VIA MEDICA

ORIGINAL ARTICLE

Agata Trafalska[1], Krzysztof Kotecki[2], Magdalena Kazimierska-Zając[1], Adam Druszcz[3], Maciej Miś[4], Luba Ślósarz[1]

[1]Wroclaw Medical University, Wroclaw, Poland
[2]Lower Silesian Specialist Hospital, Wroclaw, Poland
[3]Provincial Specialist Hospital, Legnica, Poland
[4]Health Clinic "Medic", Walbrzych, Poland

Short-term efficacy of botulinum neurotoxin for spastic cerebral palsy: A prospective and controlled study with parental feedback

Corresponding author:

Luba Ślósarz,
Wroclaw Medical University,
Wroclaw, Poland;
e-mail: luba.slosarz@umw.edu.pl

Medical Research Journal 2023;
Volume 8, Number 3, 192–202
10.5603/MRJ.a2023.0025

ISSN 2451-2591
e-ISSN 2451-4101

ABSTRACT

Introduction: Cerebral palsy (CP) is a group of disorders that affect muscle movement, tone and coordination. The reduction of spastic muscular paralysis can be obtained by intramuscular injection of botulinum neurotoxin type A (BTX-A).

Objectives: This study aimed to evaluate the antispastic effect of BTX-A in children with spastic CP and to estimate the parents' opinion about the effectiveness of BTX-A therapy.

Material and methods: A group of 40 children was divided into the study (n = 24, BTX-A + rehabilitation) and the control group (n = 16, rehabilitation). The modified Ashworth scale (MAS) was used to assess the level of muscle tone. A survey method was used to determine the subjective opinion of the children's parents regarding the effectiveness of BTX-A.

Results: The BTX-A injections significantly reduced the level of muscle spasticity in children with CP (5.5 points in the study *vs.* 2.8 points in control; p = 0.008). The analysis from the univariate linear regression model showed children from the study group (B = 1.38, p = 0.005) and older children (B = –0.30, p = = 0.046) influence the difference in obtained MAS scores. The best effect was obtained by combining the BTX-A injection with rehabilitation. Parents positively opinionated the use of BTX-A injections to improve functioning, decrease hypertonia, and facilitate carrying. 83% of parents noticed an improvement in their child's functioning after the first injection of BTX-A and 92% would recommend BTX-A injections for CP.

Conclusions: BTX-A injections lead to a reduction in spasticity in children with CP. The effects of therapy are particularly noticeable at the beginning of the treatment, and the most effective in the youngest patients. BTX-A injections combined with intensive rehabilitation contribute to an improved functional level for children with spastic CP.

Keywords: cerebral palsy, botulinum toxin, muscle spasticity, intramuscular injections, parents, family support

Med Res J 2023; 8 (3): 192–202

Introduction

Cerebral palsy (CP) is a group of permanent disorders of movement and posture causing activity restriction and is attributed to non-progressive disturbances in the developing foetal or infant brain [1]. Motor disorders in CP are often accompanied by sensory, perceptual, cognitive, communication, behavioural disorders, epilepsy, and secondary musculoskeletal difficulties [2, 3]. Despite the progress of neonatal medicine, CP is the most common cause of chronic disability in childhood, occurring in 2–2.5 per 1,000 births [4]. The risk of CP is 20–30 times more common in newborns of low birth weight [5].

Cerebral palsy is the most frequent disability among very young children [6]. Spastic CP is the most commonly diagnosed disorder among children with CP [7]. The well-known adverse effects of spastic hypertonia are muscle contractures, muscle pain, pressure sores, soft tissue shortening and stiffness, joint stiffness or dislocations, and bone torsions, of which the primary consequences are abnormal posture and difficulty in hygiene, dressing, sitting, and movement [8].

In the rehabilitation of children with CP, neurophysiological methods are most often used, especially neurodevelopmental treatment (NDT) developed by Bobath [9], dynamic neuromuscular treatment (DNT) developed by Vojta [10], proprioceptive neuromuscular facilitation (PNF) developed by Knot [11], and the sensory integration intervention concept (SI) developed by Ayres [12].

Spasticity management during CP should be comprehensive and carried out with specialists in neurology, orthopaedics, and paediatric rehabilitation. When planning spasticity therapy, attention should be paid to the impact of dysfunction of specific muscles on the patient's performance of so-called large motor activities and the gait quality. The benefits of BTX-A therapy can be divided into short-term: improving function, facilitating patient care, reducing discomfort and painful muscle hypertension, as well as long-term: preventing the development of permanent contractures, facilitating motor control, and developing correct movement patterns [13, 14].

The most important application of intramuscular BTX injections in children with CP is reducing the increased tone of spastic muscles in both the lower and upper limbs [15]. Based on spasticity and physical examination patterns, it is possible to determine which structures require injection [16]. Also, an ultrasound-guided technique for the intramuscular injection of BTX-A to treat spasticity can be utilized [17, 18]. For medical purposes, BTX-A and BTX-B are commonly used, of which BTX-A is used more often as the stronger type, whereas BTX-B is used in immunization for the first therapy [19].

BTX injections, like any other medication, may have side effects; however, despite the possible risks and side effects, the use of BTX preparations is considered safe [20]. The frequency of BTX-A administration is the key parameter of properly conducted therapy. Performing injections too frequently may lead to the patient's immunization to BTX-A and the side effects of overlapping doses. It has been shown that despite the higher requirement of mean units per appointment over time, only 19% of all treatment cycles are associated with adverse, but tolerable, side effects [21].

It should be noted that, to date, only a few studies have evaluated the efficacy of BTX-A for parents of children with CP. The current state of knowledge in this important aspect is limited. It should be remembered that these parents become the specific therapists who provide everyday care and rehabilitation for their children. Parents can systematically observe the effects of therapeutic interventions and know to what extent they impact the daily functioning of their children and the entire family.

The study aimed to evaluate the efficacy of intramuscular BTX-A in children with CP to obtain a reduction in muscle tone. The primary aim was to determine whether BTX-A injections affect the reduction in muscle tone of children with CP. The secondary aim was to identify factors affecting the efficacy of BTX-A in children with CP. The tertiary objective was to assess parents' subjective impressions regarding the effects of intramuscular BTX-A in their children with CP.

