating electrophysiological and biomechanical parameters, a more comprehensive assessment was achieved which facilitated the decomposition of the neural and non-neural components of muscle tone. Clinical scales (MAS and MTS) fail to distinguish between both components. These scales assess spasticity by subjectively interpreting the resistance felt during passive stretch. This perceived resistance may be a result of reflex muscle activity as well as of changes in visco-elastic properties of the joint and muscle and cannot discern the relative contributions of neural and non-neural components of muscle tone. The integrated instrumented spasticity measurement method could decompose the pathological response to muscle stretch into the reflex muscle activity and the visco-elastic properties, because it measures both EMG and torque. Parameters investigating the change between velocities were able to capture velocity-dependent spasticity as defined by Lance[16]. These proved most sensitive to treatment with BTX, with an average of 53% reduction in velocity-dependent RMS-EMG and a 47% reduction in torque. The moderate correlation between the change in RMS-EMG% and in torque post BTX confirms that the decrease in torque is partially influenced by velocity-dependent neurogenic factors. Torque parameters at low velocity were considered to represent intrinsic stiffness (due to secondary changes of the spastic muscle) rather than the neural components of tone, because after neuromuscular blockade by BTX no relevant change was observed.

Despite the sensitivity of the instrumented measurement method, some methodological limitations need to be considered. A first limitation is the dependence of the normalized RMS-EMG parameter on a representative maximum voluntary contraction. BTX induces temporary weakness and we therefore used the maximum contraction at baseline to quantify spasticity post-BTX. The non-normalized parameter was also reported and care was taken to minimize the disadvantage of using non-normalized EMG through standardization of the sEMG-electrode placement. Importantly, conclusions were similar for the normalized and non-normalized parameters. Secondly, in our clinical trial, we only tested the gracilis muscle. The dependence on sEMG in the outcome assessment of our patient group (children with CP) limits the measurement method to investigating surface muscles from which activity can be easily differentiated from that of neighboring muscles. Therefore, the semitendinosus and semimembranosus muscle, which lie very close to each other, were not included in our comparison of injection localizations.

In the psoas study we were confronted with the limitations of clinical research in children. Finding young children with CP who needed BTX in the psoas muscle and who were able and willing to undergo an MRI scan twice was a challenge. Therefore, as all participants were children with spastic diplegia that needed bilateral psoas injections, we started the study by randomizing the injection techniques between both sides. This would allow comparison between both sides in the same patient, thereby reducing the influence of disturbing variables. A preliminary control of the quality of the muscle volume assessment displayed a large difference between the outcome of both injections techniques. As clinicians, strongly influenced by the ethical rules aiming for the best clinical practice

for each child, we were worried of inducing asymmetry in the outcome and thus choosed to proceed only with the same infiltration technique for both psoas muscles in the same child. Unfortunately, the two children that received a bilateral distal injection were not willing the go through a second MRI. Eventually, the comparison of the five distal injected with the nine proximal injected psoas muscle showed a difference that was large enough to be significant.
Further, it would have been ideal to study children with CP without a previous history of BTX injections in the psoas. However, due to our treatment policy, all children who were old enough to cooperate for the MRI without anesthesia, had already received injections at a younger age. Including very young patients who were planned for their first psoas injection would have implied the need for sedation for the MRI study, which was not acceptable from an ethical point of view.

The results from the gracilis and psoas study have shown that BTX injections at the sites with high MEP concentrations, have an improved efficacy compared to injections more distant from these MEPs. This was documented in two prospective randomized clinical trials in children with CP. The same conclusion was made in the adult spastic biceps muscle and the extensor digitorum muscle of healthy adults. It is therefore reasonable to state that all BTX injections preferably should be given close to the MEP zone(s) of the injected skeletal muscles. We have studied this for children with CP. But BTX is also used in other pathologies presenting with spasticity such as acquired brain lesions (after stroke or cerebral trauma), spinal cord lesions, multiple sclerosis and other degenerative neurologic conditions. Using MEP targeted injections when injecting BTX in patients with these pathologies will also improve efficacy of these injections, as the same product working on the same target (MEP) is used to aim for a neuromuscular blockade.
For small muscles diffusion may be sufficient to reach the MEPs. But even in those muscles unintended spread to neighboring muscles should be avoided, which can be accomplished by more precisely target to the MEPs with lower dosages. Further, MEP targeted injections may permit the use of lower doses of BTX with still adequate tone reduction while reducing costs and minimizing the risk of high-dose BTX injections.

