HELI SÄTILÄ

Botulinum Toxin A Treatment in Children with Spastic Cerebral Palsy

Studies on Injection Techniques and Doses

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ACADEMIC DISSERTATION
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CONTENTS

ABBREVIATIONS

Ach	neurotransmitter acetylcholine
BMFM	bimanual fine motor function scale
BTXA	botulinum toxin type A
CP	cerebral palsy
EDB	extensor digitorum brevis muscle
EMG	electroneuromyography
GAS	goal attainment scale
GMFCS	gross motor function classification system
ICF	international classification of functioning, disability and health
kDa	kiloDalton
MACS	manual ability classification system
MAS	modified Ashworth scale
MTS	modified Tardieu scale
NMJ	neuromuscular junction
OGS	observational gait scale
ROM	range of movement
SCPE	the surveillance of cerebral palsy in Europe
SMC	selective motor control test
U=mu	mouse unit
U/kg	units per kilogram bodyweight
ULPRS	upper limb physician´s rating scale
UMNS	upper motor neurone syndrome

ABSTRACT

Botulinum toxin A (BTXA), an acetylcholine-blocking chemical denervant when injected into a muscle, has been used since 1993 in the management of spasticity in children with cerebral palsy (CP). The treatment indications include equinus, crouch gait and restricted hip extension and abduction in the lower limbs, and various spastic or dystonic upper limb deformities (e.g. elbow or wrist flexion, arm pronation, clenched fist, thumb-in-palm deformity). The reduction in muscle tone should be effective and selective, which requires optimal dosage and injection site for each muscle – factors not yet clearly defined even for the most commonly injected gastrocnemius muscles. The BTXA doses in children are empirical, determined by the size of the muscle with an eye to avoiding excessive weakness and deterioration of function.

In this study series, the effectiveness of two different sets of BTXA (Botox^{R}) injection techniques in the treatment of equinus gait in children with spastic CP was evaluated. In addition, the effects and detriments of low and high doses were compared in a clinical setting of equinus gait in the lower and focal spasticity in the upper limb. The studies were conducted in the units of pediatric neurology in Tampere University Hospital, Tampere, and the Central Hospital of Kanta-Häme, Hämeenlinna.

The first study compared BTXA injections given as close as possible and remote from the neuromuscular junctions (NMJs) in CP children with spastic equinus gait. Nineteen children (1.5-7 years; 9 with hemiplegia, 8 with diplegia, 2 with quadriplegia; levels I-IV by the Gross Motor Function Classification System GMFCS) with 25 treated limbs were randomized into two groups: the proximal group received a BTXA injection into the proximal part of both heads of the gastrocnemius, the distal group into the mid-belly of the muscle bulks. Single-point injection, 3 U/kg per site, was used. Assessments of active and passive ankle range of movement (ROM), ankle dynamic muscle length (Modified Tardieu Scale, MTS), calf tone (Modified Ashworth Scale, MAS), and video gait analysis (Observational Gait Scale, OGS) were made before treatment and 3, 8 and 16 weeks post-treatment. The median of changes from baseline in active and passive ROM and MAS in both groups and MTS and OGS Total scores in the distal group improved significantly at all time-points. The change from baseline in OGS Initial Foot Contact and Total scores at 8 weeks showed a statistically significant difference between the treatment groups, favoring the distal group, the clinical relevance remaining however tenuous. Thus, using the methods described, no major changes in main outcome measures were associated with injections close to or remote from the NMJs.

The second study investigated the clinical relevance of single or multiple injection sites by comparing the two techniques in children with CP and spastic equinus gait. A total of 17 children (1.8-9.4 years; 8 with hemiplegia, 8 with

diplegia, 1 with quadriplegia; GMFCS I-IV) with 25 treated lower limbs were randomized into two groups: a single-point group receiving a standard dose (4 U/kg) of BTXA into one site and a multiple-points group injected at two sites on both heads of the gastrocnemius. Active and passive ankle ROM, selective ankle dorsiflexion (SMC), ankle dynamic muscle length (MTS), calf tone (MAS), attainment of anticipated gait pattern (Goal Attainment Scale, GAS), and video-observed gait (OGS) were assessed before and 1, 2 and 4 months after the intervention. In both treatment groups the median of changes from baseline in MAS, MTS, OGS Total scores and Initial Foot Contact scores improved significantly and a similar number of children attained their goals on the GAS. The only statistically significant difference between the groups was observed at 2 months in passive ankle dorsiflexion with knee flexed, favoring the single-point group. Though not significantly, the incidence of adverse events was higher in the multiple-points group. No major changes in main outcome measures were associated with the number of injection sites. Issues other than efficacy thus guide the decision on whether to inject at single or multiple sites when treating spastic equinus with BTXA.

The third study investigated the effects of various doses of BTXA when treating equinus gait. Twenty-nine children with CP (age 1.5-9.6 years, GMFCS I-IV) met the preset inclusion criteria. The treatment sessions per child ranged from 1 to 5 and the effects on a total of 80 legs in 55 sessions were evaluated. BTXA doses injected into the gastroc-soleus muscle were divided into low- ($\leq$ 6 Units/kg) and high- (> 6 Units/kg) dose groups. The outcome measurements included active and passive ankle ROM, MAS, MTS, SMC, OGS and GAS at pre-treatment and 1, 2 and 4 months post-treatment. MAS and OGS Initial Foot Contact scores in both groups and passive ROM and SMC in the low-dose group improved significantly at all time-points. The only statistically significant inter-group differences were observed at 2 and 4 months in mean change in passive ankle ROM and at 4 months in median change in selective dorsiflexion, favoring the low-dose group. The incidence and severity of adverse events did not differ between the groups. Thus, doses over 6 Units/kg injected into the gastroc-soleus muscle do not necessarily yield superior results compared with lower doses.

The fourth study focused on the effects and adverse events of BTXA treatment on upper limb impairment and function in 18 children with spastic or dystonic hyperactivity**.** The three main treatment groups by indication were: 1) *functional*, to improve a specific function or quality of movement; 2) *pre-operative* evaluation, to postpone surgery or help the surgeon in planning; and 3) *non-functional*, to help children with no or minimal functional abilities or after sustained brain injury to improve posture or support on the extremity involved. The functional and pre-operative groups were combined into one, the *functional* group (n= 8 subjects), and the non-functional constituted one, the *non-functional* group (n=10 subjects). Each involved upper extremity was measured and analyzed. A total of 54 treated extremities were divided into low- or high-dose groups according to the dose used for the target muscles. The outcome measurements included MAS, passive ROM, various grips, bimanual functions, movement pattern, House classification of upper extremity use and subjective ratings of function and cosmetic appearance. In the functional group, children benefited in terms of reduction in muscle tone at elbow and wrist and an increase in passive wrist extension and House classification scores. No major changes in

grips were observed. A statistically significant difference between the low- and high-dose groups was noted in the House classification, favoring the low-dose group. In the non-functional group a significant difference was detected in subjective parental cosmetic ratings, favoring the high dosage. Adverse events were few and occurred mostly in the high-dose group. In conclusion, higher doses in the spastic upper limb do not necessarily yield superior results compared with lower doses but increase the incidence of adverse events.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their Roman numerals.

I. Sätilä H, Iisalo T, Pietikäinen T, Seppänen RL, Salo M, Koivikko M, Autti-Rämö I, Haataja R (2005): Botulinum toxin treatment of spastic equinus in cerebral palsy: a randomized trial comparing two injection sites. Am J Phys Med Rehabil 84: 355-365.
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II. Sätilä H, Pietikäinen T, Iisalo T, Lehtonen-Räty P, Salo M, Haataja R, Koivikko M, Autti-Rämö I: Botulinum toxin type A injections into the calf muscles for treatment of spastic equinus in cerebral palsy: a randomized trial comparing single and multiple injection sites. Accepted for publication in Am J Phys Med Rehabil. Reprinted with permission from Lippincott Williams & Wilkins.

III. Sätilä HK, Pietikäinen T, Lehtonen-Räty P, Koivikko M, Autti-Rämö I (2006): Treatment of spastic equinus gait with botulinum toxin A: does dose matter? Analysis of a clinical cohort. Neuropediatrics 37: 344-349.
Reprinted with permission from Georg Thieme Verlag KG.

IV. Sätilä H, Kotamäki A, Koivikko M, Autti-Rämö I (2006): Low- and high-dose botulinum toxin A treatment: a retrospective analysis. Pediatr Neurol 34: 285-290. Reprinted with permission from Elsevier Inc.

In addition, some unpublished data are presented.

INTRODUCTION

Cerebral palsy (CP) is the most common cause of physical disability in children, with an overall prevalence of 2-2.5 per 1000 live births in the developed countries (Stanley et al. 2000). According to a recent definition CP "describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour; by epilepsy; and by secondary musculoskeletal problems" (See Rosenbaum et al. 2007). All of these contribute to producing a clinical picture of CP unique to each child. CP is classified according to the type of motor movement disorder and topographical involvement in question and the most common movement disorder is spastic CP (Stanley et al. 2000).

Botulinum toxin A (BTXA) is produced by the anaerobic spore-forming bacterium *Clostridium botulinum* and is one of the eight immunologically distinct serotypes. It exerts its effect by blocking the release of the neurotransmitter acetylcholine (Ach) at cholinergic nerve endings. A selective and temporary chemical denervation ensues, causing clinically detectable muscle weakness (Simpson 2000, Setler 2002).

BTXA has rapidly been adopted into the pediatric armament available for the treatment of focal spasticity or dystonia of different etiology. As the neuromuscular junctions (NMJs) in the muscles are the sites of BTXA action, targeting the correct muscle and the vicinity of the NMJs is considered essential. Diffusion of BTXA and the need to inject close to the NMJs are interrelated with doses, dilutions, volumes and number of injection sites.

In theory, injections close to the NMJ zone would improve efficacy, reduce side-effects and potentially lower the required doses. There is evidence from animal models that injection distance to NMJs influences the effect of BTXA treatment (Shaari and Sanders 1993, Childers et al. 1998). However, results of human studies on adults with post-stroke spasticity have been equivocal (Childers et al. 1996, Gracies et al. 2002) and the effect of BTXA injections close to the NMJs has not been studied in children.

The use of either single or multiple BTXA injection sites has mostly evolved from the practical issue of dividing larger doses, and hence the volume injected, over multiple sites in a given muscle in order to reduce unwanted spreading and adverse effects. It has been shown that at a certain dose saturation of the NMJs occurs and a plateau is reached (Sloop et al. 1996), which may lead to spread of the overflow toxin into neighbouring structures and the systemic circulation (Graham et al. 2000). This could be avoided by splitting the dose over multiple sites. Hence in children the recommendations suggest a maximum volume of 0.5

ml (Russman et al. 2002) per injection site. Adult studies (blepharospasm, torticollis) investigating the differences between single and multiple site injection techniques have advocated the multiple site technique (Borodic et al. 1991, 1992) but no studies specifically evaluating these two injection techniques in children have been published.

Since the first report on BTXA treatment in children (Koman et al. 1993), using a total dosage of 1-2 U/kg, the toxin amounts both per single muscle and total dose per session have increased (Kinnett 2004). Guidelines for dosage have been published (Graham et al. 2000, Russman et al. 2002) based on the experience of experts in combination with research findings at the time. However, doses have for the most part been determined by "trial and error" and we still lack evidence regarding optimal dosage in both the upper and lower limbs. Studies among adults (Sloop et al. 1996, Dressler and Rothwell 2000) and CP children (Autti-Rämö et al. 2001, Baker et al. 2002, Polak et al. 2002) suggest that an optimum dose per muscle exists, after which the effect does not increase in a given muscle.

The studies in this series aimed to evaluate the effect of two different sets (close to or remote from the NMJs and single or multiple sites) of BTXA (Botox^{R}) injection techniques in the treatment of equinus gait in children with spastic CP. It was also sought to compare the effects and adverse events of low and high doses in a clinical setting treating equinus gait in the lower limb and focal spasticity in the upper limb.

REVIEW OF THE LITERATURE

1. Cerebral palsy

1.1. Definition

The concept "cerebral palsy" (CP) stems from "cerebral paralysis" or "cerebral paresis" and refers to a neurodevelopmental condition beginning in infancy or early childhood and persisting through the lifespan. Originally described by Little in 1861, CP has become familiar to most health service professionals as well as the general public.

Over the years many definitions have been proposed. Probably the most frequently cited is that by Bax (1964), stating that CP is "*a disorder of movement and posture due to a defect or lesion of the immature brain. For practical purposes it is usual to exclude from cerebral palsy those disorders of posture and movement which are of short duration, due to progressive disease, or due solely to mental deficiency.*" Subsequently, Mutch and associates (1992) revised the definition as follows: "*an umbrella term covering a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of its development*." Both definitions emphasize the motor impairment which is the hallmark for this condition.

A reformulation was recently proposed by The International Workshop on Definition and Classification of Cerebral Palsy (Rosenbaum et al. 2007): "*Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour; by epilepsy; and by secondary musculoskeletal problems*." The committee sought to promote the idea that "a comprehensive approach to CP needs to be multidimensional and that management of patients with CP almost always requires a multidisciplinary setting."(Rosenbaum et al. 2007)

1.2. Prevalence and etiology

The overall prevalence of CP has remained around 2-2.5 per 1000 live births in the developed countries. For developing countries no population-based birth prevalence data are available but rates are assumed to be higher. The longest

follow-ups from the 1950s (Western Sweden and Western Australia) show that although perinatal mortality declined from the 1960s to 1980s, the overall rate of CP has remained the same. Even though most children with CP are born at term, the risk of the disorder among very preterm births (defined as births before 32 completed weeks of gestation) may be up to 100 times that associated with term births. (Stanley et al. 2000).

In Europe, the CP prevalence for the period 1980-1990 was found to be 2.08 per 1000 live births, ranging from 1.49 to 2.63 among 11 centres (postnatal cases excluded) (Surveillance of Cerebral Palsy in Europe 2002; SCPE). The rate was higher among babies weighing less than 1500 g at birth (72.6 per 1000 live births) compared with those weighing 2500 g or more (1.2 per 1000 live births). The prevalence of CP of postnatal origin (arising over 28 days after birth and before the age of 25 months) was 1.26 per 1000 live births, the functional pattern being more severe than with pre- and perinatal etiologies (Cans et al. 2004). Recently, Himmelmann and colleagues (2005) reported a CP prevalence of 1.92 per 1000 live births in the birth-year period 1995-1998 in Western Sweden (postnatal cases included). The overall decreasing trend from the period 1991-1994 continued, but an increase in dyskinetic CP among term children raised concern. In the Finnish population, the prevalence has ranged between 1.6 and 5.7 per 1000 live births (postnatal cases included) (Tuuteri et al. 1967, Riikonen et al. 1989) and has remained 11-12% among extremely low-birth-weight infants (<1000g) (Tommiska et al. 2007).

CP is a condition with multiple etiologies and it is often impossible to determine any single causative factor in an individual patient. Indeed a new etiological model has been introduced envisaging a sequence of events cumulating in CP, “causal pathways to CP” (Stanley et al. 2000). *Prenatal factors* may include genetic and chromosomal disorders, congenital infections, cerebral or neural tube malformations/maldevelopments, and periventricular leukomalacia; *perinatal factors* include brain edema, neonatal shock, intracerebral hemorrhage, sepsis or central nervous system infection, metabolic maladjustment, and hypoxic-ischemic encephalopathy; *postnatal factors* include central nervous system infection, sepsis, vascular inflammation, infarctation or hemorrhage, and accidental or non-accidental brain injury (Krägeloh-Mann et al. 1995, Aicardi and Bax 1998).

1.3. Classification of CP

CP is not an etiologic diagnosis but a clinical descriptive term based on phenomenology. The phenotype and severity of motor involvement depend on the location and extent of the central nervous system lesion: spasticity is associated with damage to the corticospinal tracts, usually white-matter or focal cortical/subcortical damage, and dystonia with damage to basal ganglia and the thalamus (Bax et al. 2006, Woodward et al. 2006). The accompanying impairments (e.g. epilepsy, communication deficits and mental retardation) are associated with the extent of white- and gray-matter lesion and tend to accumulate in the most severely affected individuals (Nordmark et al. 2001, Beckung and Hagberg 2002, Carlsson et al. 2003, Himmelmann et al. 2006, Woodward et al. 2006).

Traditionally classifications have focused on the topographical distribution of affected limbs and describe the predominant type of muscle tone or movement abnormality. The so-called "Swedish Classification of CP" was originally reported by Hagberg and colleagues (1975) and identifies three types of movement abnormalities: *spastic* (further divided into *hemiplegic, diplegic* and *quadriplegic form*), *ataxic* (divided into *diplegic* and *congenital form*), and *dyskinetic* (divided into *mainly choreoathetotic* and *mainly dystonic form*) (Mutch et al. 1992). Of these the spastic form is the most prevalent (Stanley et al. 2000). Hemiplegia refers to unilateral involvement, diplegia to bilateral involvement with the lower limbs more affected than the upper and quadriplegia to bilateral involvement with the upper limbs more or equally involved. Sometimes the terms "monoplegia" and "triplegia" are used.

The Surveillance of Cerebral Palsy in Europe (SCPE 2000) adopted and refined this classification by retaining the three types of movement abnormalities but adding one class, "*unclassifiable*", for cases not predominantly spastic, ataxic or dyskinetic (Table 1). The typology classes "*unilateral*" and "*bilateral*" were also adopted, abandoning the term "diplegia". In addition, some authors identify a *hypotonic* (referring to abnormally low muscle tone and to be distinguished from weakness) and *mixed* (features of more than one type, usually spastic and dyskinetic) CP group (Howard et al. 2005).

Table 1. Classification of CP subtypes and definitions for movement abnormalities according to SCPE (SCPE 2000).

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1. *Spastic CP* is characterized by at least two of the following:
- abnormal pattern of posture and/or movement
- increased muscle tone (not necessarily constant)
- pathological reflexes (hyperreflexia and/or positive Babinski sign)

Spastic CP is divided into unilateral (i.e. limbs on one side of the body are involved) and bilateral (i.e. limbs on both sides of the body are involved).

2. *Ataxic CP* is characterized by both:
- abnormal pattern of posture and/or movement
- loss of orderly coordination (movements executed with abnormal rhythm, accuracy and force)

3. *Dyskinetic CP* is characterized by both:
- abnormal pattern of posture and/or movement
- involuntary, uncontrolled, recurring, occasionally stereotyped movements

Dyskinetic CP is divided into either *dystonic* (comprises both hypokinesia/stiffness and hypertonia) or *choreo-athetotic* (comprises both hyperkinesia and hypotonia) CP.

4. *Unclassifiable*

In Finland, the classification of CP according to the International Classification of Diseases (ICD-10) under code G 80 is used as follows (Stakes 1995):

G 80.0 Spastic quadriplegia
G 80.1 Spastic diplegia
G 80.2 Spastic hemiplegia
G 80.3 Dyskinetic CP
G 80.5 Other type of CP
G 80.9 Unclassified CP

The classification newly proposed by the International Workshop on Definition and Classification of Cerebral Palsy (Rosenbaum et al. 2007) covers a wide range of clinical presentations and activity limitations. An evaluation of this new classification is under way and time will tell how it is received by professionals working in the neuropediatric field and whether it will help to communicate cross-sectionally the multidimensional characteristics CP evinces. The components of this CP classification are set out in Table 2.

Table 2. The components of the CP classification by The International Workshop on Definition and Classification of Cerebral Palsy (Rosenbaum et al. 2007).

1. *Motor abnormalities*

A. Nature and typology of the motor disorder: tone abnormalities (e.g. hypertonia or hypotonia) and movement disorders (e.g. spasticity, ataxia, dystonia, athetosis).
It is recommended that cases continue to be classified by the dominant type of tone or movement abnormality. With mixed types, the additional tone or movement disorders present should be listed as secondary types.

B. Functional motor abilities: the extent of limits in function, including the upper and lower extremities and oromotor and speech function.
Objective functional scales such as the Gross Motor Function Classification System (GMFCS) for the lower extremities and the Bimanual Fine Motor Function Scale (BMFM) or the Manual Ability Classification System (MACS) for the upper extremities are recommended. There are as yet no activity limitations scales for bulbar and oromotor difficulties and for evaluation of participation restrictions.

2. *Accompanying impairments*

Observations of developing musculoskeletal problems, accompanying non-motor neurodevelopmental or sensory problems (e.g. seizures, hearing or vision impairments, attentional, behavioral, communicative and/or cognitive deficits).

3. *Anatomical and neuroimaging findings*

A. Anatomical distribution: the parts of the body affected by motor impairments or limitations (limbs, trunk, bulbar region).

B. Neuro-imaging findings: the neuroanatomic findings on computerized tomography or magnetic resonance imaging (e.g. ventricular enlargement, white matter loss or brain anomaly).

4. *Causation and timing*

Data on whether there is a clearly identifiable cause, as with postnatal CP (e.g. meningitis, head injury) or brain malformations, and the presumed time frame during which the injury occurred, if known.

1.4. Spasticity as a sign of upper motor neurone dysfunction

1.4.1. Definition

A widely accepted definition of spasticity is that given by Lance (1980): *"Spasticity is a motor disorder characterized by a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neurone syndrome."* Spasticity is more difficult to characterize and quantify than to recognize. In spasticity the muscle tone (i.e. the resistance felt when a limb is passively rotated about a joint with the subject at rest) is increased (hypertonic) and the resistance to externally imposed movement increases with increasing speed of stretch and varies with the direction of joint movement (Sanger et al. 2003). Additionally, the resistance to externally imposed movement may rise rapidly above a threshold speed or joint angle felt as "a catch", which may represent the threshold for onset of the stretch reflex (Sanger et al. 2003). Spasticity often coexists with other motor symptoms such as dystonia or athetosis.

Spasticity is but one of the many different features of the upper motor neurone syndrome (UMNS) (Table 3). Reducing spasticity will not automatically improve function and addressing the possibly more disabling negative UMNS features needs attention. They may, however, be more difficult to manage than spasticity (Boyd and Ada 2001).

Spasticity can vary depending on a child´s activity, posture and state of alertness (e.g. it may increase with anxiety, emotional stress, pain, surface contact or other sensory input) and is not specific to any particular task addressed (Sanger et al. 2003).

Table 3. Features of the upper motor neurone syndrome (adapted from Barnes 2001).

Central nervous system lesion	
1. Loss of inhibition of the lower motor neurons causing:	*2. Loss of connections to the lower motor neurons causing:*
Positive features	Negative features
Spasticity	Muscle weakness
Hyperreflexia	Fatiguability
Clonus, positive Babinski sign	Loss of dexterity
Co-contraction	Poor balance
Associated reactions	Sensory deficits

1.4.2. Pathophysiology

Spasticity and other (both positive and negative) features of the UMNS are caused by disruption of the descending corticospinal pathways (pyramidal and adjacent tracts) involved in motor control (Sheean 2001). The pyramidal fibers arise from both pre-central (primary motor cortex and pre-motor cortex) and post-central (primary somatosensory cortex and parietal cortex) cortical areas, the latter contributing by modulating sensory function with motor function (Sheean 2001). The impulse to an intended movement (supplementary motor area) is relayed to the premotor cortex (involved in the preparation for movement) and thence to the primary motor cortex, where an order is given to initiate the appropriate muscular contraction to achieve the desired goal (Peacock 2004a). The basal ganglia, cerebellum and brainstem motor nuclei contribute to motor programming and the command is delivered via the corticospinal tracts to the spinal cord, lower motor neurone, peripheral nerve and, finally, the muscle (Peacock 2004a).

From the brainstem two balanced systems arise which control the spinal reflexes: one inhibitory (the dorsal reticulospinal tract) and the other excitatory (the medial reticulospinal and lateral vestibulospinal tracts) (Sheean 2001). A lesion to these tracts above the nuclei (i.e. a lesion at cortex, internal capsule or periventricular white matter) produces spasticity. Injury at lower brainstem or spinal level to the reticulospinal and vestibulospinal tracts (i.e. below the reticular and vestibular nuclei) causes intense spinal spasticity, with a tendency to flexor spasms and a flexed posture. Isolated damage to the corticospinal tract produces loss of fine motor control in distal limb muscles, without spasticity. However, an isolated lesion to the corticospinal tract is rare; usually other adjacent motor tracts are injured as well (Sheean 2001, Peacock 2004a).

The spinal reflexes contributing to motor control may be divided into proprioceptive (detecting phasic and tonic muscle stretch, joint-movement position, change in the body´s mass center) and cutaneous/nociceptive (Babinski sign, flexor and extensor reflexes, detecting noxious stimulus, pressure) reflexes and, together with the spinal interneurons, form a complex network mediating diverse afferent input to the spinal cord (Sheean 2001, Dietz 2002). The monosynaptic reflex arc, the stretch reflex, maintains muscles at a given length. Stretch is detected by muscle spindles within the skeletal muscle and an excitatory impulse is given via the afferent posterior nerve root to the motor neurone to contract the muscle back to the appropriate length. Likewise, muscle spindle and tendon afferents connect polysynaptically with motor neurones which innervate agonist and antagonist muscles: reciprocal inhibition occurs when the afferents inhibit the neurones activating an antagonist muscle, and reciprocal excitation occurs when the afferents bring about a contraction in the agonist muscle (Peacock 2004b). These stretch reflexes are under the inhibitory influence of the upper motor neurones and muscle tone is maintained as a balance between the excitatory stretch reflex and descending inhibitory supraspinal control (i.e. presynaptic inhibition). As the inhibitory control of the upper motor neurone fails, the spinal reflexes become hyperexcitable and the positive features of the UMNS are manifested (Sheean 2001, Dietz 2002, Gracies 2005).

Irrespective of whether the basic alteration in spinal reflex transmission responsible for the increased stretch reflex is increased gain or decreased threshold (see Table 4), the common finding has been that spasticity is due to hyperactive tonic stretch reflexes which are velocity-sensitive (Lance 1980, Sheean 2001, Gracies 2005). The monosynaptic Ia hyperexcitation is the major contributor in the development of spasticity, but many other spinal reflex pathways may increase or reduce the effect of this monosynaptic excitation: excitation/inhibition from muscle spindle group II afferents, inhibition from Golgi tendon organs via Ib afferents, recurrent inhibition via motor axon collaterals and Renshaw cells, presynaptic inhibition of Ia afferent terminals and reciprocal inhibition from muscle spindle Ia afferents of the antagonist muscles (Gracies 2005, Pandyan et al. 2005, Nielsen et al. 2007) (See Table 4). Thus, spasticity is probably not caused by a single mechanism, but rather by a chain of alterations in different inter-dependent spinal networks (Nielsen et al. 2007). What kind of role each component plays remains uncertain.

1.4.3 Neural and biomechanical components of muscle hypertonia

The increased muscle tone in spasticity is attributable to a combination of the reflex (neural) component as well as to changes in muscle biomechanical properties (Sanger et al. 2003, Barnes 2001) (Table 4).

Table 4. Neural and biomechanical components of muscle hypertonia and the possible mechanisms (adapted from Lin 2000&2004, Pandyan et al.2005 and Nielsen et al.2007).

Neural components	*Biomechanical components*
Increase in stretch reflex activity:	Changes in resistance:
a) Increased gain (amplification) in the stretch reflex network e.g.	a) Elasticity: length-dependent
- increased alpha-motor neurone excitability and changes in the neurone properties	b) Viscosity: velocity-dependent
- decreased Ia presynaptic inhibition	c) Inertia: acceleration-dependent
- altered inhibition/excitation of the group II fibers	d) Friction: independent of length or velocity
- reduced reciprocal inhibition	e) Plastic: time-dependent
- impaired modulation of recurrent inhibition	f) Contracture: Short muscle or tendon; posture-dependent
- altered Ib inhibition/excitation balance	
b) Lowered threshold in the stretch receptors e.g.	
- increased receptor sensitivity	
- increased excitatory drive to the muscle spindle efferents (gamma-motor neurones)	

Biomechanical hypertonia is not velocity-dependent and restricts movement even at slow velocities (Barnes 2001). It does not respond to anti-spastic treatment with drugs or BTXA and the only treatment options relate to physiotherapy, splinting, casting, stretching, positioning and surgery. In clinical practice the neural and biomechanical components coexist and it may be difficult to determine the relative contribution of each.

1.4.4. Spastic muscle (dynamic and fixed contracture)

While skeletal muscle tissue adapts to altered neural and mechanical input, spastic muscles prefer to remain in shortened state (contracture) and need stretching. At first, they can be stretched more easily as the contracture is dynamic, but eventually, as the muscle tissue transformation continues, they become stiffer (less compliant) and a fixed contracture ensues (Lin 2004). This is felt as an increased resistance to stretch without reaching the reflex velocity threshold (no catch) and as a reduced range of movement of the joint.

On the basis of rodent studies it has been widely believed that the muscle contractures are due to a reduction in muscle fiber length, but Shortland and co-workers (2001) found no evidence of fascicle length change in the muscles of CP children compared to normal subjects. The authors measured the properties of the medial gastrocnemius of children with CP directly by ultrasound and attributed the shortening of the muscle to a decrease in the mean muscle fascicle diameter (atrophy) and subsequent contraction of the aponeurosis. They proposed that prevention or reversal of these changes cannot be achieved by stretching and serial casting only but by including strength training and/or electrical stimulation (Shortland et al. 2001).

In addition, variation in muscle fiber size, alteration in fiber type distribution (type-1 fiber predominance and type-2B deficiency), increased stiffness of muscle cells and progressive collagen accumulation, increasing with severity of motor function, have been found in the muscles of children with CP (Rose et al. 1994, Ito et al. 1996, Booth et al. 2001, Friden and Lieber 2003).

1.5. Clinical presentation of spastic CP

Lesions of cortical/subcortical areas, white matter and thalamus/basal ganglia during the fetal or neonatal period give rise to various combinations of movement disorders and gross and fine motor deficits. As already noted, the motor movement disorders are classified into spastic, dyskinetic, ataxic and unclassified (mixed), spastic CP being the most prevalent. The proportions of muscle tone subtypes have varied between 76-87% for spastic CP, 3-11% for ataxic CP and 2-15% for dyskinetic CP, and the distribution of different anatomic typologies has been 27-38% for hemiplegia, 18-45% for diplegia and 8.5-32% for quadriplegia (Stanley et al. 2000, Nordmark et al. 2001, SCPE 2002, Himmelmann et al. 2005, Howard et al. 2005).

In addition to motor disorder, associated impairments such as mental retardation, epilepsy, visual or hearing defect, hydrocephalus, deficits in speech, language, perception, attention and behavior are common and have an impact on activity and participation limitations. The proportion of children with accompanying impairments increases with severity levels, and especially the motor function and cognitive deficits are important predictors for participation restrictions in CP (Beckung and Hagberg 2002, Himmelmann et al. 2006). In European studies investigating accompanying impairments, 21-38% of participants have been reported to have active seizures, 31-52% cognitive deficit, and 11-23% severe visual impairment (Nordmark et al. 2001, SCPE 2002, Carlsson et al. 2003, Himmelmann et al. 2006).

1.5.1. Gross and fine motor deficits

Deficient postural control plays a dominant role in this disorder and to a degree which depends on the severity of motor involvement activation patterns lack the ordinary sequential distal-proximal recruitment order and show excessive co-activation of antagonistic muscles (Forssberg 1999, Lin 2004, Tedroff et al. 2006). Likewise, the ordinary walking pattern fails to develop. Prior to independent walking, children with hemiplegia and diplegia exhibit an immature locomotion pattern similar to that of their non-impaired peers but at the stage where children normally turn to plantigrade gait, CP children retain the immature pattern (Forssberg 1992). As a result of premature activation of the calf muscles during the swing phase, the foot is placed on the toes or forepart of the foot (equinus gait). The absence of calf muscle contraction at the close of the support phase results in loss of propulsive force to go forward. Persistent monosynaptic group-Ia projections to antagonistic and synergistic muscles, remaining spinal cutaneous reflexes and impaired reciprocal inhibition of antagonistic muscles during voluntary or automated movements may contribute to co-activation (i.e. co-contraction) of antagonistic muscles and thus to a defective gait pattern and walking ability (Forssberg 1999, Lin 2004, Tedroff et al. 2006). Release of tonic labyrinthine and neck reflexes (e.g. asymmetric tonic neck reflex, fisting) and exaggerated stretch reflexes due to spinal cord disinhibition (e.g. clonus) interfere with posture and gait (Lin 2004).

Together with spasticity and dyskinesia, muscle weakness and muscle imbalance across joints may hamper movement and locomotion (Wiley and Damiano 1998). Children with CP also often evince poor selective motor control (i.e. difficulty in moving an individual joint). Subsequently, trophic changes occur in muscles and limbs: muscle fiber-type transformation, reduced muscle extensibility and joint range, increased resistance to passive stretch, muscle atrophy and skin and vascular changes (Lin 2004).

In the upper limb, dexterity may be abnormal due to central dyscoordination and CP children do not develop the automatic force coordination pattern for grasping and manipulating objects (Gordon et al. 1999). They also have impaired sensory control of finger forces, this giving rise to excessive grip forces and difficulties in programming finger pressures to match the properties of the object grasped (Eliasson et al. 1995, Gordon and Duff 1999). In children with hemiplegia, the normally occurring mirror movements may persist and become

exaggerated, this being thought to reflect a plastic reorganization of the undamaged hemisphere and a projection of ipsilateral corticospinal tract neurons to the hand motoneurons (Forssberg 1999).

1.5.2. Classification of severity of gross and fine motor dysfunction

Gross motor function. The Gross Motor Function Classification System (GMFCS) was developed to provide a standardized classification of motor disability and functional limitations (Table 5). The aim was to enhance communication among professionals and families in determining a child's needs and making management decisions, describing the development and prognosis of children with CP and comparing and generalizing the results of evaluations and research (Palisano et al. 1997). The GMFCS is a five-level ordinal grading scale in which the distance between levels is not to be considered equal and children are not expected to be equally distributed between the levels. The assessment of self-initiated movement with emphasis on function during sitting, standing and walking can be graded (separate descriptions are provided for children in several age bands) and the distinctions between the levels are based on functional limitations, the need for walking aids or wheeled mobility, and quality of movement. Children at level I evince the most independent motor function and those at level V the least. The GMFCS has proved a reliable, stable and clinically relevant method for the classification and prediction of motor function in children between the ages of 2 and 12 years (Palisano et al. 1997, Rosenbaum et al. 2002, Palisano et al. 2006). Currently, a GMFCS classification for adolescents is under development.

Fine motor function. The Manual Ability Classification System (MACS) was developed as a method analogous to the GMFCS to classify the ability to handle objects in daily activities (Table 5). The MACS has been reported to have good validity and reliability (Eliasson et al. 2006, Morris et al. 2006). Another scale for classifying bimanual function, the Bimanual Fine Motor Function (BFMF)(Beckung and Hagberg 2002), was likewise developed to parallel the GMFCS but it has not gained such wide acceptance as the MACS among professionals.

Table 5. The GMFCS and MACS Classifications (adapted from Palisano et al. 1997 and Eliasson et al. 2006).

GMFCS

Level I: Walks without restriction. Limitations in more advanced gross motor skills.
Level II: Walks without restriction. Limitations walking outdoors and in the community.
Level III: Walks with assistive mobility devices, limitations walking outdoors and in the community.
Level IV: Self-mobility with limitations, children are transported or use power mobility outdoors and in the community.
Level V: Self-mobility is severely limited even with the use of assistive technology.

MACS

Level I: Handles objects easily and successfully. At most, limitations in the ease of performing manual tasks requiring speed and accuracy. However, no limitations in manual abilities restrict independence in daily activities.
Level II: Handles most objects but with somewhat reduced quality and/or speed of achievement. Certain activities may be avoided or be achieved with some difficulty; alternative modes of performance might be used but manual abilities do not usually restrict independence in daily activities.
Level III: Handles objects with difficulty; needs help in preparing and/or modifying activities. Performance is slow and achieved with limited success in terms of quality and quantity. Activities are performed independently if they have been set up or adapted.
Level IV: Handles a limited selection of easily managed objects in adapted situations. Performs parts of activities with effort and with limited success. Requires continuous support and assistance and/or adapted equipment, for even partial achievement of the activity.
Level V: Does not handle objects and has a severely limited ability to perform even simple actions. Requires total assistance.

1.5.3. Musculoskeletal aspects and gait deviations in spastic CP

Rang defined CP from the orthopedist's point of view as *"the result of damage to the developing brain producing a disorder of movement and posture that is permanent but not unchanging"* and emphasized the occurrence and change in musculoskeletal deformities during growth (Rang 1990). The primary problem, brain injury, leads to loss of selective motor control, balance problems and abnormal muscle tone, which in the course of time, through inability to stretch muscles during normal play and activity, lead to secondary problems, i.e. increased muscle-tendon unit contracture. Contractures, through abnormal skeletal forces, lead for their part to tertiary problems, i.e. bony deformities and joint deterioration (stiffness of periarticular connective tissue, degenerative arthritis), in consequence of which the child develops coping mechanisms in order to obviate these problems (Johnson et al. 1997, Bell et al. 2002, Bottos and Gericke 2003, Gage and Schwartz 2004).

The main determinants of fixed deformities are type of movement disorder (more often associated with the spastic than dystonic or ataxic forms), its

topographical distribution and the severity of involvement (Graham 2004). The prerequisite for normal muscle growth, frequent stretching of relaxed muscle, does not take place and spasticity, together with reduced activity, is considered to lead to failure of longitudinal muscle growth, contractures and fixed deformities (Rang 1990). Compared with the longitudinal growth of the bones, the longitudinal growth of the muscles is relegated to second place (Ziv et al. 1984, Wren et al. 2004) and the pace of this biological clock of dynamic contracture becoming fixed is variable and is principally thought to be related to severity of motor involvement and rate of growth (Graham 2004).

In spastic hemiplegia there is usually asymmetric growth of the limbs. The upper limb deformities typically include internal rotation and adduction at the shoulder (which may lead to anterior subluxation or dislocation of the gleno-humeral joint), flexion and pronation at the elbow (accompanied by contracture in the interosseus membrane which may lead to subluxation or dislocation of the radial head), flexion at the wrist accompanied by ulnar deviation, and flexion at the fingers (spastic flexors overpower the weaker extensors). The “thumb-in-palm” deformity is particularly common and is associated with significant functional impairment. The pronator teres is considered to be the first muscle to develop contracture because it is never stretched by the weak supinators (Chin and Graham 2003).

The most common gait deviation in spastic hemiplegia and diplegia is that known as equinus, which is a result of imbalance between the plantarflexors and the ground reaction force (Graham 2004). The progression from dynamic to fixed contracture may be rapid in the hemiplegic lower limb compared with the upper limb. The sagittal gait patterns in spastic hemiplegia (Figure 1) have been classified by Winters and colleagues (1987), further modified by Rodda and Graham (2001). These patterns are easily recognized and provide a useful scheme for management. Equinovarus and equinovalgus deformities are also common and may require tendon transfer and/or bony stabilization.