Material and methods

Study design and participants

This prospective, non-randomized, open-label and controlled study was conducted between December 2016 and April 2017 at the Paediatric Department of Nephrology with the Department of Neurology in the Provincial Specialist Hospital in Wroclaw, Poland. The study involved 40 children with CP from 3 to 15 years old, who were divided into two comparative groups. The study group consisted of 24 children, including 14 boys and ten girls ranging in age from 3 to 15 years (mean age 8.0 ± 3.6). The control group consisted of 16 children, including nine boys and seven girls ranging in age from 3 to 13 years (mean age 7.6 ± 2.9). Children from both groups were receiving rehabilitation and did not take any pharmacological agents affecting muscle tone. The children from the study group were subjected to BTX-A injections, while the control group children did not receive any injections.

Measurement tools

During both the basal and comparative tests, a modified Ashworth scale (MAS) was used, which is commonly employed to determine spasticity levels. MAS is a six-level scale, and the individual scoring values mean: 0 — no increase in muscle tone; 1 — a slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected parts are moved in flexion or extension; 1+ — a slight increase in muscle tone, manifested by a catch followed by minimal resistance through the remainder of the range of motion but the affected parts are easily moved; 2 — a more marked increase in muscle tone through most of the range of movement, but the affected parts are easily moved;

3 — considerable increases in muscle tone, passive movement difficult, and 4 — affected parts are rigid in flexion or extension [22].

The study also used a questionnaire designed by the authors and addressed it to the parents who accompanied the child with CP to the ward. This questionnaire contains six closed-ended questions that allow for the parent's subjective opinion of the effectiveness of BTX-A on their children. The questionnaire also included questions about the age of the child and the approximate date of the first treatment with BTX-A and the number of previous BTX-A injections.

It is known that the level of spasticity varies over time — both on a time of day and a long-term basis depending on the period of illness, as well as depending on many external factors. During the study, researchers tried to eliminate the possibility of confounding factors, examining the child in conditions of comfort and safety, in a state of maximum calm, excluding signs of infection and other conditions that affect the level of spasticity. Every effort was made to examine children at the same time of day, 1–2 hours after a meal, to eliminate the influence of hunger or feeding. It should be emphasized, that the child was examined each time by the same persons who did not know the patient before. This was a physician experienced in administering BTX and a physiotherapist who continued providing rehabilitation.

Study procedure

All patients were hospitalized in a paediatric rehabilitation unit. The study and control group were children with the spastic type of CP. The study group was patients to whom BTX-A was administered for the first time before the rehabilitation program, and then comprehensive rehabilitation was applied. The control group was patients to whom BTX-A was not administered; only a rehabilitation program was conducted. Nevertheless, it should be noted that in patients in the control group, the BTX-A injections were scheduled for later, or the parents refused to use the BTX-A injections. Inclusion criteria: spastic CP, no previous BTX-A treatment, parental consent for BTX-A treatment. Exclusion criteria: other forms than spastic CP, previous treatment with BTX-A, use of other pharmacological treatments for spasticity in the course of CP, and no written informed consent from parents.

Each child in the study group has been assessed with the MAS before the first BTX-A injection and after its completion. Based on the difference in the number of points between the pre- and post-injection measurements, the level of spasticity reduction, improvement, or deterioration was shown. The average point value for each treated muscle was also determined for each patient.

The first examination of each patient was performed on the day of admission to the hospital ward, and thus the day before the BTX-A administration. The second examination took place two weeks after the BTX-A injection during a neurologist check-up appointment. The term of 14 days after injection corresponds with the time of maximum treatment effects which can be observed. It is assumed that the post-test results are synonymous with the expected prognosis or its absence after a BTX-A injection.

After BTX-A administration, the children were rehabilitated in the paediatric rehabilitation unit. A comprehensive and individualized rehabilitation was carried out using the NDT and PNF methods and manual therapy. The main aims were focused on reducing muscle contractures, improving the child's functional status, and re-educating movement patterns. The rehabilitation program for each child was determined individually and modified depending on the effects achieved by the administration of BTX-A.

BTX-A dosage

The following BTX-A dosages were used for the lower limb muscles: Ankle flexors: gastrocnemius 5 to 15 IU/kg body weight (up to 4 injections per muscle), soleus 4 to 6 IU/kg body weight (up to 2 injections per muscle). Tibialis posterior 3 to 5 IU/kg body weight (up to 2 injections per muscle). Hamstrings (semimembranosus and semitendinosus) 5 to 6 IU/kg body weight (up to 2 injections per muscle). Hip adductors (longus, brevis and magnus) 3 to 10 IU/kg body weight (up to 2 injections per muscle). Gracilis 3 to 5 IU/kg body weight (up to 2 injections per muscle).

The following BTX-A dosages were used for the upper limb muscles: Biceps brachii 3 to 6 IU/kg body weight (up to 2 injections per muscle). Brachioradialis 1.5 to 3 IU/kg body weight (up to 2 injections per muscle). Pronator teres 1 to 2 IU/kg body weight (up to 1 injection per muscle). Wrist flexors (flexor carpi ulnaris) 1.5 to 3 IU/kg body weight (up to 1 injection per muscle), flexor carpi radialis 2 to 3 IU/kg body weight (up to 2 injections per muscle) and fingers flexors (flexor digitorum superficialis) 1.5 to 3 IU/kg body weight (up to 4 injections per muscle) and flexor digitorum profundus 1 to 2 IU/kg body weight (up to 1 injection per muscle).

The total dose used was according to the drug characteristics, per limb up to 15 IU/kg body weight per limb; simultaneously, the dose of 21 IU/kg was not exceeded when injected into both limbs. Thus, the dose per individual muscle depended on the child's body weight, and the amount of BTX-A per muscle was adjusted according to functional status.

Table 1. Characteristics of groups in terms of age and gender

Variable		Study group (n = 24)	Control group (n = 16)	P-value
Age [years]		M = 8.0 SD = 3.6 Me = 7.0 Min-Max = 3.5–15.0	M = 7.6 SD = 2.9 Me = 7.0 Min-Max = 3.0–13.0	0.912*
Sex	Boys	n = 15 (56%)	n = 9 (63%)	0.693**
	Girls	n = 9 (44%)	n = 7 (37%)	

* Mann-Whitney U test; ** χ^2 test
M — mean; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

Ethical considerations

The study protocol was approved by the independent Bioethics Committee of the Wroclaw Medical University in Poland (approval no. KB–280/2017). All parents of the participating children gave written informed consent to data collection, examinations, measurements, subsequent analysis, and personal data storage for purposes of this study before the study began. The study was carried out in accordance with the statements of the Helsinki Declaration and with consideration of the current Good Clinical Practices guidelines.