Recommendations for future research

Improved efficacy of MEP targeted injections has been documented and this knowledge will allow us to further improve the clinical use of BTX treatment in children with CP. However, localization of the injection is only one variable of this injection technique. Dose and dilution can also be further optimized. The newly developed outcome measures can be used to document the different injection protocols.

A further advancement of these outcome measures is also mandatory. The evaluation of muscle volume proved to be an important assessment, but was difficult to accomplish in very young CP patients and was quite expensive. 3D ultrasound images of the muscle are being proposed to evaluate the muscle volume.[81,82] This will also be implemented in our research in the near future. Correlation of change in muscle volume needs to be made to spasticity reduction and functional outcome.
Parameters of the instrumented spasticity assessment will be correlated with the dynamic markers of spasticity to produce an integrated spasticity assessment. Further validation of this concept will be done by comparing these data before and after BTX injections.

The final goal is to integrate information about muscle volume and muscle structure, spasticity and strength in order to delineate on optimal treatment for the individual child with CP.

Precise mapping of the MEP zones in the individual muscles of every single patient, instead of relying on descriptions of average localization, might further improve the role of MEP targeting. An exploration of the high density EMG mapping, proposed by Lapatkia et al.[74], to a faster assessment tool could be an option for surface muscles.

Conclusion

The overall goal of this thesis was to improve the effectiveness of BTX treatment in children with CP, by optimizing the injection location. After the identification of the MEP zones of the frequently injected lower limb muscles, the clinical importance of injecting these MEP zones in children with CP was documented for the gracilis and psoas muscle. An instrumented spasticity assessment and an MRI segmented muscle volume measurement were developed as outcome measures. The improved efficacy of injecting BTX at the sites where MEPs are concentrated will permit the use of lower dosages and decrease economic costs and the risk of side effects.

Abbreviations

3D: three dimensional

AB: adductor brevis muscle

AL: adductor longus muscle

AM: adductor magnus muscle

AOC: angle of catch

BF: biceps femoris muscle

BTX: Botulinum toxin type A

CI: confidence interval

CMAP: compound motor action potential

CP: cerebral palsy

DS: difference score

GMFCS: gross motor functional classification scale

HV: high velocity

ICC: intraclass correlation

ICF: international classification of function

ITB: Intrathecal Baclofen pump

L: point where the psoas muscle passes under the inguinal ligament

LV: low velocity

L4, L5: fourth lumbar vertebra, fifth lumbar vertebra

M: musculus

MAS: Modified Ashworth scale

MB: motor branch

MDC: minimal detectable change

MEH: Medial hamstrings

MEP: motor end plate

ML: muscle length

MLV: muscle lengthening velocity

MP: motor point

MRI: magnetic resonance imaging

MRV: mean rectified voltage

MTS: Modified Tardieu Scale

MV: medium velocity

Abbreviations

MVIC: maximal voluntary isometric contraction

MWU: Mann-Whitney U

N: nervus

NMJ: neuromuscular junction

P: promontorium

PEA : percentage exact agreement

PU: pubis

RCT: randomized controlled trials

RF: rectus femoris muscle

RMS-EMG: root mean square envelope of the surface electromyography signal

ROC: Receiver Operating Characteristic

ROM: range of motion

S: sacral

SD: standard deviation

SDR: Selective Dorsal Rhizotomy

SEM: standard error of measurement

sEMG: surface electromyography

SISA: spina iliaca anterior superior

SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein receptor

SM: semimembranosus muscle

ST: semitendinosus muscle

STIR: short tau inversion recovery

TD: typical developing

Th12: twelfth thoracic vertebra

U: units

V_{MAX} : maximum angular velocity of a passive muscle stretch

WHO: World Health Organization

WSCV: within-subjects coefficient of variation

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Summary

Cerebral palsy (CP) is the most common cause of physical disability in children. It is defined as a disorder of the development of movement and posture that is attributed to a non-progressive disturbance of the developing brain. In many CP patients this brain lesion causes spasticity and the elicited increased tone leads to contractures and bony malformations. An optimal use of spasticity reduction with Botulinum toxin type A (BTX) injections, started at a young age, can prevent these complications to some extent. While many clinical studies reported overall good results of this treatment, they also demonstrated considerable variation in outcome. This is partly due to injection variables. BTX blocks neurotransmission by inhibiting the release of Acetylcholine at the motor end plate (MEP). Animal studies already have shown that injecting the toxin near the MEP zone increases its paralytic effect. This was, so far, only confirmed in one human study on the biceps brachii muscle of adults with spastic hemiplegia after acquired brain lesion (Gracies et al, 2009). Besides the lack of strong clinical evidence of the importance of MEP targeted injections in children with CP, the clinician was confronted with the very limited information on the localization of the MEP-zones in the lower limb muscles.

