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Botulinum toxin type A for spasticity in cerebral palsy patients: Which impact on popliteal angle to hamstring length? A proof-of-concept study

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Abstract.
BACKGROUND: Cerebral palsy (CP) is the most common physical disability in childhood. It is a heterogeneous condition in terms of etiology, motor type and severity of impairments. Clinical impairments, such as increased muscle tone (spasticity), muscle weakness and joint stiffness contribute to the abnormal development of functional activities, including gait.
OBJECTIVE: The objective of this study was to investigate the popliteal angle to hamstring length after ultrasound guided Incobotulinum toxin A injections for spasticity in CP patients.
METHODS: In this proof-of-concept study, we included outpatients with CP and crouch gait correlated to hamstrings spasticity referred to the Pediatric Rehabilitation outpatient clinic of Umberto I University Hospital, Sapienza University of Rome, in the period between February and October 2018.
METHODS: Modified Ashworth Scale (MAS) of hamstring muscles, Popliteal Angle and Modified Popliteal Angle, Passive Knee Extension and 10 Meter Walk Test (10MWT) were assessed at baseline (T0) and three weeks after ultrasound guided injection (T1) of Incobotulinum Toxin A (dose weight and site dependent).
RESULTS: Thirteen patients (5 male and 8 female), mean aged 9.91 ± 3.59, were included. The clinical evaluation at T0 showed hamstring muscles spasticity, with MAS of 2.4 ± 0.6, popliteal angle $-51.7^\circ \pm 11.0^\circ$, modified popliteal angle of $-39.5^\circ \pm 11.0^\circ$, passive knee extension of $-14.0^\circ \pm 8.7^\circ$ and 10MWT of 14.3 ± 4.6 seconds. At T1, hamstring muscles MAS mean value was 1.7 ± 0.6 ($p < 0.01$), popliteal angle $41.3^\circ \pm 7.0^\circ$ ($p < 0.001$), modified popliteal angle $-32.9^\circ \pm 10.4^\circ$ ($p < 0.001$), passive knee extension $-4.0^\circ \pm 4.2^\circ$ ($p < 0.05$) and 10MWT 12.6 ± 4.8 seconds ($p < 0.05$). None of the treated patients reported any adverse event related to Incobotulinum Toxin A injection.
CONCLUSION: Incobotulinum toxin A treatment has been proven to be safe and effective for hamstring muscles spasticity management in CP patients. Further studies with larger samples and longer follow-up are warranted to assess the efficacy of this treatment on the popliteal angle.

Keywords: Cerebral palsy, popliteal angle, spasticity, rehabilitation, botulinum toxin

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1. Introduction

Cerebral palsy (CP) is the most common physical disability in childhood, with a global pooled prevalence of 2.11 per 1,000 live births stable over the past 10 years [1,2]. It is a heterogeneous condition in terms of etiology, motor type and severity of impairments. Consequently, CP is described using different classifications, primarily based on motor type, topography, and motor severity [3]. CP major motor types are spastic (85%), dyskinetic (7%), which includes dystonia and choreoathetosis, and ataxic (4%) ones [4,5].

Clinical impairments, such as increased muscle tone (spasticity), muscle weakness and joint stiffness contribute to the abnormal development of functional activities, including gait [6,7]. In this scenario, crouch gait is a common and severe gait pattern alteration among ambulant children with CP [8], characterized by increased knee flexion throughout stance. Crouch has a multifactorial aetiology which may include weakness of ankle plantar flexors or knee extensors, spasticity or contracture of hip and knee flexors, lever arm dysfunction, or combinations of the above. It is responsible of walking energy costs increase and contributes to ambulatory decline in these patients [9]. If untreated, crouch gait could be detrimental to long-term joint health, sometimes associated with knee pain [10]. Conservative treatment of crouch includes physical therapy, spasticity reduction, and orthoses, indicated for mild and flexible crouch. Surgical treatment consists of correction of joint contractures, restoration of extensor mechanism, and improvement of lever arms [8].

The popliteal angle (PA) test is a widely used ROM evaluation in CP patients [11]. It assesses the degree of retraction related to the tone of posterior thigh muscles, thus representing a good method to evaluate retraction related to spasticity during rehabilitation treatment and to support clinical decision-making [11,12]. PA is measured as the degrees left to reach a complete knee extension, the greater the retraction the greater the PA value. Hamstrings spasticity and related PA seemed to increase during adolescence in people with CP (PA range of 37–66°; mean: 51.9° [11]), implying potential relevance of the awareness on maintaining hamstrings length already at early ages, also for less involved children [13,14].

Hamstrings spasticity affects the knee active range of motion, further compromising gait pattern [15]. Moreover, hamstrings length moderately correlates with knee flexion during stance phase and at initial heel contact; short hamstrings is linked to shorter stride length and backward rotation of the pelvis.