Statistical analysis

Statistical analysis was performed using Statistica 13.0. software (StatSoft, Inc., Tulsa, OK, USA). For quantitative variables, arithmetic averages, medians, standard deviations, and range of variation (extreme values) were calculated. For quantitative variables, the frequency of their occurrence (percentage) was calculated. All quantitative variables tested were checked with the Shapiro-Wilk test to determine the type of distribution. A comparison of qualitative variables between groups (study vs. control) was made using the chi-squared test ($\chi 2$). A comparison of quantitative variables between groups (study vs. control) was made using the U Mann-Whitney test. Also, a linear regression analysis was carried out to assess the effect of selected factors on the difference in points obtained in the MAS assessment (difference in MAS grade after injection between the basal pre-injection MAS grade). A non-standardized and standardized regression coefficient, standard error, and statistical significance level were determined. The next step was to build a multifactor model (a stepwise progressive method), taking into account the following variables: gender, age, and group. The level of significance was set at $p < 0.05$.

Results

Participants' characteristics

A group of 40 children participated in the study and were assigned to two comparison groups. The study group consisted of 14 boys (56%) and ten girls (44%) between the ages of 3.5 and 15 years. The control group consisted of 16 children, including nine boys (63%) and seven girls (37%) aged from 3 to 13 years. There were no statistically significant differences between the variables assessed (Tab. 1).

Injected muscles with BTX-A

A neurologist chose the muscles subjected to BTX-A injections after a previous physical examination. Lower limb muscles accounted for 86.3% of all injections (hamstrings = 38 injections, ankle flexors 55 injections, tibialis posterior = 20 injections, hip adductors = 6 injections, and gracilis = 7 injections) while upper limb muscles accounted for 13.7% of all injections (5 injections of each muscle: biceps brachii, brachioradialis, pronator teres, wrist flexors, and fingers flexors). The muscle group most frequently subjected to injections was the ankle plantar flexors (gastrocnemius and soleus muscles), which accounted for 37.7% of all muscles injected. The less frequently injected muscle groups were the upper limbs' muscles, 3.4% on average.

Intergroup and intragroup comparisons of MAS

The intergroup and intragroup comparisons of the MAS scores were conducted. In the study group, 3 to 12 muscles were injected, with six muscles on average. The MAS score after BTX-A injection in the study group dropped by an average of 5.5 points. In the control group, the decrease was 2.8 points. The difference in results was statistically significant ($p = 0.008$). Also,

Table 2. Intergroup and intragroup comparison of the MAS score

Variable	Study group (n = 24)					Control group (n = 16)					P-value*
	M	Me	Min	Max	SD	M	Me	Min	Max	SD	
Number of injected muscles/ /examined muscles	6.1	6.0	3.0	12.0	2.6	4.8	4.0	3.0	8.0	1.9	0.122
Pre-intervention MAS score	12.9	11.8	5.5	23.5	5.4	10.1	8.0	5.5	20.0	4.7	0.080
Post-intervention MAS score	7.4	7.0	2.0	15.5	3.9	7.3	6.0	2.0	15.5	4.0	0.989
Difference in the MAS score	5.5[a]	4.8	1.0	13.5	3.5	2.8[a]	3.0	0.0	5.0	1.4	0.008
Average improvement per muscle	0.9	1.0	0.2	1.8	0.4	0.6	0.6	0.0	1.0	0.3	0.009

*Mann-Whitney U test — intergroup comparison (study group *vs.* control group)
[a]Statistically significant differences between results before and after the intervention (Wilcoxon's test; $p < 0.05$ — intragroup comparison)
M — mean; MAS — modified Ashworth scale; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

Table 3. Intergroup and intragroup comparison of the MAS score concerning the gender

Sex	Variable	Study group (n = 24)					Control group (n = 16)					P-value
		M	Me	Min	Max	SD	M	Me	Min	Max	SD	
Girls	Number of injected muscles/ /examined muscles	6.3	6.0	3.0	12.0	2.7	4.9	5.0	3.0	8.0	1.8	0.266
	Pre-intervention MAS score	14.1	14.0	7.0	23.5	5.6	9.9	9.0	6.0	16.0	3.3	0.153
	Post-intervention MAS score	7.6	6.0	2.0	15.5	4.3	7.1	7.5	3.5	11.0	2.3	0.916
	Difference in the MAS score	6.5[a]	5.0	3.0	13.5	3.8	2.7[a]	3.0	0.0	5.0	1.6	0.011
	Average improvement per muscle	1.0	1.0	0.6	1.6	0.3	0.5	0.6	0.0	0.8	0.3	0.011
Boys	Number of injected muscles/ examined muscles	5.9	6.0	3.0	10.0	2.6	4.7	4.0	3.0	8.0	2.1	0.311
	Pre-intervention MAS score	12.2	11.0	5.5	19.5	5.3	10.2	7.0	5.5	20.0	5.8	0.270
	Post-intervention MAS score	7.3	7.0	2.0	14.0	3.8	7.4	5.5	2.0	15.5	5.0	0.976
	Difference in the MAS score	4.9[a]	3.5	1.0	11.5	3.2	2.8[a]	3.0	0.0	4.5	1.4	0.233
	Average improvement per muscle	0.9	0.8	0.2	1.8	0.4	0.6	0.6	0.0	1.0	0.3	0.222

*Mann-Whitney U test — intergroup comparison (study group *vs.* control group)
[a]Statistically significant differences between results before and after the intervention (Wilcoxon's test; $p<0.05$ – intragroup comparison)
M — mean; MAS — modified Ashworth scale; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

a statistically significant higher mean improvement was reported in the study group (0.9 points) than in the control group (0.6 points). However, no statistically significant differences between the remaining variables were observed (Tab. 2).

Comparisons of MAS due to gender

Each boy from the study group had an average of six muscle injections, and the improvement observed was 0.9 points for each muscle subjected to injection. Each girl from the study group also had an average of six muscle injections, and the improvement observed was 1.0 points for each muscle subjected to injection. In turn, each boy and girl from the control group had tested an average of five muscles, and the improvement observed was 0.6 points for each tested muscle in boys and girls. A statistically significant decrease in the MAS score in both the study and control groups was observed in girls and boys. In addition, girls from the study group had a statistically significant higher decrease in the MAS score or a higher average improvement per muscle than the girls in the control group. However, there were no statistically significant differences between boys and girls in each comparative group (Tab. 3).