The overall goal of this thesis was to improve the effectiveness of lower limb treatment with intramuscular BTX injections in children with CP, by optimizing the injection location.
A thorough literature search -collecting all relevant histological and anatomical studies- provided information on the exact localization of the MEP zone or the terminal nerve ramifications of most of the frequently injected lower limb muscles. After comparing these with clinical practice, it became clear that for many muscles its location was somewhat different than the currently injected areas. In the review article, optimal injection sites in relation to external anatomical landmarks were presented. As no information was found on the innervation of the psoas muscle, a cadaver dissection study was performed on 24 adult psoas muscles. With stereoscopic microscopic dissection as far as the terminal nerve ramifications, the region of intramuscular nerve endings, corresponding with the MEP zone, was identified. For both the medial hamstrings (semitendinosus, semimembranosus and gracilis muscle) as well as the psoas muscle, there was enough evidence to conclude that current popular injection techniques were not injecting the toxin at a site close to the MEP zone. To explore the clinical importance of injecting these MEP zones in children with CP, both injection techniques (‘current’ versus MEP targeted) were compared for both muscle groups through the application of innovative assessments.

An instrumented spasticity assessment was used to evaluate the effect of BTX in the medial hamstrings. Biomechanical (position and torque) and electrophysiological signals were measured when applying passive stretches to the medial hamstrings at different velocities. First, the sensitivity of this assessment was studied on nineteen children before and after BTX injections. The biomechanical and electrophysiological parameters proved to be adequately sensitive to assess the response to treatment with BTX with an average of 53% reduction in velocity-dependent root mean square electromyography (RMS-EMG) and a 47% reduction in torque. A second methodological study was set up to assess whether parameters obtained from the instrumented spasticity assessment were more sensitive than clinical scales in detecting treatment response and whether they could

help explain response variability. Thirty-one children with CP (40 medial hamstring muscle groups) had a clinical and instrumented spasticity assessments of the medial hamstrings before and after BTX injection. It was concluded that the instrumented spasticity assessment showed higher responsiveness than the clinical scales. The amount of RMS-EMG was considered a promising parameter to predict treatment response. Following these methodological studies, a prospective randomized trial was set up, including 34 gracilis muscles which were injected with BTX in 27 children with CP (8.5±2.5y). Seventeen muscles were treated by proximal injections (at 25% of the length of the upper leg) and 17 muscles by MEP targeted injections (half the dosage at 30% and half at 60% of the upper leg). Clinical and instrumented spasticity assessments were performed before and after the injections. The MEP targeted injections showed a significantly better decline in pathological EMG signal compared to the conventional proximal injections, demonstrated by a higher reduction of the normalized RMS-EMG parameter. This difference could not be demonstrated using the clinical scale. It was concluded that BTX injection in the gracilis muscle at the sites with a high concentration of MEPs resulted in improved spasticity reduction in children with CP. Further, we demonstrated that different injection protocols could be compared sensitively and objectively using the instrumented spasticity assessment that integrates biomechanical and electrophysiological measures.
The ultimate goal is to optimize motor function and thus to understand the influence of spasticity and tone reduction treatment on functional activities, such as gait. Therefore, a study was set up to search for functional markers of spasticity of the gastrocnemius and hamstring muscles during gait. Because spasticity is a velocity dependent feature, it has been suggested that signs of spasticity during gait may be highlighted by increasing the walking velocity. Gait parameters (kinematic, kinetic and EMG parameters, muscle length and muscle lengthening velocity MLV) of 17 typical developing (TD) children (10.46±2.36y) and 53 patients diagnosed with spastic CP (9.8±3.0y) were collected during a 3D gait analysis at different walking velocities (normal, fast and as fast as possible without running) and compared at two similar non-dimensional velocities, estimated by a linear regression model. A number of gastrocnemius and hamstrings related parameters could be considered as functional markers for spasticity, due to significantly different ‘difference scores’ (between slow and fast walking velocity) between CP and TD. The spastic gastrocnemius muscle, while walking at high velocity, was characterized by a higher ankle angular velocity, plantar flexion moment and power absorption during loading response. Additionally, this muscle demonstrated an increased EMG signal during stance phase. The increased walking velocity affected the spastic hamstrings at the level of the hip and knee joints at mid-stance by a delayed maximum knee extension moment and by an increased hip extension moment and power generation. The hamstrings also presented with a lower MLV during swing phase.