In spastic diplegia there are gradually evolving deformities at all three levels: flexion/adduction/internal rotation at the hip; flexed/stiff knee at the knee; equinus, usually accompanied by valgus, at the ankle (Graham 2004, Gage and Schwartz 2004). The principal effect is loaded on the biarticular large muscles such as the hamstrings, the psoas, the rectus femoris and the gastrocnemius. The sagittal gait patterns in diplegia have been classified by Rodda and Graham (2001)(Figure 2). In addition, torsional deformities and deformation of joints are common, for example medial femoral or lateral tibial torsion, midfoot breaching with valgus hindfoot and abductus of the forefoot, and hallux valgus or flexion deformities of the other toes. Pelvic obliquity and scoliosis may also occur (Graham 2004).

Figure 1. Sagittal gait patterns in spastic hemiplegia according to Winters et al. (1987) and Rodda and Graham (2001). Reproduced from Graham and Selber (2003) with permission from the British Editorial Society of Bone and Joint Surgery. RF, rectus femoris.

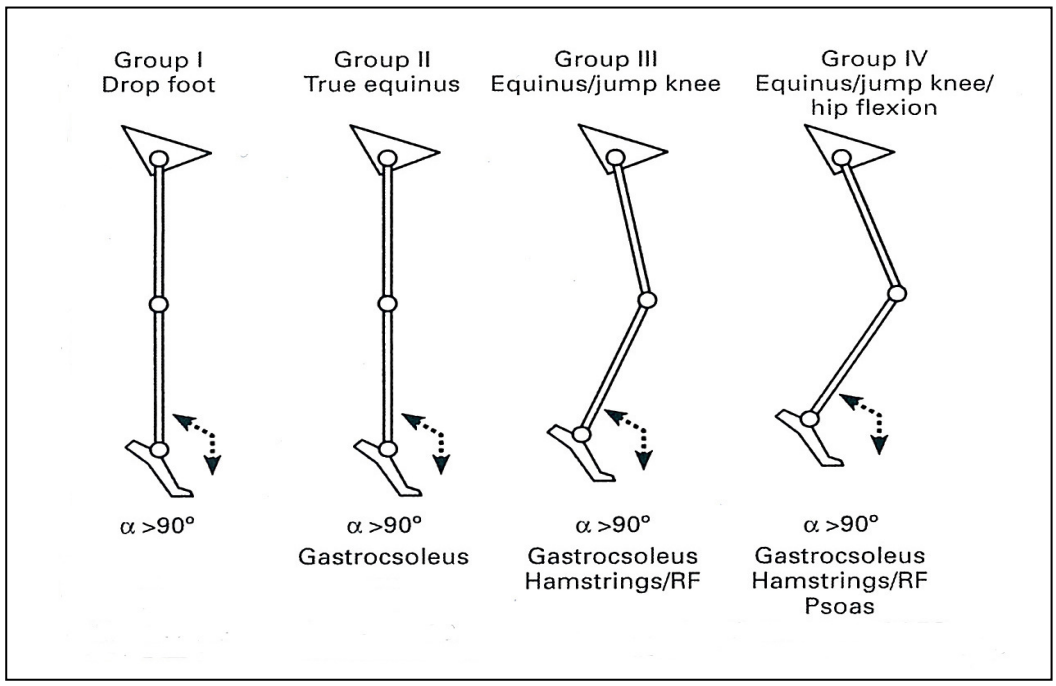
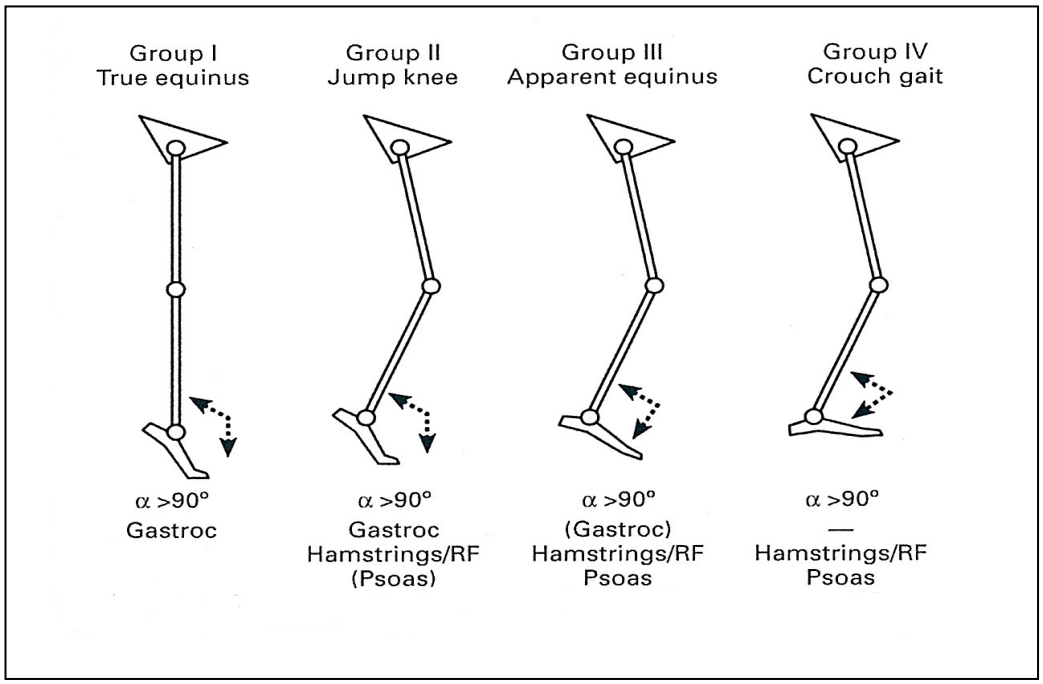


Figure 2. Sagittal gait patterns in spastic diplegia according to Rodda and Graham (2001). Reproduced from Graham and Selber (2003) with permission from the British Editorial Society of Bone and Joint Surgery. RF, rectus femoris.



In spastic quadriplegia with generalized spasticity and total body involvement the limb deformities are severe, often asymmetric, and accompanied by trunk deformities such as pelvic obliquity and spinal deformities (Graham 2004). Hip subluxation and dislocation often evolves silently and is diagnosed late due to communication difficulties and increased attention paid to other important issues (Scrutton et al. 2001, Soo et al. 2006). Soo and associates (2006) found the incidence of displacement (defined as a migration percentage of > 30%) to increase with GMFCS level: the incidence was 0% at level I, 15.1% at level II, 41.3% at level III, 69.2% at level IV and 89.7% at level V. Some children develop "windswept" hip deformity (i.e. one hip is flexed, adducted and internally rotated, the other is abducted, externally rotated and usually extended), which is difficult to manage (Graham 2004).

1.6. Methods of assessing CP by the International Classification of Functioning, Disability and Health model

The World Health Organization´s International Classification of Functioning, Disability and Health (ICF) provides a useful framework for understanding and measuring the impact of the deficits in body structures and functions (impairments) on the performance or execution of tasks (activities) and involvement in situations and activites at home and school and in the community (participation) (World Health Organization 2001). It also considers the effect of contextual factors (personal and environmental factors) on function and disability. In Table 6 methods of assessing CP used in this study series are listed and arranged under the ICF classes of impairment, activity and participation by the author. In addition, methods often preferred and quoted in the literature by physicians, physiotherapists and occupational therapists in dealing with CP children are provided, as well as novel assessments of participation.

Table 6. Assessment methods for CP according to ICF classification. Some of the measures may be used in more than one class.

A. Impairment
Active and passive range of movement (ROM; Stuber et al. 1988, Fosang et al. 2003)
Modified Ashworth Scale for measuring muscle spasticity, or more precisely, resistance to passive movement (MAS; Bohannon and Smith 1987)
Modified Tardieu Scale for measuring dynamic muscle length, i.e. dynamic spasticity or "catch" (MTS; Boyd and Graham 1999)
Selective Motor Control Test (SMC; Boyd and Graham 1999)
Goal Attainment Scale (GAS; Maloney et al. 1978)
Physician´s Rating Scale (PRS; Koman et al. 1993)
Observational Gait Scale (OGS; Boyd and Graham 1999)
Upper Limb Physician´s Rating Scale (ULPRS; Graham et al. 2000)

B. Activity
Gross Motor Function Measure (GMFM; Russell et al 1989)
Pediatric Evaluation of Disability Inventory (PEDI; Feldman et al. 1990)
The Functional Independence Measure for Children (WeeFIM; Msall et al. 1994)
Canadian Occupational Performance Measure (COPM; Law et al. 1990)
Goal Attainment Scale
Observational Gait Scale
Physician´s Rating Scale
Upper Limb Physician´s Rating Scale
Quality of Upper Extremities Test (QUEST; DeMatteo et al. 1993)
Melbourne Assessment (Randall et al. 2001)
House Classification System (House et al. 1981)
Assistive Hand Assessment (AHA; Krumlinde-Sundholm et al. 2007)

C. Participation
Goal Attainment Scale
Pediatric Evaluation of Disability Inventory
The Functional Independence Measure for Children
Canadian Occupational Performance Measure
Children´s Assessment of Participation and Enjoyment (CAPE; www.canchild.ca)
Preferences for Activities of Children (PAC; www.canchild.ca)

1.7. Treatment options for spasticity in children with CP

The aims of treatment are to reduce spasticity so as to improve function, participation and quality of life, to enable children to function optimally given their impairments, to prevent or delay secondary complications or compensatory mechanisms and to promote wellness and satisfaction. The different treatment options for spasticity in children with CP are set out in Table 7. Each treatment modality may be used alone or in various combinations depending on the goals and the severity of dysfunction.

Table 7. Treatment options for spasticity in CP children (modified from Lin 2000 and Autti-Rämö 1999).

A. General care
Nutrition and feeding
Posture, seating
Sleep pattern (melatonin)
Pain management (gastro-esophageal reflux, fractures, joint pain, dental abcesses, pressure sores, hip dislocation etc.)
Psychological contentment (frustration)
B. Physiotherapy
C. Orthotics, casting, positioning
D. Electrical stimulation
E. Oral medication
Benzodiazepins (diazepam, nitrazepam)
Baclofen
Dantrolene
Tizanidine
F. Local injections
Botulinum toxin
Alcohol or phenol
Lidocaine
G. Intrathecal baclofen
H. Surgery
Dorsal rhizotomy
Tendon lengthening, release and transfer
Osteotomy and derotation
Arthrodesis

2. Botulinum toxin type A

While botulinum toxin has long been recognized as an extremely potent poison, it has nonetheless relatively recently come to assume an important role in the treatment of spasticity and other neurological indications (Setler 2002). The toxin is produced by the anaerobic spore-forming bacterium *Clostridium botulinum,* of which eight immunologically distinct serotypes – A, B, C1, C2, D, E, F, and G – have been identified. All but C2 are neurotoxins and exert their effect by blocking the release of the neurotransmitter acetylcholine (Ach) at cholinergic nerve endings. A selective and temporary chemical denervation ensues, causing clinically detectable muscle weakness and atrophy (Simpson 2000, Setler 2002).

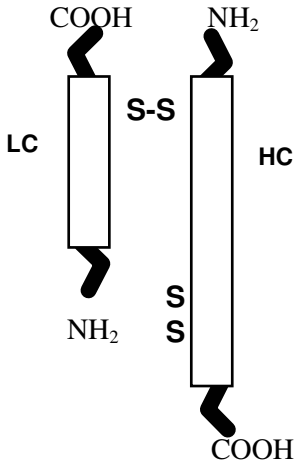
Among the seven neurotoxins, types A and B have been introduced into clinical practice (Setler 2002). After the initial demonstration in treating strabismus (Scott 1981), the use of botulinum toxins type A (available as Botox^{R} and Dysport^{R}) and B (available as NeuroBloc^{R} in Europe and as Myobloc^{R} in the US) has proved effective and safe in a variety of conditions in, for example, adult and pediatric neurology, urology, dermatology, gastroenterology and plastic surgery. This review of the literature focuses on botulinum toxin type A (BTXA), as this is the most widely used serotype in the pediatric population.

2.1. Mechanism of action

2.1.1. Structure

BTXA molecules are synthesized as single polypeptide chains of 150 kiloDaltons (kDa) which are only weakly toxic (Figure 3). The toxin molecule associates with additional non-toxin proteins to form a complex weighing between 300-900 kDa. Either in the host bacterium or at the final destination the molecule undergoes two major changes, nicking and disulfide bond reduction, both of which increase the potency of the toxin. The nicking step consists of a 50 kDa light chain connection with a 100 kDa heavy chain linked by a disulfide bond (Simpson 2000, Dolly and Aoki 2006). The heavy chain is a kind of homing device, responsible for targeting the neuromuscular junctions (NMJ): the C-terminus (carboxyl end) of the heavy chain binds the toxin molecule specifically to cholinergic neurones and the N-terminus (amino acid end) is important for translocation of the light chain from the endocytosed vesicle into the cytosol. The light chain is the toxic component in the molecule, acting as a zinc-endopeptidase responsible for the toxic intracellular activity of BTXA. Once in the neurone cytosol the disulfide bond is reduced and the toxin is activated by converting the light chain into a proteolytic enzyme (Rossetto and Montecucco 2003).

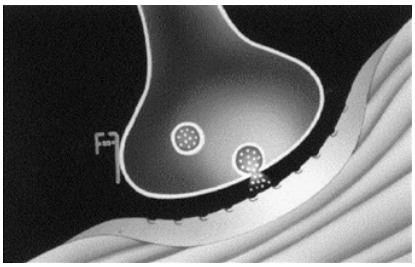
Figure 3. Structure of BTXA. LC= light chain, HC= heavy chain.



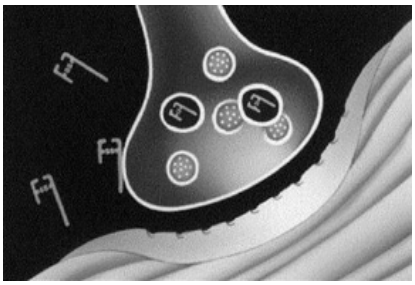
2.1.2. Mode of action at cholinergic nerve endings

From the site of adsorption (intestine, wounds or injection into the muscle), BTXA diffuses to the peripheral cholinergic nerve endings in the preganglionic sympathetic and parasympathetic nervous system, postganglionic parasympathetic nervous system and efferent motor nerves at the NMJ – the last-mentioned being the principal target for toxin action (Simpson 2000). The presynaptic blocking of the release of Ach involves four stages: *binding, internalization, translocation and action* in the cytosol (Simpson 2000, Rossetto and Montecucco 2003). These stages are presented in Figure 4.

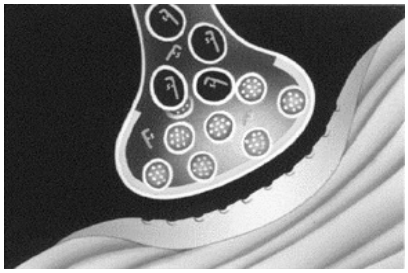
Figure 4. Blocking of the release of Ach. Reprinted with permission from Allergan.



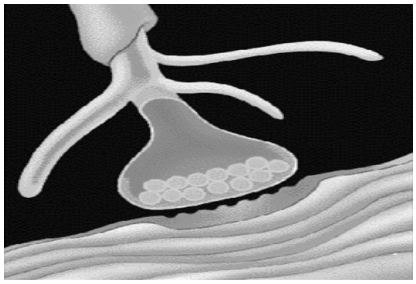
1. Rapid, specific and irreversible *binding* to acceptors on the presynaptic nerve ending surface.



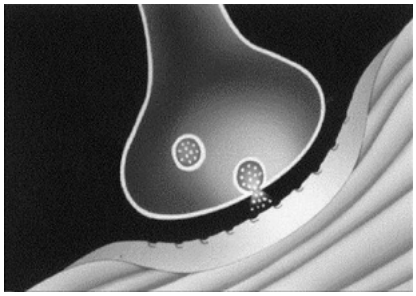
2. *Internalization* of the toxin molecule into the cell by receptor-mediated endocytosis.



3. *Translocation* in which the toxin molecule is released into the cytosol.



4. Toxin molecule *action* in the cytosol as a zinc-dependent endoprotease, cleaving polypeptides essential for the Ach release mechanism. The nerve end starts to recover by sprouting.



5. The nerve end is re-established.

BTXA (as also serotypes E and C) cleaves synaptosome-associated protein-25 (SNAP-25), one of the proteins forming the SNARE (soluble N-ethyl-maleimide-sensitive factor attachment protein receptor) complex which is essential for fusion of Ach-containing vesicles and thus for Ach release (Setler 2002, Dolly and Aoki 2006). Among the proteins in the SNARE complex are syntaxin (cleavage site of serotype C) and synaptobrevin/vesicle-associated membrane protein (VAMP; cleavage site of serotypes D, F, and G).

2.1.3. Recovery of nerve endings

BTXA does not cause cell death and the nerve remains in contact with the muscle (Moore and Naumann 2003). The human NMJs recover by developing sprouts from the end plate, the preterminal axon and adjacent nodes of Ranvier in this (Holds et al. 1990). It has been shown *in vivo* with mouse sternocleidomastoideus muscle that the new sprouts are able to activate the muscle after 28 days from BTXA injection. Eventually the sprouts degenerated and the original parent terminal axon regained its function by day 91 with normal Ach receptors and release (de Paiva et al. 1999)(Figure 4). The recovery was accompanied by synthesis of new SNARE protein. Thus, the effect of BTXA is long-lasting, but temporary.

The time course of the effects of BTXA assessed by serial electroneuromyography (EMG) recordings in healthy adults` extensor digitorum brevis (EDB) muscle shows that the entire NMJ recovery process requires in most situations 2-4 months (Hamjian and Walker 1994). The amplitude of the compound muscle action potential started to decline 48 hours after the injection, peaked between 1 and 3 weeks and gradually wore off. The compound muscle action potential reduction was accompanied by a decrease in mean rectified voltage during maximal voluntary contraction and muscle atrophy with a volume loss of approximately 40% (Hamjian and Walker 1994). The degree and to some extent the duration of the effect are dose-dependent (Sloop et al. 1996). In the autonomic nervous system recovery may take longer (6-12 months) (Naumann et al. 1999).

As a mode of treatment of symptoms repeated BTXA injections may in all likelihood be needed. Unless toxin neutralizing antibodies intervene, the muscles continue to respond well after multiple injections (Holds et al. 1990, Moore and Naumann 2003). BTXA may affect type I and type II muscle fibers differently. In mice, the soleus muscle, consisting mostly of type I slow fibers, were restored from atrophy by the 4th-6th weeks while the gastrocnemius with mostly type II fast fibers began to recover only after the 5th-6th week (Duchen 1971). However, no persistent histological changes in human orbicularis oculi muscles were detected even after multiple treatments (Harris et al. 1991, Borodic and Ferrante 1992).

The BTXA may be preferentially taken up by the most active muscle fibers, and in electrophysiological studies with rabbits (Kim et al. 2003) and human adults (Hesse et al. 1995, Eleopra et al. 1997, Hesse et al. 1998) the paralytic effect has been enhanced by electrical stimulation or stretching exercise. However, Detrembleur and colleagues (2002) could not replicate this finding in children.

2.1.4. Other sites of action

Local spreading. After local muscle injection, BTXA spreads within the muscle in question and through fascias to adjacent muscles, presumably by diffusion (Borodic et al. 1990, Shaari et al. 1991, Borodic et al. 1994, Eleopra et al. 1996, Ross et al 1997). This effect may or may not be desirable.

Distal or systemic spreading. Signs of spreading into distal muscles have been detected. Garner and colleagues (1993) carried out a single-fiber EMG of the extensor digitorum communis or tibialis anterior muscle in eight adults treated for focal head/neck dystonia and found increased jitter 3-13 days after treatment in six patients. There was no correlation between single-fiber EMG pathology and dosage, but the two patients complaining of side-effects evinced a tendency toward more pronounced pathologic findings. Likewise, in a double-blind placebo-controlled study of single-fiber EMG changes after treatment for adult idiopathic torticollis, Lange and associates (1991) noted increased jitter in distant limb muscles 2 weeks after treatment without clinical weakness.

The possible mechanisms underlying these observations may be either highly efficient uptake of BTXA at the injection site and retrograde axonal transport along the spinal motor neurons or a systemic distribution of excess toxin by the

blood circulation (Garner et al. 1993). Wiegand and associates (1976) showed in cats that ^{125}I-labeled BTXA was retrogradely transported to the ipsilateral spinal anterior horn cells after intramuscular injection. Radioactivity was also found in the corresponding contralateral spinal segment, raising the possibility of trans-synaptic transfer within the cord, passage to other spinal segments and anterogade axonal transportation to lower motor neurones and their end plates. However, the stability of the binding of intact toxin with the labelled molecule has been questioned (Aoki et al. 1997). Furthermore, the heavy chain is necessary for uptake, but is cleaved in the cell from the light chain which is responsible for the toxic action. This prompts one to ask how the light chain is transported trans-synaptically in the spinal cord and whether it retains the toxic ability (Garner et al. 1993).

Effects on muscle spindles. In the muscle spindles there are cholinergic junctions between the gamma motor neurones and intrafusal muscle fibers. As the muscle stretches, afferent signals from the muscle spindle organs travel along the Ia and II fibers and excite the alpha motor neurones (stretch reflex) as well as interneurones inhibiting the alpha motor neurones of the antagonistic muscles (reciprocal inhibition). Signals from the muscle spindles are also relayed to supraspinal structures involved in long latency responses to the stretch reflex. Filippi and colleagues (1993) demonstrated a direct BTXA blocking of cholinergic gamma endings in rat masseter muscle, and a group under Rosales (1996) showed atrophy and reduction of muscle action potentials in extrafusal fibers and concomitant atrophy and reduction of spindle afferent discharges in intrafusal fibers in the biceps femoris of Wistar rats after BTXA injection. Thus, in spasticity and dystonia, BTXA is apparently able to alter muscle spindle output both directly and indirectly (i.e. either by reducing continuous muscle tension or modifying reciprocal inhibition). The latter process has been demonstrated with adult focal dystonia (Priori et al. 1995) but not in adult spasticity (Girlanda et al. 1997). In CP children, however, a reduction in nonselective activation of agonist-antagonist muscles (i.e. co-contraction) between tibialis anterior and triceps surae muscles has been detected at 4 weeks (Hesse et al. 2000) and 3 months (Detrembleur et al. 2002) after BTXA injection into the calf muscles.

Effects on autonomic nervous system. BTXA action on the autonomic nervous system does not differ from its action on the striate muscle. BTXA has been used to treat dysfunctions in smooth muscles (e.g. detrusor sphincter dyssynergia, hyperreflexic bladder, esophageal sphincter in achalasia, sphincter Oddi dysfunction and anal fissures) or hyperactivity of secretory glands (e.g. hyperhidrosis, hypersalivation and increased tear-forming) (Naumann et al. 1999). Only mild distant effects on autonomic functions (cardiovascular reflexes) have been noted in 4 of 5 adult patients treated for focal dystonia (Girlanda et al. 1992) and the authors recommend monitoring patients who undergo high cumulative dosages or are taking drugs impairing autonomic pathways/neuromuscular transmission or suffer from diseases involving the autonomic nervous system.

Central effects. In adults, somatosensory-evoked potentials or transcranial magnetic stimulation have been used to study probable BTXA-induced central or cortical reorganization. Only a few studies have investigated children. Park and associates (2002) showed with 19 spastic CP children and 8 children with

posttraumatic brain injury spasticity that BTXA injection into the limb muscles improved the cortical somatosensory-evoked potentials by day 7 as spasticity was reduced as measured on the Modified Ashworth Scale. The results cannot be explained by assuming that the lesions of these patients may not be limited to the motor system; the authors postulated that the electrophysiological characteristics underlying spasticity might partly contribute to the abnormal cortical responses, i.e. the spindle afferent input to the spinal cord and higher central nervous system. This input was presumably altered by BTXA.

At present, there is no *in vivo* human or animal evidence of a direct BTXA effect at brainstem or cortical level after intramuscular injection at the low doses used in therapeutic indications. Valls-Sole and colleagues (1994) showed no change in brainstem interneuronal excitability in adult dystonia patients, and a group under Moreno-Lopez (1997) found no direct central effects after BTXA injections with varied doses into the cat lateral rectus eye muscle, except in the case of the highest dose (3 ng/kg) used. As a large 150 kDa molecule BTXA does not cross the healthy blood-brain barrier and hematogenous spread to the central nervous system is unlikely (Moore and Naumann 2003). Thus according to current knowledge all the possible effects caused by BTXA at cortical level must be considered indirect and secondary to modification of the peripheral afferent input.

Other probable effects. Observations of pain relief when treating painful muscle hyperactivity have led to further research on possible mechanisms besides those of cholinergic nerves. In brief, BTXA has been found to inhibit substance P release from cultured embryonic dorsal root ganglion neurones, to reduce stimulated release of calcitonin gene-related peptide from cultured trigeminal ganglia neurones, and to inhibit glutamate release, fos-like immunoreactivity in the dorsal horn and evoked activity of dorsal horn wide dynamic range neurones in a rat Formalin-pain model (reviewed in Aoki 2005). These findings suggest a direct BTXA-induced inhibition of neurotransmitters from sensory neurone terminals. Speculation on a possible consequent reduction in inflammatory pain via direct inhibition of peripheral sensitization and indirect inhibition of central sensitization is intriguing and the issue is at present under intense study (Aoki 2005, Dolly and Aoki 2006).

2.1.5. Effect of doses and volumes on diffusion

BTXA diffusion occurs both within the injected muscle and into adjacent muscles outside it.

Diffusion within the muscle. Shaari and Sanders (1993) injected rat tibialis anterior muscles with different doses ranging from 0.02 to 20 Units (U) of Botox^{R} and established that the area paralysed was equivalent to the area of glycogen-retained fibers stained with periodic acid Schiff (i.e. PAS stain). As the concentration was kept at 0.2 U/ul, increasing doses also represented increasing volumes (from 0.1 ul to 100 ul). The authors found the area of paralysis to correlate with increasing doses up to 5 U. This amount stained most of the cross-sectional area of the muscle and with increased doses staining spread no further. Likewise, they injected increasing volumes (from 1 ul to 100 ul) with a fixed dose (0.2 U) and noted the paralysed area increased concomitant with volume.

They concluded that dose is more potent than volume in producing paralysis, since a 25-fold increase in dose but a 100-fold increase in volume was needed to double the area of paralysis.

Diffusion outside the muscle. Borodic and associates (1990) observed that in adult cervical dystonia injection of the sternocleidomastoid muscle with a BTXA amount over 100 U (apparently Botox^{R}) caused more dysphagia than amounts under this. They adjusted their protocol accordingly and tested their theory of toxin spread with albino rabbit latissimus dorsi muscle. Injecting 2-3 U/kg with 1.25 U/0.1 ml and using acetylcholinesterase staining as marker, they noted a diffusion gradient over a distance of 3-4.5 cm from the injection site. Furthermore the toxin spread into the contralateral muscle 1.5-2.5 cm from the injection point, even crossing anatomic barriers such as fascias and bones. In a later study (Borodic et al. 1994), they injected 1 U, 2.5 U, 5 U and 10 U in a standard volume of 0.1 ml and a control with saline into albino rabbit longissimus dorsi muscles. After 5 weeks, the denervated area correlated with the dose used: within the injected muscle, diffusion occurred up to 1.5-3 cm with 1 U and up to 4.5 cm with 10 U, and in the contralateral muscle with 10 U the denervation area showed diffusion at 4.5 cm from the injection site. Similarly, Shaari and colleagues (1991) dropped increasing doses (0.2, 0.4, 6 and 10 U of Botox^{R}, volume kept at constant 0.1 ml) of BTXA on rat tibialis anterior muscle fascia or on muscle without fascia. After 24 hours, the toxin had easily passed through the muscle fascia even at subclinical doses (area stained for glycogen), the presence of fascia reducing toxin spread by 23%.

Whether these animal experiment results can be transferred to humans remains an open question, since there are substantial difficulties in directly documenting BTXA diffusion in human muscle. In clinical studies with adults, evidence for (Borodic et al. 1990, Eleopra et al. 1996, Ross et al. 1997) and against (Lorenzano et al. 2006) unintended spread of BTXA due to diffusion has been published. However, based on the animal and human data available and on clinical experience, BTXA diffusion is likely to take place up to a distance of 5 cm, depending on the dosage and volume injected (Schroeder et al. 2006).

2.2. Potency, dose equivalency, safety and immunogenicity

2.2.1. Potency and dose equivalency

In the earliest studies, doses were expressed in nanograms of toxin-hemagglutinin complex. Later the standard unit of potency used for all preparations was chosen to be 1 mouse unit (mu or at present U). One mouse unit is the amount of BTXA which kills 50% of mice injected into the intraperitoneum (lethal dose 50, LD50, or mouse lethality test) (Moore and Naumann 2003). However, as the mouse units of the two products currently available and most frequently used (*Botox*^{R}, Allergan Inc., Irvine, California, USA, and *Dysport*^{R}, Ipsen Limited, Berkshire, UK) are not equivalent and the doses are not straightforwardly interchangeable, comparisons of potency and adverse event ratios are difficult. BTXA is also marketed in China (*Prosigne*^{R}, Lanzhou Institute of Biological Products, China) and in Germany (a highly purified form *Xeomin*^{R}, Merz Pharmaceuticals, Germany).

The characteristics of the two most frequently used BTXA products are summarized in Table 8. The size of the molecule complex appears to affect the fluid-based diffusion of BTXA within the target muscle; the larger the molecule, the more limited diffusion (Aoki et al. 2006). This migration pattern, in turn, influences the adverse effect profile and safety margin. Moreover, the non-toxin accessory proteins, comprising hemagglutinins and non-hemagglutinins, stabilize biological activity and thus add to adherence to the muscle tissue (Aoki et al. 2006). No substantial differences between the two products have been detected in respect of potency or diffusion in animal and human models (Wohlfarth et al. 1997, Rosales et al. 2006).

Both animal (mouse, rat) (Aoki et al. 2006, Rosales et al. 2006) and human (adult focal dystonia) (Sampaio et al. 1997, Odergren et al. 1998) studies have sought to establish a conversion ratio between the two BTXA products. No studies with young animals or children have been conducted. The Botox^{R}:Dysport^{R} ratio is probably between 1:3 and 1:4 in most indications, but no consensus has been reached as to a set dose (Aoki et al. 2006). Apart from the intrinsic properties of the product (i.e. protein load), many other factors influence comparative data on potency and diffusion, for example muscle configuration, fascial planes, dilutions, the underlying condition being treated, and individual predispositions (Rosales et al. 2006).

Table 8. Characteristics of the two BTXA formulations commercially available (adapted from Moore and Naumann 2003)

Parameter	Botox[R]	Dysport[R]
LD50 for primates with systemic injection (U/kg)	30-40	90-120
Human food poisoning: mild (U)	3500	10 000
Human food poisoning: severe (U)	3500-30 000	30 000-90 000
Safe range per session in children, total dose (U)[1]	400-600	900
Safe range per session in children, dose in U/kg bw[1]	6-25	15-25
Maximum recommended dose per injection site (U)	50	150
Package size (U)[2]	100	500
Molecular mass of complex (kDa)[3]	900	500-700
Amount of neurotoxin (ng/vial) = protein load per dose[3,4]	5 ng in 100 U	5 ng in 500 U
Presentation	Powder Reconstitute with 0.9% saline	Powder Reconstitute with 0.9% saline
Excipients[2]	Human albumin, NaCl	Human albumin, lactose
Shelf life unopened[2]	3 years	1 year
Shelf life opened/reconstituted[2]	24 hours stored at 2-8 C	8 hours stored at 2-8 C

Data from [1]European Consensus guidelines by Heinen et al. 2006, [2]manufacturer guidelines in CD-Pharmaca Fennica 2007, [3] Aoki et al. 2006, [4]Pickett et al. 2003

2.2.2. Safety and adverse events

Confidence in the long-term safety of BTXA has built up as a result of many years´ therapeutic use of the toxin in a variety of indications: blepharospasm since 1980, cervical dystonia since 1985, oromandibular and limb muscles since 1989 and spastic/dystonic CP since 1988 (Koman et al. 1993, Nauman et al. 2006). Also, histological studies confirming full recovery of end plates and muscle tissues, combined with clinical observations of reappearance of symptoms, bespeak the safety of BTXA (Harris et al. 1991, Borodic and Ferrante 1992, De Paiva et al. 1999).

Safety in both short- and long-term use is associated with spread of the toxin from the target muscle leading to systemic adverse effects, and in long-term use also with the development of neutralizing antibodies. Local adverse reactions at the injection site may include bruising, pain, edema and erythema; systemic adverse reactions comprise flu-like symptoms, fatigue, generalized weakness, malaise and nausea (Naumann et al. 2006). Sometimes it may be difficult to separate systemic adverse events from local diffusion into the autonomic nervous system nerve endings (urinary or fecal incontinence, dry mouth). The extent and frequency of adverse events depend on the injection site and technique and the indication; e.g. injections into the sternocleidomastoideus muscle bilaterally at multiple sites may induce dysphagia, but injections into gastrocnemius muscles

by the same technique may only cause slight tripping and clumsiness. Thus clinical experience leading to optimal muscle targeting and knowledge of the properties of the BTXA product used will minimize toxin spread and improve safety (Naumann et al. 2006).

The safety of Botox^{R} was assessed in a meta-analysis comprising 36 randomized clinical trials across a variety of indications with 2309 subjects, both adults and children (Naumann and Jankovic 2004). The incidence of adverse events was 25% for Botox^{R} and 15% for controls (placebo or other control group for comparison). Focal weakness was the specific adverse event occurring significantly more frequently in Botox^{R} treatments, being thus related to the mechanism of action. All adverse events were rated mild to moderate; no severe or systemic adverse events were reported.

Safety is well documented for the treatment of CP in children (Gormley et al. 1997, Boyd et al. 1999, Bakheit et al. 2001, Koman et al. 2001, Mohamed et al. 2001, Goldstein 2006). As spasticity and dystonia are often combined and multiple muscles are affected, a multilevel and multimuscle integrated approach is often warranted. The clinical picture alters as the child grows and the motor pattern matures. In earlier studies lower doses were used, but as clinical experience grew dosage was increased. Doses up to 31 U/kg body weight of Botox^{R} have been reported (Desloovere et al. 2007), the total dose being distributed over multiple muscles and sites per muscle.

In studies investigating the incidence and profile of adverse events in CP treatments, the overall incidence has been reported as 22% (Gormley et al. 1997; doses up to 10 U/kg of Botox^{R}), 5% (Boyd et al. 1999; doses up to 16U/kg of Botox^{R}), 27% (Koman et al. 2001; up to 4U/kg of Botox^{R}), 35% (Mohamed et al. 2001; doses up to 29U/kg of Dysport^{R}) and 3% (Goldstein 2006; doses up to 19 U/kg or 930 U of Botox^{R}) of treated children. Bakheit and colleagues (2001) reported adverse events in 7% of treatments (mean dose per session 23 U/kg of Dysport^{R}) and noted that the incidence was related to the total dose rather than the dose calculated per body weight. In double-blind placebo-controlled studies the incidence has been reported as 17% for Botox^{R} and 4% for placebo (Koman et al. 2000), 27% for Dysport^{R} and 5% for placebo (Ubhi et al. 2000) and 51% for Dysport^{R} and 32% for placebo (Baker et al. 2002). The follow-up ranged from 3 months to 4.5 years.

The adverse events most frequently reported in these previous studies were focal weakness, soreness at injection site, bruising, tripping or falling, local rash and influenza-like illness. Fewer reports include symptoms such as urinary or fecal incontinence, generalized weakness, worsening of strabismus or dysphagia, irritability or constipation. Neither death nor systemic anaphylaxis has been officially reported in children.

Caution is nonetheless recommended in the BTXA treatment of children with pre-existing bulbar symptoms, gastro-esophageal reflux or frequent chest infections, as these conditions expose to aspiration pneumonia (Boyd et al. 1999). Other contraindications include a history of neuromuscular disease such as myasthenia gravis, and the simultaneous use of amino glycoside antibiotics and non-depolarizing muscle relaxants.

2.2.3. Immunogenicity

The major factor affecting the efficacy of long-term BTXA treatment (i.e. repeated injections) is the development of neutralizing antibodies and induction of secondary non-responsiveness. This latter is defined as a failure to respond to BTXA treatment in two subsequent treatment sessions, after at least two previous successful injections and/or typical BTXA-related adverse events; some definitions also specify failure despite an increase in BTXA dose (Moore and Naumann 2003). The antibodies in question are not dangerous in themselves, but are long-lasting and, even when they subside, are easily reactivated. Antibodies formed against non-toxin proteins do not interfere with BTXA activity (Dressler and Hallett 2006).

Tests used to detect secondary non-responsiveness comprise both laboratory (enzyme-linked or sphere-linked immunosorbent assay, immunoprecipitation assay, mouse protection assay, Western blot assay, mouse hemidiaphragm-phrenic nerve bioassay) and clinical tests (frontalis antibody test, EDB test, sweating test) (Moore and Naumann 2003). The mouse protection bioassay and the mouse phrenic nerve hemidiaphragm test have been used with children (Koman et al. 2001, Herrmann et al. 2004) and the mouse protection assay with adults (Dressler and Hallett 2006). However, in the clinical setting the frontalis antibody test may be the easiest and cheapest (Moore and Naumann 2003).

The overall rate of antibody formation in adults across the indications has been reported to be approximately 5-10% (Dressler and Hallet 2006). In CP child studies with the earlier batch of Botox^{R}, the neutralizing antibodies were detected in 28% (Koman et al. 2001; the mouse protection bioassay) and 32% (Herrmann et al. 2004; the mouse phrenic nerve hemidiaphragm test) of treated children. The latter study used both Botox^{R} and Dysport^{R}, and no significant difference was noted between the two products. After the introduction of the new Botox^{R} batch in 1999, with an 80% reduction in total protein content, the neutralizing antibody formation has been approximately 1% (Naumann et al. 2006). Short intervals (< 3 months) between injections, administration of booster injections, use of high doses (U/kg) at each injection, a high cumulative BTXA dose, early introduction of BTXA therapy, number of treatments and male sex have been found to correlate with the formation of neutralizing antibodies (Herrmann et al. 2004, Naumann et al. 2006). Certain individuals may have a predisposition to produce neutralizing antibodies (Dressler and Hallett 2006, Naumann et al. 2006). Presumably only high antibody titers result in failure of therapy (Naumann et al. 2006). When there is - despite increasing doses - failure to respond to BTXA treatment, secondary non-responsiveness should be suspected and tested for. Switching to an antigenically distinct preparation such as BTX type B may be necessary (Moore and Naumann 2003).