Comparisons of MAS due to age group

The test results were also compared according to the children's age. The group was divided into three age

Table 4. Intergroup and intragroup comparison of the MAS score concerning the age group

Age group	Variable	Study group (n = 24)					Control group (n = 16)					P-value
		M	Me	Min	Max	SD	M	Me	Min	Max	SD	
3–6	Number of injected muscles/ examined muscles	7.2	7.0	3.0	12.0	2.8	4.2	3.5	3.0	6.0	1.5	0.031
	Pre-intervention MAS score	15.0	16.0	7.0	23.5	5.2	8.3	6.0	5.5	15.0	4.0	0.014
	Post-intervention MAS score	7.5	9.0	2.0	11.0	3.1	5.7	4.5	2.0	11.5	3.5	0.340
	Difference in the MAS score	7.4[a]	6.0	2.5	13.5	4.0	2.6[a]	3.0	0.0	4.0	1.5	0.012
	Average improvement per muscle	1.0	1.0	0.4	1.6	0.3	0.6	0.6	0.0	1.0	0.3	0.039
7–10	Number of injected muscles/ /examined muscles	5.1	5.0	3.0	9.0	2.5	4.3	4.0	3.0	8.0	1.8	0.609
	Pre-intervention MAS score	11.3	9.0	5.5	20.0	6.1	9.5	8.0	7.0	20.0	4.7	0.701
	Post-intervention MAS score	6.4	4.0	2.0	15.5	5.3	7.1	6.0	4.0	15.5	3.9	0.371
	Difference in the MAS score	4.9[a]	5.0	2.0	7.5	1.8	2.4[a]	3.0	0.0	4.5	1.5	0.018
	Average improvement per muscle	1.0	1.0	0.6	1.8	0.4	0.6	0.6	0.0	1.0	0.3	0.030
11–15	Number of injected muscles/ examined muscles	5.2	5.5	3.0	8.0	1.9	7.0	8.0	5.0	8.0	1.7	0.302
	Pre-intervention MAS score	11.0	10.5	6.0	18.0	4.2	15.0	16.0	11.0	18.0	3.6	0.245
	Post-intervention MAS score	8.3	7.5	3.5	14.0	4.0	11.0	11.0	7.5	14.5	3.5	0.366
	Difference in the MAS score	2.8[a, b]	3.0	1.0	4.0	1.0	4.0	3.5	3.5	5.0	0.9	0.093
	Average improvement per muscle	0.6	0.6	0.2	1.0	0.3	0.6	0.6	0.4	0.7	0.1	0.897

*Mann-Whitney U test — intergroup comparison (study group *vs.* control group)
[a]Statistically significant differences between results before and after the intervention (Wilcoxon's test; $p < 0.05$ — intragroup comparison)
[b]Statistically significant differences between the results in relation to the age groups (ANOVA Kruskal-Wallis test; $p < 0.05$ — intergroup comparison)
M — mean; MAS — modified Ashworth scale; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

subgroups: 3–6 years, 7–10 years, and 11–15 years. In children from the 3–6 age group as well as the 7–10 age group, a one-point improvement was demonstrated for each muscle subjected to the BTX-A injection. A significantly smaller improvement was observed in children from the 11–15 age subgroup, which was 0.6 points for each muscle subjected to BTX-A injection. Children from the control group showed similar improvement values, regardless of their age. The reduction of muscle spasticity oscillated around 0.6 points for each muscle tested. In all subgroups for both the study and control groups, the decreases were statistically significant. There was no statistically significant decrease in MAS score observed in the age subgroup 11–15 years (control group). In the intergroup comparisons (study vs. control), higher statistically significant differences were presented in the study group. These results concern the age subgroups 3–6 years and 7–10 years. Also, it was observed in the study group that the difference in the MAS score was statistically highest in the age group 3–6 years in relation to the other age subgroups (Tab. 4).

Comparisons of MAS due to injected limb

The effectiveness of BTX-A injections in relation to particular muscle groups of the upper or lower limbs was considered. In the study group, there was a comparable reduction of MAS scores after BTX-A injections in the muscles of the lower and upper limbs. A similar result was found in the control group. However, in the lower limbs of the study group, differences in the MAS score were significantly higher than in the control group ($p = 0.020$). However, it should be noted that in the study group, the initial MAS scores of the lower limb muscles were also significantly higher ($p = 0.034$) (Tab. 5).

Another factor is the number of muscles subjected to BTX-A injections in each child. Three subgroups were adopted: 3–5 muscles, 6–8 muscles, and 9–12 muscles. In all three subgroups, there was a statistically significant reduction in MAS scores after the intervention. The largest decrease was recorded in the subgroup where most muscles received BTX-A injections. The highest differences in MAS scores were recorded in this subgroup (Tab. 6).

Comparisons of MAS due to amount of injections

The relationship between variables such as study vs. control group, sex, and age was carried out using the MAS. The analysis from the univariate linear regression model showed children from the study group ($B = 1.38$, $p = 0.005$) and older children ($B = -0.30$, $p = 0.046$)

Table 5. Intergroup and intragroup comparison of the MAS score concerning the evaluated muscle (lower *vs.* upper limb)

Limb	Variable	Study group (n = 24)					Control group (n = 16)					P-value
		M	Me	Min	Max	SD	M	Me	Min	Max	SD	
Lower limb	Number of injected muscles/ /examined muscles	6.3	6.0	3.0	12.0	2.4	4.6	4.0	3.0	8.0	1.9	0.053
	Pre-intervention MAS score	13.5	12.5	6.0	23.5	5.5	9.6	8.0	5.5	18.0	4.3	0.034
	Post-intervention MAS score	8.2	8.0	2.0	15.5	4.3	6.9	5.5	2.0	14.5	3.7	0.475
	Difference in the MAS score	5.3[a]	5.0	1.0	13.5	3.4	2.7[a]	3.0	0.0	5.0	1.5	0.020
	Average improvement per muscle	0.9	0.8	0.2	1.8	0.5	0.6	0.6	0.0	1.0	0.3	0.167
Upper limb	Number of injected muscles/ /examined muscles	5.7	5.0	3.0	10.0	3.0	5.3	5.0	3.0	8.0	2.5	1.000
	Pre-intervention MAS score	11.8	11.0	5.5	19.5	5.4	12.2	9.0	7.5	20.0	6.8	0.853
	Post-intervention MAS score	6.1	5.5	2.0	10.0	3.0	9.2	6.0	6.0	15.5	5.5	0.267
	Difference in the MAS score	5.8[a]	4.0	2.5	12.5	3.7	3.0[a]	3.0	1.5	4.5	1.5	0.309
	Average improvement per muscle	1.0	1.0	0.7	1.6	0.3	0.6	0.6	0.5	0.6	0.1	0.016