To evaluate both injection techniques for the psoas muscle, a quantitative evaluation using muscle volume assessment by digital magnetic resonance imagination (MRI) segmentation was done. The temporary chemical denervation caused by BTX injections leads to muscle atrophy. MRI sensitively identifies these changes in muscle volume as was confirmed by a good intra-class correlation (0.988) and within-subject coefficient of variation of 3.506% in our study. In seven spastic diplegic children, the MEP targeting versus a widely used more distal injection technique were compared. Five patients received two different injection techniques randomly applied to both psoas muscles and in two patients a bilateral MEP targeting technique was used. MRI images of the psoas were taken before, two months and -in three patients- six months after the injections. The average injection volume two months after the injection (in relation to pre-injection volume) for the nine MEP targeted muscles

was 79,5% versus 107.8% for the five distal injected psoas muscles. This difference was statistically significant. In all five asymmetric injected patients, the MEP targeted psoas had an average of 27% (range 9-37%) larger volume reduction than the more distal injected psoas muscle. This atrophy remained even six months after the treatment. We therefore concluded that injections in the MEP zone of the muscle, which is the more proximal part of the psoas muscle, caused muscle atrophy -as a demonstration of the effect of the toxin-, in contrary to more distal injections were this atrophy was not observed.

The newly developed assessment tools (the instrumented spasticity assessment and the digital MRI segmentation muscle volume assessment) proved to be reliable and valid to compare different BTX injection protocols. The results from the gracilis and psoas study have shown that BTX injections at the sites with high MEP concentrations, have an improved efficacy compared to injections more distant from these MEPs. It is therefore reasonable to state that all BTX injections preferably should be given close to the MEP zone(s) of the injected skeletal muscles. The effect on function of the child with CP when using these more efficient MEP targeted BTX injections will be further explored by studying the effect on the functional spasticity markers during gait. Future studies comparing different dosage and dilution protocols injected at these MEP zones, documented by the sensitive instrumented spasticity assessment and muscle volume measurement, can further improve the treatment efficacy. This can eventually lead to the use of lower dosages thus decreasing economic costs and the risk of side effects.

Samenvatting

Cerebrale parese (CP) of hersenverlamming is de meest voorkomende oorzaak van een lichamelijke handicap bij kinderen. CP is een aandoening gekenmerkt door verstoorde houdings- en bewegingsontwikkeling ten gevolge van een niet-progressief letsel van de zich ontwikkelende hersenen. Bij veel CP patiënten veroorzaakt dit hersenletsel spasticiteit en de hierdoor verhoogde spierspanning leidt tot contracturen en beenderige misvormingen. Een optimaal gebruik van spasticiteitsvermindering door intramusculaire Botuline toxine type A (BTX) injecties kan deze complicaties ten dele voorkomen. Meerdere klinische studies hebben goede resultaten met deze behandeling gerapporteerd, maar deze studies toonden ook een aanzienlijke variatie in de resultaten. Dit is gedeeltelijk het gevolg van injectievariabelen. BTX blokkeert neurotransmissie door de vrijstelling van Acetylcholine ter hoogte van de motorische eindplaat (MEP) te inhiberen. Dierstudies toonden reeds aan dat injecties van het toxine nabij de MEP zone het paralytisch effect verbeteren. Dit was, tot op heden, enkel bevestigd in één humane studie op de biceps brachii spieren van volwassenen met spastische hemiplegie na een verworven hersenletsel (Gracies et al., 2009). Naast het gebrek aan overtuigend klinisch bewijs van het belang van injecties gericht op de MEP zone, werd de clinicus ook geconfronteerd met een zeer beperkte hoeveelheid informatie over de lokalisatie van de MEP zones van de spieren van het onderste lidmaat.