2.3. Injection techniques

Since the NMJs in the muscles are the sites of BTXA action, targeting the right muscle and close to the motor end plates is considered essential. Diffusion of BTXA and the potential need to inject close to NMJs are interrelated with doses, dilutions, volumes and number of injection sites. Localization techniques have evolved over time, but each technique has its own limitation in locating the motor end plates. Injection methods may also be determined by a number of other factors such as the patient's age and diagnosis, the anatomic site (accessibility) of the target muscle, and the training and preferences of the treating clinician.

2.3.1. Site of injections: near the motor end plate zone

In mammalians and humans, the NMJs or motor end plates have been found to be located at the midpoint of each muscle fiber, forming a distinct clustering called the NMJ zone or end plate zone (Coers and Woolf 1959, Christensen 1959). The shape of this zone depends on muscle configuration and fiber length and should be uniform in a given muscle, but differs in different muscles (Coers and Woolf 1959, Christensen 1959). The NMJs may either be scattered diffusely or form one or multiple detectable zone-like patterns (Coers and Woolf 1959). Thus, if the muscle fiber architecture is known, the location of the end plate zone is predictable.

Attempts have been made to locate and map the motor end plate bands in different human muscles. Christensen (1959) studied stillborn infants and located the end plates by means of cholinesterase staining. Saitou and colleagues (2000) investigated 17 muscles in the upper and lower limb in three subjects by detecting the innervation zones with the bi-directional propagation of motor unit action potentials in a multichannel surface electrode array. Parratte and associates (2002) dissected the motor nerves in 40 human limbs and found that in the triceps surae muscle the motor end plates were distributed in characteristic zones confined mostly to the mid-part of the muscle belly (Figure 5). A group under Deshpande (2006) published an excellent work mapping the end plate zones in a total of 30 muscles in upper and lower limbs. They examined the muscle fiber orientation in adult cadavers, determined the relationship of the muscle with surface landmarks and developed figures showing the approximated end plate zones (Figure 6). Their work enables the clinician working with children to extrapolate the maps to patients of varying sizes.

Figure 5. Schematic drawing of the intramuscular nerve distribution in the gastrocnemius muscle. Reprinted from Parratte et al. 2002 with permission from Springer.

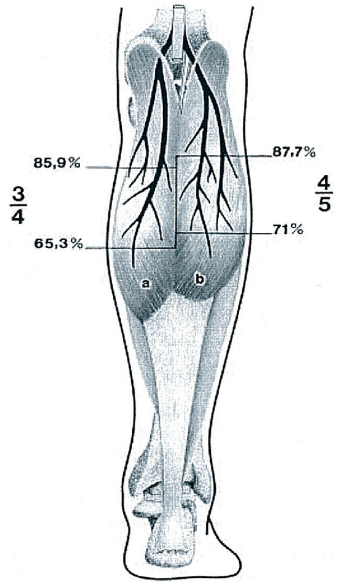
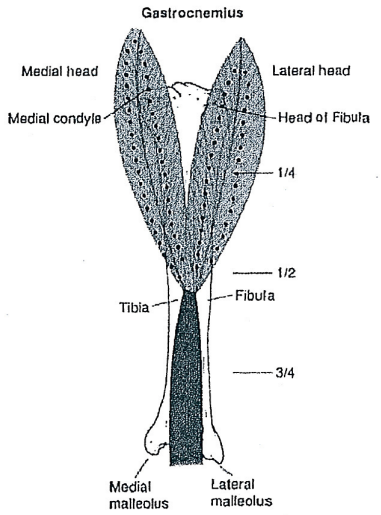


Figure 6. The approximated NMJ zones forming parabole bands in the convergent bipennate gastrocnemius muscle. Reprinted from Deshpande et al. 2006 with permission from F.P.Graham Publishing Co.



The effect of BTXA injections close to the NMJs has not been studied in children. In theory, injections close to the NMJ zone would improve efficacy, reduce side-effects and potentially reduce the required doses. There is evidence from animal models that injection distance to NMJs influences the effect of BTXA treatment. Shaari and Sanders (1993) showed with the rat tibialis anterior muscle that BTXA injections given closest to the NMJ zone produced the greatest paralysis, measured as an area of glycogen retention. Injections 0.5 cm from the NMJ zone produced 50% less paralysis, and no paralysis was observed in injections 1.0 cm from the NMJs. The authors suspected that the toxin spread further than the area of glycogen retention measured, but in quantities insufficient to cause paralysis. The animals were sacrificed after 24 hours and more paralysis could have occurred. Likewise, it is not known how well this indirect histochemical model tallies with functional paralysis.

In a canine model, six dogs were injected into either the right or left gastrocnemius with 10 U/kg Botox^{R} divided over four sites per muscle (Childers et al. 1998). The other leg was injected with saline. Three dogs were injected at the NMJ zone located by EMG, while three were treated similarly without EMG (by palpation). Leg extension and flexion forces were measured on days 0, 2, 5, 14, 35 and 70. The outcome variable was the ratio of measurements from the BTXA-treated leg to the saline-treated leg. In both groups the extension ratio decreased on days 2, 5, 14 and 35. On days 14 and 35 the extension ratios in the BTXA-treated legs were reduced significantly better in the EMG-guided limbs injected near the NMJs than in anatomic-guided limbs. Both groups recovered by day 70, though there was still a tendency for the EMG-guided (near the NMJ) group to have weaker extension compared with the other group.

Only two human adult studies have been published comparing injection sites close to or remote from the NMJs. Childers and associates (1996) conducted a double-blind, randomized, placebo-controlled study comparing two different injection techniques in 15 ambulant adults with spastic hemiplegia. One group received a single-point injection of BTXA (50 U of Botox^{R}) into the proximal part near the motor point using surface electric stimulation, the other 50 U divided over three sites in the mid-belly part near the assumed NMJ zone of the gastrocnemius using palpation. Only one (the largest) of the two heads of the gastrocnemius was injected with BTXA and the other with placebo. No difference in reduced muscle tone (Ashworth scale), increased ankle ROM (goniometry) or functional outcome (Fugl-Meyer score, a timed distance walk) was detected between the groups. The fact that only one of the two heads of the gastrocnemius was injected may have affected the results. In a double-blind study with 21 hemiplegic adults, published only as an abstract with sparse details, Gracies and colleagues (2002) injected 160 U of Botox^{R} divided over four sites of the biceps brachii muscle. The patients were randomized into three groups: one receiving 100U/ml dilution (0.4 ml/site) into four quadrants, one receiving 100U/ml dilution (0.4 ml/site) near the end plate zone and one being injected with 20U/ml dilution (2 ml/site) into four quadrants. Using reduction in mean rectified voltage during maximal voluntary contraction by EMG, spasticity according to the Tardieu Scale and strength with a strain gauge in the elbow flexors as outcomes, they showed that a small volume of BTXA near the NMJ was more effective than the same volume injected distantly. Injecting with a high-volume dilution distant from the NMJ was as effective as the low-volume close to the NMJ. However, no functional outcomes were used and the authors do not specify whether EMG was used in targeting the end plate zone.

2.3.2. Single or multiple injection sites

The number of injection sites per muscle may be determined by the morphology (and hence to the end plate zone configuration) of a given muscle. Borodic and associates (1991) observed the human orbicularis oculi muscle in adults to possess a diffuse innervation architecture and injected BTXA (Botox^{R}) either into a single (at the motor point) or multiple sites on separate sides in 10 adults with blepharospasm. Eight patients reported significantly better relief after 2-4 weeks with the multiple-site method, while two showed no difference between the methods. The outcome was judged by direct observation of muscle weakness by the clinician and by the subjective opinion of the patient. When treating adult spasmodic torticollis, Borodic and associates (1992) showed the multiple-point per muscle injection strategy to be superior to the single-point per muscle in terms of pain reduction, improvement in posture and range of motion, and improvement in activity endurance, but not in hypertrophy reduction or involuntary movements. The investigators injected several muscles involved in the dystonic deforming pathology, without knowing the exact pattern of the end plate zones. The doses were chosen on an individual basis and no evaluation of the incidence of side-effects was presented. In the aforementioned double-blind, placebo-controlled study by Childers and associates (1996) comparing two different injection techniques, one group receiving a single-point injection of

BTXA (50 U of Botox^{R}) into the proximal part and the other 50 U divided over three sites (i.e multiple-point injection) in the mid-belly area, no difference emerged between the groups. It might be suitable to divide the toxin even when injecting muscles with one innervation zone, as was done in the study by Gracies (2002).

The number of injection sites may also vary according to the size of the muscle. In a small muscle (e.g. in the upper limb), the toxin presumably diffuses well through the whole muscle and the risk of spread into adjacent muscles is increased with larger doses. In larger muscles (e.g. in lower limb or biceps brachii in the upper limb), larger doses may have to be used and dividing the dose along the assumed end plate zone might increase the effect.

In children, one or two injection sites in the adductors, the lateral hamstrings, soleus and each head of the gastrocnemius, and two to four sites in the medial hamstrings has been recommended (Graham et al. 2000, Russman et al. 2002). The use of either single or multiple BTXA injection sites has mostly evolved from the practical issues of dividing the larger dose, and hence the volume injected, into multiple sites in a given muscle in order to reduce unwanted spreading and adverse effects (Graham et al. 2000). It has been shown that at a certain dose saturation of the NMJs occurs and a plateau is reached (Sloop et al. 1996), which may allow spread of the toxin overflow into neighbouring structures and the systemic circulation (Graham et al. 2000). This could be avoided by splitting the dose over multiple sites. Hence for children the recommendations suggest a maximum dose of 50 U of Botox^{R} (Graham et al. 2000, Russman et al. 2002), or a maximum volume of 0.5 ml (Russman et al. 2002) per injection site. In practice, the number of injection sites used may also depend on the total number of muscles needing treatment and the availability of general anesthesia or sedation (Graham et al. 2000). However, no studies have been published specifically evaluating the single or multiple site injection technique and the related incidence of side-effects in children.

2.3.3. Techniques for localizing muscles and end plate zones

Young children dislike situations which require holding them still and are rarely co-operative. For this reason, the localizing techniques often used with adult patients (for example motor point stimulation or searching for the motor end plate zones with EMG or electric stimulation) are not particularly suitable without sedation. Also, CP children have limited control of specific muscles, which affects their ability to activate a given muscle for EMG location.

Manual needle placement, which takes advantage of surface landmarks, palpation of the muscle bellies and passive movement of the distal joints, remains the most common technique (Chin et al. 2005, Schroeder et al. 2006). It may be used with large, long and superficially located muscles such as the gastrocnemius but is not usually sufficient with deep-seated, small and slender muscles (Schroeder et al. 2006). Manual approaches may be combined with electrophysiological techniques such as passive or active EMG detecting the motor units or active electrical stimulation of a muscle. The drawbacks in both manual and electrophysiological techniques are the uncertainty of controlling the depth of the needle tip and ascertaining the correct placement after finishing the

injection. Sedation may reduce the EMG signal amplitude. EMG verifies that the needle is in the muscle but not always that it is in the right muscle. Electrical stimulation verifies the right muscle and thus increases accuracy in needle placement but may need multiple replacements and is usually painful, requiring at least adequate analgesia and sedation in children.

In a study of 226 children with 502 muscles injected and a total of 1372 injections administered, the manual needle placement was checked with electrical stimulation when children were injected with BTXA under general anesthesia (Chin et al. 2005). Manual placement was executed by three experienced pediatric orthopaedists. The accuracy of the placement was 78% in the gastroc-soleus, 67% in the hip adductors, 46% in the medial hamstrings, 11% in the tibialis posterior, 62% in the biceps brachii, 35% in the adductor pollicis, 22% in the pronator teres, 16% in the flexor carpii ulnaris and 13% in the flexor carpii radialis. As the procedures were carried out using general anesthesia, the spastic muscles were relaxed and soft, which is likely to have reduced the ability to palpate the target muscles before electrical stimulation. The authors recommended using electrical stimulation for all muscles except the gastroc-soleus.

Ajax and companions (1998) investigated the accuracy of needle placement in 45 adult dystonic or spastic patients without sedation. An electromyographer inserted the needle first with the preamplifier turned off and then switched it on to verify placement. No electrical stimulation was used. The authors do not specify the muscles, but the initial placement was correct in 77% in “large thigh muscles”, 85% in “paraspinal muscles”, 77% in “facial muscles”, 86% in “large arm muscles”, 56% in “hand muscles”, 68% in “forearm extensors”, 73% in “foot muscles”, 68% in “forearm muscles”, and 62% in “small leg muscles”. The average accuracy rate was 71%. Likewise, Molloy and colleagues (2002) showed that in the absence of EMG guidance, only 37% of needle placements reached the appropriate muscle, 47% were placed in an unintended muscle, and 16% were outside a muscle altogether in the treatment of adult focal limb dystonia. No studies have been published investigating the accuracy of needle placement under conscious sedation.

Recently, ultrasound guidance has been advocated by Berweck and colleagues (2002), offering a visually controlled alternative to potentate the accuracy of BTXA treatments in children. The advantages of this method are painlessness, the possibility to control needle tip placement even with anatomic variations, differentiation of neighbouring structures (muscles, vessels, nerves, bones), independency of co-operation or sedation and the possibility to document the site and volume of the injected toxin (Schroeder et al. 2006). However, ultrasound does not help in defining the NMJs. Ultrasound has been used by several other authors in iliopsoas injections either solely (Westhoff et al. 2003) or combined with EMG/electrical stimulation (Willenborg et al. 2002) and with salivary gland injections (Banerjee et al. 2006), but so far no pediatric studies comparing the accuracy of needle placement with or without ultrasound guidance have been published.

2.3.4. Dosage

Doses, dilutions, volumes and diffusion are interrelated. Dose refers to the amount of diluted BTXA as measured in units (U) of activity. Volume is the amount of diluted BTXA as measured in milliliters of saline used for reconstitution. The concentration is the dose divided by the volume. In children, the total amount of BTXA is indicated either in units per body weight (U/kg) or as an absolute total dose in units (Kinnett 2004).

As already mentioned, in the rodent study of Shaari and Sanders (1993) the paralysis area doubled with a 25-fold increase in dose (constant volume) and with a 100-fold increase in volume (constant dose). Sloop and colleagues (1996) injected saline or seven varying doses of Botox^{R} (1.25, 2.5, 5, 7.5, 10, 15, or 20 U; constant volume 0.2 ml) into the EDB muscles in healthy volunteers and obtained a significant dose-dependent relationship between the dose and the decrement in EDB M-response amplitude, area and mean rectified voltage. They showed denervation up to 85% at a medium range dosage of 7.5 U per EDB, after which the logarithmic curve reached a plateau. Additionally, they indicated that in humans a doubling of paralysis was obtained by a 4-fold increase in dose in the lower dose range up to 7.5 U. These findings were later replicated by Dressler and Rothwell (2000) in adult cervical dystonia patients, using amplitude reduction of the maximal voluntary contraction of the sternocleidomastoid muscle. The reduction was as follows: with Botox^{R} 20 U 80%, 40 U 84%, 60 U 85% and 80 U 91%, and with Dysport^{R} 100 U 70%, 200 U 85%, 300 U 83%, 400 U 78% and 500 U 91%. Autti-Rämö and colleagues (2001) obtained a statistically significant relationship between the dose/kg of Botox^{R} and the weakness of the flexor carpi radialis measured by the reduction in M-response area on EMG. In the spastic upper limb in nine children they showed denervation up to 94% at a dose of 1.4 U/kg and recommended injecting the wrist flexors with fewer than 1.5 U/kg per muscle. These results suggest that an optimum dose per muscle exists, after which the effect does not increase in a given muscle.

Since the first report of Koman and colleagues (1993) on BTXA treatment in children, using a total dosage of 1-2 U/kg, the amounts used at a single session have increased (Kinnett 2004), doses over 16 U/kg now being frequently administered. There has been a corresponding increase in the amounts used in single muscles. This elevation is, however, less pronounced than in the total dose, which may reflect injection of more muscles per session (Kinnett 2004). Guidelines for treating children have been published by groups under Russman (2002) and Graham (2000) based on the experience of experts in combination with research known at that time. Recently, the European Consensus Table (Heinen et al. 2006) has been developed, the maximum total amount being set at 400-600 U or 25 U/kg for Botox^{R} and 900 U or 15-25 U/kg for Dysport^{R} (Heinen et al. 2006)(See Table 8). Adult dosing recommendations are to be substituted for children heavier than 60 kg (Russman et al. 2002).

Lower limb. Russman and associates (2002) proposed for the gastrocnemius (medial or lateral part) a dose of 3-6 U/kg (Botox^{R}). Likewise, Graham and colleagues (2000) supported a dosage of 3-6 U/kg (Botox^{R}) for the lower limb, but the muscles in question are not specified. Studies focusing on the effects of different doses in children are few. Wissel and associates (1998) investigated the effect of a low dose (100 U Botox^{R} per treated leg, with a mean of 6 U/kg) and

high dose (200 U per treated leg, with a mean of 12 U/kg) in a study in which all injections were given at two levels (gastrocnemius and hamstrings or gracilis). Both groups benefited in terms of reduction in muscle tone, ankle and knee ROM, stride length and velocity, but the improvement was significantly more marked in the high-dose group. However, the use of two fixed absolute total doses may have influenced the results in favor of patients with lower body weights. In a study by Polak and colleagues (2002) the two doses selected for comparison were smaller (8 U/kg and 24 U/kg of Dysport^{R}; with a conversion ratio of 1:4 corresponding to approximately 2 and 6 U/kg of Botox^{R}), given into the gastroc-soleus muscle for hemiplegic children. They found the effect of the higher dose to be more pronounced and long-lasting and suggested that the absolute dose optimal range should remain between 200 and 500 U per gastroc-soleus muscle (50-125 U of Botox^{R}). Baker and associates (2002) studied calf muscle injections at three dose levels (10, 20, and 30 U/kg of Dysport^{R}; approximately 2.5, 5, and 7.5 U/kg of Botox^{R}) and placebo, divided evenly over both legs for diplegic children. All BTXA groups benefited compared to placebo, but the effect was most pronounced in the 20 U/kg groups.

Upper limb. Doses of 0.5-1 U/kg for the adductor pollicis, 1-2 U/kg for the forearm muscles and 2-3 U/kg for the arm muscles have been recommended (Graham et al. 2000, Russman et al. 2002). Recently, Kawamura and colleagues (2007) in their double-blind randomized trial of high- and low-dose groups obtained no between-group difference in MAS, QUEST, GAS or PEDI. They proposed dosage as follows: biceps 1 U/kg, brachioradialis/pronator teres 0.75 U/kg, finger/wrist flexors 1.5 U/kg, adductor/opponens pollicis 0.3 U/kg (max. 10U).

The dosages used in various upper and lower limb studies with CP children are depicted in Tables 9-11. Detailed dosage guidelines for each muscle are also available in WeMove webpages at http://www.mdvu.org/resource library/ dosingtables.

2.3.5. Dilution

Animal studies have demonstrated that increasing injection volume increases muscle paralysis (Shaari and Sanders 1993 in rat tibialis anterior muscle, Kim et al. 2003 in rabbit gastrocnemius muscle). In rabbits, high-dilution preparations (Botox^{R} 10U/0.5 ml), with or without electrical stimulation and exercise, have resulted in more extended gastrocnemius paralysis and histological changes compared with low-dilution preparations (10 U/0.1 ml) (Kim et al. 2003).

In a study by Francisco and associates (2002), 13 adults who had both a wrist and finger flexor spasticity score of 3 on the Modified Ashworth Scale were randomized to be injected with dilutions of either 100 U/1 ml or 100 U/2 ml of Botox^{R}. The dose was kept at 60 U per muscle. No statistically significant difference between the groups was detected in muscle tone (Modified Ashworth Scale) and the Global Rating Scale, though there was a trend toward greater improvement in the more diluted preparation group. The sample size was small and the dilution difference between the two preparations might not have been large enough to exert the desired effect. In the aforementioned study by Gracies and colleagues (2002), even more diluted preparations were used, showing

significant improvement in strength and electrophysiological measurements. A group under Lee (2004) investigated 17 children whose calf muscle spasticity score on the Modified Ashworth Scale was 2-3. The right gastrocnemius was injected with a high-volume (100 U/4 ml) and the left gastrocnemius with a low-volume (100 U/1 ml) preparation of Botox^{R}. No significant differences between the groups were noted in spasticity (measured on the Modified Ashworth Scale and as dynamic muscle length), passive ROM, and amplitude and area of compound muscle action potential or pain at injection site. The total dose for each subject was not standardized and ranged from 25 to 140 U, and the follow-up was only 4 weeks. Since the muscles of children may be smaller, the BTXA dose may reach the endplates despite the volume used.

The most common concentrations used in pediatric studies have been 100 U/ml or 50 U/ml for Botox^{R} and 200 U/ml for Dysport^{R} (Tables 10-11).

2.4. BTXA treatment in children with spastic CP

2.4.1. Indications and outcome

BTXA has rapidly been adopted into the armament for treating focal spasticity or dystonia of different etiology. In Finland – as in most other European and North-American countries – BTXA has been registered for the treatment of spastic equinus in children with CP from 2 years of age, at a maximum dose of 4U/kg Botox^{R} or 10 U/kg Dysport^{R} per gastrocnemius muscle in hemiplegia and 6 U/kg Botox^{R} or 20 U/kg Dysport^{R} divided between the two limbs in diplegia (CD Pharmaca 2007). Thus at present, treatment of other spastic muscles in the upper or lower limb or with other indications is off-label. Physicians administering BTXA must have adequate training and experience in diagnosing CP spasticity and using BTXA (CD-Pharmaca 2007).

In CP with generalized abnormality of muscle tone, BTXA is used as focal treatment for a dynamic muscle imbalance interfering with function or causing pain, in the absence of fixed contracture (Cosgrove et al. 1994). The general aims of the treatment are 1) through weakening of the spastic muscle to enhance muscle growth and allow the weak antagonists to strengthen, 2) to enhance the training of functional goals and 3) in the long run to prevent the development of secondary problems such as bony deformities (Chin and Graham 2003, Koman et al. 2003). Temporary relief of muscle hyperactivity gives an opportunity to ease passive stretch whether through ground reaction force for the calf muscle while walking, antagonistic activity for the elbow or wrist extensors, or use of stretching therapies (splints, orthoses, casting, manual stretch, posturing). The ultimate aim is to improve the quality of life for the child and caregivers: to improve function (use of arm/hand and lower limb), to ease activities of daily living, to ease hygiene and caretaking, to enhance social participation by improving cosmesis and execution of activities, and to reduce pain (Chin and Graham 2003, Koman et al. 2003). Common patterns of spasticity, specific goals for treatment, muscles involved and recommended doses and numbers of injection sites for each muscle are shown in Table 9.

Table 9. Specific indications, goals, muscles involved, doses and number of injection sites for each muscle. Adapted from Russman et al. 2002, Chin et al. 2003 and Koman et al. 2003. Doses are for Botox^{R}.

Indication/ Clinical pattern	Goals	Muscles involved	DoseU/kg of Botox^{R}	Number of sites
Equinovarus foot	Correct excessive plantarflexion	Gastrocnemius mediale	1-3	1-2
	Allow heel strike	Gastrocnemius laterale	1-3	1-2
	Improve balance and gait	Soleus	1-3	1-2
	Help wearing orthoses	Tibialis posterior	1-2	1-2
Toe clawing	Improve balance and gait	Flexor digitorum longus /brevis	1-2	1
	Prevent toe-turning	Flexor hallucis longus	1-2	1
Striatal toe	Allow footwear	Extensor hallucis longus	1-2	1
Flexed knee	Improve balance, gait, stride length	Semitendinosus	1-3	1-2
Crouch gait	Allow heel strike	Semimembranosus	1-3	1-2
	Improve sitting	Biceps femoris	1-3	1-2
		Gastrocnemius mediale /laterale	3-6	1-4
Scissoring gait,	Improve balance, scissoring gait	Adductor longus	1-4	1-2
adducted thighs	Ease perineal hygiene	Adductor brevis	1-4	1-2
	Help wearing abductor braces	Adductor magnus	1-4	1-2
	Improve sitting	Gracilis	1-2	1-2
	Ease post-operative pain/spasms			
Stiff knee	Ease knee flexion at swing phase	Rectus femoris	1-3	2
Flexed hip	Improve gait	Iliacus/Psoas	2-4	1-2
Crouch gait	Ease hip extension	Rectus femoris	1-3	2
Thumb-in-palm	Improve thumb abduction	Adductor pollicis brevis	0.5-1	1
	Improve pinch, opposing	Flexor pollicis brevis	0.5-1	1
	Help wearing orthoses	Flexor pollicis longus	0.5-1	1
	Improve hand opening	Interosseus dorsalis I	0.5-1	1
Clenched fist/	Improve hand opening	Interosseus muscles	0.5-1	1
hand	Help wearing orthoses	Lumbricales muscles	0.5-1	1
	Ease skin hygiene in palm	Flexor digitorum superf.	1-2	1-4
		Flexor digitorum prof.	1-2	1-4
		Flexor pollicis longus	1-2	1-2
Flexed wrist	Allow wrist extension	Flexor carpi radialis	1-2	1
	Help wearing orthoses	Flexor carpi ulnaris	1-2	1
Pronated forearm	Allow supination	Pronator teres	1	1
	Improve dexterity	Pronator quadratus	1	1
Flexed elbow	Allow elbow extension	Biceps brachii	2-3	2-4
	Improve reaching	Brachialis	2	1-2
		Brachioradialis	1-2	1
Adducted/	Allow shoulder abduction	Pectoralis major	2	2-3
internally	Improve balance and gait	Subscapularis	1	1-2
rotated shoulder	Ease dressing	(Teres major)	1	1
		(Latissimus dorsi)	1-4	2
Externally rotated	Allow shoulder adduction	Infraspinatus	1-2	1-2
shoulder	Improve balance and gait	Teres minor	1	1
	Ease dressing	Long head of triceps	2-3	2-3
	Improve reaching	Latissimus dorsi	1-4	2

The lower limb. In the lower limb, a number of randomized, double-blind, placebo-controlled and other controlled trials (see Table 10) have established the short-term (3-6 months) effect on active and passive ROM, reduction in muscle tone, improvement in gait pattern and safety. The dynamic equinus gait is the most common indication and in a recent meta-analysis comprising six double-blind placebo-controlled studies with a mean follow-up of 3.25 months (range 6 weeks-6 months) BTXA was shown to be superior to placebo (Cardoso et al. 2006). In consequence of the heterogeneity of patients and indications, the variety and insufficient sensitivity of outcome measures applied, the variety of doses and injection techniques used and the alternation of follow-up periods, it has been difficult to draw clear-cut conclusions as to the effect of BTXA treatment on function in other indications than equinus gait (Berweck et al. 2003). In a Cochrane systematic review including only three controlled studies, the authors found "no evidence to support or refute the efficacy of BTXA in improving function" (Ade-Hall and Moore 2000).

The upper limb. In the upper limb, the only randomized, double-blind, placebo-controlled study, that by Corry and associates (1997), clearly showed a BTXA short-term (12 weeks) effect on muscle tone reduction compared to placebo, while the effect on fine motor functions was modest (see Table 11). However, the cosmetic effect was valued by both participants and parents.

In the upper limb it has been even more difficult to verify the BTXA effect on function than in the lower limb. Cochrane´s systematic review including two controlled studies found "no evidence to support or refute the efficacy of BTXA as an adjunct to managing the upper limb in children with spastic CP" (Wasiak et al. 2005). Pain and spasm relief in the arm, relaxation of elbow, wrist and finger flexion, and better posturing of the arm have been reported, but this does not necessarily translate into better dexterity (Autti-Rämö et al. 2000, Wallen et al. 2004). The relaxation of spasticity may be hampered by deficits in motor control or sensation, learned non-use of the affected arm or intellectual problems. Groups under Fehlings (2000) and Lowe (2006) both demonstrated an improvement on the QUEST, a test performed under verbal instruction and illustrating what the child can do. This, however, tells nothing of what the children actually do in real-life situations. Two recent randomized controlled studies showed BTXA to enhance the attainment of functional goals in GAS or COPM over a short term of 3 months, but children on occupational therapy likewise improved (Wallen et al. 2007, Russo et al. 2007)(See Table 11). The authors emphasized the careful selection of patients and definition of specific goals for the treatment.

Other indications for BTXA treatment in CP include control of post-operative pain (Barwood et al. 2000) and help in pre-operative decision-making in upper and lower limb surgery (Autti-Rämö et al. 2000).

Table 10. Lower limb studies in children with CP in order of publication year.

Study (N) *Design*	*Age (years)* *Patient description* *Severity*	*Dosage* *Muscles* *Dilution*
Single level treatment		
Koman et al. 1993 (n=27) Open label, prospective	3-16 H, D, Q Moderate to severe	Botox 1-4 U/kg/gastroc-soleus, also Botox 1-5 U/kg given into paraspinals, adductors or hamstrings Dilution not reported
Cosgrove et al. 1994 (n=26) Open label, prospective	2-17 H, D, Q Mild to severe	Dysport 5-28 U/kg into gastroc-soleus (+tib.post), medial hamstrings Doses per muscle not defined 200U/ml
Koman et al. 1994 (n=12) Double-blind placebo-controlled RCT	4-11 H, D Unknown	Botox 1 U/kg/gastroc-soleus Dilution not reported
Corry et al. 1998 (n=20) RCT BTXA vs casts 4-6 wks	2-9 H, D, Q Ambulatory	Botox 6-8U/kg/gastroc-soleus Dysport 15 U/kg/gastroc-soleus For two legs the dose was divided Botox 100U/ml, Dysport 200U/ml
Flett et al. 1999 (n=20) RCT BTXA vs casts 2x2 wks Night splints after 8 wks	2-5 H, D, Q Ambulatory	Botox 4-8 U/kg/gastrocs For two legs the dose was divided Dilution not reported
Sutherland et al. 1999 (n=20) Double-blind placebo-controlled RCT	2-12.5 H, D, Q Ambulatory	Botox 4 U/kg/gastrocs at 0 and 4 wks For two legs the dose was divided Dilution not precisely defined
Koman et al. 2000 (n=114) Double-blind placebo-controlled RCT	2-16 H, D Ambulatory	Botox 4 U/kg/gastrocs at 0 and 4 wks For two legs the dose was divided 25U/ml for H; 12.5U/ml for D
Ubhi et al. 2000 (n=40) Double-blind placebo-controlled RCT	2-16 H, D Ambulatory	Dysport 25U/kg for diplegic, 15U/kg for hemiplegic Gastroc-soleus (3 hamstrings) 200U/ml
Love et al. 2001 (n=24) RCT BTXA vs control	3-13 H GMFCS I	Botox 2.5 - 4.7U/kg/gastroc-soleus 100U/1 ml (3 had 100U/5 ml)
Hamstring studies		
Corry et al. 1999 (n=10) Open label, prospective	4-11 H, D, Q Ambulatory	Botox 5-8U/kg or Dysport 6U/kg Hamstrings not specified Dilution not reported
Thompson et al. 1998 (n=10) Retrospective	4-12 D, Q Ambulatory	Botox 5-8U/kg or Dysport 6U/kg Hamstrings not specified Dilution not reported

Table 10 Continued

Number of sites *Injection techniques* *Assessments*	*Outcome measures: Change*
2-4 sites/muscle Palpation Pre, every 2 wks ad 27 mos	PRS: Improvement ++ Muscle tone: Reduced + PROM: Increased +
4 sites/gastroc-soleus 1 site/hamstring Palpation Pre, ad 26 wks	3D gait analysis: ++ Muscle tone: Reduced + PROM: Increased +
2-4 sites/muscle Palpation Pre, 2, 6 wks	PRS: BTXA + Energy cost: both 0 Parental rating: BTXA +
4 sites/gastroc-soleus Palpation Pre, 2, 12 wks	3D-gait analysis: BTXA ++ PRS: both ++ PROM: both ++ MAS: BTXA ++ at 2 wks
$\geq$ 2 sites/muscle Palpation Pre, 2, 4, 6 mos	PRS & GMFM: both ++ MAS & PROM: both ++ Global scoring: both 0 Parental satisfaction: both ++
2 sites/muscle Palpation Pre, 8 wks	3D gait analysis: BTXA ++ PRS: BTXA ++ PROM & EMG: both 0
Unknown Palpation Pre, 2, 4, 8, 12 wks	PROM: both 0 AROM & PRS & EMG/M-response: Improvement BTXA ++
Unknown Palpation Pre, 2, 6, 12 wks	VGA: BTXA ++ at 6 and 12 wks GMFM: BTXA ++ at 12 wks Physiological cost index: both 0 PROM: both +
"Several sites" Palpation Pre, 1, 3, 6 mos	GMFM: BTXA ++ at 3 and 6 mos PROM: BTXA ++ at 3 and 6 mos MAS: BTXA ++ at 3 and 6 mos MTS: BTXA ++ at 3 and 6 mos Parental satisfaction: BTXA ++
Not reported Palpation Pre, 2,12 wks	3D-gait analysis: knee extension, knee angle at initial contact and walking speed: ++ at 2 wks Popliteal angle: ++ at 2 and 12 wks Energy cost: 0
2-3 sites at the junction of the prox.1/4 & dist. 3/4 of the muscle length Palpation 2 wks	Walking speed: Increase ++ Excursion of short muscles: Increase ++ Knee extension: Improvement ++

Table 10 Continued

Study (N) *Design*	*Age (years)* *Patient description* *Severity*	*Dosage* *Muscles* *Dilution*
Adductor studies		
Barwood et al. 2000 (n=16) Double-blind placebo-controlled RCT	Mean 4.7 D, Q GMFCS IV-V Hips at risk	Botox 8U/kg/child into adductors 100U/ml
Boyd et al. 2001 (n=39) RCT BTXA + Hip orthosis vs. best clinical practice	1.6-4.8 D, Q GMFCS II-V	Botox 16U/kg/child into adductors and med.hamstrings at 6 mo intervals 100U/ml
Mall et al. 2006 (n=61) Double-blind placebo-controlled RCT	3-9 D, Q GMFCS I-V	Dysport 30U/kg (max. 1500U) into adductors (2/3 of the dose) and medial hamstrings (1/3) 250U/ml
Multilevel treatment		
Reddihough et al. 2002 (n=49) RCT BTXA + PT vs PT alone Cross over after 6 mo	2-7 D, Q GMFCS I-IV	Botox 8-20 U/kg into multiple muscles and into 1-2 levels, 3-6U/kg/muscle Dilution not reported
Scholtes et al. 2006 & 2007 (n=46) RCT BTXA + intensive PT vs PT alone	4-11.5 H, D GMFCS I-IV	Botox 5.6-27.1 U/kg total into multiple muscles Multilevel treatment 4-6U/kg/muscle 50U/ml
Desloovere et al. 2007 (n=60) Retrospective case-control BTXA and best clinical practice vs. best clinical practice Treated between 1987-2001	3-9 H, D GMFCS I-III	Botox 6-23 U/kg for H, 14-31 U/kg for D into multiple muscles Multilevel treatment 50U/ml

Abbreviations in Table 10.
0, no change; +, trend of improvement; ++, significant improvement ($p < 0.05$).
RCT, randomized controlled trial; H, hemiplegic; D, diplegic; Q, quadriplegic; GMFCS, Gross Motor Function Classification System; U, units; Pre, pre-treatment assessment; PT, physiotherapy; PRS, Physician's Rating Scale; PROM, passive range of movement; AROM, active range of movement; MAS, Modified Ashworth Scale; 3D, three-dimensional; GMFM, Gross Motor Function Measure; EMG, electromyography; MTS, Modified Tardieu Scale; VAB, Vulpe Assessment Battery; VGA, video gait analysis; GAS, Goal Attainment Scale.

Table 10 Continued

Number of sites *Injection techniques* *Assessments*	*Outcome measures: Change*
2 sites/muscle Palpation 3 mos	Mean pain scores: BTXA ++ Length of admission: BTXA ++ Analgesic requirments: BTXA ++
2 sites per muscle Palpation Pre, 6, 12 mos	GMFM: both 0 5% (2 children) in BTXA group and 18% (7 children) in the control group progressed to surgery
Not reported Palpation Pre, 4, 12 wks	MTS: BTXA ++ at 4 and 12 wks MAS: BTXA ++ at 4 and 12 wks GAS: BTXA ++ at 4 and 12 wks GMFM: both 0
2 sites/hamstring and adductors 4 sites/gastroc-soleus Palpation Pre,3, 6, 12 mos	GMFM: both + VAB: BTXA ++ at 3 mos MAS: BTXA + PROM: + Parental satisfaction: BTXA ++
2 sites/muscle Palpation or stimulation Pre x 2, 6, 12, 24, 48 wks	GMFM: BTXA ++ at 12 and 24 wks PROM: BTXA ++ ad 24 wks "Problem score": BTXA ++ at 12 and 24 wks MTS: BTXA ++ at 6 and 12 wks VGA: BTXA ++ Energy cost: both 0
Multiple sites/muscle Palpation At least one 3D-gait analysis between ages 5-10 years	3D-gait analysis: BTXA ++ towards more normal gait pattern No differences in kinetics and EMG Controls:Anterior pelvic tilt increase

Table 11. Upper limb studies in children with CP in order of publication year.