*Mann-Whitney U test — intergroup comparison (study group *vs.* control group)
[a]Statistically significant differences between results before and after the intervention (Wilcoxon's test; $p < 0.05$ — intragroup comparison)
M — mean; MAS — modified Ashworth scale; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

Table 6. Intergroup and intragroup comparison of the MAS score concerning the number of injected muscles

Number of injected muscles	Variable	Study group (n = 24)				
		$\bar{x}$	Me	Min	Max	SD
3–5	Number of injected muscles/examined muscles	4.7	5.0	3.0	8.0	1.8
	Pre-intervention MAS score	10.0	9.0	5.5	20.0	4.2
	Post-intervention MAS score	6.3	5.0	2.0	15.5	4.3
	Difference in the MAS score	3.7[a]	3.5	1.0	6.0	1.4
	Average improvement per muscle	0.9	0.8	0.2	1.8	0.4
6-8	Number of injected muscles/examined muscles	7.0	7.0	6.0	8.0	1.0
	Pre-intervention MAS score	15.6	16.0	12.5	18.5	2.4
	Post-intervention MAS score	8.3	10.0	3.5	11.0	3.1
	Difference in the MAS score	7.3[a]	6.0	2.5	12.5	4.5
	Average improvement per muscle	1.0	1.0	0.4	1.6	0.5
9-12	Number of injected muscles/examined muscles	10.3[b]	10.0	9.0	12.0	1.3
	Pre-intervention MAS score	20.3[b]	19.5	18.5	23.5	2.2
	Post-intervention MAS score	10.3	10.0	9.0	12.0	1.3
	Difference in the MAS score	10.0[a,b]	9.5	7.5	13.5	2.5
	Average improvement per muscle	1.0	1.0	0.8	1.1	0.1

[a]Statistically significant differences between results before and after the intervention (Wilcoxon's test; $p < 0.05$ — intragroup comparison)
[b]Statistically significant differences between the results in relation to the number of injected muscles (ANOVA Kruskal-Wallis test; $p < 0.05$ — intergroup comparison)
M — mean; MAS — modified Ashworth scale; Max — maximum value; Me — median; Min — minimum value; n — number of persons; SD — standard deviation

influence the difference in obtained MAS scores. These variables have been confirmed in a multifactor model. The results of children who underwent BTX-A injections ($B = 0.89$, $p = 0.007$) increase the difference in obtained MAS scores. Also, the older the children are, the smaller the difference ($B = -0.28$, $p = 0.003$) (Tab. 7).

Table 7. Simple and multiple linear regression model assessing the association of predictors with the difference in the MAS score

Variable		The difference in the MAS score									
		Simple linear regression analysis					Multiple linear regression analysis				
		B	SE	t	p-value	ß	B	SE	t	p-value	ß
Group	Control			–					–		
	Study	1.38	0.46	3.01	0.005	0.44	0.89	0.31	2.9	0.007	0.28
Gender	Boy			–					–		
	Girl	0.37	0.51	0.73	0.47	0.12	–	–	–	–	–
Age		–0.30	0.14	–2.07	0.046	–0.32	–0.28	0.09	–3.1	0.003	–0.30

B — unstandardized regression coefficient; ß — standardized regression coefficient; MAS — modified Ashworth scale; SE — standard error; t — B/standard error

Parental feedback on BTX-A effects

The results obtained are compatible with parents' opinions of children with CP who were involved in the study. Most parents (83%) noticed an improvement in their child's functioning after the first injection of BTX-A. On the other hand, 13% of parents could not comment on this relationship, and 4% did not notice improvement after the injections.

Most parents (88%) noticed a reduction in muscle tone in their child after BTX-A injections (42% "definitely" and 46% "slightly"). However, 12% of parents were not able to clearly state whether such improvement occurred. It is worth emphasizing that only 4% did not notice an improvement in muscle tone.

Most parents indicated an answer confirming the improvement in caring for their children, 54%, of which 25% is significant, and 29% is a slight improvement. Only 13% of parents were unable to determine if child-care improved. A large group, 29% are self-dependent children, which is reflected in selecting the answer "not applicable". Only 4% of parents believe there is no difference in their child's daily care since the injection. Most parents (75%) perceived BTX-A injections as adjunctive and support for their child's rehabilitation. According to 13% of parents, injections were the only and the best way to reduce muscle tone, and 8% of parents could not determine their opinion. Only 4% of parents believe BTX-A did not improve the level of the child's functioning.

Most parents (92%) would recommend BTX-A injections to other parents of children with CP in order to effectively reduce spastic muscle tone. Only 8% of parents were unable to answer the question unambiguously; however, they did not deny its effectiveness.

Discussion

One of the aims of this study was to assess parental subjective perceptions of the effects of intramuscular BTX-A administration in their CP children. So far, only in a few studies, the effectiveness of BTX-A was evaluated by parents. This is an important aspect of therapy but an unreliable way to evaluate treatment. Given the goals of spasticity treatment, including that facilitating patient care, and improving patient independence, a parental subjective assessment has significant practical value. In the study by Papavasiliou et al. [23], parental assessment reflects the effects of therapy confirmed by clinical tests. In contrast, a study by Slawek and Klimont [24] showed a lack of correlation between parental assessment and medical findings. The present study also correlated the results with parental opinions. The results obtained are consistent with the opinions of parents of children with MPD who participated in the study. Most parents (83%) noted an improvement in their children's functioning after the first BTX-A injection.