Het globale doel van dit proefschrift was de verbetering van de efficiëntie van de behandeling met intramusculaire BTX injecties van het onderste lidmaat bij kinderen met CP door de injectielokalisatie te verbeteren. Een grondige literatuurstudie -waarbij alle relevante histologische en anatomische studies werden verzameld- zorgde voor informatie over de exacte localisatie van de MEP zone of de zone met de zenuw-eindvertakkingen van de meeste regelmatig geïnjecteerde spieren van het onderste lidmaat. Na het vergelijken van deze zones met de klinische praktijk, werd duidelijk dat voor veel spieren deze locatie verschillend was dan de momenteel gebruikte injectie regio. In het overzichtsartikel werden optimale injectieplaatsen in relatie tot uitwendige anatomische punten voorgesteld. Aangezien er geen informatie werd gevonden over de innervatie van de psoas spier, werd een kadaverdissectiestudie uitgevoerd op 24 volwassen psoas spieren. Door middel van stereoscopische microscopische dissectie tot op het niveau van de eindvertakkingen van de zenuwen, werd de zone geïdentificeerd met de intramusculaire eindtakjes, overeenstemmend met de MEP zone. Zowel voor de mediale hamstrings (semimembranosus, semitendinosus en gracilis spier) als voor de psoas spier, waren er genoeg aanwijzingen dat de huidige populaire injectietechnieken het toxine niet injecteren op een plaats nabij de MEP zone. Ten einde het klinische belang van het injecteren van de MEP zones bij kinderen met CP aan te tonen, werden beide injectietechnieken (‘huidige’ versus MEP gerichte) in beide spiergroepen vergeleken door middel van innovatieve meetmethoden.

Een geïnstrumenteerde spasticiteitmeting werd gebruikt om het effect van BTX op de mediale hamstrings te meten. Hierbij werden biomechanische (positie en moment) en elektrofysiologische signalen gemeten tijdens het uitvoeren van passieve rekbewegingen van de mediale hamstrings aan verschillende snelheden. Vooreerst werd de gevoeligheid van deze meetmethode bestudeerd bij negentien kinderen voor en na BTX injecties. De biomechanische en elektrofysiologische parameters

bleken voldoende gevoelig te zijn om de reactie van de BTX behandeling te evalueren met een gemiddelde reductie van 53% in snelheidsafhankelijk kwadratisch gemiddeld electromyografisch signaal (RMS-EMG) en een vermindering in moment van 47%. Een tweede methodologische studie werd uitgevoerd om te evalueren of de parameters bekomen met de geïnstrumenteerde spasticiteitsmeting meer gevoelig waren dan de klinische evaluatieschalen bij het detecteren van reactie op behandeling en of deze konden bijdragen tot het verklaren van de reactievariabiliteit. Eenendertig kinderen met CP (40 mediale hamstrings spiergroepen) kregen een geïnstrumenteerde en klinische spasticiteitmeting van de mediale hamstrings voor en na BTX injectie. Er kon geconcludeerd worden dat de geïnstrumenteerde meting een betere gevoeligheid vertoonde dan de klinische schalen. De hoeveelheid RMS-EMG was een veelbelovende parameter om het effect van de behandeling te voorspellen. Na deze methodologische studies werd een prospectieve gerandomiseerde studie opgestart, waarbij 34 gracilis spieren met BTX geïnjecteerd werden in 27 kinderen met CP (8.5 ± 2.5jaar). Zeventien spieren werden behandeld met proximale injecties (ter hoogte van 25% van de bovenbeenafstand) en 17 spieren kregen een MEP gerichte injectie (de helft van de dosis op 30% en de helft op 60% van de bovenbeenafstand). Klinische en geïnstrumenteerde spasticiteitmetingen werden uitgevoerd voor en na de injecties. De MEP gerichte injecties vertoonden een significant betere vermindering in pathologisch EMG signaal in vergelijking met de conventionele proximale inspuitingen. Dit verschil kon niet aangetoond worden met de klinische schaal. Het besluit was dat BTX inspuiting van de gracilis spier op plaatsen met een hoge concentratie aan MEPs resulteert in een verbeterde spasticiteitsvermindering bij kinderen met CP. Daarnaast kon aangetoond worden dat verschillende injectietechnieken gevoelig en objectief konden vergeleken worden met de geïnstrumenteerde spasticiteitsmeting die biomechanische en elektrofysiologische metingen integreert.