Study (N) *Design*	*Age (years)* *Patient description*	*Dosage & Dilution* *Injection techniques*
Corry et al. 1997 (n=14) Double-blind placebo-controlled RCT	4-19 H, D, Q Dynamic contracture	Botox 4-7 U/kg total 100U/ml Dysport 8-9 U/kg total 200U/ml 1-2 sites per muscle Palpation only
Fehlings et al. 2000 (n=30) Single-blind RCT BTXA+OT vs OT only	2.5-10 All H MAS $\geq$ 2, full PROM Ability to initiate voluntary movement	Botox 2-6.6 U/kg total injected into 1-3 muscle groups Dilution not reported 1-2 sites per muscle Palpation only
Wallen et al. 2004 (n=16) Open-label case series	2-12 H, D, Q MAS 2-3 Ability to initiate voluntary movement	Botox 0.5-2 U/kg/muscle 100U/ml Palpation + EMG + muscle stimulation
Speth et al. 2005 (n=20) RCT (non-blinded) Matching for age and Zancolli grade BTXA+OT+PT vs OT+PT	4-16 All H Zancolli I to IIB	Botox 1-3 U/kg/muscle 50 U/ml & 30 min PT+ 30 min OT 3 X weekly for 6 mos for both groups & night splints Palpation + muscle stimulation
Lowe et al. 2006 (n=42) Single-blinded RCT BTXA+OT vs OT only	2-8 All H MAS $\geq$ 2 Ability to initiate voluntary movement	Botox 0.5-2 U/kg/muscle 200U/ml & OT + individualized home programmes Palpation + EMG + muscle stimulation
Wallen et al. 2007 (n=80) RCT (non-blinded) BTXA + OT or OT only or BTXA only (+ ordinary OT) or no-treatment group (i.e. ordinary OT)	2-14 H, D, Q MAS 2-3 Ability to initiate voluntary movement	Botox 0.5-2 U/kg/muscle 100U/ml OT groups: 1 h/wk for 12 wks Palpation + EMG + muscle stimulation
Kawamura et al. 2007 (n=39) Double-blind RCT High- vs low-dose groups	3-10 H, D MAS $\geq$ 2 Ability to initiate voluntary movement	High: Botox 50-200 U/ml; biceps 2U/kg; brachioradialis 1.5U/kg; common flexor origin 3U/kg; pronator teres 1.5U/kg; adductor/opponens pollicis 0.6U/kg to a max. 20U Low: 50% of these doses

Table 11 Continued

Assessments *Outcome measures: Change*
Pre, 2, 12 wks AROM & tone: BTXA active elbow/thumb extension, wrist/elbow tone ++ at 2 wks Grasping & releasing: BTXA ++ at 12 wks Finger-thumb pinch/coin transfer: 0 General impression of parent: Pleased with cosmesis in BTXA group Wrist resonant frequency: BTXA ++ at 2 and 12 wks
Pre, 1, 3, and 6 mos QUEST: BTXA ++ at 1 mo, but not at 3 or 6 mos PEDI: BTXA + at 1 mo and 6 mos PROM & Grip strength: 0 MAS at elbow, wrist, supination and thumb: 0
Pre, 2 wks, 3 and 6 mos MAS: elbow, pronator and wrist ++ at 2 wks, remaining ad 3 mos for wrist MTS: elbow and pronator ++ at 2wks, remaining ad 6 mos for elbow COPM: ++ at 3 and 6 mos GAS: Increase in T-score generally; 8/16 at 6 mos attained their goals Melbourne & CHQ & AROM & PROM: 0 Caregivers´ subjective ratings: ++ at 2 wks and 3 mos, and return to baseline by 6 mos
Pre, 2 and 6 wks, 3, 6, and 9 mos AROM & PROM: BTXA active wrist extension, thumb abduction +; supination ++ Ashworth scale: BTXA wrist and elbow tone reduction ++ at 2 wks; control + Melbourne & PEDI: both 0; Nine hole peg test: BTXA +, control 0 Satisfaction: BTXA children considered their hand function better at 3, 6 and 9 mos, but parents noted no difference in function 9/10 in BTXA and 6/10 in control group reached their goals
Pre, 1, 3, 6 mos QUEST & MAS: BTXA ++ at 1 and 3 mos, but not 6 mos COPM & PEDI: Both groups improved, but more so in BTXA group GAS/family & therapy: Both groups improved ad 6 mos, but more so in BTXA group
Pre, 2 wks, 3 and 6 mos QUEST & PEDI & CHQ & PROM: All groups 0 MTS: BTXA+OT and BTXA ++ at 2 wks and 3 mos, but not at 6 mos COPM: BTXA+OT ++ at 3 mos, but not at 6 mos GAS: BTXA+OT ++ at 3 mos and all but control group ++ at 6 mos Caregivers´ subjective ratings: BTXA+OT at 2 wks and all but control group at 6 mos AROM: Active supination ++ in BTXA and BTXA+OT groups at 6 mos Melbourne: BTXA+OT + at 3 mos, OT + at 6 mos
Pre, 1 and 3 mos QUEST & PEDI & PROM: No between-group difference GAS: Both groups attained T score 50 Grip strength & MAS: Decrease in both groups, no between-group difference

Table 11 Continued

Study (N) *Design*	*Age (years)* *Patient description*	*Dosage & Dilution* *Injection techniques*
Russo et al. 2007 (n=43) Single-blind RCT BTXA + OT vs OT only	7-10 H MAS $\geq$ 2 Ability to initiate voluntary movement	Botox 5-11.6 U/kg total dose (doses per muscle not spesified) 100 U/ml & OT 1x weekly for 4 weeks Palpation + EMG + muscle stimulation

Abbreviations in Table 11.
0, no change; +, trend of improvement; ++, significant improvement ($p< 0.05$).
RCT, randomized controlled trial; H, hemiplegic; D, diplegic (triplegic); Q, quadriplegic; U, units; Pre, pre-treatment assessment; PT, physiotherapy; OT, occupational therapy; PROM, passive range of movement; AROM, active range of movement; MAS, Modified Ashworth Scale; QUEST, Quality of Upper Extremities Test; PEDI, Pediatric Evaluation of Disability Inventory; EMG, electromyography; MTS, Modified Tardieu Scale; COPM, Canadian Occupational Performance Measure; Melbourne, Melbourne Assessment; GAS, Goal Attainment Scale; CHQ, Child Health Questionnaire; PEDsQL, Pediatric Quality of Life Inventory; AMPS, Assessment of Motor and Process Skills.

Table 11 Continued

Assessments *Outcome measures: Change*
Pre, 3 and 6 mos
MTS & MAS: BTXA+OT ++ at 3 and 6 mos
Subjective evaluation on function: BTXA+OT ++ at 3 and 6 mos
Subjective evaluation on cosmesis: BTXA+OT ++ at 3 mos, 0 at 6 mos
GAS & Global Self-Worth: BTXA+OT ++ at 3 mos, 0 at 6 mos
PEDI & PEDsQL & AMPS: Increase in both groups, no between-group-difference

2.4.2. Effects of repeated injections

The long-term effects of repeated injections of BTXA in CP spasticity are as yet unknown. It is hoped that regular injections may delay or reduce the need for surgical interventions (Eames et al. 1999). Such assumptions are based on findings in a hereditary spastic mouse model (Cosgrove and Graham 1994), where BTXA injected into the gastrocnemius of infant mouse before they developed spasticity prevented the development of muscle contracture. BTXA administered into the gastrocnemius or hamstrings has been shown to increase the length of these muscles in CP children (Thompson et al. 1998, Eames et al. 1999). The maturing and growth of a child takes a much longer time compared with the mouse, and the long-term results after repeated injections and follow-up are as only emerging as the children first treated are now reaching their teens. However, there is a growing body of evidence to suggest that BTXA, combined with other treatment modalities, may have postponed the age for surgery, reduced the incidence of re-operations and lowered the prevalence of surgical procedures (Garcia Ruiz et al. 2000, Metaxiotis et al. 2002, Hägglund et al. 2005, Molenaers et al. 2006). In most of these studies no control group has been used and improvements over time are confounded with natural development and a combination of other treatment modalities. Thus, the effect cannot be attributed to one treatment only.

2.4.3. Post-treatment modalities

Studies of BTXA post-treatment modalities are gathered in Table 12. An occupational or physiotherapy programme with targeted motor training and stretching aiming to achieve carry-over improvement persisting beyond the pharmacologic effect of the treatment is regarded as central (Graham et al. 2000). No comprehensive recommendations on the frequency of post-treatment therapy are to hand. Orthoses are used in conjunction with therapy and are considered particularly useful with muscles not stretched by weight-bearing alone, for example in upper limb (thumb abduction splints), adductors (hip abductor braces) and hamstrings (knee orthoses) (Graham et al. 2000, Berweck et al. 2003). However, no systematic studies have been conducted on this subject.

There is evidence that BTXA alone is as effective as casting alone (Corry et al. 1998, Flett et al. 1999), but the optimal timing and the value of combining casting with BTXA remains open. Some clinicians cast immediately after a BTXA injection, others prefer to delay casting for 2 weeks to distinguish the effect of BTXA and to facilitate tolerance of casting (Graham et al. 2000). Casting without BTXA usually requires 4-6 weeks, but with BTXA 1-3 weeks is sufficient (Berweck et al. 2003).

Electrical stimulation combined with BTXA has not been found to enhance the treatment effect in spastic equinus gait (Detrembleur et al. 2002), but further studies are needed on this issue. At present, there are no published data on activation of the weak antagonists with electrical stimulation after BTXA treatment of the agonists in children.

Abbreviations in Table 12.
RT, randomized trial; H, hemiplegic; D, diplegics; Q, quadriplegic; U, units; Pre, pre-treatment assessment; Post, post-treatment; GMFCS, Gross Motor Function Classification System; PROM, passive range of movement; AROM, active range of movement; PRS, Physician's Rating Scale; MAS, Modified Ashworth Scale; 3D, three dimensional; MTS, Modified Tardieu Scale; GMFM, Gross Motor Function Measure; Hz, hertz; msec, milliseconds; mA, milliamperes

Table 12. Studies of post-treatment modalities in children with CP in order of publication year.

Study (N) *Design*	*Age (years)* *Patient description*	*Dosage & Dilution* *Other intervention*	*Assessments* *Outcome measures*	*Results*
Desloovere et al. 2001 (n=34) Prospective RT 2 groups: casts before or after BTXA injection	4-10 H, D Ambulant Equinus	Botox 12-31U/kg; 50U/ml Multilevel injections into several muscles Pre- or post-BTXA casting for 10-28 days	Pre, 1, 2, 6 mos 3D-gait analysis Parental satisfaction	Post-BTXA casting slightly better than pre-BTXA casting in dynamic equinus
Bottos et al. 2003 (n=10) Prospective RT 2 groups: BTXA only or BTXA + casts	4-11 All D Ambulant Equinus	Dysport 15-20 U/kg/gastroc-soleus Dilution not reported Casts applied immediately for 3 wks	Pre, 1, 4, 12 mos PROM & MAS GMFM 3D-gait analysis	BTXA+casting better than BTXA only in dynamic equinus
Kay et al. 2004 (n=23) Prospective RT 2 groups: BTXA + casts or casts only	4-14 H, D, Q Ambulant Equinus	Botox 4-8U/kg/gastrocnemius Dilution not reported Casts 1-3 wks after BTXA or only casts immediately; changed every 2 wks until 5^{o} dorsiflexion was achieved	Pre, 3, 6, 9, 12 mos PROM MAS GMFM 3D-gait analysis	Casting alone better than BTXA+casting in fixed equinus
Glanzman et al. 2004 (n=55) Retrospective 3 groups: BTXA only, BTXA + casts or casts only	3-12 H, D, Q GMFCS I-V	Botox 1.8-7.6U/kg; dilution not reported Multiple muscles injected Casts changed weekly until 10^{o} dorsiflexion achieved	Wks to receive 10^{o} dorsiflexion PROM	BTXA+casting & casting alone equally better than BTXA alone in dynamic equinus
Ackman et al. 2005 (n=39) Double-blind placebo-controlled 3 groups: BTXA only or BTXA+ casts or casts + placebo	3-9 H, D GMFCS I-II	Botox 4U/kg/gastrocs; 100U/ml Only BTXA or BTXA + casts 3 for wks or placebo+ casts; three injections/ castings repeated every 3 mos	Pre, 3, 6, 7.5, 12 mos 3D-gait analysis MAS & MTS AROM & PROM	BTXA+casting & placebo+casting equally better than BTXA alone in dynamic equinus
Detrembleur et al. 2002 (n=12) Prospective RT 2 groups: electrical stimulation or non-stimulation	4-6 H, D Ambulant Equinus	Botox 2-5 U/kg/muscle; 50U/ml Electrical stimulation 20 hz, 0.2 msec, 50-90 mA for 30 min x 6 per day, for 3 days, beginning on BTXA treatment day	Pre, 1, 3, 6 mos 3D-gait analysis MAS & PROM PRS	No significant difference between the treatment groups in any parameter

2.4.4. Role of BTXA treatment in spastic CP

BTXA has rapidly been accepted in the neuropediatric rehabilitation field and has found its place in the management of CP. The role of BTXA is flexible but limited by the temporary mode of action as a focal spasticity treatment and restrictions on the total dose deliverable per visit. BTXA should always be regarded as an adjunctive therapy to be combined with other therapies and treatment modalities to fit the child's situation. Thus, formulation of long-term goals for the entire rehabilitation plan for a given CP child determines in part the short-term goals, timing and number of treatments for BTXA therapy.

The indications and short-term goals have already been mentioned in paragraph 2.4.1 and in Table 9. As younger children are regarded as having more dynamic spasticity and less fixed contracture than older, they respond better to BTXA treatment (Graham et al. 2000, Preiss et al. 2003). In childhood, the walking pattern often continues to mature until the age of 7 years and bimanual functions up to 10 years, taking an even longer time with some CP children (Johnson et al. 1997, Forssberg 1999).

In the lower limb, BTXA is often used as a means of "buying time" until the child is sufficiently mature for more definitive procedures. It is thus considered that e.g. spastic equinus management with BTXA is useful from age 2 to 6 years, but that equinus in most children over the age of 6 years requires muscle-tendon lengthening (Graham 2004, Gage and Schwartz 2004). Some orthopedic teams advocate the age of 1 to 5 in the lower limb as the best timing for BTXA injections in children (Graham et al. 2000). This does not however preclude subsequent use of BTXA in selective muscles following surgical procedures. Later treatment may still yield benefit in terms of pain relief, ease of care and posture, and more specific functional goals such as standing, sitting and reaching (Graham et al. 2000).

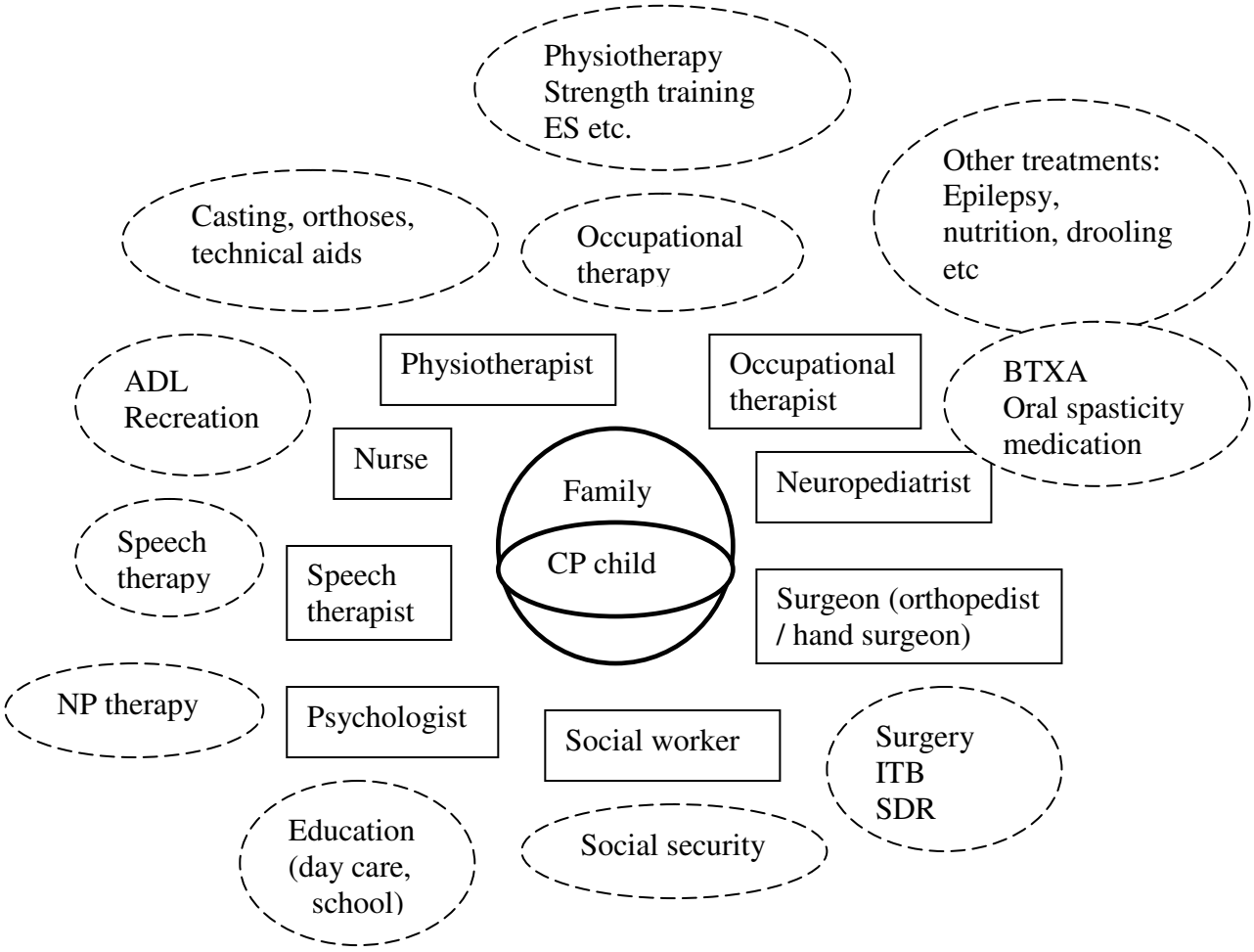
In the upper limb, it is thought that children with dynamic spastic deformities, with moderate spasticity (Modified Ashworth Scale 2-3), with the ability to initiate voluntary movements in wrist or fingers, with good motivation level and with a capacity for motor learning benefit most from BTXA treatment (Autti-Rämö et al. 2001, Chin and Graham 2003). Adequate grip strength prior to injection was found to correlate with a positive functional response (Fehlings et al. 2001). Age over 4 years is regarded as the best timing for upper limb BTXA injections in children (Graham et al. 2000).

As in orthopedic surgery in the lower limb and in hand surgery of the upper limb, BTXA can be administered as a single or multilevel treatment (Chin and Graham 2003, Molenaers et al. 2006). The objective of the single-level injection is by weakening the spastic muscle to "break" the abnormal strict movement pattern and allow the training and strengthening of the weak antagonists (Chin and Graham 2003). The multilevel treatment, in contrast focuses on enabling overall flexibility and better alignment (Molenaers et al. 2006, Desloovere et al. 2007). BTXA has proved useful in pre-operative decision-making particularly in upper limb surgery (Autti-Rämö et al. 2000), but is also used in this indication with the lower limb.

The management of spasticity, as well as management of CP as whole, is complex and calls for a multidisciplinary input from neuropediatricians, physio- and occupational therapists, orthopedic and hand surgeons, orthosists and other

allied health specialists (psychologists, speech therapists, social workers, nurses)(Thylefors et al. 2000). The multiple issues needing attention, related treatments and health specialists involved with the rehabilitation are collected by the author in Figure 7, illustrating the "network of rehabilitation" surrounding the CP child and family. Although not every profession may be required, a multifaceted and integrated approach is best in providing optimal functional outcomes and in reaching goals. In BTXA treatment, as in overall CP management, the role of occupational and physiotherapists is important in patient selection, goal-setting, pre- and post-treatment assessment of functional level, education of patient and parents and planning of adjunctive therapies (Berweck et al. 2003). The neuropediatrician is crucial in communicating the goals between patient, family and multidisciplinary team, incorporating different treatment modalities and overseeing the whole rehabilitation process.

Figure 7. The network of rehabilitation. ADL, activities of daily living; ES, electrical stimulation; NP, neuropsychological; BTXA, botulinum toxin A; ITB, intrathecal baclofen; SDR, selective dorsal ritsotomy.



Spasticity is only one component in the UMNS and frequently not the most important factor contributing to function. Other elements such as weakness, impaired selective motor control and poor perception may have a major effect on functional abilities. Some children may even need their spasticity, which can be helpful for locomotion or weightbearing (Berweck et al. 2003). As gait and upper limb problems are considered progressive, they alter with maturation (Rang 1990), and the clinician should be aware of the natural history of both the movement disorder and the child and assess the situation regularly. More important than any assessment test is the ability to interpret test results, to observe movement patterns and trends in growth, and assess family and child dynamics (Boyd 2004). To rephrase a comment by Mercer Rang (1990): “After you *inject*, patients still have cerebral palsy”.

AIMS OF THE STUDY

The general aim of the present study was to assess the effect of different BTXA injection techniques and doses in terms of reducing muscle tone, enhancing function and inducing adverse events in children with spastic CP.

The specific aims and hypotheses were as follows:

1. To prospectively explore whether injections directed as close to the NMJ zone as possible are more effective in reducing muscle tone, increasing passive ROM and improving spastic equinus gait pattern than injections given into a remote site. We also sought to compare the incidence of side-effects in the two treatment groups (I). We hypothesized that, if toxin diffusion occurs parallel to gastrocnemius fibers, the effect on calf tone and passive dorsiflexion will appear in both groups but be more pronounced in subjects distally injected. The two injection sites were chosen for their clear anatomical appearance and easy accessibility, the intervening distance being sufficient (i.e. 4 to 5 cm despite the size of the child) to be at the upper limit of possible diffusion up to 4.5 cm from the injection site, as shown in animal models (Borodic et al. 1994).
2. To prospectively assess whether the multiple-site injection technique is associated with better outcomes in reducing muscle tone, increasing passive ROM and improving spastic equinus gait pattern compared with the single-site injection method. We also examined whether the multiple-site technique was associated with fewer adverse events (II). We predicted that, if the end plates in the gastrocnemius muscle form a parabolic pattern and toxin diffusion occurs parallel to muscle fibers, the effect on calf tone, passive dorsiflexion and gait pattern will appear in both groups but be more pronounced in the multiple-points group.
3. To retrospectively evaluate the effects of and adverse events associated with individually adjusted doses in treating spastic equinus gait in a cohort of CP children, using 6 U/kg of Botox^{R} per gastrocnemius-soleus muscle as cut-off point (III). It was hypothesized that doses over this limit would not yield better results in terms of muscle tone, ROM and gait pattern.
4. To retrospectively compare the effects of and adverse events associated with low and high doses in a clinical setting of treating upper limb spasticity in a cohort of CP children with individually adjusted doses (IV). It was hypothesized that the higher doses would yield better results in terms of muscle tone, passive ROM, hand use, fine motor functions and change in movement pattern but produce more negative side-effects.

PATIENTS AND METHODS

1. Patient series and study designs

1.1. Definitions

CP was defined as "an umbrella term covering a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of its development" (Mutch et al. 1992) and classified according to Hagberg and associates (1975) and ICD-10 into spastic hemiplegia, diplegia or quadriplegia. Spastic equinus gait was defined as walking tiptoe or on the forefoot because of spasticity in the calf muscles. Spasticity was defined as "a velocity-dependent increase in tonic stretch reflexes (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex, as one component of the upper motor neurone syndrome" (Lance 1980). Fixed contracture was defined as passive plantarflexion remaining more than 15 degrees under "neutral null" position (Wissel et al. 1998).

1.2. Subjects and study designs in the injection technique studies: Near vs remote from NMJs (I) and Single vs multiple sites (II)

A total of 19 (I) and 17 (II) subjects were recruited from Tampere University Hospital, Tampere, and the Central Hospital of Kanta-Häme, Hämeenlinna, both in Finland. Two participants in each study were recruited from Hämeenlinna and the rest from Tampere.
Inclusion criteria were:
1) Diagnosis of cerebral palsy
2) Ambulation with or without devices
3) Spastic and dynamic equinus gait (i.e. no fixed contracture)
Exclusion criteria were:
1) Age over seven (I) or twelve (II) years
2) Previous serial casting or BTXA treatment within 6 months prior to enrolment
3) Previous surgery on the lower limbs

Detailed data on the treatment groups are presented in the original publications in Tables 1/**I** and 2/**II**. In study I one subject from each treatment group failed to attend the 4-month post-treatment assessment and was assessed 6 months post-injection. The intention-to-treat analysis did not alter the results and the data presented include all the 6 months´ measurements (as in the original

article). In study I three children and in study II four children were enrolled twice, with at least one year between treatments.

In both studies, a prospective, randomized, two-parallel-treatment-groups design was utilized. After the baseline measurements and informed written consent from the parents, subjects meeting the study criteria were randomized into one of the two treatment groups. These comprised the proximal (remote from the NMJ zone) and distal (as close as possible to the NMJ zone) groups in study I and the single- (injection into two sites per gastrocnemius muscle) and the multiple-points (injection into four sites per gastrocnemius mucle) groups in study II. The treating physician (HS) allocated each child by tossing a coin with face predetermined for each treatment group. Unilaterally involved (hemiplegic) vs. bilaterally involved (diplegic and quadriplegic) children were enrolled in separate categories and then paired on a calf spasticity grade measured by the modified Ashworth Scale so that after randomization both treatment groups included the same number of hemiplegics/diplegics with the same spasticity grade at the outset.

Clinical assessments were made at baseline and 3, 8 and 16 weeks post-treatment (I) or at baseline and 1, 2 and 4 months post-treatment (II) by the same pediatric physiotherapist, who continued with the child throughout the research period and was blinded to the treatment group. The treating physician assisted with the measurements by holding ankle and leg. Data on each leg were collected and analyzed: a total of 25 legs in study I and 25 legs in study II**.**

1.3. Subjects and study designs in the dose studies: Lower limb dose study (III) and Upper limb dose study (IV)

Study III. Records of 61 children treated for equinus gait between March 2000 and March 2006 at the Department of Pediatric Neurology, Tampere University Hospital, were reviewed. The inclusion criteria were:

1) Diagnosis of cerebral palsy
2) Spastic and dynamic equinus gait
3) Ambulation with or without devices
4) Treated with BTXA injections into the gastroc-soleus muscle. Additionally, a maximum of two other functional muscle groups could have been treated at the same session. Only single- or two-level treatments were accepted.
5) Having individually set goals on the Goal Attainment Scale (GAS)
6) No oral or intrathecal antispasticity medication, rhizotomy or previous surgery on the Achilles tendon
7) Baseline measurements before treatment and one to three times post-treatment
8) A questionnaire on side-effects completed by the caregivers

Twenty-nine patients fulfilling the inclusion criteria and a total of 80 legs in 55 treatments were retrospectively divided into low- or high-dose groups according to the BTXA dose used for the gastroc-soleus muscles as follows: low-dose group $\leq$ 6 U/kg per leg and high-dose group > 6 U/kg per leg. This cut-off point was chosen in keeping with the doses used in the gastroc-soleus muscle in earlier studies (Corry et al. 1998, Flett et al. 1999, Eames et al. 1999, Sutherland et al. 1999, Koman et al. 2000, Ubhi et al. 2000, Love et al. 2001),

recommendations (Graham et al. 2000, Russman et al. 2002) and observations of absence of further effect with doses over 6 U/kg (Polak et al. 2002, Baker et al. 2002). Thirty-two subjects were excluded due to failure to fulfil the inclusion criteria or having inadequate data. This study also includes the 19 treatments in study I and seven treatments from study II. Table 3/**III** shows the data on patients in the low- and high-dose groups. Data on the 80 legs were collected and analyzed**.** All children were whenever possible assessed by the same physiotherapist.

The material in ***study IV*** comprised a cohort of all the 18 children treated for upper limb spasticity at the Department of Pediatric Neurology, Tampere University Hospital, between February 1999 and October 2004 (including one child treated by an adult neurologist in September 1996). The neurologic diagnosis included prematurity in three (17%), pre- or post-partum cerebrovascular incident in four (22%), hypoxic encephalopathy in six (33%), meningomyelocele and hydrocephalus in one (5.5%), hydrocephalus in one (5.5%), and head injury in three (17%). Retrospective anonymized data gathered routinely as part of an ongoing clinical management protocol were used. The upper limb functional abilities ranged from independence in bimanual activities to a poor supportive extremity.

The three main treatment groups by indication were: 1) *functional*, to improve a specific function or quality of movement; 2) *pre-operative* evaluation, to postpone surgery or help the surgeon in planning; and 3) *non-functional*, to help children with no or minimal functional abilities or after sustained brain injury to improve posture or support on the extremity involved. The functional and pre-operative groups were combined into one, the *functional* group (n= 8 subjects), and the non-functional constituted one, the *non-functional* group (n=10 subjects). Each involved upper extremity was measured and analyzed. A total of 54 upper extremities were treated in 46 sessions: 27 extremities in the functional and 27 in the non-functional group. Under the categories functional and non-functional, children, or more precicely, the treated extremities, were retrospectively allocated to low- or high-dose groups according to the botulinum toxin dose used for the target muscles as follows:

1. Low-dose group: adductor pollicis 5 U or finger/wrist flexors/pronator teres $\leq$ 1 U/kg or arm flexors $\leq$ 1.4 U/kg
2. High-dose group: adductor pollicis 10 U or finger/wrist flexors/pronator teres $\geq$ 1.1 U/kg or arm flexors $\geq$ 1.5 U/kg

Table 2/**IV** gives the detailed data on patients in the low- and high-dose groups. The assessing occupational therapist was unaware of the doses but not the treated muscles or time of treatment.

2. Interventions

All BTXA treatments were given on an outpatient basis by the same physician (HS). With local lidocaine cream and light conscious sedation with midazolam (0.3 mg/kg, maximum 10 mg per child), the injections were administered with a

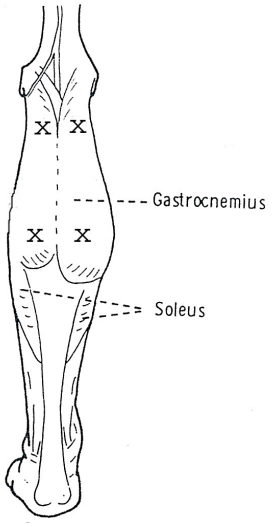
22-gauge needle and 1 ml syringes under sterile conditions. A constant dilution of 100 U in 1 ml 0.9% saline (Botox^{R}, Allergan Inc, Irvine, CA, USA) was used.

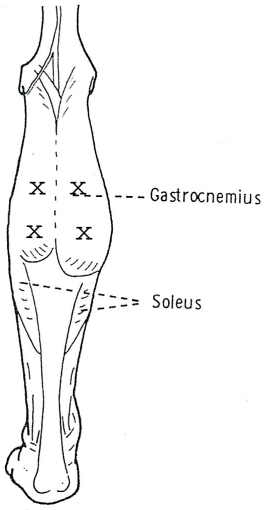
In study I, the proximal group received a BTXA injection into the proximal part of both heads of the gastrocnemius (near the muscle origin or motor point where the motor nerve enters the muscle) and the distal group into the mid-belly of the muscle bulk (near the assumed NMJ zone) (Figure 1). A constant dose of 3 U/kg per site (6 U/kg per gastrocnemius) was used. A single-point injection technique was employed except for doses over 50 U, which were divided over two adjacent sites (distance 0.5-1 cm) corresponding to the single-point injection (5 children in the proximal and 4 in the distal group). In addition to gastrocnemius injections, five patients (3 in the proximal and 2 in the distal group) received treatment of the medial hamstring muscles.

In study II, the single-point group received a BTXA injection into the mid-belly of both heads of the gastrocnemius and the multiple-point group into the mid-belly and about 5 cm proximally towards the popliteal fossa (Figure 2) at a constant dose of 4 U/kg per head of the gastrocnemius muscle**.** For the single-point technique the dose was injected into one point per muscle head, except for doses over 50 U (0.5 ml), which were divided over two adjacent sites (distance 0.5-1 cm) (in 11 out of 12 treated legs). For multiple-point injections the dose was divided over two sites per muscle head. In addition to gastrocnemius injections, in one hemiplegic patient (in single-point group) the medial hamstrings, in two diplegics (one from both groups) the medial hamstring-adductors and in five diplegics the adductors (2 in the single-point and 3 in the multiple-points group) were treated.

Figure 1. Proximal and distal injection sites in study I: the distance between the proximal and distal sites was kept at a minimum of 4 to 5 cm.

Figure 2. The single- and multiple-point injection sites in study II: the single-point group was injected at the two distal sites and the multiple-point group at all four sites.





In both studies, the injection sites were determined according to anatomic landmarks and palpation and no EMG or motor point stimulation was used. The needle was inserted into the muscle (depth 5-7mm) with the ankle in neutral position. The needle position was checked by flexing-extending both knee and ankle joint as described by Corry and colleagues (1998). After injection, the flexing-extending movement was continued to enhance the spread of BTXA in the muscle bulk. The injection sites were not entered in any official record and the therapists involved in assessment were blinded to the injection technique used. The parents were instructed not to reveal the injection technique after treatment during the assessments.

The content and frequency of physiotherapy continued unchanged throughout the study period. In study I every child received night splints at the 3-week assessment. If the participant already had a splint, he/she was asked to have at least a two-month pause in wearing it before entering the trial. In study II all children had worn a night splint on the affected limb for at least 2 months before enrolment.

In study III, the muscles needing treatment were determined during the baseline assessment and identified by anatomic knowledge and palpation, and the injections were administered either with (low: n=1; high: n=1) or without (low: n=31; high: n=22) EMG guidance. BTXA was injected into one or two sites in a given muscle and doses over 50 U were always divided over two adjacent sites. The doses were calculated according to clinical evaluation. Thirteen children underwent BTXA treatment once, nine twice, five three times, and two four to five times with a mean interval of 11 months (range 4-44 months). In total, 156 muscles were injected: 80 gastrocnemius, 10 soleus, 3 tibialis posterior, 36 hamstring, 25 adductor, 1 peroneus longus and 1 rectus femoris. At the first post-treatment assessment, children received night splints on the treated limb to potentiate the treatment effect. In cases where the subject already had a night splint, the angle of dorsiflexion was checked and a new splint provided if required. The frequency and extent of post-treatment physiotherapy continued unchanged, except in the case of four children in the low dose group: for two the frequency was increased by one session per week and for two the therapy was resumed after a pause.

In study IV, at least one out of six functionally different muscle groups (a total of 154 muscles) was injected: in persistent shoulder abduction/flexion, the deltoideus (anterior and/or medial parts); for elbow flexion, the biceps and/or brachialis and/or brachioradialis; for forearm pronation, the pronator teres; in wrist flexion, the flexor carpi radialis and/or ulnaris; in finger flexion, the flexor carpii superficialis and/or profundus; and in thumb adduction, the adductor pollicis muscle. The muscles were identified on the basis of anatomic knowledge and by palpation, and the injections administered either with or without EMG guidance. In the functional group the use of EMG was distributed evenly between the low- (EMG n=6, no-EMG n=6) and high-dose (EMG n=7, no-EMG n=8) groups. In the non-functional group the use of EMG was not equal (low: EMG n=1, no-EMG n=11; high: EMG n=1, no-EMG n=14). BTXA was injected into one or two sites in a given muscle. Occupational and physical therapy mostly continued with the same frequency as before treatment. Facilitating or stretching splints were used individually when appropriate. Indication for new

treatment was return of the motor pattern deformity, range of movement or motor function (e.g. pen grip) to pre-treatment level. The treatment was given once to five children, twice to five, three times to three, four times to three, and five times to two children with a mean interval of 9.6 months (range 2-24 months).

3. Assessment methods

3.1. The lower limb studies (I-III)

The severity of gross motor function was classified by GMFCS (Palisano et al. 1997) (See Table 5/Review of literature). The outcome measures used are set out in Table 1.

Table 1. Assessment methods in studies I-III.

Physiotherapist (blinded to the treatment groups and doses)
- *Active and passive ankle ROM* (Stuber et al. 1988). Active ankle dorsiflexion with knee extended (I-III), passive dorsiflexion with knee extended (I-III) and flexed (I-II) was measured by manual goniometry with the "neutral-null" method: dorsiflexion angle over the neutral position was counted in positive degrees, under the neutral in negative degrees.
- *Muscle tone (spasticity)* on the Modified Ashworth Scale (MAS) (Bohannon and Smith 1987) (Table A/Appendix).
- *Dynamic muscle length* (dynamic spasticity) on the Modified Tardieu Scale (MTS) (Boyd and Graham 1999). The ankle was dorsiflexed as fast as possible and the "catch" angle measured by manual goniometry.
- *Selective Motor Control test* (SMC) (Boyd and Graham 1999) (Table B/Appendix). The child was asked to dorsiflex the ankle by trying to touch the finger of the examiner with the big toe (II-III).
- *Observational Gait Scale* (OGS) (Boyd and Graham 1999) (Table C/Appendix). The gait pattern was recorded on video in sagittal and coronal planes, with the child walking barefoot. The Initial Foot Contact and Total scores were noted.
Physiotherapist or physician
- *Goal Attainment Scale* (GAS) (Maloney et al. 1978) (Table D/Appendix). Each limb was scored either by the research physiotherapist at each post-treatment assessment (II) or by the physician from the videotapes (III) on a Goal Attainment Scale**,** which was used for all children to allow comparison in gait improvement.

The passive ankle ROM and muscle tone on MAS were taken as primary and dynamic muscle length and gait pattern as secondary outcomes. In OGS, a pediatric physiotherapist not involved with the measurements and blinded to the treatment groups or time sequences scored each treated leg from compiled video recordings.

3.2. The upper limb dose study (IV)

Each child underwent a physical examination to determine the severity and distribution of spasticity involvement and a test battery to define functional motor problems. The assessment protocol is shown in Table 2.

Table 2. Assessment methods in study IV.

Physician
- *Passive ROM* with manual goniometry: elbow, wrist, fingers
- *Muscle tone* (spasticity) with the MAS: elbow, wrist and finger flexors, pronator teres
- *Active thumb abduction* with a 4-point Corry scale (Corry et al. 1997) as follows: 0 = 0-0.9 cm distance, 1 = 1-1.9 cm distance, 2 = 2-2.9 cm distance, and 3 = over 3 cm distance from thumb interphalangeal fold to edge of palm.
- *House Classification of upper extremity use* (House et al. 1981) (Table E/Appendix)
- *Change in movement pattern:* scored from the videotape using the Upper Limb Physician's Rating Scale (ULPRS) (Graham et al. 2000). ULPRS Change score was noted. (Table F/Appendix)
Occupational therapist (blinded to the doses)
- *Grips:* pinch (cucumber slice), key grip (zip), 3-finger grip (holding paper or sleeve), narrow cylinder grip (jug, stick), wide cylinder grip (glass), pen grip (pencil) and diagonal grip (cutting with knife); grasping, releasing; pronation-supination
- *Bimanual tasks*: putting on a jacket with a zipper, drawing a circle with the help of a glass and cutting out the circle with scissors, cutting and buttering a slice of bread, cutting cucumber

Fine motor and bimanual functions were evaluated using standardized grips and specific tasks. A summary grip test score for the affected extremity was calculated for each child by scoring each of 10 grip items with a quantitative 4-point scale as follows: 0= cannot grip, 1= grips but cannot perform the given task, 2= grips using an awkward grip and performs the given task, 3= grips using a normal grip and performs the given task (10 items, max.3 points/item) (Eliasson et al. 1998). Scoring from videotape was done by the occupational therapist. As the MACS or BMFM were not available at the time of study, based on a total score of 30 in the standardized grips and tasks, the children were categorized as having a mild (21≥ points), moderate (11-20 points) or severe (≤ 10 points) impairment of the affected limb.