Also, this study's results show a comparison of the MAS scores between the first test (before the injection) and post-treatment (two weeks after the injections). It was demonstrated that the applications of intramuscular BTX-A injections significantly reduced spastic muscle tone in the examined group of children. The MAS scores obtained after injections indicate improvement in muscle tone ranging from minimal to a complete abatement of spasticity. However, it is worth noting that in all the examined children, including the control group, there was a reduction in muscle tone.

Beneficial results of using BTX-A for antispastic purposes in children with CP were previously presented by many other researchers [25–29]. On this basis, comprehensive evaluations and systematic reviews [30–33], and meta-analyses [34] were also developed. It was also observed that the BTX-A affects an increase in the range of mobility of both small and gross motor skills in the limbs of children with CP [35, 36]. It was confirmed that BTX-A injections are a valuable treatment option to improve gait function in children with CP [37, 38].

Analysis of the results in terms of factors that may affect the effectiveness of BTX-A injections showed

the child's age, and the number of treatments children were subjected to are the most important. The study suggests that the most responsive to BTX-A were the youngest children in the age group 3–6 years. The oldest children's scores were almost 50% worse compared to younger children. A similar difference in effectiveness occurred among children after their first injection. After three or more injections, the scores showed significantly declining results. This conclusion corresponds to the results of Hong et al. [39], who also noticed an improvement in children who did not have an injection before the observation or only had one. Therefore, it is worth emphasizing the maximum effect of the first injection and carefully considering whether increasing the number of injections is necessary [40].

Similar conclusions regarding the age of children were demonstrated by researchers Linder et al. [41]. Their study compared results to a placebo group and showed that the efficacy of BTX is strongly correlated with younger age. Similarly, the study by Mirska et al. [42] noted that young age and a low degree of disability are predictors of long-term improvement after BTX-A administration. Kalinowski et al. [43] also stated that a single BTX injection could bring effects lasting as long as 18 months, and suggested the age range of 1–5 years was optimal for injections.

Many researchers extend their research to observe parents' opinions [44], who have a great deal of insight into the child's current development and spend more time with the children than the researchers. In the present study, a subjective assessment of the effects of BTX-A treatment by parents indicates positive results of this therapy. Most parents noticed an improvement in their children's functioning since the first injections were applied. Parents also declared that lowering muscle tone leads to more comfortable daily care. Almost all parents would recommend using BTX-A to other parents of children suffering from CP to reduce muscle tension.

As shown in the study conducted by Gugała and Snel [45], as many as 96.7% of parents noticed a therapeutic effect after the administration of BTX-A to their children. Of these, 80% of positive responses concerned a noticeable reduction in muscle tone. In turn, a study by Wilk et al. [46] on the efficacy of BTX-A in therapy for children with spastic CP indicates that 90% of parents notice improvement after treatment.

Therapy with BTX-A is an effective and safe method for reducing muscle tone in children with spastic CP. It is important to note that serious side effects may occur in approximately 3% of patients, in which case injections should be discontinued [47].

Many factors influence the effectiveness of BTX-A injections, but a two-way improvement should be considered, supplemented with rehabilitation. The implementation of therapy with BTX-A should occur as soon as possible, even beginning in the child's first year. With this approach, the formation of muscle contractures, secondary bone deformities, and an increase in the overall disability can be avoided. It can also significantly facilitate the child's daily care.

Studies assessing the impact of BTX-A should be continued on a larger population using a range of other measurement tools, thus enabling more accurate data to be collected. Despite many existing studies on the impact of BTX-A injections, further research is worthwhile to improve individual treatment programs for children with CP. The aspects in need of optimization include the selection of appropriate muscles, the dose (including all treatments) and doses for individual muscles, the time between injections, calculations taking into account the weight of the child, the initial degree of spasticity, and the level of development of motor skills.

Also, injections can be painful for children, and the first impressions of pain affect the degree of pain perception in subsequent injections. It is worth considering modern solutions to reduce pain and minimize the accompanying stress, such as video games or virtual reality.

Study limitations

This study has some potential limitations which should be discussed. First of all, the size of the experimental group. This number is sufficient to observe the overall impact of BTX-A injections but may be considered insufficient when comparing a subsequent subgroup (e.g., in relation to children's age). In the larger study population, the conclusions about the differences between the subgroups would be more confident. Another limitation is that the study only shows results obtained after two weeks of BTX-A treatment. The authors did not carry out any further measurements to observe the effectiveness of the therapy (follow-up evaluation). However, in this case, it is complicated to evaluate the long-term clinical effects without the potential risk of bias, because once the observation is completed, the subjects undergo different influences (other treatments, pharmacotherapy, and rehabilitation) which may be of importance for the results (disturbing them). Another limitation which should be emphasized is the fact that apart from BTX-A injection, both groups were subjected to physiotherapeutic procedures. The quality of such procedures may affect clinical findings and modify the results obtained in other facilities, which should be taken into account in future studies. The last limitation is that the MAS assessor knew the allocation of patients to each group so future studies should consider blinding the assessor.

Conclusions

The conducted research concludes that intramuscular injections with BTX-A cause a reduction of spastic hypertonia in children with CP. The effects of BTX-A therapy are particularly noticeable at the beginning of the treatment and are most effective in the youngest patients. BTX-A injections, combined with intensive rehabilitation contribute to the functional improvement of children with CP.

Article information

Ethical approval: *This study was approved by the independent Bioethics Committee of the Wroclaw Medical University in Poland (approval no. KB–280/2017). All parents of the participating children gave written informed consent.*

Conflict of interest: *None.*

Funding: *The authors received no specific funding for this work. The APC was funded by the Ministry of Health subventions according to the number of SUBZ from the IT Simple system of the Wroclaw Medical University in Poland.*