Het uiteindelijke doel is om bewegingsfunctie te verbeteren en dus om het effect van spasticiteit en tonusreductie op functionele activiteiten, zoals het stappen, te begrijpen. Daarom werd een studie opgezet ter opsporing van de functionele merktekens van spasticiteit in gastrocnemius en hamstringspieren tijdens het stappen. Aangezien spanning een snelheidsafhankelijk fenomeen is, werd gesuggereerd dat tekens van spasticiteit tijdens het stappen tot uiting zouden komen wanneer de gangsnelheid wordt verhoogd. Gangparameters (kinematische, kinetische en EMG parameters, spierlengte en spierverlengingssnelheid) van 17 normaal ontwikkelende kinderen (TD) (10.46 ± 2.36 jaar) en 53 kinderen met spastische CP (9.8 ± 3.0 jaar) werden verzameld tijdens een 3D ganganalyse aan drie verschillende gangsnelheden (normaal, snel en zo snel mogelijk zonder te rennen) en werden vergeleken op twee gelijke niet-dimensionele snelheden, geschat via een lineair regressiemodel. Een aantal gastrocnemius en hamstrings gerelateerde parameters konden beschouwd worden als functionele merktekens voor spasticiteit omdat ze een significant verschillende ‘verschilscore’ (tussen trage en snelle stapsnelheid) vertoonden tussen CP en TD. De spastische gastrocnemius werd gekarakteriseerd, bij het stappen aan hogere snelheid, door een hogere enkel-hoeksnelheid, plantair flexie moment en krachtabsorptie tijdens opvang van belasting (loading response). Daarnaast vertoonde deze spier ook een toegenomen EMG signaal tijdens de steunfase. De verhoogde stapsnelheid beïnvloedde de spastische hamstrings ter hoogte van heup- en kniegewrichten bij het midden van de steunfase met een verlaat maximum knie-extensiemoment en een verhoogd heup-extensiemoment en krachtopwekking. De hamstrings vertoonden ook een lagere spierverlengingssnelheid tijdens de zwaaifase.

Om beide injectietechnieken te evalueren in de psoasspier werd een kwantitatieve evaluatie met spiervolume-meting door middel van digitale segmentatie van magnetische resonantie beeldvorming (MRI) gebruikt. De tijdelijke chemische denervatie veroorzaakt door de BTX injecties leidt tot spieratrofie. MRI is zeer gevoelig om deze spiervolumeveranderingen te beoordelen, zoals kon bevestigd worden door de goede correlatie binnen de groep (ICC 0.988) en de variatiecoëfficiënt binnen het subject van 3.506% in onze studie. Bij zeven kinderen met spastische diplegie werden de MEP gerichte en een veelvuldig gebruikte distale injectietechniek vergeleken. Vijf kinderen kregen twee verschillende injectietechnieken toegepast, ad random verdeeld over beide psoasspieren en bij twee patiënten werd bilateraal een MEP gerichte injectietechniek uitgevoerd. MRI beelden van de psoas werden voor, twee maanden na en –bij drie patiënten- 6 maanden na de injecties genomen. Het gemiddelde volume twee maanden na de injectie (in relatie tot het pre-injectievolume) was voor de negen MEP gerichte spieren 79,5% ten opzichte van 107,8% voor de vijf distaal ingespoten spieren. Dit verschil was statistisch significant. Bij alle vijf asymmetrisch ingespoten patiënten vertoonde de MEP gerichte spier een gemiddeld 27% (spreidingsbreedte 9-37%) grotere volume vermindering dan de distaal ingespoten psoas spier. Deze atrofie bleef aanwezig tot zes maanden na de behandeling. We konden daaruit besluiten dat inspuitingen in de MEP zone van de spier (voor de psoasspier is dit het proximale gedeelte) spieratrofie veroorzaken. Dit demonstreert het effect van het toxine in tegenstelling tot meer distale injectie waarbij deze atrofie niet werd geobserveerd.

De nieuw ontwikkelde meetinstrumenten (de geïnstrumenteerde spasticiteitsmeting en de digitale segmentatie MRI spiervolume meting) maakten zich waar als betrouwbare en valabele methoden om verschillende injectiemethoden te vergelijken. De resultaten van de gracilis en psoas-studie toonden aan dat BTX injecties op plaatsen met een hoge concentratie MEPs een verbeterde efficiëntie hebben in vergelijking met inspuitingen uitgevoerd op een afstand van deze MEPs. Het is daarom gefundeerd om te stellen dat alle BTX injecties bij voorkeur nabij deze MEP zones gegeven worden. Het effect op de functie van de patiënt bij het gebruik van deze efficiëntere MEP gerichte BTX injecties zal verder geëxploreerd worden door een evaluatie van het effect op de functionele spasticiteitsparameters bij het stappen. Toekomstige studies die verschillende dosissen en diluties geïnjecteerd ter hoogte van deze MEP zones vergelijken, zullen de behandelingsefficiëntie verder optimaliseren. Dit kan dan leiden tot het gebruik van lagere dosissen en aldus de economische kosten en het risico op nevenwerkingen verminderen.