Subjective ratings of the child's overall response in both function and cosmetic appearance. At each post-treatment session the caregiver and treating physician rated the child using the following scoring: "deteriorated" (situation worse than pre-treatment, score -1), "no change" (situation same as pre-treatment, score 0), "slight improvement" (better than pre-treatment, score +1), "clear improvement" (much better than pre-treatment, score +2).

The passive ROM and muscle tone on MAS were primary and hand use on House classification and fine motor functions (grips and bimanual tasks) were secondary outcomes.

In all studies (I-IV). Caregivers received a *questionnaire* asking them to report the timing and duration of beneficial and adverse effects of treatment. The adverse events were classified as "severe", "moderate" or "mild" by the caregivers. Adverse events were also actively asked by the physician at each assessment. (Table G/Appendix)

4. Statistical analyses

SPSS for Windows versions 11.5 (I) and 12.0 (II-IV) and StatXact version 4.0.1 (I-IV) were used in data analyses and Solo Power Analysis version 1.0 in power calculations. Significance was assumed at $p < 0.05$.

Studies I-II, IV. Continuous data with skew distribution and ordinal data within groups were tested using Friedman`s test and further analysis by Wilcoxon test. Differences between groups were tested by Mann-Whitney U test and Fisher´s exact test.

In study I, the needed sample size was estimated when each group had 6 to 8 treated limbs. By reason of the skew continuous distributions, calculations were made within groups using Friedman`s test and between groups at 8 weeks assessment using t-test (as an estimate of the Mann-Whitney U test) with alpha 0.05 and power 80%. A sample size of 11 legs per group was taken to detect a between-group-difference of 5 or more degrees in passive dorsiflexion. The power calculation with the final data with alpha 0.05 gave the following results: 0.61 at 3 weeks, 0.40 at 8 weeks, and 0.26 at 16 weeks.

In study II, the needed sample size was estimated when each group had 8 treated limbs and the power calculation using t-test (as an estimate of the Mann-Whitney U test) to detect a difference of 5 or more degrees in passive dorsiflexion with knee extended between the treatment groups at 2 months with an alpha of 0.05 and power 80% gave 15 legs per group. In the final data the power calculation gave the following results: 0.42 at 1 month, 0.70 at 2 months, and 0.95 at 4 months.

In studies I and II, the power of the Mann-Whitney U test might be even greater than that of the t-test, on account of the skew continuous distributions.

Study III. Continuous data with normal distribution were tested using analysis of variance for repeated measures and two-way analysis of variance with night splints as covariate. Changes in ordinal data within groups were tested using Friedman`s test and further analysis by Wilcoxon test. Differences between groups were tested by Mann-Whitney U test and Fisher´s exact test. The power calculation using two-way analysis of variance with an alpha of 0.05 to detect a difference between treatment groups of 5 or more degrees in passive dorsiflexion with knee extended gave the following results: 0.30 at 1 month, 0.95 at 2 months, and 0.75 at 4 months.

5. Ethical aspects

The protocols of Studies I-II were approved by the Ethical Committees of Tampere University Hospital and the Central Hospital of Kanta-Häme and the National Agency for Medicines. Written informed consent was obtained from the caregivers before enrolment. The protocols for the retrospective studies III-IV were approved by the Ethical Committee and local research authorities of Tampere University Hospital. According to the practice of Tampere University Hospital, informed consent from the caregivers was not needed.

RESULTS

1. The injection technique studies (I-II)

1.1. Near vs remote from NMJs (I)

Study I comprised 19 children (13 males, 6 females aged 1.5 to 7 years; 9 with hemiplegia, 8 with diplegia, 2 with quadriplegia; levels I to IV on GMFCS) with 25 treated lower limbs randomized into two treatment groups: the proximal group with 12 and the distal group with 13 treated legs. The treatment groups were similar in all parameters (See Table 1/**I** for details).

1.1.1. Primary outcomes (ROM, MAS)

At baseline, the difference between the groups approached significance in median passive ankle dorsiflexion with knee extended, the distal group having slightly better passive ROM ($p=0.065$). In both proximal and distal groups, a significant improvement was noted in the median of changes in active ankle dorsiflexion with knee extended ($p<0.05$) and in passive ankle dorsiflexion with knee both extended ($p<0.01$) and flexed ($p<0.01$) at 3, 8 and 16 weeks assessments. No differences between the groups were detected in active or passive ROM (Table 1). Muscle tone by MAS scores improved significantly in both treatment groups ($p<0.001$). Calf tone decreased slightly later in the proximal (peak at 8 weeks) compared with the distal group (already low at 3 weeks), but no statistical differences emerged between the treatment groups (Table 1).

1.1.2. Secondary outcomes (MTS, OGS)

No significant differences between baseline MTS or OGS median values were observed. Dynamic muscle length by MTS increased at all assessment points in both groups, but was significant in the distal group ($p<0.05$). No differences emerged between treatment groups in MTS (Table 1). In the distally injected group at all time-points, a significant improvement was noted in the median of changes in Total scores ($p<0.05$), and that in Initial Foot Contact subscore approached significance ($p=0.075$). The difference between the groups in change in Total and Initial Foot Contact scores was significant at 8 weeks, favoring the distal group (Table 1), but this difference disappeared by 16 weeks. An at least

Table 1. Primary and secondary outcome data in study I (median of change from baseline). Improvement in parameters is marked positive, decline negative.

	Proximal site		***Distal site***	
	n	*Median (range)*	*n*	*Median (range)*
Active dorsiflexion knee extended				
Baseline	10	-25 (-40 - -10)	11	-20 (-35 - -15)
3 wk	10	**+5 (-2 - +10)**	11	**0 (-10 - +15)**
8 wk	10	**+7.5 (-15 - +22)**	11	**+15 (-10 - +40)**
16 wk	10	**+5 (-30 - +25)**	11	**0 (-8 - +15)**
Passive dorsiflexion knee extended				
Baseline	12	-1 (-15 - +15)	13	+5 (-13 - +27)
3 wk	12	**+11.5(-10 -+25)**	13	**+9 (-2 - +25)**
8 wk	12	**+9.5 (0 - +35)**	13	**+15 (-5 - +27)**
16 wk	12	**+9.5 (-1 - +25)**	13	**+10 (-5 - +30)**
Passive dorsiflexion knee flexed				
Baseline	12	+3 (-10 - +30)	13	+20 (-10 - +35)
3 wk	12	**+11.5 (-5 - +20)**	13	**+5 (-4 - +20)**
8 wk	12	**+10.5 (+3- +35)**	13	**+10 (-10 - +31)**
16 wk	12	**+7.5 (-2 - +20)**	13	**+10 (-5 - +30)**
Calf tone				
Baseline	12	3 (2 - 4)	13	3 (2 - 4)
3 wk	12	**+0.75 (0 - +2)**	13	**+1 (0 - +2)**
8 wk	12	**+1 (+0.5 - +3)**	13	**+1 (0 - +3)**
16 wk	12	**+1 (0 - +2)**	13	**+1 (0 - +2.5)**
Dynamic muscle length				
Baseline	12	-12.5 (-35 - 0)	13	-15 (-40 - 0)
3 wk	12	+4 (-15 - +20)	13	**+8 (-8 - +30)**
8 wk	12	+3 (-2 - +22)	13	**0 (-20 - +35)**
16 wk	12	0 (-15 - +15)	13	**+5 (0 - +35)**
Initial Foot Contact score				
Baseline	12	0.5 (0 - 2)	13	0 (0 - +2)
3 wk	9	0 (-1 - +2)	13	0 (0 - +2)
8 wk	12	0 (-1 - +1)[a]	11	+1 (-1 - +2)[a]
16 wk	11	0 (-1 - +2)	11	0 (-1 - +2)
Total score				
Baseline	12	9 (3 - 17)	13	9 (3 - 20)
3 wk	9	+1 (0 - +7)	13	**+1 (-2 - +7)**
8 wk	12	+0 (-2 - +5)[b]	11	**+2 (-1 - +7)[b]**
16 wk	11	+2 (-2 - +8)	11	**0 (-3 - +11)**

[a] p=0.025, [b]p=0.049, significant difference between treatment groups (Mann-Whitney U test). In bold, significant improvement at all assessment points within the treatment groups (Friedman´s test).

one-grade improvement in Initial Foot Contact scores was noted in 48% of the treated legs at 3 weeks (proximal: 50%; distal 46%), in 52% at 8 weeks (proximal: 25%; distal 77%), and in 40% at 16 weeks (proximal: 50%; distal 31%). These intergroup differences were significant at 8 weeks (p=0.017). In study I, a few recordings were inadvertently lost due to technical errors in the editing process (data on 3 legs in proximal group at 3 weeks, 2 legs in distal group at 8 weeks, and 1 leg in proximal and two legs in distal at 16 weeks).

1.2. Single vs multiple sites (II)

Study II involved a total of 17 children (9 males, 8 females aged 1.8 to 9.4 years; 8 hemiplegics, 8 diplegics, 1 quadriplegic; levels I to IV on GMFCS) with 25 treated lower limbs randomized into two groups: the single-point group with 12 and the multiple-points group with 13 treated legs. The treatment groups were similar at baseline, except that the subjects in the multiple-points group were lighter and younger compared with those in the single-point group (See Table 2/**II** for details). The doses were calculated based on units per kg body weight and the total dose (U) for the gastrocnemius muscles did not differ between the groups (p= 0.139).

1.2.1. Primary outcomes (ROM, MAS)

Baseline passive ankle dorsiflexion with knee both extended (p=0.01) and flexed (p=0.001) was better in the multiple-points group. However, the improvements in median passive ROM expecially with knee extended were of similar magnitude in both treatment groups at each assessment. The only statistically significant difference emerged in passive dorsiflexion with knee flexed at 2 months, favoring the single-point group. Active ROM improved significantly at 1, 2 and 4 months in the single-point group ($p<0.05$) (Table 2).

A significant improvement was seen in both treatment groups in MAS scores ($p<0.001$) and no differences were detected between the groups (Table2).

1.2.2. Secondary outcomes (MTS, OGS, GAS, SMC)

There were no significant differences between baseline MTS, OGS or SMC median values. A significant improvement was seen in both treatment groups in dynamic muscle length ($p<0.05$) and no differences emerged between the treatment groups (Table 2).

Both the Total and Initial Foot Contact scores improved significantly in both single- and multiple-point groups ($p<0.05$) and no differences were noted between the groups (Table 2). An at least one-grade improvement in Initial Foot Contact scores was noted in 44% of the treated legs at 1 month (single: 42%; multiple 46%), in 52% at 2 months (single: 50%; multiple 54%), and in 44% at 4 months (single: 42%; multiple 46%). These intergroup differences were not significant.

Table 2. Primary and secondary outcome data in study II (median of change from baseline). Improvement in parameters is marked positive, decline negative.

	Single-point		***Multiple-point***	
	n	*Median (range)*	*n*	*Median (range)*
Active dorsiflexion knee extended				
Baseline	12	-11 (-25 - +2)	11	-10 (-50 - +10)
1 mo	12	**+7 (-10 - +17)**	11	+2 (-10 - +23)
2 mo	12	**+8 (-4 - +20)**	11	+5 (-15 - +45)
4 mo	10	**+5.5 (-16 - +28)**	11	+5 (-20 - +18)
Passive dorsiflexion knee extended				
Baseline	12	+15 (0 - +40)[a]	13	+25 (+15 - +39)[a]
1 mo	12	+4 (-10 - +15)	13	+5 (-8 - +12)
2 mo	12	+4 (-10 - +10)	13	0 (-14 - +9)
4 mo	12	+4 (-15 - +22)	13	+4 (-14 - +14)
Passive dorsiflexion knee flexed				
Baseline	12	+23.5 (+15 - +40)[b]	13	+38 (+23 - +45)[b]
1 mo	12	+6 (-10 - +16)	13	-1 (-10 - +12)
2 mo	12	+1.5 (-5 - +10)[c]	13	-2 (-10 - +10)[c]
4 mo	12	+1 (-12 - +14)	13	-3 (-14 - +12)
Calf tone				
Baseline	12	2.25 (2 - 3)	13	2.5 (1 - 3)
1 mo	12	**+1 (0.5 - +2)**	13	**+1 (0.5 - +3)**
2 mo	12	**+1 (+0 - +2)**	13	**+1 (0 - +2)**
4 mo	12	**+1 (0 - +2)**	13	**+1 (0 - +2)**
Dynamic muscle length				
Baseline	12	-12 (-22 - 0)	13	-5 (-45 - 0)
1 mo	12	**+7.5 (-10 - +22)**	13	**+4 (0 - +30)**
2 mo	12	**+8 (-7 - +16)**	13	**+5 (-3 - +25)**
4 mo	12	**+6.5 (-5 - +17)**	13	**+5 (-5 - +25)**
Selective dorsiflexion				
Baseline	12	3 (1 - 4)	13	3 (0 - 4)
1 mo	12	0 (0 - 0)	13	0 (0 - +1)
2 mo	12	0 (0 - 0)	13	0 (0 - +1)
4 mo	12	0 (0 - +1)	13	0 (0 - 0)
Initial Foot Contact score				
Baseline	12	1 (0 - 2)	13	1 (0 - 2)
1 mo	12	**0 (0 - +1)**	13	**0 (-1 - +2)**
2 mo	12	**+0.5 (0 - +2)**	13	**+1 (0 - +2)**
4 mo	12	**0 (0 - +2)**	13	**0 (-1 - +2)**
Total score				
Baseline	12	7 (4 - 13)	13	9 (3 - 15)
1 mo	12	**+2 (-2 - +6)**	13	**+2 (-4 - +10)**
2 mo	12	**+2 (-1 - +8)**	13	**+2 (-1 - +10)**
4 mo	12	**+1.5 (-2 - +5)**	13	**+1 (-3 - +6)**

[a] p=0.010, [b] p=0.001, [c] p=0.046, significant difference between treatment groups (Mann-Whitney U test). In bold, significant improvement at all assessment points within the treatment groups (Friedman´s test).

On the Goal Attainment Scale the functional goal in gait pattern was achieved in 75% of the treated legs in the single-point group and 69% in the multiple-points group at 1 month, in 50% and 69% at 2 months, and in 50% and 69% at 4 months, respectively. These intergroup differences were not significant.

No notable improvement was observed in selective dorsiflexion.

1.3. Time course of the change in muscle tone in studies I-II

In both studies, the differences in time courses between the groups were not statistically significant.

Study I. Caregivers perceived a change in muscle tone within a mean 4.1 days (range 2-7) in the proximally injected group and 5.8 (range 1-20) in the distally injected group. A rise in muscle tone commenced between 3 and 8 weeks in two children (one child in each group), and between 8 and 16 weeks in 11 children (proximal: 5; distal: 6). A good response was still observed in six children at 16 weeks (three in both groups).

Study II. Caregivers observed a change in muscle tone within a mean 3.6 days (range 1-7) in the single-point group and 3.5 (range 1-7) in the multiple-points group. A rise in muscle tone commenced between 2 and 4 months in two children (one child in each group) and good response was still observed in all the remaining children at 4 months.

1.4. Adverse events in studies I-II

Study I. Parents reported a total of 19 adverse events (proximal: nine events; distal: ten events; total incidence in 68% of treatments) in 13 children. No difference between the groups was detected in adverse event parameters. Mild symptoms were tenderness in the calf in seven (proximal: 5; distal: 2), tiredness in three (proximal: 1; distal: 2), irritability in one (distal), clumsiness in three (proximal: 1; distal: 2), and fever or flu-like symptoms in two (one in each group). Moderate symptoms were irritability in two (one in each group) and tenderness in the calf in one (distal). All symptoms resolved within 1 to 7 days, except that one child in the proximal group remained clumsy for 16 days. Three adverse events were judged not to be related to the treatment: flu and fever (n=2; both having common cold in their family) and calf pain (n=1) occurring after one week after a long walk.

Study II. A total of eight adverse events (single: two events; multiple: six events; total incidence in 35% of treatments) were reported in six children: tenderness of the injected calf for 1 to 2 days after the injection in three children (single: 1; multiple: 2), both tenderness in the calf and clumsiness lasting 2 to 7 days in two (both in the multiple-points group), and spasms in the injected calf lasting 10 days in one child (in the single-point group). Though the difference was not significant, the incidence was higher in the multiple-points group. All adverse events were considered mild.

2. The dose studies (III-IV)

2.1. Lower limb dose study (III)

The low-dose (32 subjects, 46 treated lower limbs) and high-dose (23 subjects, 34 treated lower limbs) groups were similar in all other baseline parameters except that the high-dose group used night splints more before treatment (p=0.000) (See Table 3/**III** for details). The age range of the participants was 1.5 to 9.6 years and GMFCS levels I to IV. The mean total amount administered and the mean total dose per kg body weight for the gastroc-soleus muscle in the high-dose group were 1.6-fold those in the low-dose group (Table 3). In both treatment groups the first post-treatment assessment was at a mean of 1.1 month (range 1-2; n=55 sessions), the second at 2.1 months (range 2-3; n=34 sessions), and the third at 4.0 months (range 4-4.5, n=33 sessions).

Table 3. Doses in study III, mean (range) (n= treated limbs).

	Low-dose (n=46)	***High-dose (n=34)***
Total amount/gastroc-soleus (U)	91.9 (30-144)	143.1 (80-240)
Dose/gastroc-soleus (U/kg bw)	5.2 (2-6)	8.2 (7.2-11)
Total amount (U)	201 (70-480)	280 (80-600)
Total dose (U/kg bw)	11.6 (4-24.5)	16.0 (7.2-27.5)

2.1.1. Primary outcomes (ROM, MAS)

The baseline mean passive ankle ROM was better in the high-dose group (p=0.003). The improvement was significant at all assessment points in MAS (p<0.001) in both groups and in passive ROM knee extended (p<0.01) in the low-dose group. A significant difference between the treatment groups emerged in mean change in passive ankle ROM at 2 and 4 months, favoring the low-dose group (Table 4).

2.1.2. Secondary outcomes (MTS, OGS, SMC)

No differences prevailed between the groups at baseline. The mean change in MTS improved in both, though not significantly, and no differences were noted between the treatment groups. The improvement was significant at all assessment points in SMC (p<0.05) in the low-dose group. The difference in median change between groups in SMC at 4 months became significant, favoring the low-dose group (Table 4).

Both treatment groups improved in equinus gait pattern, the median change being significant at all measurement points in the OGS Initial Foot Contact scores (p<0.005) (Table 4). At least a one-grade functional improvement in gait pattern on the GAS was achieved in 59% of the treated legs at 1 month (n= 68; low: 63%; high: 55%), in 58% at 2 months (n=40; low: 57%; high: 58%), and in

49% at 4 months (n=37; low: 52%; high: 44%). These differences between groups were not significant.

Table 4. Primary and secondary outcomes in study III (mean and median of changes from baseline). Improvement in parameters is marked positive, decline negative.

		Low dose		***High dose***
	n	*Mean ± SD (range)*	*n*	*Mean ± SD (range)*
Active dorsiflexion knee extended				
Baseline	29	-20.4 ± 11.8 (-50 - 0)	28	-18.5 ± 12.8 (-50 - +10)
1 mo	28	+5.0 ± 7.7 (-10 - +33)	27	+3.3 ± 8.0 (-10 - +20)
2 mo	22	+9.9 ± 11.6 (-15 - +40)	15	+5.9 ± 14.7 (-15 - +45)
4 mo	17	+5.4 ± 13.9 (-30 - +25)	18	+3.5 ± 14.9 (-25 - +28)
Passive dorsiflexion knee extended				
Baseline	46	+5.8 ± 11.6 (-15 - +35)[a]	34	+18.3 ± 14.1(-20 - +40)[a]
1 mo	46	**+7.9 ± 10.3 (-16 - +25)**	34	+2.8 ± 8.9 (-15 - +27)
2 mo	30	**+13.5 ± 9.5 (-5 - +35)[b]**	17	+1.1 ± 5.1 (-10 - +10)[b]
4 mo	25	**+11.0 ± 8.4 (-5 - +30)[c]**	23	+1.4 ± 8.2 (-15 - +15)[c]
Dynamic muscle length				
Baseline	40	-12.5 ± 13.1 (-40 - 0)	34	-14.1 ± 11.7 (-45 - 0)
1 mo	37	+6.5 ± 8.8 (-8 - +30)	34	+7.1 ± 12.3 (-10 - +35)
2 mo	25	+4.0 ± 12.1 (-10 - +35)	16	+8.1 ± 8.7 (-7 - +25)
4 mo	20	+7.4 ± 11.5 (-10 - +35)	23	+2.0 ± 19.7 (-40 - +35)
OGS/Total scores				
Baseline	39	8.1 ± 4.1 (2 - 17)	33	7.4 ± 2.8 (3 - 13)
1 mo	33	+2.1 ± 2.0 (-2 - +7)	33	+2.1 ± 3.1 (-2 - +11)
2 mo	26	+2.3 ± 1.8 (-1 - +7)	12	+2.8 ± 3.9 (-1 - +11)
4 mo	21	+1.4 ± 2.2 (-2 - +7)	16	+1.6 ± 3.2 (-2 - +7)
		Median (range)		*Median (range)*
Ashworth				
Baseline	46	3 (1 - 4)	34	2.8 (1 - 4)
1 mo	46	**+1 (0 - +2.5)**	34	**+1 (-1 - +3)**
2 mo	30	**+1 (0 - +3)**	17	**+1 (0 - +2)**
4 mo	25	**+1 (0 - +2.5)**	23	**+1 (-1 - +2)**
OGS/Initial Foot Contact scores				
Baseline	39	1 (0 - 2)	33	1 (0 - 2)
1 mo	33	**0 (-1 - +1)**	33	**0 (-1 - +2)**
2 mo	26	**+1 (-1 - +1)**	12	**+1 (0 - +2)**
4 mo	21	**0 (-1 - +1)**	16	**0 (0 - +2)**
Selective Motor Control/ankle				
Baseline	42	2 (0 - 4)	34	2 (0 - 4)
1 mo	42	**0 (0 - +2)**	34	0 (0 - +1)
2 mo	28	**0 (-1 - +2)**	17	0 (0 - +1)
4 mo	27	**0 (-1 - +2)[d]**	23	0 (-1 - +1)[d]

[a] p=0.003, [b] p=0.001, [c] p=0.010, [d] p=0.018, significant difference between groups (two-way analysis of variance or Mann-Whitney U-test). In bold, significant improvement at all assessment points within the treatment groups (analysis of variance for repeated measures or Friedman´s test). Use of night splints was taken as covariate.

2.2. Upper limb dose study (IV)

In the *functional* group, the data on median change from baseline to 1 (3-4 weeks) and 3 months (12-14 weeks) are reported. In the *non-functional* group only the House classification score and subjective ratings by parents and physician at 1 month were noted, as the results for spasticity scores were not recorded systematically and the grip and bimanual function tests could not be performed for all of these children.

At baseline, the functional low-dose (12 extremities) and high-dose (15 extremities) groups were similar in all parameters, while in the non-functional category the low-dose (12 extremities) and high-dose (15 extremities) groups differed significantly in terms of impairment of the affected limb (p=0.018), most of the severely involved children belonging to the high-dose group (See Table 2**/IV** for details). The mean dose for the target muscles in the functional and non-functional high-dose groups was 2-fold and the mean total amount 1.4-fold those in the low-dose groups (Table 5).

Table 5. Doses in study IV, mean (range) (n= treated extremities).

	Low-dose (n=12)	***High-dose (n=15)***
Functional group		
Total amount (U), mean (range)	81 (22-155)	114 (8-250)
Total dose (U/kg), mean (range)	3.2 (1.3-6.5)	4.2 (0.2-9.5)
Dose (U/kg/target muscle), mean (range)	0.9 (0.7-1.0)	1.8 (1.5-2.6)
Add. pollicis (U), mean (range)	None	8.8 (7.5-20.0)
	Low-dose (n=12)	***High-dose (n=15)***
Non-functional group		
Total amount (U), mean (range)	142.5 (20-460)	228 (30-640)
Total dose (U/kg), mean (range)	4.1 (1.1-11.5)	6.9 (1.9-14.2)
Dose (U/kg/target muscle), mean (range)	0.8 (0.4-1.0)	2.1 (1.1-3.4)

2.2.1. Primary outcomes (ROM, MAS)

In the functional group, the improvement at all time-points was significant in passive wrist extension and pronator spasticity scores in both the low- and high-dose groups ($p<0.05$), in passive elbow extension and wrist spasticity scores in the high-dose group ($p<0.05$) and in elbow flexor spasticity scores in the low-dose group ($p<0.05$), but no significant differences between the treatment groups were detected in passive ROM or MAS scores (Table 6).

Table 6. Treatment results in the functional low- and high-dose groups in study IV (median of change from baseline). Improvement in parameters is marked positive, decline negative.

	Baseline		**1 mo**		**3 mo**	
	n	**Median (range)**	**n**	**Median (range)**	**n**	**Median (range)**
House scores						
Low dose	10	4 (3-6)	10	**+1 (0- +1)**[a]	6	**+0.5 (0- +1)**
High dose	14	3,5 (1-8)	14	**0 (-1- +1)**[a]	11	**+1 (0- +3)**
ULPRS/Change						
Low dose	12	NA	12	+1 (0- +2)	6	+1 (0- +2)
High dose	13	NA	13	+1 (-1- +2)	9	0 (0- +2)
Grip scores						
Low dose	12	11 (3-27)	11	0 (-1- +4)	6	0 (-1- +2)
High dose	13	13 (3-26)	13	0 (-4 - +5)	9	0 (-1- +1)
Bimanual function scores						
Low dose	9	4 (0-11)	8	0 (-3- +2)	3	0 (0- +1)
High dose	12	3,5 (0-11)	12	0 (-1- +4)	7	0 (0- +3)
Thumb abduction/ Corry scores						
Low dose	7	1 (0-2)	7	0 (-2- +2)	6	+1.25 (-2- +2)
High dose	8	1 (0-3)	8	0 (-1- +1)	6	0 (0- +2)
Ashworth/elbow						
Low dose	6	2,5 (2-4)	6	**+1.5 (0- +3)**	6	**+1 (0- +2)**
High dose	8	3 (2-4)	8	+1.15 (-1- +3)	7	+1 (-1- +2)
Ashworth/pronator						
Low dose	7	3,5 (2-4)	8	**+1 (0- +2)**	6	**0 (-1- +2)**
High dose	8	3 (3-4)	8	**+0.75 (0- +2)**	7	**0 (0- +2)**
Ashworth/wrist						
Low dose	7	1 (0-3)	8	+1 (0- +2)	6	+0.9 (0- +2)
High dose	7	2 (0-5)	7	**+1 (0- +2)**	6	**+1 (0- +2)**
Elbow extension/passive (°)						
Low dose	6	140,5 (120-153)	6	+1 (-13- +10)	6	-1.5 (-8- +17)
High dose	6	134 (103-140)	6	**+8.5 (0- +20)**	5	**+2 (2- +20)**
Forearm supination/ passive (°)						
Low dose	8	+52,5 (0- +90)	9	+10 (0- +70)	6	+7.5 (-35- +30)
High dose	8	+29 (-30- +90)	7	+15 (-8- +30)	7	+13 (-8- +30)
Wrist extension/passive (°)						
Low dose	8	+75,5 (+20-+90)	9	**7.5 (0- +45)**	6	**6 (0- +22)**
High dose	7	+88 (+3- +90)	6	**11 (0- +37)**	6	**3.5 (0- +17)**

[a] = $p<0.01$, significant difference between groups (Mann-Whitney U-test)
In bold, significant improvement at all time-points (Friedman`s test)
ULPRS, Upper Limb Physician´s Rating Scale; NA, not applicable

2.2.2. Secondary outcomes (House classification, fine motor functions, Corry scores, ULPRS)

In the functional group, the improvement in House classification scores was significant in both treatment groups ($p<0.05$). The difference between groups

was significant at 1 month, favoring the low-dose group. No significant changes within or between the functional low- and high-dose groups were detected in grip or bimanual function, Corry or ULPRS Change scores (Table 6).

In the non-functional group, improvement in House classification scores at 1 month was significant only in the low-dose group ($p<0.05$). No intergroup differences emerged.

2.2.3. Subjective ratings on function and cosmetical appearance

In the functional group, parental ratings revealed at least a one-grade improvement in function for 96% (n=27; low: 100%; high: 93%) of treatments at 1 month and for 78% (n=18; low: 71%; high: 82%) at 3 months, and the physician ratings for 93% (n=27; low: 100%; high: 87%) and 78% (n=18; low: 71%; high: 82%), respectively. The parental ratings in cosmetic appearance were: for 92% of treatments (n=24; low: 100%; high: 86%) at 1 month and for 65% (n=17; low: 67%; high: 64%) at 3 months, and the physician ratings for 92% (n=24; low: 100%; high: 86%) and 65 % (n=17; low: 67%; high: 64%), respectively. The differences between the treatment groups were not significant.

In the non-functional group, the parents rated at least a one-grade improvement for 100% of treatments in function (n=20; low: 100%; high 100%) and for 71% (n=21; low: 44%; high: 92%) in cosmetic appearance, and the physician for 85% (n=26; low: 82%; high: 87%) in function and for 62% (n=26; low: 36%; high: 80%) in cosmetic. A significant difference between the groups, favoring the high dosage, was detected in subjective parental cosmetic appearance ratings ($p=0.047$).

2.3. Time course of the change in muscle tone in studies III-IV

In neither study was the difference between groups significant.

Study III. The onset of reduction in muscle tone was noted by the caregivers within a mean of 4.3 days (range 1-14) in the low-dose and 3.9 (range 1-10) in the high-dose group, and the effect was observed to last for a mean 3.9 (range 2-8) and 3.8 months (range 2-6), respectively. There was a similar portion of children in both groups still evincing at least a one-grade improvement in MAS scores at 4 months.

Study IV. In the functional group, the muscle tone reduction was seen to commence within a mean of 5.0 days (range 2-14) in the low-dose group and 2.7 (range 1-5) in the high-dose group. The effect was observed to last for a mean 4.3 (range 1-12) and 4.8 months (range 1-8), respectively. In the non-functional group, reduction in muscle tone set in within a mean 3.7 days (range 3-5) and lasted for 2.7 months (range 0.7-4.5) in the low-dose group, and 3.3 days (range 1-6) and 3.6 months (range 0.5-6), respectively, in the high-dose group.

2.4. Adverse events in studies III-IV

Study III. A total of 41 adverse events in 22 children (low: 26; high: 15; total incidence 51% of treatments) were reported: bruising or soreness at the injection site (low: 9, high: 9), clumsiness (low: 5, high: 1), irritability (low: 4, high: 2), tiredness (low: 3, high: 1), constipation or diarrhea (low: 2), flu-like symptoms (low: 3, high:1), and pain in the sole of the foot (high:1). All adverse events were considered mild or moderate and no significant differences in incidence emerged between the groups.

Study IV. In the functional group, six adverse events (22% of treatments) were reported: bruising (n=1), tiredness (n=1), constipation (n=1), reduction in finger grip strength for four weeks (n=1), and reduction in thumb motility for two weeks (n=1) in the high-dose group, and flu-like symptoms (n=1) in the low-dose group. Five adverse events were considered mild and one (thumb motility reduction) moderate. In the non-functional group, no adverse events were reported.

DISCUSSION

1. Results

1.1. Near vs remote from NMJs (I)

Our findings demonstrated that both groups benefited from the treatment in terms of passive and active dorsiflexion, dynamic muscle length, calf tone reduction, and function (Initial Foot Contact score per treated leg), and that the differences between groups were not substantial. In the distally injected (as close to the NMJs as possible) group, video gait analysis showed a slightly greater improvement in equinus gait (Initial Foot Contact and Total score in OGS) at 8 weeks, this then declining by 16 weeks.

The present results did not support the hypothesis that BTXA injections directed as close to the NMJ zone as possible are more effective in reducing muscle tone and produce better functional results in spastic equinus gait than injections into a remote site. The sample size (n=treated legs) was small and the calculated power varied from 0.61 at 3 weeks to 0.40 and 0.26 at 8 and 16 weeks, respectively. This increases the risk of type II error, i.e. obtaining false-negative results. Considering that the intergroup differences in median ROM just exceeded 5 degrees and that the range was wide, it is unlikely that increasing the sample size would have yielded clinically significant results. Variations of up to 10 to 15^{o} by a single tester have been found in assessments of children with CP (Stuberg et al. 1988) and an intergroup difference over 10^{o} could be considered clinically meaningful.

The minimal difference between the groups in our study indicates efficient toxin diffusion within the muscle and avid binding to the NMJs. Judging by the findings of Deshpande and colleagues (2006; Figure 6/Review of literature) it may be suspected that some NMJs are also located in the upper part of the medial and lateral gastrocnemius muscle. Thus, with proximal injections some amount of the toxin might have bound to these NMJs and some diffused along the muscle fibers downwards. Directing the injections as close to the NMJs as possible should still be considered desirable, but it may suffice to inject into the target muscle in order to reduce muscle tone. The distal group´s slightly faster reduction in muscle tone at 3 weeks, the period of the pharmacological peak effect of BTXA, and the tendency towards better gait pattern at 8 weeks advocates injection as close as possible to the NMJs. Also, the superiority may reflect diffusion or inadvertent injection into the soleus. The charts of Deshpande and colleagues (2006) of approximated patterns of end plates in different muscles were not available at the time of the study, but are a valuable tool for today´s clinician.

Due to the different outcome measures used (ankle ROM, MAS, MTS, Initial Foot Contact score) our results are not directly comparable with animal models using histochemical staining technique or limb force decrement. We did not duplicate the results of Shaari and Sanders (1993), who in their rat experiment noted a decline in paralysis with injections given far from the NMJ zone. The rat tibialis anterior muscle has a band-like NMJ zone at the mid-belly. However, the rats used were sacrificed after 24 hours, possibly leaving too short a time for further diffusion and additional paralysis to occur. Likewise, it is not known how well this indirect histochemical model is equivalent to functional paralysis. Our findings did not agree with those of Childers and associates (1998), who in their canine model obtained better force decrements (i.e. weakness) in end-plate-targeted versus anatomically guided injections.

Our results are on the other hand in agreement with those of Childers and colleagues (1996), in finding no significant differences in muscle tone and functional parameters between treatment groups. They injected only one of the two heads of the gastrocnemius and studied hemiplegic adults with varying etiology and duration of central nervous system injury, which makes comparison of the two sets of results complex. Also, our findings correlate well with the clinical observations of Westhoff and associates (2003), who under ultrasound-guidance injected the iliopsoas muscle from the groin, a site remote from the NMJ zone.

1.2. Single vs multiple sites (II)

In previous studies on BTXA treatment for spastic equinus gait in children with CP either a single- or a multiple-points technique has been used (Cosgrove et al. 1994, Corry et al. 1998, Hesse et al. 2000, Baker et al. 2002, Scholtes et al. 2007). In most cases, the single-point technique has included injections into two sites per gastrocnemius muscle and the multiple-points technique into four sites per muscle, but also six injection sites per muscle have been utilized (Polak et al. 2002).

Our findings demonstrated that both groups benefited equally from the treatment and the only statistically significant difference was detected in passive dorsiflexion with knee flexed at 2 months, favoring the single-point group. The effect declined by 4 months. This difference in median ROM (3.5 degrees) cannot be considered clinically significant. The minute difference between the groups in our study may again indicate efficient toxin diffusion within the muscle and avid binding to the end plates or diffusion and/or unintended injection to the soleus muscle.

The results did not support the hypothesis that multiple injections are more effective in reducing muscle tone and produce better functional results in spastic equinus gait than injections given into a single site. As in study I, the sample size (n=treated legs) in study II was small but power calculations gave better results (0.42 at 1 month, 0.70 at 2 months, and 0.95 at 4 months), thus reducing the risk of type II error. Nonetheless the effect of chance increases when sample size is small.

The treatment groups were similar at baseline except for mean age and weight. As the doses were calculated based on units per kg body weight and the

total dose for the gastrocnemius muscles did not differ between the groups, it is unlikely that this baseline difference affected the results. However, it may well have caused the toxin volumes to exceed the recommendations of 0.5 ml per site in the single-point group. The injected volumes per site ranged from 0.52 to 1.2 ml in this group and from 0.23 to 0.43 ml in the multiple-points group. This means that almost every injection in the single-point group was given divided between two adjacent sites in order to adhere to the recommended maximum of 0.5 ml per site. It may thus be that the increased incidence of calf soreness in the multiple-point group occurred not because of the volume injected but because of the number of injection sites.

The significant difference between the treatment groups in passive dorsiflexion at baseline may reflect a more dynamic spasticity (and thus less potential for increase in ROM) in younger patients in the multiple-points group. It is unlikely that the difference in baseline passive ROM affected the results, as median changes from baseline were similar in both injection groups.

We are not aware of any studies comparing single- vs multiple-sites techniques in children. In adults, Borodic and associates (1991) injected BTXA (Botox^{R}) either into a single (at the motor point) or multiple sites on separate sides in ten patients with blepharospasm and reported significantly better relief in eight subjects after 2-4 weeks with the multiple-site method, while two showed no difference between the methods. The outcome was judged by direct observation of muscle weakness by the clinician and the subjective opinion of the patient. When treating adults with spasmodic torticollis, Borodic and colleagues (1992) showed the multiple points per muscle injection strategy to be superior to the single point in terms of pain reduction, improvement in posture and range of motion, and improvement in activity endurance, but not in reduction of hypertrophy or involuntary movements. Our results did not parallel those of Borodic (1991, 1992). They are however in accord with those of the aforementioned study by Childers and associates (1996), who found no significant difference between single- and multiple-site injection groups.

In addition to dose and volume, the number of injection sites per muscle may be determined by the morphology and the end plate configuration of a given muscle. We injected the bipennate gastrocnemius muscle with parabole end plate zones, while the Borodic group (1991) treated the orbicularis oculi muscle with diffuse innervation. The patterns of the end plate zones in the neck muscles are as yet unknown. The number of injection sites used may also depend on the size of the muscle, the total number of muscles needing treatment and the availability of general anesthesia or sedation (Graham et al. 2000).

1.3. Lower limb dose study (III)

This study evaluated the effect of individually adjusted doses in a clinical setting of treating equinus gait in CP children. Though the baseline passive ankle ROM knee extended was better in the high-dose group, both treatment groups improved in each outcome measure. Even though the change in passive ankle ROM at 2 and 4 months and selective dorsiflexion at 4 months were better in the low-dose group, these did not translate into significantly better gait parameters. As a whole in this patient group use of high doses did not bring better effects

compared with lower doses, which was in accord with the hypothesis whereby no better improvement in passive ROM and gait pattern is to be obtained with doses over 6 U/kg per gastrocnemius-soleus muscle.

The less satisfactory results than anticipated in the high-dose group passive ankle ROM might be attributable to having treated the possibly fixed component with pre-treatment night splints, the toxin thus affecting only the remaining dynamic component and passive ROM (i.e. the high-dose group had less potential for change in ROM), and a lower dose might well have been sufficient. Another possible explanation for the tenuous change in the high-dose group is saturation of neuromuscular junctions and spread of the remaining toxin into adjacent muscles.