References

1. Richards CL, Malouin F. Cerebral palsy: definition, assessment and rehabilitation. Handb Clin Neurol. 2013; 111: 183–195, doi: 10.1016/B978-0-444-52891-9.00018-X, indexed in Pubmed: 23622163.
2. Agarwal A, Verma I. Cerebral palsy in children: An overview. J Clin Orthop Trauma. 2012; 3(2): 77–81, doi: 10.1016/j.jcot.2012.09.001, indexed in Pubmed: 26403442.
3. Leclercq C. Cerebral palsy: a comprehensive review. BMC Proceedings. 2015; 9(S3), doi: 10.1186/1753-6561-9-s3-a70.
4. Stavsky M, Mor O, Mastrolia SA, et al. Cerebral palsy-trends in epidemiology and recent development in prenatal mechanisms of disease, treatment, and prevention. Front Pediatr. 2017; 5: 21, doi: 10.3389/fped.2017.00021, indexed in Pubmed: 28243583.
5. Pavone V, Testa G, Restivo DA, et al. Botulinum toxin treatment for limb spasticity in childhood cerebral palsy. Front Pharmacol. 2016; 7: 29, doi: 10.3389/fphar.2016.00029, indexed in Pubmed: 26924985.
6. Rosenbaum P. Cerebral palsy: what parents and doctors want to know. BMJ. 2003; 326(7396): 970–974, doi: 10.1136/bmj.326.7396.970, indexed in Pubmed: 12727772.
7. Bar-On L, Molenaers G, Aertbeliën E, et al. Spasticity and its contribution to hypertonia in cerebral palsy. Biomed Res Int. 2015; 2015: 317047, doi: 10.1155/2015/317047, indexed in Pubmed: 25649546.
8. Trompetto C, Marinelli L, Mori L, et al. Pathophysiology of spasticity: implications for neurorehabilitation. Biomed Res Int. 2014; 2014: 354906, doi: 10.1155/2014/354906, indexed in Pubmed: 25530960.
9. Tekin F, Kavlak E, Cavlak U, et al. Effectiveness of Neuro-Developmental Treatment (Bobath Concept) on postural control and balance in Cerebral Palsied children. J Back Musculoskelet Rehabil. 2018; 31(2): 397–403, doi: 10.3233/BMR-170813, indexed in Pubmed: 29171980.
10. Ha SY, Sung YH. Effects of Vojta approach on diaphragm movement in children with spastic cerebral palsy. J Exerc Rehabil. 2018; 14(6): 1005–1009, doi: 10.12965/jer.1836498.249, indexed in Pubmed: 30656162.
11. Shamsoddini A, Amirsalari S, Hollisaz MT, et al. Management of spasticity in children with cerebral palsy. Iran J Pediatr. 2014; 24(4): 345–351, indexed in Pubmed: 25755853.
12. Labaf S, Shamsoddini A, Hollisaz MT, et al. Effects of neurodevelopmental therapy on gross motor function in children with cerebral palsy. Iran J Child Neurol. 2015; 9(2): 36–41, indexed in Pubmed: 26221161.
13. Dressler D, Johnson EA. Botulinum toxin therapy: past, present and future developments. J Neural Transm (Vienna). 2022; 129(5-6): 829–833, doi: 10.1007/s00702-022-02494-5, indexed in Pubmed: 35396965.
14. Multani I, Manji J, Hastings-Ison T, et al. Botulinum toxin in the management of children with cerebral palsy. Paediatr Drugs. 2019; 21(4): 261–281, doi: 10.1007/s40272-019-00344-8, indexed in Pubmed: 31257556.
15. Flemban A, Elsayed W. Effect of combined rehabilitation program with botulinum toxin type A injections on gross motor function scores in children with spastic cerebral palsy. J Phys Ther Sci. 2018; 30(7): 902–905, doi: 10.1589/jpts.30.902, indexed in Pubmed: 30034093.
16. Strobl W, Theologis T, Brunner R, et al. Best clinical practice in botulinum toxin treatment for children with cerebral palsy. Toxins (Basel). 2015; 7(5): 1629–1648, doi: 10.3390/toxins7051629, indexed in Pubmed: 25969944.
17. Depedibi R, Ünlü E, Çevikol A, et al. Ultrasound-guided botulinum toxin type A injection to the iliopsoas muscle in the management of children with cerebral palsy. NeuroRehabilitation. 2008; 23(3): 199–205, doi: 10.3233/nre-2008-23301.
18. Sconfienza LM, Perrone N, Lacelli F, et al. Ultrasound-guided injection of botulinum toxin A in the treatment of iliopsoas spasticity. J Ultrasound. 2008; 11(3): 113–117, doi: 10.1016/j.jus.2008.05.002, indexed in Pubmed: 23396653.
19. Peyronnet B, Gamé X, Vurture G, et al. Botulinum toxin use in neurourology. Rev Urol. 2018; 20(2): 84–93, doi: 10.3903/riu0792, indexed in Pubmed: 30288145.
20. Naumann M, Jankovic J. Safety of botulinum toxin type A: a systematic review and meta-analysis. Curr Med Res Opin. 2004; 20(7): 981–990, doi: 10.1185/030079904125003962, indexed in Pubmed: 15265242.
21. Ramirez-Castaneda J, Jankovic J. Long-term efficacy, safety, and side effect profile of botulinum toxin in dystonia: a 20-year follow-up. Toxicon. 2014; 90: 344–348, doi: 10.1016/j.toxicon.2014.07.009, indexed in Pubmed: 25130293.
22. Mutlu A, Livanelioglu A, Gunel MK. Reliability of Ashworth and Modified Ashworth scales in children with spastic cerebral palsy. BMC Musculoskelet Disord. 2008; 9: 44, doi: 10.1186/1471-2474-9-44, indexed in Pubmed: 18402701.
23. Papavasiliou AS, Rapidi CA, Filiopoulos C, et al. Evaluation of a multimodal management of prematurity-related spasticity. Pediatr Neurol. 2006; 35(6): 400–407, doi: 10.1016/j.pediatrneurol.2006.08.003, indexed in Pubmed: 17138009.
24. Sławek J, Klimont L. Functional improvement in cerebral palsy patients treated with botulinum toxin A injections - preliminary results. Eur J Neurol. 2003; 10(3): 313–317, doi: 10.1046/j.1468-1331.2003.00582.x, indexed in Pubmed: 12752407.
25. Criswell SR, Crowner BE, Racette BA. The use of botulinum toxin therapy for lower-extremity spasticity in children with cerebral palsy. Neurosurg Focus. 2006; 21(2): e1, doi: 10.3171/foc.2006.21.2.2, indexed in Pubmed: 16918222.
26. Keren-Capelovitch T, Jarus T, Fattal-Valevski A. Upper extremity function and occupational performance in children with spastic cerebral palsy following lower extremity botulinum toxin injections. J Child Neurol. 2010; 25(6): 694–700, doi: 10.1177/0883073809344621, indexed in Pubmed: 20508235.
27. Picelli A, La Marchina E, Gajofatto F, et al. Sonographic and clinical effects of botulinum toxin Type A combined with extracorporeal shock wave therapy on spastic muscles of children with cerebral palsy. Dev Neurorehabil. 2017; 20(3): 160–164, doi: 10.3109/17518423.2015.1105320, indexed in Pubmed: 26890193.
28. Tedroff K, Löwing K, Haglund-Akerlind Y, et al. Botulinum toxin A treatment in toddlers with cerebral palsy. Acta Paediatr. 2010; 99(8): 1156–1162, doi: 10.1111/j.1651-2227.2010.01767.x, indexed in Pubmed: 20222884.
29. Ubhi T, Bhakta BB, Ives HL, et al. Randomised double blind placebo controlled trial of the effect of botulinum toxin on walking in cerebral palsy. Arch Dis Child. 2000; 83(6): 481–487, doi: 10.1136/adc.83.6.481, indexed in Pubmed: 11087280.
30. Albavera-Hernández C, Rodríguez JM, Idrovo AJ. Safety of botulinum toxin type A among children with spasticity secondary to cerebral palsy: a systematic review of randomized clinical trials. Clin Rehabil. 2009; 23(5): 394–407, doi: 10.1177/0269215508099860, indexed in Pubmed: 19389744.
31. Baird MW, Vargus-Adams J. Outcome measures used in studies of botulinum toxin in childhood cerebral palsy: a systematic review. J Child Neurol. 2010; 25(6): 721–727, doi: 10.1177/0883073809346846, indexed in Pubmed: 19808988.