Curriculum vitae & Publication list

Anja Van Campenhout was born in Mortsel (Antwerp), Belgium, July 2 nd 1969. She and her partner Frank Baart have two children, Jari and Naomi.

After her studies Latin-Mathematics at the Onze-Lieve-Vrouw Presentatie, Boom, she graduated cum magna lauda in Medical Sciences at the University of Antwerp in 1994. First practical experiences were acquired in local Antwerp hospitals and at the St. Georges Hospital, London and Karl University Hospital, Prague. She specialised in Orthopaedic Surgery from 1994 to 2000 under the supervision of Prof. Dr. J. Verstreken (University Hospital Antwerp) with resident training years in AZ Middelheim, University Hospital Antwerp, APRA Hospital and University Hospitals Leuven. In 2001, she further specialised in Paediatric Orthopaedics by completing a fellowship at Leuven University Hospitals under the supervision of Prof. Dr. G. Fabry. During this training, she spent one month in the Hospital for Special Surgery, New York, USA with Dr. Boachie-Adjei, renowned for correcting spinal deformities.

She was appointed as a staff member of the Department of Orthopaedics of the University Hospital Antwerp in 2001, where she worked until 2004. During this period, she also gave courses at the University of Antwerp ('Vaardighedenlabo': training of medical students in the practice of clinical examination) and was consultant at the Gaitlab of St. Jozef institute, Antwerp. She further immersed in the care for children with CP and gait analysis by regularly visiting the orthopaedic team and gait lab at Gilllette's Children Specialty Healthcare, Saint Paul, Minnesota, USA with Prof. Dr. J. Gage and Dr. T. Novacheck. In 2004, she received a grant from the Foundation Horlait-Dapsens (Belgium) which encourages medical research.

In September 2004, she joined the Department of Orthopaedics of the University Hospitals Leuven, where she currently takes care of neuromuscular pathologies and foot abnormalities in children. Together with Prof. G. Molenaers, she is responsible for the orthopaedic management of children with cerebral palsy. In 2010, she received a 2-year grant from FWO Flanders (Foundation for Scientific Research).

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American Academy for Cerebral Palsy and Developmental Medicine (AACPDM), Annual Meeting 2007, Vancouver, Canada. Instructional course: After injections with BTX-A – What happens now? Specific after care to improve the effect of BTX-A injections in the lower limbs for children with cerebral palsy.
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Gait course 2010, Leuven, Belgium. Spasticity management; Physiotherapy and gait analyses.
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1er curso neuro-ortopedia y analysis de marcha, 2011, Bogota, Colombia. Can BTX change the natural history of CP? ; Long term use of BTX; Do clinical measurements correlate with gait analysis parameters in children with CP; Spasticity management in CP; Case reports on multilevel CP surgery.

AACPDM, Annual meeting 2011, Las Vegas, USA. Anatomical guidelines for the injection with botulinum toxin: localization of the motor end plate zone in human skeletal muscles of the lower limb & Instructional course: Integrated quantitative spasticity assessment.

EPOS, Annual meeting 2012, Helsinki, Finland. The use of goal attainment scores to evaluate the effect of repeated BTX-A treatments in the lower limb of children with cerebral palsy.

Gillette's Gait Course 2012, St Paul, Minneapolis, USA. Anatomical guidelines for injection with botulinum toxin: localization of the motor end plate zone in muscles of the lower limb; SEMLS cases; Tone Management cases; Surgical techniques in CP.

AACPDM, Annual meeting 2012, Toronto, Canada. Botulinum toxin type A injections in the psoas muscle of children with CP: MRI documented muscle atrophy after motor endplate-targeted injections.

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Spasticity symposium 2013, Leuven, Belgium. MEP targeted BTX injections; MEP targeted BTX injections of the gracilis muscle in children with CP; MEP targeted injections of the psoas muscle.

Gait course 2013, Leuven, Belgium. Spasticity management.

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