Our results are in line with those reported by groups under Polak (2002) and Baker (2002), who found most pronounced change in gastrocnemius muscle length with doses between 20 to 24 U/kg of Dysport^{R}. With a ratio of 1:4 this corresponds to approximately 5 to 6 U/kg of Botox^{R}. In the studies in question the beneficial effect seemed to plateau or even decline with higher doses, only increasing the rate of adverse events. Further, Bakheit and colleagues (2001), in their study on single- and multilevel treatments, noted that children receiving a moderate dose of BTXA (10 to 40 U/kg Dysport^{R}; approximately 2.5 to 10 U/kg of Botox^{R}) benefited the most, but that doses over 40 U/kg did not yield better results.

Our results also tally with the findings of Sloop and colleagues (1996), who in their study of varying BTXA doses (Botox^{R}) injected into the EDB muscles obtained a significant dose-dependent relationship between the dose and the decrement in M-response amplitude, area and mean rectified voltage during maximal voluntary contraction. They showed denervation up to 85% at a medium range dosage of 7.5 U per EDB, after which the curve reached a plateau. This finding, together with those of Polak (2002) and Baker (2002), suggest that an optimum dose per muscle exists, after which the effect does not increase in a given muscle.

In an animal model, Shaari and Sanders (1993) found that a 25-fold increase in dose was needed to double the paralysing effect in rats, this being quantified by glycogen staining. In humans, Sloop and colleagues (1996) showed that, at least in the lower dose range, the dose had to be 4-fold to double the paralysing response, as quantified by mean rectified voltage decrement in EDB. To our knowledge, no dose-response curves for the gastroc-soleus muscle are available. As the dose-response curve is logarithmic, it could be argued that the paralysing response in the present study might have been good enough (that is, reaching the plateau) with the 6 U/kg per gastrocnemius-soleus muscle and no further response could be elicited with doses over this. Also, the 1.6-fold difference between the low- and high-dose groups could have been too small and at least a 3- or 4-fold difference might have been needed in order to achieve a clear intergroup difference. This could not be studied in our retrospective cohort as the number of observations was too small to allow statistical analysis of a third dose group under 4 U/kg.

Children may require relatively higher doses than adults. Ma and colleagues (2002) studied the density, distribution and morphometry of NMJs in the biceps and gastrocnemius muscles of juvenile (1-month-old) and adult (6-month-old) rats. They found that while the NMJs are of smaller size in juveniles their density

within the muscle is higher than in adults (4 times greater in gastrocnemius and 2.6 times greater in biceps). If this is representative of age-dependent differences, then relatively higher doses in children may be appropriate.

The duration of treatment response has been found to correlate with the dose. In the study by Polak and associates (2002) the maximum gastrocnemius length and dorsiflexion in stance at 12 weeks remained significantly better compared to baseline values in the high-dose but not in the low-dose group. This difference declined by 24 weeks. Baker and colleagues (2002) found the change in gastrocnemius muscle length still to be significantly better at week 16 in the dose group 20 U/kg but not in the other active treatment groups. A prolonged treatment effect with higher doses has likewise been observed with adults, measured with finger/wrist/elbow flexor MAS (Childers et al. 2004, Suputtitada and Suwanwela 2005), ankle plantar flexor MAS and gait (Mancini et al. 2005) and decrement in M-response amplitude and mean rectified voltage during maximal voluntary contraction in the EDB muscle (Wohlfarth et al. 2007). In the present study, the duration of treatment effect was investigated by asking the caregivers´ subjective observations on muscle tone return to pretreatment level and the number of children still evincing good response in MAS (change of ≥ 1 compared with baseline) and gait pattern (at least a one-grade improvement on the GAS) at 4 months. No differences between groups were detected, which may reflect the too narrow dose difference between the low- and high-dose groups or the inaccurateness of the outcome measures to detect change.

1.4. Upper limb dose study (IV)

In this study the doses were determined individually by the author, as no comprehensive guidelines for optimal dosing in the upper limb were available. A variety of doses were used even with the same children, adjusting subsequent dosage according to experiences accruing with previous injections.

In the functional group the only difference between the treatment groups was detected at 1 month in House classification score. In the non-functional group the House classification scores at 1 month were better in the low-dose group, but the difference between the treatment groups did not reach significance. The bias of having more children with mild upper extremity involvement selected in the non-functional low-dose group is likely to have affected the results. One explanation for this is that the more severely involved (and more severely spastic) children were thought to benefit from a higher dose to begin with. However, the parents were highly satisfied with both the functional and cosmetic achievements.

The total doses used in the present high-dose groups corresponded to those in the randomized controlled series reported by Corry and associates (1997) (4-7 U/kg Botox^{R} or 8-9 U/kg Dysport^{R}), and those in the low-dose groups to those used by Fehlings and colleagues (2000) (2-6 U/kg Botox^{R}). Our results are in agreement with those of Corry and colleagues (1997), who found minimal change in grasp-and-release and fine motor functions despite a reduction in muscle tone at elbow and wrist and an increase in elbow and thumb extension. In their study, the beneficial cosmetic effect was valued by the patients and caregivers. Fehlings´ study (2000) showed no significant difference between treatment and control groups in passive ROM, spasticity scores or grip strength,

but on the other hand improvement in function as assessed by the QUEST and the Self Care Domain of the PEDI. The results in the present study are not directly comparable with those of Fehlings by reason of different outcome measures. Some upper limb studies have reported better functional results using more individually oriented measures such as COPM and GAS (Wallen et al. 2004, Lowe et al. 2006, Wallen et al. 2007, Russo et al. 2007), while others have obtained no functional improvement on more global measurements such as PEDI or the Melbourne test (Speth et al. 2005, Wallen et al. 2007, Russo et al. 2007). These outcomes were not evaluated in the present study. In the studies in question, the doses varied between 3-10 U/kg Botox^{R}.

A recent double-blind randomized controlled trial compared high and low doses in the upper limbs of spastic CP children (Kawamura et al. 2007). The doses in the higher dosage group were twice those of the lower dose group, parallelling fairly closely those used in our study. In order to keep the injector blinded, the authors used varied dilutions of 50-200U/ml Botox^{R}, which may have affected the results in favor of the low-dose group. Their study showed no differences between treatment groups in QUEST, PEDI Self Care Domain, GAS, ROM, MAS and grip strength. As a conclusion the authors recommended the following doses: biceps brachii 1 U/kg, wrist/finger flexors 1.5 U/kg (as total), brachioradialis and pronator teres 0.75 U/kg and adductor/opponens pollicis 0.3 (total max. dose 10 U). Our own findings are in agreement with these.

1.5. Adverse events (I-IV)

In general, the great majority of adverse events in this study series were mild, occurred within 3-4 weeks (by the first assessment), and were transient and resolved within a couple of weeks. No severe adverse events were encountered. The total incidence of adverse events ranged from 26% to 68%, thereby in two studies (I and III) exceeding the limits reported in previous papers (3 to 35%) (Gormley et al. 1997, Boyd et al. 1999, Koman et al. 2001, Mohammed et al. 2001, Goldstein 2006). The higher incidence in studies I and III is difficult to explain but may be related to methodological factors, the physician systematically asking the parents about any events they thought could be connected to the injections. This may increase the reporting of symptoms not related to the treatment but reduce the chance of forgetting. Symptoms most frequently reported in the present series were leg pain or bruising, clumsiness, fatigue because of transient weakness, and flu-like symptoms.

In the upper limb, most of the side-effects in the functional group occurred with high doses. One child receiving injections into the flexor digitorum superficialis at a dose of 2 U/kg/muscle evinced decline at 1 month's assessment in finger strength, not being able to hold objects for four weeks. In the same session, the pronator (1.5 U/kg) and flexor carpi ulnaris (1.5 U/kg) muscles were injected and the declined ability to stabilize the wrist added to the problem. The subject did not have these problems when injected into the same muscles with doses of 1 U/kg and 0.75 U/kg, though she reported mild clumsiness in the fingers. Another child had weakness of the thumb lasting for two weeks after treatment of the adductor pollicis with 20 U (0.6 U/kg) of Botox^{R}. No such weakness occurred with adductor pollicis injections at doses of 5-10 U (0.2-0.4

U/kg). As functional improvement was the goal, doses over 1.5 U/kg into the forearm and over 10 U to the adductor pollicis appeared to be too high. Our observations parallel those of Autti-Rämö and colleagues (2001), who obtained a significant relationship between the dose/kg of Botox^{R} and weakness of the flexor carpi radialis measured by reduction in area in the M-response. In the spastic upper limb of nine children, denervation was seen in up to 94% at a dose of 1.4 U/kg and the authors adjusted their own practice of injecting wrist flexors under a dose of 1.5 U/kg. Our findings also agree with those of Kawamura and associates (2007).

Of note is the absence of observed adverse events in the non-functional group in study IV. Though looked for and asked after, the parents had not noticed any adverse events even with higher doses. This may indicate the difficulty of interpreting probable side-effects in more severely involved patients. However, this patient group is inclined to bulbar symptoms, pre-existing gastro-esophageal reflux and frequent chest infections such as pneumonia, and caution with BTXA treatments is recommended (Boyd et al. 1999).

2. Methodological considerations

Children with CP constitute a heterogeneous patient population. We sought to address this circumstance by limiting the sample in studies I-III to those having the spastic form of CP, being ambulatory and under 12 years of age. Nevertheless, the children belonged to different GMFCS levels (I-IV) and showed variety in their cognitive limitations and performance. In study IV the patient sample was more heterogeneous in etiology, severity of impairment, cognitional abilities and type of movement pattern, some subjects having a dystonic component along with spasticity. Hand function and particularly the assessment of hand function are influenced by cognition and motivation level. However, in the study designs in both the prospective studies I-II and the retrospective studies III-IV we preferred not to set too many limitations so as to reflect the actual "natural" situation in clinical practice.

The randomization in studies I and II was done by flipping a coin with sides predetermined for each treatment group (heads for proximal/single-point group and tails for distal/multiple-point group). Subjects having the same diagnosis (that is, hemiplegics and diplegics, were enrolled in their own categories, quadriplegics with diplegics) and calf tone spasticity grades (measured by the MAS) were randomized in pairs, which ensured that both treatment groups had the same proportion of patients with hemiplegia and diplegia with the same spasticity grade to begin with. As more severely involved patients usually have greater MAS scores, this tallied quite well with the severity graded by GMFCS. However, baseline passive ankle ROM was not taken into account in the randomization as a possible prognostic factor affecting the outcome, but was instead addressed both in the inclusion criteria (i.e. no fixed contracture) and statistical analysis (using change from baseline). In a small sample excessive stratification may weaken the randomization. Nevertheless, the inclusion criteria in dynamic equinus gait could have been stricter (e.g. passive ankle ROM of 0^{o}

or more) than that used in this series. The randomization by coin tossing could have been replaced by generating random numbers, but the problems with variation in passive ROM would have remained the same.

In the retrospective studies, the selection of target muscles and the choice of dose were made on subjective clinical grounds. Especially in the upper limb this approach, while appropriate, introduces a covariable which makes analysis of outcome more difficult. It should also be noted that, in both the lower and possibly even more so in the upper limb, spasticity is not the only component of the UMNS affecting performance in assessments. BTXA reduces spasticity by lowering the total force produced by the injected muscle, thus tending to further, albeit temporarily, impair the performance. The functional results may emerge later than the follow-up period of 3-4 months. In addition, the use of splints and orthoses other than night splints was not standardized.

BTXA reduces muscle tone and increases dynamic muscle length and the outcome measures were chosen to detect changes in these parameters. They thus focused on impairment and to some extent activity. The reliability of the MTS, passive ROM and the MAS has been assessed in the lower limb of CP children (Fosang et al. 2003, Yam and Leung 2006). In spite of their limitations, they are at present the best clinical methods available for measuring impairments in CP. We did not use GMFM in studies I-II because, firstly, we felt it would not discriminate equinus gait between the two treatment groups, and secondly, we evaluated the treated legs and felt that OGS was better for this purpose.

Different gait rating scales have been used in previous studies (Corry et al. 1998, Flett et al. 1999, Ubhi et al. 2000, Koman et al. 2001). The interrater and intrarater reliability of the OGS subscales were found to be moderate to substantial in children with diplegia aged six years or more (Mackey et al. 2003). We found foot contact at midstance and timing of heel rise subscores difficult to rate from the video recordings of young children lacking compliance and decided to stay with the initial foot contact section, which would be comparable with previous studies (Corry et al. 1998, Flett et al. 1999, Ubhi et al. 2000, Koman et al. 2001). The validity or reliability of the standardized grips and bimanual functions, House classification and ULPRS has not been studied.

Measurements made by the therapists (I-IV) and the physician (IV) constitute a possible source of variation. Considering the subjectivity of the measurement tools used and differences in interrater and intrarater reliabilities, we sought to maximize reliability by having the same therapist assess the same children throughout the study (see Stuber et al. 1988, Fosang et al. 2003). In ROM measurements, an increase over 10-15^{o} by a single tester is generally considered to be the limit for a clinically significant change (Stuber et al.1988). The therapists assessing the outcomes were blinded to the treatment groups (I-II) and doses (III-IV), but not to the time of treatment, except for the physiotherapists scoring the videos (I-III).

Documenting the placement of the needle by EMG or ultrasound might have increased the accuracy of injections. Accuracy of needle placement has been shown to be 78% in the gastrocnemius, 62% in the biceps brachii and less than this in other upper limb muscles as assessed by manual palpation only (Chin et al. 2005). However, Chin and associates (2005) delivered their injections to children under general anesthesia and with muscles relaxed. This often hampers the reliability of palpating the muscles in question. We used conscious sedation,

which leaves the spastic muscles tighter and enables the injector to differentiate the muscles more accurately. Nevertheless there remains the challenge of the depth of the needle. Although in studies I-II we took care to inject only the gastrocnemius muscle, there is no guarantee that in all instances the soleus muscle was not injected.

In study IV both manual palpation and EMG-guided techniques were used. It has been postulated that poor functional results in earlier BTXA upper limb studies might be due partly to injections by manual palpation only (Chin et al.2005). This may be the case, as studies using EMG and electrical stimulation guidance have been able to yield better functional results, albeit mostly with outcome measures using individual goals such as GAS and COPM (Wallen et al. 2004, Lowe et al. 2006, Wallen et al. 2007, Russo et al. 2007). In addition, as BTXA seems to affect the quality of hand movement, the present quantitative, ordinal-scaled or more global measures may not be sensitive enough to detect subtle changes. When evaluating the ease of hand use or cosmetic appearance, subjective ratings of parents and child have their place.

The present studies, like many in the field of pediatric rehabilitation, were hampered by small sample size, and thus tended to involve the risk of type II error, not gaining significant results when in fact there are such. Additionally, the risk of bias due to chance is increased. We encountered substantial difficulties in recruiting more national centers into these studies by reason of the demanding protocols and wide variation in current clinical practice in treating children with CP. This variation was shown very clearly in two studies nationally investigating current Finnish practices in CP rehabilitation both generally (Autti-Rämö et al. 2007) and concerning BTXA treatment (Sätilä 2006) in children. In the study by Autti-Rämö and colleagues (2007) short written summaries and videos of three children with diplegia of varying severity were sent to 20 university and central hospital CP rehabilitation teams, asking them to suggest interventions, therapies and three most important goals for the next year. The duration of recommended individual therapy sessions ranged from 45 to 60 minutes and frequency from once a month to three times weekly, and the total amount increased with the severity of disability. The goals were mostly for body structure and function, with lesser orientation towards activity and participation. BTXA treatment has been widely and quickly adopted in Finland and the indications for the treatment seem fairly similar, but each rehabilitation team has formed its own practice in toxin administration, dosaging and post-treatment modality policy (Sätilä 2006, Autti-Rämö et al. 2007).

In the field of pediatric rehabilitation, and particularly in a small country like Finland, there are many specific questions which are only addressed by multicenter studies with sufficient numbers of subjects included. This, again, requires fairly uniform application of outcome measurements and agreement on indications and different treatments and therapies.

3. Clinical implications and suggestions for further studies

When treating spastic equinus gait, it seems that BTXA diffuses efficiently within the muscle and binds avidly to the NMJs. Thus, injection into the target muscle suffices to reduce muscle tone. Nevertheless, targeting as close to the NMJs as possible is considered to be desirable. As more information on the location of NMJs in different muscles in both the lower and upper limb has become available, correct targeting of BTXA injections with better probability even by palpation only is possible. Further study on the larger and differently structured lower limb muscles such as the semitendinosus/semimembranosus or adductors may be required in order to define whether injections near the end plate zones are important. In the smaller upper limb muscles the toxin presumably diffuses sufficiently throughout the muscle length, but this issue is no less in need of further studies.

The present findings would also indicate that increasing the dose over 6 U/kg or splitting the dose volume across the gastroc-soleus muscle does not enhance the effect. Additionally, increasing the doses in the upper limb over 1.5 U/kg in the arm, over 1 U/kg in the forearm and over 10 U in the adductor pollicis muscle yields no better results in reducing tone and increasing function. Children responding poorly to lower doses do not seem to benefit from higher doses but should be carefully evaluated for fixed muscle contractures and treated accordingly. Also, clinicians should be aware of the possibility of a non-linear dose response to BTXA, and carefully monitor the effect of their treatment practice. A potential advantage of using lower doses is reduced drug cost and the possibility to inject additional muscle groups within the safety limits. The decision to inject into multiple sites per muscle may be guided by other factors such as the morphology and end plate configuration of a given muscle, the size of the muscle, the total number of muscles needing treatment and the availability of general anesthesia or sedation.

As increasing doses yields no additional benefit, changing the dilution might be an important factor in both lower and upper limb injections. So far studies with different dilutions have yielded inconclusive results (Francisco et al. 2002, Gracies et al. 2002, Lee et al. 2004). Only one such study investigated CP children (Lee et al. 2004). Further trials with constant dose but various volumes are needed in treating spasticity with BTXA in children with CP.

CONCLUSIONS

1. In clinical practice the gastrocnemius muscles are the most common targets for BTXA treatment and are considered easy to inject without technical guidance. In this present series, study I was conducted to establish whether injections directed as close to the NMJs as possible are more effective in reducing muscle tone, increasing passive ROM and improving spastic equinus gait than injections given into a remote site. The results suggest that, using the methods described, no major changes in main outcome measures or occurrence of adverse events were associated with changing the injection site.

2. Study II evaluated whether multiple-site injections are associated with better outcomes in reducing muscle tone, increasing passive ROM and improving spastic equinus gait compared with single-site injections. Our results suggest that, using the methods described, no major changes in main outcome measures or occurrence of adverse events were associated with the number of injection sites and that the decision on injecting into single or multiple sites may be guided by issues other than efficacy.

3. In study III the choice of doses reflected actual clinical practice. Our findings would indicate that use of doses higher than 6 U/kg body weigth in the gastroc-soleus muscle do not yield superior results compared with lower doses. In the present study the number, type and severity of adverse events did not differ between the dose treatment groups**,** suggesting that BTXA is a safe modality for treating local spasticity in equinus gait.

4. In study IV the doses reflected actual clinical practice, having been determined by the size of the muscle, the degree of spastic hypertonia and the previous response to therapy, seeking to avoid excessive weakness and deterioration of function. Improvement in muscle tone, passive ROM and, to some extent, function was achieved even with lower doses. Thus, the use of high doses (forearm muscles $\geq$ 1 U/kg, arm muscles $\geq$ 1.5 U/kg or adductor pollicis $\geq$ 10 U) yielded no better results compared with lower doses. The incidence of adverse events increased with higher doses, highlighting the importance of careful consideration when functional improvements in the upper extremity are desired.

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ERRATA

Original article I: Botulinum toxin treatment of spastic equinus in cerebral palsy. A randomized trial comparing two injection sites.
Page 359 Table 2 total score maximum: 23 points should read 22 points.

Original article III: Treatment of spastic equinus gait with botulinum toxin A: Does dose matter? Analysis of a clinical cohort.
Page 347 in Results, paragraph starting “The onset of reduction…” line 10: flulike symptoms (n=3 in low-dose, n=1 in low-dose) should read flulike symptoms (n=3 in low-dose, n=1 in high-dose).

Original article IV: Low- and high-dose botulinum toxin A treatment: a retrospective analysis.
Page 287 Table 3, under Functional group/High-dose adductor pollicis (U), mean (range) 8.8 (7.5, 10.0) should read 8.8 (7.5, 20.0).

APPENDIX

Table A. Modified Ashworth Scale (Bohannon and Smith 1987).

Scoring	*Definition*
0	No increase in muscle tone
1	Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion
2	Slight increase in muscle tone shown as a catch and followed by minimal resistance throughout less than half the range of motion
3	Marked increase in muscle tone through most of the range of motion, the affected part being easily moved
4	Considerable increase in muscle tone, passive movement difficult
5	Affected part remaining rigid

Table B. Selective Motor Control Test in ankle (Boyd and Graham 1999).

Scoring	*Definition*
0	No ability to activate dorsiflexion of the foot
1	Predominantly extensor hallucis and/or extensor digitorum activated
2	Extensor hallucis and some activity of tibialis anterior
3	Dorsiflexion with effective activation of tibialis anterior with knee and/or hip flexion
4	Isolated selective dorsiflexion with knee extended

Table C. Observational Gait Scale (Boyd and Graham 1999). Reprinted with permission from Georg Thieme Verlag KG.

Gait parameter	*Definition*	*Right*	*Left*
Knee position in midstance	Crouch: Severe > 15 degrees	0	0
	Moderate 10-15 degrees	1	1
	Mild < 10 degrees	2	2
	Neutral	3	3
	Recurvatum: Mild < 5 degrees	2	2
	Moderate 5-10 degrees	1	1
	Severe > 10 degrees	0	0
Initial foot contact	Toe	0	0
	Forefoot	1	1
	Foot-flat	2	2
	Heel	3	3
Foot contact at midstance	Toe/toe (equinus)	0	0
	Foot-flat/early heel rise	1	1
	Foot-flat/no early heel rise	2	2
	Occasional heel/foot-flat	3	3
	Heel/toe (normal roll-over)	4	4
Timing of heel rise	No heel contact (fixed equinus)	0	0
	Before 25% stance (very early)	1	1
	Between 25-50% (slightly early)	2	2
	At terminal stance	3	3
	No heel rise (after foot-flat, i.e. crouch)	0	0
Hindfoot at midstance	Varus	0	0
	Valgus	1	1
	Neutral	2	2
Base of support	Frank scissoring	0	0
	Narrow base (poor knee clearance)	1	1
	Wide base	2	2
	Normal base (width of shoulders)	3	3
Gait assistive devices	Walker (forward/posterior) with assistance	0	0
	Walker (independent)	1	1
	Crutches, sticks	2	2
	None, independent for 10 m	3	3
Overall change	Worse	-1	-1
	None	0	0
	Better	1	1
Total score max. 22 points			

Table D. Goal Attainment Scale used for scoring gait improvement. Reprinted with permission from Georg Thieme Verlag KG.

Score	Definition for the goal
-1	Worse: Limb weakness with clumsiness
0	Start: Defined individually for each leg per child
1	Moderate change: Flat foot contact in about 50% of steps
2	Goal: Flat foot contact at every step
3	Better than anticipated: Heel strike at every step

Table E. House Classification System. Reprinted with permission from House JH, Gwathmey FW and Fidler MO (1981): A dynamic approach to the thumb-in-palm deformity in cerebral palsy. The Journal of Bone and Joint Surgery Am, Vol 63-A, No 2, pp. 216-225.

Level	**Category**	**Description**
0	Does not use	Does not use
1	Poor passive assist	Uses as stabilizing weight only
2	Fair passive assist	Can hold onto object placed in hand
3	Good passive assist	Can hold onto object and stabilize it for use by other hand
4	Poor active assist	Can actively grasp object and hold it weakly
5	Fair active assist	Can actively grasp object and stabilize it well
6	Good active assist	Can actively grasp object and manipulate it against other hand
7	Partial spontaneous use	Can perform bimanual activities easily and occasionally uses the hand spontaneously
8	Complete spontaneous use	Uses hand completely independently without reference to the other hand

Table F. Upper Limb Physician´s Rating Scale (Graham et al. 2000). Reprinted with permission from Elsevier Inc.

Parameter	*Definition*	*Right*	*Left*
Active elbow extension (normal 180°)	> 10 degrees reduction	0	0
	0-10 degrees reduction	1	1
	No reduction	2	2
Active supination elbow extended (Mid-position: palm 90° to horizontal)	None	0	0
	Under mid-position	1	1
	To mid-position	2	2
	Past mid-position	3	3
Active supination elbow flexed	None	0	0
	Under mid-position	1	1
	To mid-position	2	2
	Past mid-position	3	3
Active wrist dorsiflexion (forearm supported)	None	0	0
	Under mid-position	1	1
	To mid-position	2	2
	Past mid-position	3	3
Wrist dorsiflexion	With ulnar deviation	0	0
	With radial deviation	0	0
	Neutral	1	1
Finger opening	Only with wrist flexion	0	0
	With wrist in neutral position	1	1
	With wrist in dorsiflexion	2	2
Thumb in function	Within palm	0	0
	Pressed laterally against index finger	1	1
	Partly assists in grasp	2	2
	Thumb-finger grasp possible	3	3
	Active abduction	4	4
Associated increase in muscle tone	In all manipulative function	0	0
	Only with fine motor manipulation	1	1
	Only with walking or running	2	2
	None	3	3
Two-handed function	None	0	0
	Poor, no use of hidden functions	1	1
	Use of all functions, but limited in activities in daily living	2	2
	Use of all functions, not limited in activities in daily living	3	3
Total score max. 24 points			
Overall change	Worse	-1	-1
	None	0	0
	Slight improvement	1	1
	Clear clinical improvement	2	2

Table G. Questionnaire for caregivers.

1. When did you notice the muscle tone reduction? (day from injection)
2. What kind of functions did the child do better than before injection?
3. Did some functions get worse? How did it show?
4. During what time course did the worsened function recover?
5. When did you notice the muscle tone return? (month from injection)
6. Did the child have: (please underline)
Bruising/soreness at the injection site
Fatigue
Headache
Sense of dry mouth
Changes in urination or fecation (e.g. diarrhea, constipation)
Palpitation
Dizziness
Vomiting
Changes in appetite
Fever
Something else that you associated with the treatment?
7. The adverse events lasted _____ days and were: 1. Mild 2. Moderate 3. Severe
8. Other comments:

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Botulinum Toxin Treatment of Spastic Equinus in Cerebral Palsy

A Randomized Trial Comparing Two Injection Sites

ABSTRACT

Sätilä H, Iisalo T, Pietikäinen T, Seppänen RL, Salo M, Koivikko M, Autti-Rämö I, Haataja R: Botulinum toxin treatment of spastic equinus in cerebral palsy: A randomized trial comparing two injection sites. Am J Phys Med Rehabil 2005;84:355–365.

Objective: To explore the clinical relevance of injection site by comparing two different injection techniques in children with cerebral palsy who have spastic equinus gait.

Design: A total of 19 children (13 boys, 6 girls; range, 1 yr 6 mos to 7 yrs; nine hemiplegics, eight diplegics, two quadriplegics; levels I to IV with the Gross Motor Function Classification System) participated in the study. The children were randomized into two groups: the proximal group received a botulinum toxin A injection into the proximal part of both heads of the gastrocnemius, and the distal group received a botulinum toxin A injection into the mid-belly of the muscle bulks. A single-point injection of BOTOX, 3 units/kg per site, was used. Assessments of active and passive range of motion, dynamic muscle length (modified Tardieu scale), calf tone (modified Ashworth scale), and video gait analysis (Observational Gait Scale) were performed before treatment and 3, 8, and 16 wks posttreatment.

Results: Active and passive dorsiflexion and calf tone in both groups and Observational Gait Scale total scores in the distal group improved at all time points. The median change from baseline values in Observational Gait Scale initial foot contact and total scores at 8 wks showed a significant difference favoring the distal group, but the clinical relevance remained tenuous.

Conclusions: Using the methods described, no major changes in main outcome measures were associated with changing the injection site.

Key Words: Botulinum Toxin Type A, Cerebral Palsy, Spastic Equinus, Injection Site

Botulinum toxin A (BTX-A), an acetylcholine-blocking, denervating chemical when injected into a muscle, has been used in children since 1993 in the management of spasticity in cerebral palsy.[1] The most common indication is spastic equinus gait.[2] Botulinum toxin is an expensive and potentially toxic agent. The reduction in muscle tone should be effective and selective, which requires both optimal dosage and injection site for each muscle—not yet clearly defined factors, even for the most commonly injected gastrocnemius muscles.

Various techniques to improve verification of needle placement in the correct muscle comprise palpation and anatomic landmarks, neurophysiologic methods (nerve or motor-point stimulation, electromyographic guidance), ultrasound, and computed tomography.[3–5] Furthermore, the importance of localizing the neuromuscular junctions (NMJs) within the muscle has been stressed.[6]

In humans and experimental animals, BTX-A spreading occurred into nearby muscles or into contralateral muscles through fascia and bony structures, leading to undesired excessive weakening.[7,8] These results suggest that a dosage of 2–3 units/kg body weight of BTX-A (BOTOX; concentration, 1.25 units/0.1 ml injected into rabbit longissimus dorsi muscle) spreads up to 4.5 cm from the injection site. Quantification of BTX-A–induced muscle paralysis with varying injection sites in rat tibialis anterior muscle showed that injection directly into the NMJ zone gave the best results: when the toxin was injected 0.5 cm from the NMJ zone, paralysis was decreased by 50%.[9] To avoid excessive spread, the authors recommended toxin administration into the center of a muscle, as near the NMJ zone as possible.

In the 1950s, Coers[10] in Belgium and Christensen[11] in Denmark studied dissected mammalian and fetal striated muscles, staining them with cholinesterase. They noticed that the NMJs were situated in the middle of each extrafusal muscle fiber. Depending on the structure of the muscle, the NMJs were either scattered diffusely or formed one or multiple detectable zone-like patterns. Gastrocnemius is a bipennate muscle and, according to work of Christensen,[11] the NMJ zones form oval patterns (Fig. 1). NMJ zones can be detected with electromyography needles (miniature end-plate potentials), but this procedure can be painful.

Childers et al.[12] conducted a double-blind, placebo-controlled study comparing two different BTX-A injection techniques in 15 ambulant adults (aged 19–76 yrs) with spastic hemiplegia. One group received a single-point injection of 50 units of BTX-A (BOTOX) into the proximal part (near motor point) and another group received 50 units divided at three sites into the mid-belly part (near the NMJ zone) of the gastrocnemius. No difference in reduction in muscle tone or functional outcome was detected. Only one (the largest) of the two heads of the gastrocnemius was injected, which may have affected the results.

Little is known about the importance of injection site in children. Compared with adults, the muscles are smaller in size, and the toxin may diffuse more easily throughout the muscle. Therefore, it might be enough to inject into the target muscle. In pediatric clinical practice, the gastrocnemius is the most often treated muscle in cerebral palsy, and due to its clear anatomic appearance, it is also mostly injected by palpation. In the present randomized study, we used the model described by Childers et al.,[12] modified for children. A standard dose of BTX-A (6 units/kg/gastrocnemius, BOTOX) was injected either as near to the NMJ zone as possible or into the proximal part of each gastrocnemius muscle near the origin to discover whether the injection site is of clinical relevance in regard to tone reduction and walking pattern.

METHODS

Patient Sample

Patients were recruited over a 39-mo period from Tampere University Hospital and Central

Objectives: On completion of this article, the reader should be able to (1) recognize the pros and cons of different techniques to improve verification of needle placement in the correct muscle, (2) identify a) the parameters that showed improvement in both groups after botulinum toxin A treatment and b) the parameters that showed no differences in median changes between groups after botulinum toxin A treatment, and (3) realize the shortcomings in various measurement tools used with CP children after botulinum toxin A treatment.

Level: Advanced.

Accreditation: The Association of Academic Physiatrists is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians.

The Association of Academic Physiatrists designates this continuing medical education activity for a maximum of 1.5 credit hours in Category 1 of Physician's Recognition Award of the American Medical Association. Each physician should claim only those hours of credit that he or she actually spent in the education activity.

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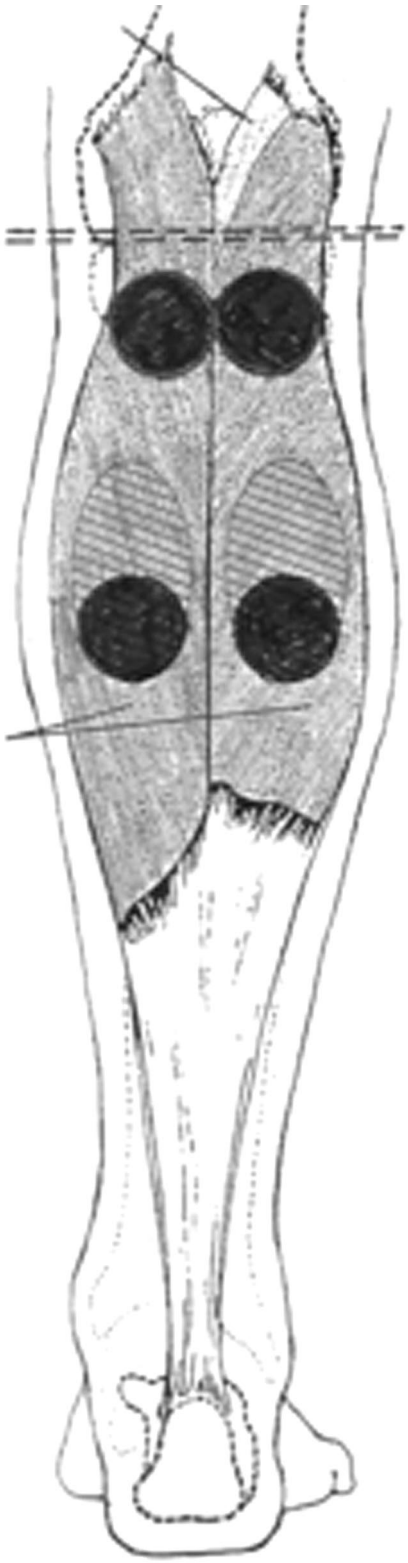

FIGURE 1 *Proximal and distal injection sites. The distance between the popliteal fossa and proximal injection site is approximately 3–4 cm, and the distance between the proximal and distal injection sites is approximately 4–5 cm.* Dashed line*, popliteal fossa;* dotted line*, neuromuscular junctions zone.*

Hospital of Kanta-Häme, Hämeenlinna, both in Finland. Inclusion criteria were: 1) diagnosis of cerebral palsy verified by a child neurologist, 2) ambulation with or without devices, and 3) spastic equinus gait. Exclusion criteria were: 1) age of >7 yrs, 2) previous serial casting or BTX-A treatment within 6 mos before enrolment, and 3) previous surgery on the lower limbs.

Patient characteristics are presented in Table 1.[13] Two patients, one from each treatment group, failed to attend the 4-mo posttreatment assessment and were assessed 6 mos postinjection. Their 6-mo data are included in the intention-to-treat analysis. Three children were enrolled twice, with 1 yr between the treatments. The study was approved by the local Research Ethics Committees and the National Agency for Medicines.

After the baseline measurements, informed written consent was obtained from parents or guardians. Patients meeting the study criteria were randomized into one of the two treatment groups. The treating physician (H. Sätilä) allocated each child by tossing a coin with predetermined sides for each treatment group (heads for the proximal group and tails for the distal group). Unilaterally involved (hemiplegic) *vs.* bilaterally involved (diplegic and quadriplegic) subjects were enrolled in separate categories and then paired on calf tone spasticity grade measured by the Ashworth scale so that after randomization, both treatment groups had the same amount of hemiplegics/diplegics with the same spasticity grade to begin with. The proximal group received BTX-A injection into the proximal part of both heads of the gastrocnemius (near the muscle origin or motor point where the motor nerve enters the muscle), and the distal group received BTX-A injection into the mid-belly of the muscle bulk (near the assumed NMJ zone) (Fig. 1).

Location of the injection site was determined according to anatomic landmarks and palpation. No electromyography or motor-point stimulation was used. In addition to gastrocnemius injections, five patients received hamstring injections due to excessive spasticity. All injections were administered by the same physician (H. Sätilä). Site of injection was not entered in any official record, and the therapists involved in assessment and parents were blinded to the injection site throughout the study.

Clinical assessments were performed at baseline and at 3, 8, and 16 wks posttreatment by one of the two research physiotherapists (T. Pietikäinen or T. Iisalo) in Tampere University Hospital or one research physiotherapist (M. Salo) in Central Hospital of Kanta-Häme, Hämeenlinna, all having 7–15 yrs experience with handicapped children. The same examiner continued with the child throughout the research period.

TABLE 1 Patients' characteristics in proximally (PROX) and distally (DIST) injected groups

	PROX	DIST
Patients, *n*	9	10
Age in yrs, median (range)	4.9 (3.3–7.1)	4.7 (1.5–6.2)
Weight in kg, mean (SD)	17.9 (1.6)	16.7 (3.8)
Sex, female/male	3/6	3/7
Type of cerebral palsy, *n*		
Hemiplegics (right/left)	4 (1/3)	5 (4/1)
Diplegics	3	5
Quadriplegic	2	0
GMFCS[13], *n*		
Level I	6	6
Level II	1	2
Level III	0	1
Level IV	2	1
Children with previous BTX-A injections, *n*	4	3
Children with injected muscles, *n*		
Gastrocnemius only	6	8
Gastrocnemius and hamstrings	3	2
Legs injected, *n*	12	13

GMFCS, Gross Motor Function Classification System; BTX-A, botulinum toxin A.

The definition for each level of the GMFCS according to Palisano et al.[13]: I = walks without restrictions, limitations in more advanced gross motor skills; II = walks without assistive devices, limitations walking outdoors and in the community; III = walks with assistive mobility devices, limitations walking outdoors and in the community; IV = self-mobility with limitations, transported or uses power mobility outdoors and in the community; V = self-mobility is severely limited, even with assistive technology.

Throughout the study, the content and frequency of physiotherapy continued unchanged. Parents and personal physiotherapists were instructed not to introduce any new programs or activities. At the 3-wk assessment, every child received night splints (Fig. 2) on the treated limb to potentiate the BTX-A effect. If the participant already had a splint, he or she was asked to have at least a 2-mo pause in wearing it before entering the trial (three legs in the proximal group, four legs in the distal group).