32. Juneja M, Jain R, Gautam A, et al. Effect of multilevel lower-limb botulinum injections & intensive physical therapy on children with cerebral palsy. Indian J Med Res. 2017; 146(Supplement): S8–SS14, doi: 10.4103/ijmr.IJMR_1223_15, indexed in Pubmed: 29578189.
33. Wasiak J, Hoare B, Wallen M. Botulinum toxin A as an adjunct to treatment in the management of the upper limb in children with spastic cerebral palsy. Cochrane Database Syst Rev. 2004(3): CD003469, doi: 10.1002/14651858.CD003469.pub2, indexed in Pubmed: 15266487.
34. Cardoso ES, Rodrigues BM, Barroso M, et al. Botulinum toxin type A for the treatment of the spastic equinus foot in cerebral palsy. Pediatr Neurol. 2006; 34(2): 106–109, doi: 10.1016/j.pediatrneurol.2005.08.010, indexed in Pubmed: 16458821.
35. Kahraman A, Seyhan K, Değer Ü, et al. Should botulinum toxin A injections be repeated in children with cerebral palsy? A systematic review. Dev Med Child Neurol. 2016; 58(9): 910–917, doi: 10.1111/dmcn.13135, indexed in Pubmed: 27103334.
36. Lukban MB, Rosales RL, Dressler D. Effectiveness of botulinum toxin A for upper and lower limb spasticity in children with cerebral palsy: a summary of evidence. J Neural Transm (Vienna). 2009; 116(3): 319–331, doi: 10.1007/s00702-008-0175-8, indexed in Pubmed: 19142573.
37. Galli M, Cimolin V, Valente EM, et al. Computerized gait analysis of botulinum toxin treatment in children with cerebral palsy. Disabil Rehabil. 2007; 29(8): 659–664, doi: 10.1080/09638280600948136, indexed in Pubmed: 17453987.
38. Nieuwenhuis MMW, Jaarsma T, van Veldhuisen DJ, et al. Self-reported versus 'true' adherence in heart failure patients: a study using the Medication Event Monitoring System. Neth Heart J. 2012; 20(7-8): 313–319, doi: 10.1007/s12471-012-0283-9, indexed in Pubmed: 22527915.
39. Hong BoY, Chang HJ, Lee SJ, et al. Efficacy of repeated botulinum toxin type A injections for spastic equinus in children with cerebral palsy-a secondary analysis of the randomized clinical trial. Toxins (Basel). 2017; 9(8), doi: 10.3390/toxins9080253, indexed in Pubmed: 28825663.
40. Kolaski K. Botulinum toxin: did the black box warning change how we treat children with cerebral palsy? Dev Med Child Neurol. 2018; 60(5): 440–441, doi: 10.1111/dmcn.13719, indexed in Pubmed: 29504648.
41. Linder M, Schindler G, Michaelis U, et al. Medium-term functional benefits in children with cerebral palsy treated with botulinum toxin type A: 1-year follow-up using gross motor function measure. Eur J Neurol. 2001; 8 Suppl 5: 120–126, doi: 10.1046/j.1468-1331.2001.00044.x, indexed in Pubmed: 11851740.
42. Mirska A, Kułak W, Okurowska-Zawada B, et al. Effectiveness of multiple botulinum toxin sessions and the duration of effects in spasticity therapy in children with cerebral palsy. Childs Nerv Syst. 2019; 35(1): 141–147, doi: 10.1007/s00381-018-3923-6, indexed in Pubmed: 30058050.
43. Kalinowski Z, Bonikowski M. The effect of botulinum toxin A in the treatment of dynamic contracture of the triceps surae muscle in small and older children with cerebral palsy. Neurol Dziec. 2003; 23: 29–40, doi: 10.1111/dmcn.44_13511.
44. Nguyen L, Di Rezze B, Mesterman R, et al. Effects of botulinum toxin treatment in nonambulatory children and adolescents with cerebral palsy: understanding parents' perspectives. J Child Neurol. 2018; 33(11): 724–733, doi: 10.1177/0883073818786567, indexed in Pubmed: 30039731.
45. Gugała B, Snela S. Effectiveness of botulinus toxin therapy in treatment of functional contracture of joints in observations of parents of children with cerebral palsy. Eur J Clin Exp Med. 2008; 2: 175–178.
46. Wilk T, Malinowska-Matuszewska M, Bilski J, et al. Evaluation of the effects of botulinum toxin in unaided-walking children with spastic diplegia and hemiplegia form of cerebral palsy. Sci Arch Vincent Pol Univ Lub. 2013; 17(2): 129–134, indexed in Pubmed: 22873758.
47. Blaszczyk I, Foumani NP, Ljungberg C, et al. Questionnaire about the adverse events and side effects following botulinum toxin A treatment in patients with cerebral palsy. Toxins (Basel). 2015; 7(11): 4645–4654, doi: 10.3390/toxins7114645, indexed in Pubmed: 26561833.