Injection Technique

A constant dose (100 units in 1 ml of 0.9% saline, BOTOX, Allergan, Irvine, CA) was used for every patient: 3 units/kg per site (6 units/kg per gastrocnemius), making the total dose 12 units/kg for the diplegics and 6 units/kg for the hemiplegics. A single-point injection was used except for doses of >50 units, which were divided into two nearby sites (distance, 0.5–1.0 cm) corresponding to the single-point injection (five children in the proximal group, four children in the distal group).

With local lidocaine cream applied to the injection sites and light oral sedation with midazolam (0.3 mg/kg, maximum of 10 mg per child), the injections were performed with a 22-gauge needle and 1-ml syringes under sterile conditions. The needle was inserted into the muscle (depth, 5–7 mm) with the ankle in neutral position. The needle position was checked by flexing-extending both knee and ankle joint as described by Corry et al.[14] After injection, the flexing-extending movement was continued to enhance the spreading of BTX-A in the muscle bulk. Treatments were given on an outpatient basis; no immediate side effects were observed.

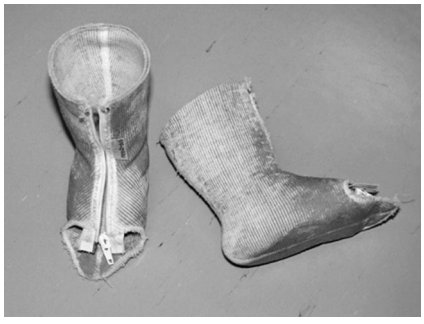

FIGURE 2 *Night splints.*

Outcome Measures

Primary outcome measures were:

(1) Active and passive ankle dorsiflexion with knee extended and flexed measured by manual goniometry with the "neutral-null" method: dorsiflexion angle over the neutral position was counted in positive degrees, under the neutral in negative degrees.

(2) Calf muscle tone measured by modified Ashworth scale.[15] The scoring was as follows: 0 =

TABLE 2 Observational Gait Scale[16]

Gait Parameter	Definition	Right	Left
Knee position in midstance	Crouch: Severe, >15 degrees	0	0
	Moderate, 10–15 degrees	1	1
	Mild, <10 degrees	2	2
	Neutral	3	3
	Recurvatum: Mild, <5 degrees	2	2
	Moderate, 5–10 degrees	1	1
	Severe, >10 degrees	0	0
Initial foot contact	Toe	0	0
	Forefoot	1	1
	Foot-flat	2	2
	Heel	3	3
Foot contact at midstance	Toe/toe (equinus)	0	0
	Foot-flat/early heel rise	1	1
	Foot-flat/no early heel rise	2	2
	Occasional heel/foot-flat	3	3
	Heel/toe (normal rollover)	4	4
Timing of heel rise	No heel contact (fixed equinus)	0	0
	Before 25% stance (very early)	1	1
	Between 25% and 50% (slightly early)	2	2
	At terminal stance	3	3
	No heel rise (after foot-flat, i.e., crouch)	0	0
Hindfoot at midstance	Varus	0	0
	Valgus	1	1
	Neutral	2	2
Base of support	Frank scissoring	0	0
	Narrow base (poor knee clearance)	1	1
	Wide base	2	2
	Normal base (width of shoulders)	3	3
Gait assistive devices	Walker (forward/posterior) with assistance	0	0
	Walker (independent)	1	1
	Crutches, sticks	2	2
	None, independent for 10 m	3	3
Overall change	Worse	−1	−1
	None	0	0
	Better	1	1
Total score maximum: 23 points			

no increase in muscle tone; 1 = slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion; 2 = slight increase in muscle tone shown as a catch and followed by minimal resistance throughout less than half the range of motion; 3 = marked increase in muscle tone through most of the range of motion, the affected part being easily moved; 4 = considerable increase in muscle tone, with passive movement difficult; and 5 = affected part remaining rigid.

Secondary outcome measures were:

(1) Dynamic ankle range of motion with knee extended assessed by modified Tardieu scale.[16] The ankle was dorsiflexed as fast as possible and the catch angle was measured by manual goniometry.

(2) Video gait analysis by Observational Gait Scale[16] (Table 2). Video recordings were performed in sagittal and coronal planes, with the child walking barefoot. A senior pediatric physiotherapist (R. L. Seppänen) not involved with the measurements and unaware of the treatment groups or time sequences scored each treated leg from compiled video recordings. The initial foot contact and total scores were noted.

A questionnaire for parents asked them to report timing and duration of beneficial effects and side effects of the BTX-A treatment. The physician actively asked about adverse events at each assessment, and classified them as severe, moderate, or mild.

Statistical Analysis

The needed sample size was estimated when each group had 6–8 treated limbs. Because of the skew continuous distributions, calculations were done within groups using Friedman's test and between groups at the 8-wk assessment using the *t* test (as an estimate of Mann-Whitney *U* test), with alpha of 0.05 and power of 80%. Sample size of 11 legs per group was required to detect a difference in passive dorsiflexion. The changes of skew continuous and ordinal data within groups were tested using Friedman's test and paired comparisons us-

ing Wilcoxon's test. Mann-Whitney *U* test was used to compare differences of the same type of data between groups. Differences in cross-tables were tested using Fisher's exact test. Significance was assumed at $P < 0.05$. Statistical analysis was performed using SPSS for Windows (version 11.5) and Solo Power Analysis (version 1.0).

RESULTS

Clinical Examination Data

The treatment groups were similar in age, sex, diagnosis, and Gross Motor Function Classification System score (Table 1). At baseline, the difference between the groups approached significance only in passive dorsiflexion with knee extended ($P = 0.065$) (Table 3). At all time points, the active dorsiflexion with knee extended (proximal group, $P = 0.030$; distal group, $P = 0.014$), passive dorsiflexion both with knee extended (proximal group, $P = 0.004$; distal group, $P = 0.004$) and flexed (proximal group, $P = 0.000$; distal group, $P = 0.006$), and modified Ashworth scale scores (proximal group, $P = 0.000$; distal group, $P = 0.001$) improved significantly in both groups. The dynamic muscle length increased significantly at all assessment points in the distal group ($P = 0.048$) but not in the proximal group ($P = 0.074$). Table 3 shows the changes in absolute scores from baseline to those at 3, 8, and 16 wks after treatment. A significant difference between the treatment groups was detected in passive dorsiflexion with knee extended at 3 wks and in passive dorsiflexion with knee flexed at 16 wks, favoring the distal group. However, no differences between the groups were detected when median changes from baseline were used (Table 4). The calf tone decreased slightly later in the proximal group (peak at 8 wks) compared with the distal group (already low at 3 wks) (Table 3).

Observational Gait Scale

No significant differences between baseline values existed. A significant improvement was found at all measurement points in total scores in the distal group (proximal group, $P = 0.122$; distal group, $P = 0.014$) but not in initial foot contact subscore (proximal group, $P = 0.548$; distal group, $P = 0.075$). Table 3 shows the Observational Gait Scale variable absolute score changes from baseline to those at 3, 8, and 16 wks after treatment. No differences between the groups existed. However, in median changes from baseline, a significant difference between the groups was detected in initial foot contact and total scores at 8 wks, favoring the distal group (Table 4). This difference disappeared by 16 wks.

Subgroup Analysis

A subgroup of eight children had passive dorsiflexion of <0 degrees at baseline. Five of these children (six treated legs; two hemiplegics, two quadriplegic, one diplegic) were enrolled into the proximal group and three children (three treated legs; two hemiplegics, one diplegic) into the distal group (difference between groups, $P = 0.226$). No significant differences between groups were detected in any variable. In the subgroup of children having passive dorsiflexion of ≥ 0 degrees at baseline, the only significant difference between groups was found in median change of active dorsiflexion knee extended at 16 wks, favoring the distal group ($P = 0.044$). When comparing the less involved children (Gross Motor Function Classification System I–II) with more severely involved (Gross Motor Function Classification System III–IV) ones, significant differences between groups were found in median change of passive dorsiflexion knee extended at 3 wks ($P = 0.011$), active dorsiflexion knee extended at 8 wks ($P = 0.021$), total scores ($P = 0.037$), and foot contact at 3 wks ($P = 0.022$), favoring the less involved children.

Parental Perception and Adverse Events

Parents noticed a change in muscle tone within a few days: mean of 4.1 days (range, 2–7 days) in the proximal group and mean of 5.8 days (range, 1–20 days) in the distal group. An increase in muscle tone started between 3 and 8 wks in two children (one child in both groups) and between 8 and 16 wks in 11 children (five in the proximal group, six in the distal group). A good response was still being observed in six children at 16 wks (three in both groups). Parents reported a total of 19 adverse events (proximal group, nine events; distal group, ten events), out of which 16 events were considered mild and only three moderate. Adverse events were: tenderness in calf ($n = 8$), tiredness ($n = 3$), irritability ($n = 3$), clumsiness ($n = 3$), and fever or flu-like symptoms ($n = 2$). Moderate symptoms were irritability ($n = 2$) and tenderness in calf ($n = 1$). All symptoms resolved within 1–7 days, except in one child in the proximal group who experienced clumsiness for 16 days. Three adverse events were judged not to relate to the treatment: flu and fever ($n = 2$; both children having the common cold in their family) and calf pain ($n = 1$) occurring after 1 wk, following a long walk. No one withdrew because of these symptoms. No difference between the groups was detected in parental perception or adverse event variables. After the trial period, ten children required a new BTX-A treatment during the following 8 mos (five in both groups), eight children continued with conservative treatment (three in the proximal group, five in

TABLE 3 Clinical examination and Observational Gait Scale results in proximally (PROX) and distally (DIST) injected groups (absolute scores)

	n	PROX Group Median (Range)	*P* Value W	*n*	DIST Group Median (Range)	*P* Value W	*P* Value MWU
Active dorsiflexion knee extended							
Pretreatment	10	−25 (−40 to −10)		11	−20 (−35 to −15)		0.943
3 wks	10	−20 (−35 to −10)	0.017[a]	11	−20 (−35 to −10)	0.202	0.772
8 wks	10	−15 (−35 to 0)	0.049[a]	11	−10 (−30 to +10)	0.014[a]	0.355
16 wks	10	−15 (−45 to 0)	0.313	11	−25 (−40 to 0)	0.400	0.453
Passive dorsiflexion knee extended							
Pretreatment	12	−1 (−15 to +15)		13	+5 (−13 to +27)		0.065
3 wks	12	+5 (−10 to +20)	0.018[a]	13	+15 (−4 to +30)	0.005[a]	0.023[a]
8 wks	12	+7.5 (−5 to +35)	0.003[a]	13	+20 (0 to +35)	0.002[a]	0.091
16 wks	12	+10 (−10 to +25)	0.007[a]	13	+15 (0 to +35)	0.006[a]	0.194
Passive dorsiflexion knee flexed							
Pretreatment	12	+3 (−10 to +30)		13	+20 (−10 to +35)		0.080
3 wks	12	+19.5 (−3 to +30)	0.005[a]	13	+29 (−4 to +38)	0.017[a]	0.274
8 wks	12	+15 (0 to +45)	0.002[a]	13	+30 (0 to +48)	0.020[a]	0.623
16 wks	12	+15 (0 to +30)	0.012[a]	13	+36.5 (+5 to +45)	0.005[a]	0.019[a]
Calf tone							
Pretreatment	12	3 (2 to 4)		13	3 (2 to 4)		0.708
3 wks	12	2 (1 to 3)	0.011[a]	13	2 (1 to 3)	0.003[a]	0.192
8 wks	12	1.8 (1 to 3)	0.002[a]	11	1.5 (0.5 to 3)	0.005[a]	0.710
16 wks	12	2 (1 to 3)	0.010[a]	13	2 (0.5 to 2)	0.010[a]	0.172
Dynamic muscle length							
Pretreatment	12	−12.5 (−35 to 0)		13	−15 (−40 to 0)		0.616
3 wks	12	−15 (−20 to 0)	0.878	13	0 (−30 to 0)	0.021[a]	0.156
8 wks	12	−8.5 (−19 to 0)	0.017[a]	13	0 (−55 to 0)	0.726	0.953
16 wks	12	−10 (−30 to 0)	0.440	13	−2.5 (−35 to 0)	0.063	0.499
Initial foot contact							
Pretreatment	12	0.5 (0 to 2)		13	0 (0 to 2)		0.420
3 wks	9	1 (0 to 2)	0.257	13	2 (0 to 2)	0.023[a]	0.238
8 wks	12	0.5 (0 to 2)	1.000	11	1 (0 to 3)	0.021[a]	0.069
16 wks	11	1 (0 to 3)	0.084	11	0 (0 to 3)	0.257	0.682
Total score							
Pretreatment	12	9 (3 to 17)		13	9 (3 to 20)		0.805
3 wks	9	11 (4 to 18)	0.016[a]	13	9 (7 to 19)	0.106	0.946
8 wks	12	8.5 (5 to 18)	0.226	11	11 (5 to 22)	0.009[a]	0.137
16 wks	11	11 (4 to 19)	0.044[a]	11	8 (2 to 22)	0.441	0.765

Median and range at 3, 8, and 16 wks with significance of difference from pretreatment values (W, Wilcoxon's test) and significance of difference between groups (MWU, Mann-Whitney *U* test).

[a] Significant difference from pretreatment values (W) and between treatment groups (MWU).

TABLE 4 Clinical examination and Observational Gait Scale results in proximally (PROX) and distally (DIST) injected groups (median change from pretreatment values)

	n	PROX Group Median (Range)	*n*	DIST Group Median (Range)	*P* Value MWU
Active dorsiflexion knee extended					
Pretreatment	10	−25 (−40 to −10)	11	−20 (−35 to −15)	0.943
3 wks	10	+5 (−2 to +10)	11	0 (−10 to +15)	0.490
8 wks	10	+7.5 (−15 to +22)	11	+15 (−10 to +40)	0.520
16 wks	10	+5 (−30 to +25)	11	0 (−8 to +15)	0.258
Passive dorsiflexion knee extended					
Pretreatment	12	−1 (−15 to +15)	13	+5 (−13 to +27)	0.065
3 wks	12	+11.5 (−10 to +25)	13	+9 (−2 to +25)	0.935
8 wks	12	+9.5 (0 to +35)	13	+15 (−5 to +27)	0.381
16 wks	12	+9.5 (−1 to +25)	13	+10 (−5 to +30)	0.913
Passive dorsiflexion knee flexed					
Pretreatment	12	+3 (−10 to +30)	13	+20 (−10 to +35)	0.080
3 wks	12	+11.5 (−5 to +20)	13	+5 (−4 to +20)	0.127
8 wks	12	+10.5 (+3 to +35)	13	+10 (−10 to +31)	0.339
16 wks	12	+7.5 (−2 to +20)	13	+10 (−5 to +30)	0.171
Calf tone					
Pretreatment	12	+3 (+2 to +4)	13	+3 (+2 to +4)	0.708
3 wks	12	+0.75 (0 to +2)	13	+1 (0 to +2)	0.385
8 wks	12	+1 (+0.5 to +3)	13	+1 (0 to +3)	0.674
16 wks	12	+1 (0 to +2)	13	+1 (0 to +2.5)	0.362
Dynamic muscle length					
Pretreatment	12	−12.5 (−35 to 0)	13	−15 (−40 to 0)	0.616
3 wks	12	+4 (−15 to +20)	13	+8 (−8 to +30)	0.457
8 wks	12	+3 (−2 to +22)	13	0 (−20 to +35)	0.540
16 wks	12	0 (−15 to +15)	13	+5 (0 to +35)	0.132
Initial foot contact					
Pretreatment	12	+0.5 (0 to +2)	13	0 (0 to +2)	0.420
3 wks	9	0 (−1 to +2)	13	0 (0 to +2)	0.302
8 wks	12	0 (−1 to +1)	11	1 (−1 to +2)	0.025[a]
16 wks	11	0 (−1 to +2)	11	0 (−1 to +2)	0.448
Total score					
Pretreatment	12	9 (+3 to +17)	13	+9 (+3 to +20)	0.805
3 wks	9	1 (0 to +7)	13	+1 (−2 to +7)	0.708
8 wks	12	0 (−2 to +5)	11	+2 (−1 to +7)	0.049[a]
16 wks	11	2 (−2 to +8)	11	0 (−3 to +11)	0.446

Median and range at 3, 8, and 16 wks, with significance of difference between groups (MWU, Mann-Whitney *U* test).
[a] Significant difference between treatment groups (MWU).

the distal group), and one child in the proximal group was referred to surgery.

Intention to Treat

The intention-to-treat analysis with the same variables did not alter the results.

DISCUSSION

We are not aware of any studies on BTX-A diffusion in human muscles, but we predicted that if toxin diffusion occurs parallel to gastrocnemius fibers, the effect on calf tone and passive dorsiflexion will occur in both groups but be more pronounced in the distal group. The two injection sites were chosen because of their clear anatomic appearance, easy accessibility, and the in-between distance being long enough (4–5 cm, despite the size of the child). Our findings demonstrated that both groups benefited from the treatment in terms of passive and active dorsiflexion, dynamic muscle length, calf tone reduction, and function (initial foot contact per treated leg) and that the differences between groups were not robust. In the distal group, the video gait analysis showed a slightly better improvement in equinus gait at 8 wks, declining by 16 wks. These results did not support the hypothesis that BTX-A injections directed as near the NMJ zone as possible are more effective in reducing the muscle tone and produce far better functional results in spastic equinus gait than injections given into a remote site.

The nearly significant difference between the treatment groups in passive dorsiflexion knee extended pretreatment values was unexpected. However, it is unlikely that this affected the results, as median changes from baseline were used, showing similar changes in both injection groups. An extended analysis revealed that the legs with baseline values of <0 degrees accumulated to the most severely involved children (Gross Motor Function Classification System IV) and into the proximal group (three out of four legs). They all benefited in terms of passive dorsiflexion and foot contact. When analyzing the whole group, the less involved children had better change in passive dorsiflexion at 3 wks, active dorsiflexion at 8 wks, and foot contact and total scores at 3 wks, but they were equally distributed between the treatment groups.

Because of different outcome measures, our results are not directly comparable with the animal models. Our findings did not agree with those of Childers et al.,[6] who in their canine model received better force decrements (i.e., weakness) in endplate-targeted *vs.* anatomically guided injections. We did not duplicate the results of Shaari and Sanders,[9] who in their rat experiment noticed paralysis decline with injections given away from the NMJ zone. Rat tibialis anterior muscle has a bandlike NMJ zone at the mid-belly and thus resembles human gastrocnemius muscle. The rats were killed after 24 hrs, which may have been too short a time for additional paralysis to occur. The minimal difference between the groups in our study may indicate efficient toxin diffusion and avid binding to the NMJs within the muscle.

Compared with the proximal group, the modified Ashworth scale scores decreased slightly earlier in the distal group. This may indicate either BTX-A diffusion through fascia to soleus or unintended injection into soleus. Although we were careful to inject only the gastrocnemius muscle, we could not ensure that in all instances the soleus muscle was not injected. Accuracy of the needle placement by manual palpation was found to be 78% in gastrocnemius muscle injections among 226 children.[17] Documenting the place of needle with ultrasound or electromyography might have increased reliability. In animal experiments by Borodic et al.,[7] histochemical changes were noticed within 2-cm distances in adjacent, noninjected muscles, suggesting that diffusion to soleus could occur, which is in line with our findings. The temporary improvement in initial foot contact scores at 8 wks in the distal group, in comparison with the proximal group, may also reflect diffusion to soleus. However, Desloovere et al.[18] found no evidence in three-dimensional gait analysis of remarkable diffusion from gastrocnemius to soleus in a subgroup of children with injections only to gastrocnemius.

Our results are in agreement with those of Childers et al.,[12] who found no significant differences in muscle tone and functional variables between treatment groups. They injected only one of the two heads of gastrocnemius and studied hemiplegic adults with varying pathogeneses and duration of central nervous system injury, which makes the comparisons of these two study results complex. Our findings correlate well with the clinical observations of Westhoff et al.,[5] who under ultrasound guidance injected the iliopsoas muscle from the groin, a site far from the NMJ zone.

Our study population comprised both hemiplegics and diplegics. Equinus gait in hemiplegia is usually due to spasticity in the gastrocnemius, whereas in diplegia, the involvement of hamstrings is more frequent.[2] The hemiplegics might have benefited from soleus injections, which the study protocol did not allow. Eight children had passive dorsiflexion of <0 degrees at baseline—a group not so often treated with BTX-A only. All three children in the distal group and three children (one hemiplegic and two quadriplegics) in the proximal group benefited from the injection. In children with cerebral palsy, the reduction in muscle excursion may rather be progressive than sud-

den, having a transition period of coexisting spasticity and mild shortening of muscle.[19] Thus, these children may benefit from BTX-A injections, but the effect is not long lasting. Combining the treatment with serial casting is thought to be more effective than BTX-A alone.[18,20] Examination of these children after sedation could have revealed whether the stiffness in the ankle was because of fixed shortening.

BTX-A reduces muscle tone and increases dynamic muscle length, and the outcome measures were chosen to detect changes in these variables. Different gait rating scales have been used in previous studies.[14,21–23] The interrater and intrarater reliability of the Observational Gait Scale subscales were found to be moderate to substantial in diplegics aged ≥6 yrs.[24] We found the foot contact at midstance and timing of heel rise subscores to be difficult to rate from the video recordings of our young children lacking compliance and decided to stay with the initial foot contact section, which would be comparable with previous studies.[14,21–23] The measurements of the three research physiotherapists are a possible source of variation. The reliability of the modified Tardieu scale, passive range of movement, and the modified Ashworth scale were recently assessed in the lower limb of children with cerebral palsy.[25] Considering the subjectivity of the measurement tools used and differences in interrater and intrarater reliabilities, we tried to maximize the reliability by having the same physiotherapist assess the same children throughout the study.[25,26]

The dosage of 3 units/kg body weight for each muscle half was chosen according to recommendations[27] and was thought to be effective enough to reduce muscle tone. Higher dosage might have increased BTX-A spreading but also might have resulted in better functional improvement. Despite the rather low dosage, the prevalence of adverse events (68% of participants) in this present study was unexpectedly high. This may be related to injection technique but also to the methodologic reason of the physician actively asking the parents about any events that they thought could be connected to the injections. This may increase reporting symptoms not related to the treatment but decrease the chance of forgetting. Adverse events have varied between 5% and 85% in children, and the most frequent symptoms have been leg pain or bruising, falling, flu-like symptoms, and transient weakness.[23,28,29] Adverse events, like pain at the injection site, may be underreported. In this study, all adverse events occurred within 3 wks, and the majority resolved within a week. The hemiplegic child with prolonged clumsiness had poor active dorsiflexion and sensory problems in the treated limb and, according to the parents, had easily stumbled (e.g., on mats) before the injection. Parents considered her clumsiness to be mild and were not concerned. She received her second treatment a year later (same dose) without any adverse events.

In clinical practice, the gastrocnemius muscles are the most common targets for BTX-A injections and are considered easy to inject without any technical guidance. This present preliminary study was conducted to find out whether the injection site is of clinical importance in situations in which no electromyographic or ultrasound guidance is available. These results suggest that, using the methods described, no major changes in main outcome measures were associated with changing the injection site. In muscles with more complex fiber configuration, or in large muscles, injecting into multiple sites may be more effective than injecting into a single site. The clinical significance of multiple-site injection technique in children deserves further investigation.

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Botulinum Toxin Type A Injections Into the Calf Muscles for Treatment of Spastic Equinus in Cerebral Palsy:
A Randomized Trial Comparing Single and Multiple Injection Sites

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Running head: Comparing single and multiple injection sites

Botulinum Toxin Type A Injections Into the Calf Muscles for Treatment of Spastic Equinus in Cerebral Palsy:
A Randomized Trial Comparing Single and Multiple Injection Sites

ABSTRACT

Objective: To investigate the hypothesis that the multiple-site injection technique is associated with better outcomes than the single-point injection method in children with cerebral palsy and spastic equinus gait.

Design: A total of 17 children (9 boys, 8 girls aged 1.8 to 9.4 years; 8 hemiplegics, 8 diplegics, 1 quadriplegic; levels I to IV with the Gross Motor Function Classification System) with 25 treated lower limbs were randomized into two groups: a single-point group receiving a standard dose of botulinum toxin A injection into one site and a multiple-points group into two sites on both heads of the gastrocnemius. Active and passive range of movement, selective dorsiflexion, dynamic muscle length (modified Tardieu Scale), calf tone (modified Ashworth Scale), attainment of anticipated gait pattern (Goal Attainment Scale), and video gait analysis (Observational Gait Scale, OGS) were assessed before and 1, 2, and 4 months after intervention.

Results: Both groups improved in dynamic muscle length, muscle tone, Observational Gait Scale Total scores and Initial Foot Contact scores and a similar number of children attained their goals on the Goal Attainment Scale. The only significant difference between the groups was observed at 2 months in passive dorsiflexion with knee flexed, favouring the single-point group. Though not significantly, the incidence of adverse effects was higher in the multiple-points group.

Conclusions: Using the methods described, no major changes in main outcome measures were associated with the number of injection sites. Issues other than efficacy guide the decision on whether to inject in single or multiple sites when treating spastic equinus with botulinum toxin.

Key Words: Botulinum Toxin, Cerebral Palsy, Spasticity, Single or Multiple Injection Site

Botulinum toxin A (BTX-A), an acetylcholine-blocking chemical denervant when injected into a muscle, has become the treatment of choice in the management of dynamically spastic equinus gait in cerebral palsy (CP).[1,2] The reduction achieved in muscle tone and the increase in ankle range of movement enables the child to walk in a more efficient pattern.[1] Also, an improvement in the inappropriate phasic co-contraction pattern seen between the tibialis anterior and triceps surae muscles in some children with CP has been reported.[3]

BTX-A is a potent toxic agent and requires both optimal dosage and administration for each muscle – factors not yet clearly defined even for the most commonly injected gastrocnemius muscles. In the course of time, as experience with BTX-A has increased, both the amount injected into a given muscle and the total dose per session have risen.[4] Doses, dilutions, volumes and diffusion are interrelated. Dose refers to the amount of diluted BTX-A as measured in units (U) of activity. Volume is the amount of diluted BTX-A as measured in milliliters of saline used for reconstitution. The concentration is the dose divided by the volume. In children, the total amount of BTX-A is indicated either in units per body weight (U/kg) or as an absolute total dose in units.[4] In animal models, increasing the dose with constant volume (increasing concentration)[5], increasing the volume with fixed dose (decreasing concentration)[6] or increasing the dose with constant concentration (increasing volume)[6] have been found to associate with increased paralysis, measured as acetylcholinesterase or glycogen staining.

The use of either single or multiple injection sites has mostly evolved from practical issues of dividing larger doses, and hence the volume injected, into multiple sites in a given muscle in order to reduce unwanted spreading and adverse effects. BTX-A has been shown to spread up to 4.5 cm from the injection site.[5] It has also been shown that, at a certain dose, saturation of the nerve end plates occurs and a plateau is reached,[7] which may allow spread of the toxin overflow into neighbouring structures and the systemic circulation.[8] This could be avoided by splitting the dose over multiple sites. In children a maximum dose of 50 U of Botox^{R} or a maximum volume of 0.5 ml per injection site is therefore recommended.[8,9]

In practice, the number of injection sites used may also depend on the size of the muscle, the total number of muscles needing treatment and the availability of general anesthesia or sedation.[8] In children, one or two injection sites in larger lower limb muscles such as the adductors, the lateral hamstrings, the soleus and each head of the gastrocnemius, and two to four sites in the medial

hamstrings have been recommended.[8,9] However, no studies have been published specifically evaluating the clinical relevance of single or multiple site injection technique in children.

The number of injection sites per muscle may also be determined by the morphology (and hence the end-plate zone configuration) of a given muscle. In adults, Borodic and associates[10] observed the human orbicularis oculi muscle to have diffuse innervation architecture and injected BTX-A (Botox^{R}) either into a single (at the motor point) or multiple sites on separate eyes in ten patients with blepharospasm. Eight subjects reported significantly better relief after 2-4 weeks with the multiple-site method, while two showed no difference between the methods. The outcome was judged by direct observation of muscle weakness by the clinician and the subjective opinion of the patient. In another study, when treating adult patients with spasmodic torticollis, Borodic and colleagues[11] showed the multiple points per muscle injection strategy to be superior to the single point in terms of pain reduction, improvement in posture deformity and range of motion, and improvement in activity endurance, but not in reduction of hypertrophy or involuntary movements. They injected several muscles involved in the dystonic deforming pathology, without knowing the exact pattern of the end-plate zones. The doses were chosen on an individual basis and no evaluation of the incidence of side-effects was reported. Childers and associates[12] conducted a double-blind, placebo-controlled study comparing two different injection techniques in 15 ambulant adults with spastic hemiplegia. One group received a single-point injection of BTX-A (50 U of Botox^{R}) into the proximal part near the motor point using surface electric stimulation and another group 50 U divided over three sites into the mid-belly part near the assumed neuromuscular junction (NMJ) zone of the gastrocnemius using palpation. Only one (the largest) of the two heads of the gastrocnemius was injected with BTX-A and the other with placebo. No difference in reduced muscle tone (Ashworth scale), increased ankle ROM or functional outcome (Fugl-Meyer score, a timed 50-ft fastest walk) was detected between the groups. The fact that only one of the two heads of the gastrocnemius was injected may have affected the results.

In pediatric clinical practice, the gastrocnemius is the muscle most often treated in CP, and due to its clear anatomical appearance it is also mostly injected by palpation.[4] Studies on BTX-A in the treatment of equinus gait in children with CP have used either two (i.e. single-point)[3,13,14] or four (i.e. multiple-point)[15-17] injection sites per gastrocnemius muscle, but little is known as to the effects of single or multiple injection sites in children. Coers[18] and Christensen[19] have shown that in the bipennate gastrocnemius muscle the end-plate zones form parabole patterns. Recently, Parratte and colleagues [20] noted that most end plates are likely to be located in the distal part of the upper third of

the gastrocnemius muscle bulk**.** Since the NMJs in the muscles are the sites of BTX-A action, targeting the right muscle and as close to the motor end plates as possible is considered essential.[21] Diffusion of BTX-A and the potential need to inject close to NMJs are also interrelated with doses, dilutions, volumes and number of injection sites.[4] In theory, injections close to the NMJ zone would improve efficacy, reduce adverse effects and the required doses.[21] There is evidence from animal models that injection distance to NMJs influences the effect of BTX-A treatment. [5,6,22]

In the present randomized study we hypothesized that, if the end plates in the gastrocnemius muscle form a parabole pattern and toxin diffusion occurs parallel to muscle fibres, multiple injection sites would be associated with better outcomes in reducing muscle tone, increasing active and passive ankle ROM and improving equinus gait compared with the single-site method. We also assumed that the multiple-site technique would be associated with fewer adverse effects. A standard dose of BTX-A was injected into either one or two sites on each gastrocnemius muscle to establish whether the number of injection sites is of clinical relevance in regard to tone reduction, walking pattern and incidence of adverse effects.

METHODS

Patients. Patients were recruited from two centres treating CP children if they had a diagnosis of cerebral palsy, were ambulant with or without devices, and had dynamic equinus gait. Exclusion criteria were age over twelve years, previous serial casting or BTX-A treatment within 6 months before enrolment, or previous surgery on the lower limbs. The study was approved by the local Research Ethics Committees and the National Agency for Medicines.

After the baseline measurements, informed written consent was obtained from caregivers. Patients meeting the study criteria were randomized into one of the two treatment groups. The treating physician (HS) allocated each child by tossing a coin with sides predetermined for each treatment group: heads for the single-point group and tails for the multiple. Unilaterally involved (hemiplegic) vs. bilaterally involved (diplegic and quadriplegic) children were enrolled in separate categories and then paired on calf tone spasticity grade measured by the modified Ashworth Scale so that after randomization both treatment groups included the same number of hemiplegics/diplegics with the same spasticity grade at the outset.

Injection technique. The single-point group received a BTX-A injection into the mid-belly of both heads of the gastrocnemius and the multiple-points group into the mid-belly and about 5 cm proximally towards the popliteal fossa (Figure 1)**.** The injection site was determined according to anatomic landmarks and palpation and no electromyography or motor point stimulation was used. All injections were given by the same physician (HS), at a constant dose of 4 U/kg per head of the gastrocnemius muscle (100 U/ml 0.9% saline, BOTOX^{R} , Allergan Inc., Irvine, CA, USA), making a total dose of 16 U/kg for the diplegics and 8 U/kg for the hemiplegics. For single-site injections the dose was injected into one point per muscle head, except for doses over 50 U (0.5 ml), which were divided into two adjacent sites (distance 0.5-1 cm) corresponding to the single-point injection. For multiple-site injections the dose was divided over two sites per muscle head. Treatment was given on an outpatient basis.

With local lidocaine cream and light conscious sedation with midazolam (0.3 mg/kg, maximum 10 mg per child), the injections were administered with a 22-Gauge needle and 1 ml syringes under sterile conditions. The needle was inserted into the muscle (depth 5-7mm) with the ankle in neutral position. The needle position was checked by flexing-extending both knee and ankle joint as described by Corry.[16] After injection, the flexing-extending movement was continued to enhance the spreading of BTX-A in the muscle bulk. The number of injection sites per calf muscle was not entered in any official record. The parents or patients were not blinded to the injection site and received instructions not to reveal the injection sites after treatment during the assessments.

Clinical assessments. Clinical assessments were made at baseline and 1, 2 and 4 months post-treatment by the same pediatric research physiotherapist (TI or TP in Tampere University Hospital, Tampere, or MS in Central Hospital of Kanta-Häme, Hämeenlinna), who continued with the child throughout the research period (Figure 2). All three physiotherapists had 10-18 years experience with CP children and were blinded to the injection site. The content and frequency of physiotherapy continued unchanged throughout the study period and all children had worn a night splint on the affected limb for at least 2 months before enrolment.

Outcome measures on impairment and function. Active and passive ankle range of movement (ROM) and calf muscle tone were primary and dynamic ankle range of motion, selective ankle movement, equinus gait with Observational Gait Scale (OGS) and Goal Attainment Scale (GAS) were secondary outcomes. Active and passive ankle ROM with knee extended and flexed was measured by manual goniometry with the "neutral-null" method (dorsiflexion angle over the neutral

position was counted in positive degrees, under the neutral in negative degrees).[23] Calf muscle tone was measured with the modified Ashworth Scale (MAS).[24] The dynamic ankle range of motion with knee extended was assessed by modified Tardieu Scale (MTS).[25] The ankle was dorsiflexed as fast as possible and the "catch" angle measured by manual goniometry. The selective movement of the ankle was tested by the Selective Motor Control test (SMC).[25] The child was asked to dorsiflex the ankle by trying to touch the finger of the examiner with his/her big toe. Dorsiflexion was scored as follows: 0 = no ability to activate dorsiflexion of the foot, 1 = predominantly extensor hallucis and/or extensor digitorum activated, 2 = extensor hallucis and some activity of the tibialis anterior, 3 = dorsiflexion with effective activation of the tibialis anterior with knee and/or hip flexion, 4 = isolated selective dorsiflexion with knee extended. The physiotherapist evaluating the child scored each limb at each post-treatment assessment on a Goal Attainment Scale[26] (Table 1)**,** which was used for all children to allow comparison in gait improvement.

The gait pattern was recorded on video in sagittal and coronal planes, with the child walking bare-foot. A pediatric physiotherapist (PL-R) not involved with the measurements and blinded to the treatment groups (i.e. injection sites) or time sequences scored each treated leg from compiled video recordings with the Observational Gait Scale.[25] The Initial Foot Contact scores and Total scores were noted. An open questionnaire for parents asked them to report the timing and duration of beneficial and adverse effects of the treatment. Adverse effects were also actively asked by the physician at each assessment. The parents classified the adverse effects as "severe", "moderate" or "mild".

Statistical Analysis. Continuous data with skew distribution and ordinal data within groups for multiple time points were tested using Friedman`s test and further analysis was made with Wilcoxon test. Differences between groups were tested by Mann-Whitney *U* test and Fisher´s exact test. Significance was assumed at $p < 0.05$. The needed sample size was estimated when each group had 8 treated legs and a sample size of 15 legs per group was required to detect a difference of 5 or more degrees in passive dorsiflexion with knee extended between the treatment groups at 2 months, using t-test (as an estimate of the Mann-Whitney U test) with an alpha of 0.05 and power 80%. The power calculation with the final data with alpha 0.05 gave the following results: 0.42 at 1 month (p=0.070), 0.70 at 2 months (p=0.012), and 0.95 at 4 months (p=0.001). Statistical analysis was carried out using SPSS for Windows (version 12.0).

RESULTS

The treatment groups were similar at baseline, except for mean age and weight (Table 2). The doses were calculated based on units per kg body weight and the total dose for the gastrocnemius muscles did not differ between the groups (p= 0.139). In addition to gastrocnemius injections, one hemiplegic patient received medial hamstring injections, two diplegics medial hamstring-adductor injections and five diplegics adductor injections due to excessive spasticity, these multilevel treatments being distributed evenly between the two groups (Table 2). The total doses including the two-level treatments did not differ between the groups (p= 0.114).

At baseline, passive dorsiflexion with knee both extended and flexed was significantly better in the multiple-points group (Table 3). However, the median improvement in passive ROM from baseline was similar in both groups at each assessment and the only statistically significant difference was detected in passive dorsiflexion with knee flexed at 2 months (p= 0.046), favoring the single-point group.

Within both treatment groups at all measurement points, a significant improvement was seen in dynamic muscle length (single: p= 0.032; multiple: p= 0.006), muscle tone (single: p= 0.000; multiple: p= 0.000), OGS Total scores (single: p= 0.028; multiple: p= 0.032), and Initial Foot Contact scores (single: p= 0.021; multiple: p= 0.004), and in the single-point group in active dorsiflexion with knee extended (p= 0.010). An at least one-grade improvement in Initial Foot Contact scores was noted in 44% of the treated legs at 1 month (single: 42%; multiple 46%), in 52% at 2 months (single: 50%; multiple 54%), and in 44% at 4 months (single: 42%; multiple 46%). On the GAS the functional goal in the gait pattern was achieved in 75% of the treated legs in the single-point group and 69% in the multiple-points group at 1 month, in 50% and 69% at 2 months, and in 50% and 69% at 4 months, respectively. These between-group differences were not significant.

Caregivers observed a change in muscle tone within a mean of 3.6 days (range 1-7) in the single-point group and 3.5 (range 1-7) in the multiple-points group. A rise in muscle tone started between 2 and 4 months in two children (one child in both groups) and good response was still being observed in all the remaining children at 4 months. A total of eight adverse events (single:2; multiple: 6) in six children were reported: tenderness of the injected calf for 1 to 2 days after the injection in three children (single: 1; multiple: 2), both tenderness in calf and clumsiness lasting 2 to 7 days in two (both in the multiple-points group), and spasms in the injected calf in one child lasting

10 days (in the single-point group). Though the difference was not significant (p= 0.337), the incidence was higher in the multiple-points group. No one withdrew because of these symptoms and all adverse events were considered mild by the caregivers.

DISCUSSION

In the literature, studies on BTX-A treatment for spastic equinus gait in children with cerebral palsy have reported using either a single- or a multiple-points technique.[3,13-17] In most cases, the single-point technique has included injections into two sites per gastrocnemius muscle[3,13,14] and the multiple-points technique into four sites per muscle,[15-17] but also six injection sites per muscle have been utilized.[28] We are not aware of any studies comparing these two techniques in children, but we predicted that, if the nerve end plates in the gastrocnemius muscle form an parabole pattern and toxin diffusion occurs parallel to muscle fibres, the effect on calf tone, passive dorsiflexion and gait pattern will appear in both groups but be more pronounced in the multiple-sites group. Our findings demonstrated that both groups benefited equally from the treatment and the only statistically significant difference was detected in passive dorsiflexion with knee flexed at 2 months, favoring the single-point group. This difference (3.5 degrees) cannot be considered clinically significant and the effect declined by 4 months.

These results did not support the hypothesis that multiple injections are more effective in reducing muscle tone and produce better functional results in spastic equinus gait than injections given into a single site. The results must be interpreted with cautiousness as the sample size (n=treated legs) was small, this increasing the effect of chance. However, the power calculations gave fairly good results (0.42 at 1 month, 0.70 at 2 months, and 0.95 at 4 months), thus reducing the risk of type II error (not noticing a difference when there is one) at 2 and 4 months assessments. Our results are in agreement with those of Childers and associates[12], who found no significant difference between single- and multiple-site injection groups. Because of different outcome measures and treated muscles, our results are not directly comparable with those of the groups under Borodic[10,11].

We did not anticipate systemic side-effects in gastrocnemius muscle injections at these low doses but predicted that the negative adverse effects would be more pronounced in the single-point method, especially those due to spread into adjacent muscles (i.e. excessive focal muscle weakness) as the whole volume is injected into a limited area. Though BTX-A reduces spasticity by weakening

the muscle, excessive focal muscle weakness is regarded as an adverse effect.[29] The parents reported eight adverse effects in six children: two in the single- and six in the multiple-points group. Even though the difference in incidence between the treatment groups was not significant, the higher incidence of calf soreness and clumsiness in the multiple-points group is worth noting. Pain in calf may be associated with the volume injected as well as the puncture. In this study, the injected toxin volumes per site ranged from 0.52 to 1.2 ml in the single-point group and from 0.23 to 0.43 ml in the multiple-points group. This means that almost every injection in the single-point group was given divided between two adjacent sites in order to adhere to the recommended maximum of 0.5 ml per site. It may be that calf soreness occurred not because of the volume injected but because of the number of injection sites. Clumsiness may have occurred due to excessive muscle weakness and reflect the pharmacological efficacy of BTX-A. However, this efficacy did not translate into a between-group difference in the outcome measures in favor of the multiple-site group.

The minimal difference between the groups in our study may indicate efficient toxin diffusion within the muscle and avid binding to the end plates, so-called neurospecific binding or autofocusing.[30] In addition, toxin diffusion through fascial planes or unintended injection into the soleus might have occurred. Although we took care to inject only the gastrocnemius muscle, there is no guarantee that in all instances the soleus muscle was not injected. Documenting the place of the needle by EMG or ultrasound might have increased the accuracy which has been shown to be 78% in the gastrocnemius by manual palpation only. [31] The dosage of 4 U/kg body weight for each muscle half was chosen according to recommendations[8,9], and was thought to be effective enough to reduce muscle tone. Nonetheless, lower doses might have caused less toxin spreading. It has been postulated that less accuracy in the injection technique may be compensated for by increasing the dose and, to some extent, the volume.[6,31] However, in the present study the dosage was calculated by body weight and was constant for all participants.

We found measurement of injection pain at the moment of treatment to be of no use in differentiating between the groups, firstly, because most of the patients were too young to give answers and, secondly, because all were under conscious sedation. In multilevel treatments, where increasing the total dose and hence the volume injected may require dividing the toxin over multiple sites in order to avoid systemic toxin spread, general anesthesia may be an option for children.[8]

The significant difference between the treatment groups in passive dorsiflexion at baseline was unexpected and may reflect a more dynamic spasticity in the younger patients in the multiple-points

group. However, no child had fixed contracture at baseline. It is unlikely that the difference in passive range of movement affected the results, as median changes from baseline were used, showing similar changes in both injection groups. Likewise, the two-level treatments were distributed evenly between the treatment groups. Additionally, it is unlikely that treatment of the adductor compartment contributes much to the improvement of equinus gait.

The measurements of the three research physiotherapists constitute a possible source of variation, but we sought to obviate the problem by having the same physiotherapist assess the same children throughout the study. The inter- and intrarater reliability of the OGS has been found to be moderate to substantial [32] and we sought to maximize it by using one blinded rater.

In children with juvenile cerebral palsy, the gastrocnemius muscles are the most common targets for BTX-A treatment and in the midst of busy clinical practice considered easy to inject without technical guidance.[31] This preliminary study was conducted to explore the clinical relevance of single or multiple injection sites in situations where no EMG or ultrasound guidance is available. Our results suggest that, using the methods described, no major changes in main outcome measures or occurrence of adverse events were associated with the number of injection sites. Thus, the decision on injecting into single or multiple sites may be guided by issues other than efficacy, such as dividing larger volumes/doses, muscle size, configuration of motor end plates, total number of muscles needing treatment, use of sedation/general anesthesia or preferences of the clinician. The clinical significance of multiple sites in larger muscles and the occurrence of adverse effects in children warrant further investigation in larger samples and preferably with EMG- or ultrasound guidance.

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FIGURE 1: The injection sites. The distance between the popliteal fossa (dashed line) and the proximal injection site, and between proximal and distal injection sites is approximately 5 cm.

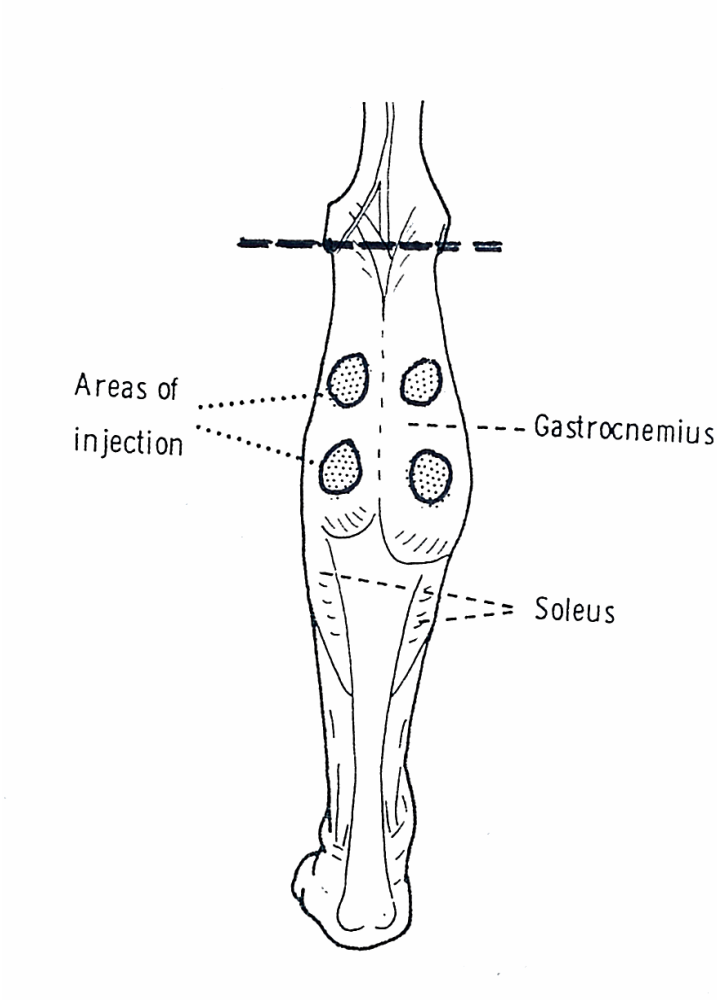
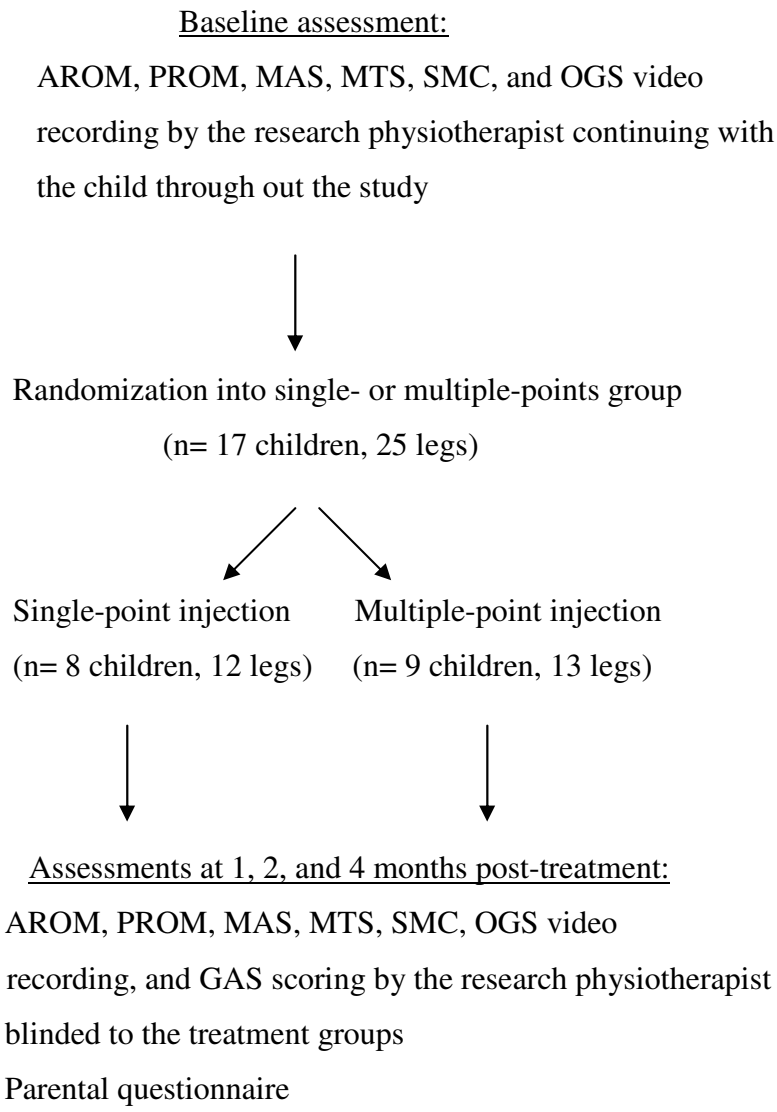


FIGURE 2 Study flow chart



After the study period independent blinded physiotherapist scored the videos with OGS

AROM, active ankle range of movement; PROM, passive ankle range of movement; MAS, Modified Ashworth Scale; MTS, Modified Tardieu Scale; SMC, selective motor control in ankle; OGS, Observational Gait Scale; GAS, Goal Attainment Scale.

TABLE 1 Goal Attainment Scale for gait improvement

Score	Definition for the goal
-1	Worse: Limb weakness with clumsiness
0	Start: Defined individually for each leg per child
1	Moderate change: Flat foot contact in about 50% of steps
2	Goal: Flat foot contact at every step
3	Better than anticipated: Heel strike at every step

TABLE 2 Data on subjects in single- and multiple-points groups

	SINGLE	*MULTIPLE*
Patients (n)	8	9
Age (y), mean (range)[a]	5.5 (2.7–9.4)	3.2 (1.8–5.4)
Weight (kg), mean (range)[a]	20.9 (13-30)	15.2 (11-21)
Sex (F/M)	5/3	3/6
Type of CP (n)		
Hemiplegic (R/L)	4 (3/1)	4 (3/1)
Diplegic	4	4
Quadriplegic	0	1
GMFCS[23] (n)		
Level I	4	4
Level II	1	3
Level III	2	1
Level IV	1	1
Children with injected muscles (n)		
Gastrocnemius only	4	5
Gastrocnemius+ adductors	2	3
Gastrocnemius+ hamstrings/adductors	2	1
Legs injected (n)	12	13

[a] = $p < 0.05$, significant difference between treatment groups.

Gross Motor Function Classification System according to Palisano et al 1997. [27] The definition for each level: I = walks without restrictions; limitations in more advanced gross motor skills; II = walks without assistive devices; limitations walking outdoors and in the community, III = walks with assistive mobility devices; limitations walking outdoors and in the community, IV = self-mobility with limitations; transported or uses powered mobility outdoors and in the community, V = self-mobility is severely limited even with assistive technology

TABLE 3 Results in single- and multiple-points groups (median change from baseline)

	SINGLE		*MULTIPLE*		*p value*
	n	*Median (range)*	*n*	*Median (range)*	
Active dorsiflexion knee extended					
Baseline	12	-11 (-25 - +2)	11	-10 (-50 - +10)	0.928
1 mo	12	+7 (-10 - +17)	11	+2 (-10 - +23)	0.118
2 mo	12	+8 (-4 - +20)	11	+5 (-15 - +45)	0.608
4 mo	10	+5.5 (-16 - +28)	11	+5 (-20 - +18)	0.557
Passive dorsiflexion knee extended					
Baseline	12	+15 (0 - +40)	13	+25 (+15 - +39)	0.010[a]
1 mo	12	+4 (-10 - +15)	13	+5 (-8 - +12)	0.295
2 mo	12	+4 (-10 - +10)	13	0 (-14 - +9)	0.470
4 mo	12	+4 (-15 - +22)	13	+4 (-14 - +14)	0.769
Passive dorsiflexion knee flexed					
Baseline	12	+23.5 (+15 - +40)	13	+38 (+23 - +45)	0.001[a]
1 mo	12	+6 (-10 - +16)	13	-1 (-10 - +12)	0.186
2 mo	12	+1.5 (-5 - +10)	13	-2 (-10 - +10)	0.046[a]
4 mo	12	+1 (-12 - +14)	13	-3 (-14 - +12)	0.168
Calf tone					
Baseline	12	+2.25 (+2 - +3)	13	+2.5 (+1 - +3)	0.769
1 mo	12	+1 (0.5 - +2)	13	+1 (0.5 - +3)	0.689
2 mo	12	+1 (+0 - +2)	13	+1 (0 - +2)	0.611
4 mo	12	+1 (0 - +2)	13	+1 (0 - +2)	0.936
Dynamic muscle length					
Baseline	12	-12 (-22 - 0)	13	-5 (-45 - 0)	0.538
1 mo	12	+7.5 (-10 - +22)	13	+4 (0 - +30)	0.852
2 mo	12	+8 (-7 - +16)	13	+5 (-3 - +25)	0.810
4 mo	12	+6.5 (-5 - +17)	13	+5 (-5 - +25)	1.000
Selective dorsiflexion					
Baseline	12	+3 (+1 - +4)	13	+3 (0 - +4)	0.320
1 mo	12	0 (0 - 0)	13	0 (0 - +1)	0.769
2 mo	12	0 (0 - 0)	13	0 (0 - +1)	0.347
4 mo	12	0 (0 - +1)	13	0 (0 - 0)	0.728
Foot contact score					
Baseline	12	+1 (0 - +2)	13	+1 (0 - +2)	0.852
1 mo	12	0 (0 - +1)	13	0 (-1 - +2)	0.894
2 mo	12	+0.5 (0 - +2)	13	+1 (0 - +2)	0.650
4 mo	12	0 (0 - +2)	13	0 (-1 - +2)	0.979
Total score					
Baseline	12	+7 (+4 - +13)	13	+9 (+3 - +15)	0.894
1 mo	12	+2 (-2 - +6)	13	+2 (-4 - +10)	0.810
2 mo	12	+2 (-1 - +8)	13	+2 (-1 - +10)	0.936
4 mo	12	+1.5 (-2 - +5)	13	+1 (-3 - +6)	0.611

[a] Significant difference between treatment groups (Mann-Whitney U).

ELSEVIER

Low- and High-Dose Botulinum Toxin A Treatment: A Retrospective Analysis

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Upper limb botulinum toxin A doses in children are empirical, determined by the size of the muscle, seeking to avoid excessive weakness and deterioration of function. This study reports the effects and side effects of botulinum toxin treatment on upper limb impairment and function in 18 children with spastic or dystonic hyperactivity. A total of 54 treatments were divided into low-dose or high-dose groups according to the dose used for the target muscles. The outcome measurements included modified Ashworth Scale, passive range of movement, various grips, bimanual functions, movement pattern, House classification of upper extremity use, and subjective ratings of function and cosmetic appearance. In the functional goal group, children benefited in terms of reduction in muscle tone at elbow and wrist, and increase in passive wrist extension and House classification scores. A significant difference between the groups was observed in the House classification, favoring the low-dose group. In the nonfunctional goal group, a significant difference was detected in subjective parental cosmetic ratings, favoring the high dosage. Side effects were few and occurred mostly in the high-dose group. In conclusion, the use of higher doses in the spastic upper limb does not necessarily yield superior results compared with lower doses but increases the incidence of side effects.

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Introduction

Spasticity, defined as a velocity-dependent increase in muscle resistance to stretch, contributes to both reduced longitudinal muscle growth and impaired function [1]. Muscle weakness, agonist-antagonist co-activation affecting movement pattern and coordination, and defects in sensory perception add to upper limb difficulties, reducing the use of the upper extremity in daily activities [2]. Botulinum toxin type A is a widely used modality for focal management of spasticity with cerebral palsy children [3]. Botulinum toxin A works by blocking the presynaptic cholinergic nerve endings and causing a temporary relaxation of the muscle which lasts for approximately 12 weeks and is reversed by a biphasic repair process [4]. The treatment indications include equinus and crouch gait, hip flexion, and hip adduction in the lower limbs, and various spastic or dystonic upper limb deformities (e.g., elbow or wrist flexion, arm pronation, clenched fist, thumb-in-palm deformity) [2].

The few studies investigating the effectiveness of botulinum toxin in the spastic upper limb have reported a reduction in spasticity, an increase in range of movement, and improvement in function, cosmetics, and flexibility of movement pattern [5-10]. Botulinum toxin has been valuable in assessing the possible effect of surgery and in postponing operation to a more appropriate age [11]. With varying doses the side effects have been minor and transient. By reason of the short-lived effect of the toxin protein, serial injections are required. However, little is known as to the possible effect of low and high dosing in upper limb botulinum toxin treatment in children. The present aim was to investigate retrospectively the effects and side effects of botulinum toxin treatment in various dosages on upper limb impairment and function, in terms of range of movement, spasticity, various grips, bimanual functions, classification of upper extremity use, movement pattern, and cosmetic appearance.

Patients and Methods

Botulinum toxin A has been used to manage spasticity at the Department of Paediatric Neurology, Tampere University Hospital,

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Table 1. House classification of upper extremity functional use [Data from House, Gwathmey, Fidler, (14)]

Level	Category	Description
0	Does not use	Does not use
1	Poor passive assist	Uses as stabilizing weight only
2	Fair passive assist	Can hold object placed in hand
3	Good passive assist	Can hold object and stabilize it for use by other hand
4	Poor active assist	Can actively grasp object and hold it weakly
5	Fair active assist	Can actively grasp object and stabilize it well
6	Good active assist	Can actively grasp object and manipulate it
7	Partial spontaneous use	Can perform bimanual activities and occasionally uses the hand
8	Complete spontaneous use	Uses hand completely independently without reference to the other hand

Finland, since 1999. All children are treated according to individually set goals. Upper limb functional abilities have ranged from independence in bimanual activities to a poor supportive extremity, and cognitive level from normal to moderate intellectual impairment. The three main treatment groups are by indication: (1) functional, to improve a specific function or quality of movement; (2) preoperative evaluation, to postpone surgery or help the surgeon with planning; and (3) nonfunctional, to help children with no or minimal functional abilities or after acquired brain injury to improve posture or support on the extremity involved. This study group comprises all 18 children treated between February 1999 and October 2004 (including one child who was treated by an adult neurologist at our hospital in September 1996). Retrospective anonymized data gathered routinely as part of ongoing clinical management protocol were used. The study was approved by the local Research Ethics Committee.

For the functional and preoperative groups, the clinical practice and evaluation protocol evolved during the first year to include measurements of range of movement with goniometry and spasticity on the Modified Ashworth Scale [12] at elbow, wrist, and fingers, and active thumb abduction (a 4-point scale) with the Corry scale [5]. The fine motor functions were assessed using standardized grips (pinch, key grip, 3-finger grip, narrow cylinder grip, wide cylinder grip, pen grip, diagonal grip, grasping, releasing, pronation-supination) and the bimanual functions by specific tasks (putting on a jacket with a zipper, putting on socks, drawing a circle with the help of a glass and cutting out the circle with scissors, cutting and buttering a slice of bread, cutting cucumber) which were recorded on video. Change in movement pattern was analyzed and scored from the videotape using the Upper Limb Physician's Rating Scale [13]. Classification of upper extremity functional use was scored according to House et al. (Table 1) [14]. Each involved upper extremity was measured. At each posttreatment session, the caregiver and treating physician subjectively rated the child's overall response in both function and cosmetic appearance using the following scoring: "deteriorated" (situation worse than pretreatment, score –1), "no change" (situation same as pretreatment, score 0), "slight improvement" (better than pretreatment, score +1), "clear improvement" (much better than pretreatment, score +2). In the functional (n = 4) and preoperative (n = 4) groups children underwent the same assessments at baseline, 1 month (3-4 weeks), 3 months (12-14 weeks), and 6 months posttreatment. The 1-month and 3-month results are reported, as the number of measurements at 6 months were too few for statistical analysis. In the nonfunctional group (n = 10), only the House classification and subjective ratings by parents and physician at 1 month were recorded, as the results for spasticity scores were not recorded systematically and the grip and bimanual function tests could not be performed for all of these children.

The treating physician (H.S.) determined which muscles to inject during baseline function tests (in the functional and preoperative groups), or the reach-and-grasp test or by the appearance of the involved limb (in the nonfunctional group). At least one of six functionally different muscle groups was injected: in persistent shoulder abduction/flexion, the deltoideus (anterior or medial parts, or both); for elbow flexion, the biceps and/or brachialis and/or brachioradialis; for forearm pronation, the pronator teres; in wrist flexion, the flexor carpi radialis or ulnaris, or both; in finger flexion, the flexor carpii superficialis or profundus, or both; and in thumb adduction, the adductor pollicis muscle. Informed consent was obtained from parents or guardians after explaining in detail the effects of botulinum toxin. With local lidocaine cream and light sedation with oral midazolam (0.3 mg/kg, maximum 10 mg per child), the muscles were identified by anatomic knowledge and palpation and the injections were administered either with or without electromyographic guidance. The botulinum toxin (Botox; Allergan Inc., Irvine, CA; dilution 100 U in 1 mL 0.9% saline) was injected in the mid-part of each muscle, where the motor end plates are known to be located. A single or two-point injection was used. Doses over 50 U were always divided over two nearby sites. Occupational and physical therapy mostly continued with the same frequency as before treatment. Facilitating or stretching splints were used individually when appropriate. Indication for new treatment was return of the motor pattern deformity, range of movement, or motor function (e.g., pen grip) to the pretreatment level.

In data analysis, the functional and preoperative groups were combined into one group and the nonfunctional constituted one. According to the botulinum toxin dose used for the target muscles, children were allocated to low-dose or high-dose groups as follows:

(1) Low-dose group: adductor pollicis 5 U or finger/wrist flexors/pronator teres $\leq$ 1 U/kg or arm flexors $\leq$ 1.4 U/kg

(2) High-dose group: adductor pollicis 10 U or finger/wrist flexors/pronator teres $\geq$ 1.1 U/kg or arm flexors $\geq$ 1.5 U/kg

Changes in skew continuous and ordinal data within groups were tested using Friedman's test and paired comparisons using Wilcoxon test. The differences in changes between groups were tested with Mann-Whitney *U* test and with Fisher's Exact Test. Significance was assumed at $P < 0.05$. Statistical analysis was performed using SPSS for Windows (version 12.0).

Results

Of the 18 patients (age range 2-17 years at the time of their first treatment), 10 (56%) were male and 8 (44%) female. The distribution and type of spasticity involvement was as follows: hemiplegia eight (44%), diplegia three (17%), spastic quadriplegia five (28%), and dystonic quadriplegia two (11%). The neurologic diagnosis included prematurity in three (17%), pre- or postpartum cerebrovascular incident in four (22%), hypoxic encephalopathy in six (33%), meningomyelocele and hydrocephalus in one (5.5%), hydrocephalus in one (5.5%), and head injury in three (17%). The botulinum toxin treatment was administered once to five children, twice to five children, three times to three children, four times to three children, and five times to two children with a mean interval of 9.6 months (range 2-24 months). During the study period, a total of 54 upper extremities (154 muscles) were treated in 46 sessions: 27 extremities in the functional/preoperative group and 27 in the nonfunctional group. The mean total amount was 138 U (range 8-640) at a mean total dose of 4.6 U/kg (range 0.2-14.2). The injections were administered on an outpatient basis and were well tolerated. Basic demographic data on the low-dose and high-dose treat-

Table 2. Baseline demographic data

	Functional Group		Nonfunctional Group	
	Low-dose	High-dose	Low-dose	High-dose
Extremities (n) (R/L)	12 (6/6)	15 (11/4)	12 (6/6)	15 (11/4)
Age at treatment (yr), mean (range)	7.8 (3.4, 10.9)	8.5 (1.9, 12.6)	10.7 (6.2, 15.1)	12.2 (4, 17)
Weight (kg), mean (range)	28 (11.4, 45)	32.3 (13.2, 56)	33.3 (15, 60)	30.5 (16, 45)
Sex (F/M)	5/7	8/7	3/5	5/6
Type of involvement (n)				
Hemiplegic	8	11	2	3
Diplegic	1	3	2	0
Quadriplegic, spastic	3	1	3	4
Quadriplegic, dystonic	0	0	1	4
Impairement of the affected limb				
Mild	1	2	2	0
Moderate	5	4	2	0
Severe	6	9	4	11

ment groups by indication are presented in Table 2, and the doses used are summarized in Table 3. At baseline, the treatment groups were similar in age, sex, diagnosis, and impairment of the affected limb.

Functional Group

In the entire group, the improvement was significant at all assessment points in the House classification score, passive wrist extension, and spasticity scores in elbow flexors, pronator, and wrist flexors. The grips, bimanual functions, and passive supination at 1 month from baseline, and the Upper Limb Physician's Rating Scale total scores at both 1 and 3 months from baseline improved significantly. When analyzed by doses, the improvement at all time points was significant in the House classification scores, passive wrist extension, and pronator spasticity scores in both groups, in passive elbow extension and wrist spasticity scores in the high-dose group, and in elbow flexor spasticity scores in the low-dose group. The only significant difference between the treatment groups was detected in House classification median change from baseline to 1 month assessment ($P = 0.005$), favoring the low-dose group (Table 4).

Parental ratings revealed at least a one-grade improvement in function for 96% (n = 27; low: 100%; high: 93%) of treatments at 1 month and for 78% (n = 18; low: 71%; high: 82%) at 3 months, and in cosmetic appearance for 92% (n = 24; low: 100%; high: 86%) at 1 month and for 65% (n = 17; low: 67%; high: 64%) at 3 months. Physician ratings revealed at least a one-grade improvement in function for 93% (n = 27; low: 100%; high: 87%) and 78% (n = 18; low: 71%; high: 82%), and in cosmetic appearance for 92% (n = 24; low: 100%; high: 86%) and 65% (n = 17; low: 67%; high: 64%), respectively. The differences between the treatment groups at 1 or 3 months were not significant.

The onset of reduction in muscle tone was observed within a mean of 5.0 days (range 2 to 14) in the low-dose group, and 2.7 (range 1 to 5) in the high-dose group. The effect was observed to last for a mean of 4.3 (range 1 to 12) and 4.8 months (range 1 to 8), respectively. Parents reported a total of six adverse events: bruising (n = 1), tiredness (n = 1), constipation (n = 1), reduction in finger grip strength for 4 weeks (n = 1), and reduction in thumb motility for 2 weeks (n = 1) in the high-dose group, and flu-like symptoms (n = 1) in the low-dose group. Five adverse events were considered mild and one (thumb motility reduction) moderate. No child or parent was concerned. Of the four preoperative evaluation children, three were predicted to benefit from surgery and were operated successfully.

Nonfunctional Group

At baseline, the difference between the treatment groups was significant in terms of impairment of the affected limb ($P = 0.018$), most of the severely involved children being located in the high-dose group (Table 2). Improvement in House classification scores at 1 month was observed in both groups, but was significant in the low-dose group ($P = 0.046$). The parents rated at least a one-grade improve-

Table 3. The used doses in units (U)

	Functional Group		Nonfunctional Group	
	Low-dose	High-dose	Low-dose	High-dose
Total amount (U), mean (range)	81 (22, 155)	114 (8, 250)	142.5 (20, 460)	228 (30, 640)
Total dose (U/kg), mean (range)	3.2 (1.3, 6.5)	4.2 (0.2, 9.5)	4.1 (1.1, 11.5)	6.9 (1.9, 14.2)
Dose (U/kg/target muscle), mean (range)	0.9 (0.7, 1.0)	1.8 (1.5, 2.6)	0.8 (0.4, 1.0)	2.1 (1.1, 3.4)
Adductor pollicis (U), mean (range)	None	8.8 (7.5, 10.0)		

Table 4. Results in the functional low-dose and high-dose groups (median changes from baseline)

	One Month		Three Months	
	n	Median (range)	n	Median (range)
House scores				
Low-dose	10	1 (0, 1)*†	6	0.5 (0, 1)†
High-dose	14	0 (−1, 1)*†	11	1 (0, 3)†
ULPRS/change				
Low-dose	12	1 (0, 2)	6	1 (0, 2)
High-dose	13	1 (−1, 2)	9	0 (0, 2)
Grip scores				
Low-dose	11	0 (−1, 4)	6	0 (−1, 2)
High-dose	13	0 (−4, 5)	9	0 (−1, 1)
Bimanual function scores				
Low-dose	8	0 (−3, 2)	3	0 (0, 1)
High-dose	12	0 (−1, 4)	7	0 (0, 3)
Thumb abduction/Corry scores				
Low-dose	7	0 (−2, 2)	6	1.25 (−2, 2)
High-dose	8	0 (−1, 1)	6	0 (0, 2)
Ashworth/elbow				
Low-dose	6	1.5 (0, 3)†	6	1 (0, 2)†
High-dose	8	1.15 (−1, 3)	7	1 (−1, 2)
Ashworth/pronator				
Low-dose	8	1 (0, 2)†	6	0 (−1, 2)†
High-dose	8	0.75 (0, 2)†	7	0 (0, 2)†
Ashworth/wrist				
Low-dose	8	1 (0, 2)	6	0.9 (0, 2)
High-dose	7	1 (0, 2)†	6	1 (0, 2)†
Elbow extension/passive (degrees)				
Low-dose	6	1 (−13, 10)	6	−1.5 (−8, 17)
High-dose	6	8.5 (0, 20)†	5	2 (2, 20)†
Forearm supination/passive (degrees)				
Low-dose	9	10 (0, 70)	6	7.5 (−35, 30)
High-dose	7	15 (−8, 30)	7	13 (−8, 30)
Wrist extension/passive (degrees)				
Low-dose	9	7.5 (0, 45)†	6	6 (0, 22)†
High-dose	6	11 (0, 37)†	6	3.5 (0, 17)†

* $P < 0.01$, significant difference between groups (Mann-Whitney U test).
† $P < 0.05$, significant improvement at all time points (Friedman's test).

Abbreviation:
ULPRS = Upper Limb Physician's Rating Scale

ment for 100% (n = 20; low: 100%; high 100%) of treatments in function and for 71% (n = 21; low: 44%; high: 92%) in cosmetic appearance, and the physician for 85% (n = 26; low: 82%; high: 87%) in function and for 62% (n = 26; low: 36%; high: 80%) in cosmetic. A significant difference between groups, favoring the high dosage, was detected in subjective parental cosmetic appearance ratings ($P = 0.047$). The physician's cosmetic rating approached significance ($P = 0.066$). Reduction in muscle tone began within a mean of 3.7 days (range 3 to 5) and lasted for 2.7 months (range 0.7 to 4.5) in the low-dose group, and 3.3 days (range 1 to 6) and 3.6 months (range 0.5 to 6), respectively, in the high-dose group. No adverse effects were reported.

Discussion

In the functional group, the only difference between the treatment groups was detected at 1 month in the House classification score, which is an ordinal scale defining hand use in cerebral palsy [14]. The mean dose in the high-dose group for the target muscles was 2-fold and the mean total amount 1.4-fold of the doses in the low-dose group. This finding suggests that in this patient group use of high doses did not bring superior effects compared with lower doses. In addition, the onset of tone reduction reported by the caregivers commenced a few days earlier in the high-dose group but did not last significantly longer compared with the low-dose group. In the nonfunctional group, the House classification scores at 1 month were better in the low-dose group, the difference between the treatment groups not reaching significance. The bias of having more children with better functional upper extremity use selected in the low-dose group is likely to have affected these results. One explanation is that the more severely involved (and more spastic) children were thought to benefit from a higher dose to begin with. However, the parents were satisfied with both the functional and cosmetic achievements.

The doses used in the functional high-dose group correspond to those in the randomized controlled series reported by Corry et al. [5] (4-7 U/kg Botox or 8-9 U/kg Dysport), and those in the low-dose group to those used by Fehlings et al. [6] (2-6 U/kg Botox). Our results are in agreement with those of Corry et al. [5] who found minimal change in grasp-and-release and the fine motor functions despite a reduction in muscle tone at elbow and wrist and an increase in elbow and thumb extension. In their study, the beneficial cosmetic effect was valued by the patients and parents. Fehlings and associates [6] reported no significant difference between the treatment and control group in passive range of movement, spasticity scores, or grip strength, but on the other hand improvement in function assessed by the Quality of Upper Extremity Skills Test and the self-care domain of the Pediatric Evaluation of Disability Inventory. The results in the present study are not directly comparable with those of Fehlings by reason of different outcome measures. Additionally, the methodological limitations of a retrospective study compared with those of a randomized, controlled trial should be observed.

Most of the side effects in the functional group occurred with high doses. One child receiving injections into the flexor digitorum superficialis at a dose of 2 U/kg/muscle (treatment 2: without electromyography) evinced decline at 1 month's assessment in finger strength, not being able to hold objects for 4 weeks. In the same session, the pronator (1.5 U/kg) and flexor carpi ulnaris (1.5 U/kg) muscles were injected, and the decreased ability to stabilize the wrist added to the problem. She did not have these problems when injected into the same muscles with doses of 1 U/kg (treatment 3: without electromyography) and 0.75 U/kg (treatment 4: with electromyography), though she reported mild clumsiness in the fingers. Another child reported weakness of the thumb lasting for 2 weeks after treatment of the adductor pollicis with 20 U (0.6 U/kg) of Botox. No such weakness occurred with adductor pollicis injections at doses of 5-10 U (0.2-0.4 U/kg). As functional improvement was the goal, doses over 1.5 U/kg into the forearm and over 10 U to the adductor pollicis appeared to be too high, but with nonfunctional goals almost total denervation of the muscle may sometimes be desirable. Our observations parallel those of Autti-Rämö et al. [15], who obtained a statistically significant relationship between the dose/kg of Botox and the weakness of flexor carpi radialis measured by the reduction of area in the M response. In the spastic upper limb of nine children, they documented denervation up to 94% at a dose of 1.4 U/kg and adjusted their own practice of injecting wrist flexors under a dose of 1.5 U/kg.

During the study period, doses were determined empirically, as no guidelines for optimal dosing in the upper limb were available. A variety of doses were used, even with the same children, with subsequent dosage adjusted according to experiences achieved with the previous injections. There was no period of using only either low or high doses, but in many cases the higher doses were administered for more severely affected children in the belief that it was necessary, as observed in the nonfunctional group. As all children were divided into low-dose and high-dose groups according to the botulinum toxin dose used for the target muscles, in some treatments slightly higher or lower doses were administered into 1-2 other muscles (functional group: low n = 2, high n = 4; nonfunctional group: low n = 5, high n = 7). Our practice is also to measure the active range of movement, but owing to differences in cooperation and selective movements in cerebral palsy children the measurements are inclined to errors and fluctuation. We decided to report the passive range of movement as being more reliable, repeatable, and comparable, even though changes in active range of movement may better reflect improvement in function. Using electronic goniometry would have improved the reliability of the measurements. In our experience, these children prefer to use their better hand, and the motivation to practice the affected hand and thus the active range of movement is poor.

Typically, cerebral palsy children have a total flexor pattern which requires multilevel treatment. In the upper limb, the total doses usually remain within safe limits. In our series, one severely involved child in the nonfunctional group received total doses over 400 U of Botox, which is the recommendation for the upper limit per session [16]. The amount (treatment 1: 460 U, 11.5 U/kg; treatment 2: 640 U, 14.2 U/kg) was distributed over 7-9 different muscles. Multilevel injections of smaller doses into single muscles are thought to be safer and to result in better response than single-level high doses [17]. Notably, none of the parents in the nonfunctional group reported adverse effects, which may reflect the difficulty of noticing probable side effects in more severely involved patients.

In the spastic upper limb, botulinum toxin injections have been administered either by anatomic landmarks and manual palpation [5,6,8], or using electromyography or electrical stimulation [7,10,11]. No consensus prevails in this matter, but using electromyography or electrical stimulation may be helpful in locating small muscles in the forearm and hands [15]. In this study, the low-dose and high-dose treatments in the functional group were distributed evenly between the electromyography (low n = 6, high n = 7) and no-electromyography (low n = 6, high n = 8) groups, and no differences were detected in any parameter between these two groups. In the nonfunctional group, the use of electromyography was not equal (electromyography: low n = 1, high n = 1; no-electromyography: low n = 11, high n = 14), which may have affected the results. With higher doses, however, the possible diffusion of botulinum toxin into adjacent muscles may negate the benefits of targeting muscles accurately.

In pediatric practice, the recommendations on botulinum toxin dosage for the treatment of upper limb spasticity are fairly wide and the doses have been determined

empirically by the size of the muscle, the degree of spastic hypertonia, and the previous response to therapy, seeking to avoid excessive weakness and deterioration of function [2]. In this present study, the choice of doses reflects actual clinical practice. The improvement in muscle tone and function was achieved even with lower doses suggesting that use of high doses may not yield superior results compared with lower doses. The incidence of side effects increased with higher doses, highlighting the importance of careful consideration when functional improvements are desired. Also the increases in costs must be taken into account. Further investigation is required in controlled trials with fixed doses and muscles.

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