Systematic rehabilitative programs, including musculoskeletal complications early detection and interventions, are crucial to prevent progressive gait deterioration and to delay, avoid or reduce the number of surgical interventions [16]. Approved treatments for spasticity include systemic ones (oral medications), focal ones, as neuromuscular blockade via injection of botulinum toxin and phenol, orthopedic surgery, selective dorsal rhizotomy and intrathecal baclofen [8,17,18], serial casting, orthoses, and physiotherapy [19,20]. NICE clinical guidelines on spasticity management in under 19s suggest that patients should have access to a network of care that follows agreed care pathways and a multidisciplinary approach and expertise. The management programs should be individualized and goal focused, developed in partnership with the patients and his/her care-givers. General principles of Physical therapy (physiotherapy and/or occupational therapy) provide "enhancing skill development, function and ability to participate in everyday activities and preventing consequences such as pain or contractures" [21]. In this context, focal Botulinum toxin type A (BoNT-A) injections have been widely accepted as a safe and effective intervention to control lower limb spasticity in children with spastic CP [21–23]. Botulinum toxin injection in children with hamstrings spasticity has a significant effect in muscle-tendon length and gait parameters in children with a spastic CP and crouch gait. A significant increase in knee extension and anterior pelvic tilt with an increase in lengthening velocity of semimembranosus muscle have been observed after hamstrings BoNT-A injection in spastic CP with flexed knee gait [24].

Botulinum toxin blocks acetylcholine release from presynaptic terminals at neuromuscular junctions' level. It determines a partial denervation with consequent reduction of spasticity, which occurs 48–72 hours after the procedure and can last from 3 to 6 months [25–27]. The main side effect is a temporary weakness of the muscles adjacent to the injection site, due to potential BoNT-A spreading through the fascia. Systemic side effects are rare: dysphagia, local and/or generalized weakness and fatigue have been reported in less than 1% of children undergoing treatment. Also fever and pain limited to the injection site are rarely reported. Except for the aforementioned side effects, BoNT-A can be considered a safe and reliable treatment [28–30].

Among commercial forms of botulinum toxin type A, Incobotulinum Toxin A (NT 201, Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt, Germany), has the lowest immunogenicity. The absence of complexing proteins reduces the risk of developing autoantibodies,

responsible for secondary non-response to the treatment. Several studies demonstrated its efficacy in focal treatment of spasticity in adults and its safety in terms of side effects [31,32].

However, literature on its use for spastic forms in infant and children with CP is currently poor: Angel León-Valenzuela et al. indicated in a recent retrospective study that Incobotulinum toxin A is a well-tolerated treatment option for focal spasticity in children with CP [33].

Therefore, this proof-of-concept study aimed to evaluate the safety and efficacy of ultrasound-guided infiltrative treatment of Incobotulinum toxin A on spasticity of hamstring muscles, in patients with CP and crouch gait, aged between 3 and 18 years, considering PA improvement as primary outcome, also assessing the usefulness and efficacy of this evaluation method.

2. Methods

2.1. Participants

We recruited consecutive patients diagnosed CP with crouch gait correlated to hamstring spasticity in a proof-of-concept prospective study, conducted according to the Strengthening and the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [34]. Selected outpatient subjects referred to the Pediatric Rehabilitation outpatient clinic of Umberto I University Hospital, Sapienza University of Rome.

Inclusion criteria: age between 3 and 18 years; independent unassisted walking not using orthoses, with crouch – Gross Motor Function Classification System (GMFCS) I and II [35], hamstring muscles spasticity $\geqslant 1+$, according to the Modified Ashworth Scale (MAS) [36].

Exclusion criteria: elapsed time since previous botulinum toxin injections > 6 months; MAS spasticity grade equal to 4; fibro-adipose muscle involution degree equal to 4, according to the Heckmatt scale [37]; inability to walk independently (GMFCS III-IV-V) [35]; previously referred adverse reactions to Incobotulinum Toxin A or other Botulinum Toxin types; orthopedic surgery within the previous 12 months; previous selective dorsal rhizotomy or intrathecal baclofen treatment; 15° of plantar flexion at maximum passive ankle dorsiflexion, measured with extended knee (absence of structured equinus foot); maximum passive hip extension in prone position of 15° (absence of iliopsoas muscle spasticity and/or retraction); behavior disorders that would make difficult for the child to understand the tests or to cooperate during the study; poor patients' cooperation during the evaluation.

The study was approved by the Institutional Review Board of Sapienza University of Rome, Italy. The study was performed according to the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki. All study participants were fully informed about all experimental procedures and signed a written informed consent form prior to participation.

2.2. Intervention

All patients were already being treated with botulinum toxin injection (in the same locum) from the age of 2–4 years; the treatment was repeated on average 2 times a year. No reduction in efficacy was observed even after several inoculations.

An ultrasound-guided injection of Incobotulinum Toxin A at hamstring muscles' level [38] have been performed to all the enrolled patients (Figs 1 and 2). The dosage of the inoculated toxin per single muscle was calculated based on current international recommendations, with 1:1 ratio between Incobotulinum Toxin A and Onabotulinum Toxin A [31,32].

A maximum body dosage of botulinum toxin of 12–14 units per kg or a maximum of 400 units per session has been used, calculating a dosage of 4–6 units per kg for medial hamstrings and of 2–4 units for the lateral hamstrings. The maximum dose per injections was 50 units. 2 to 4 injection sites per muscle group have been performed. Drug dilution varied from 1 to 2 ml per 100 units, depending on body weight and size of the patient's muscles.

Ultrasound guidance allowed to minimize inoculation errors by visualizing muscle bellies. Ice has been applied on the inoculation sites before the injections to reduce pain [39].

All patients, already following physiotherapy programs, have continued to perform three physiotherapy sessions per week even after botulinum toxin injection. Physiotherapy included hamstring lengthening, antigravity muscles strengthening, core stability exercise to improve balance and proprioception and walking training with adaptations when necessary. All physiotherapy sessions were carried out under the supervision of physiotherapists experienced in this field.

2.3. Outcome measures

More in detail, the outcome measures assessed were:

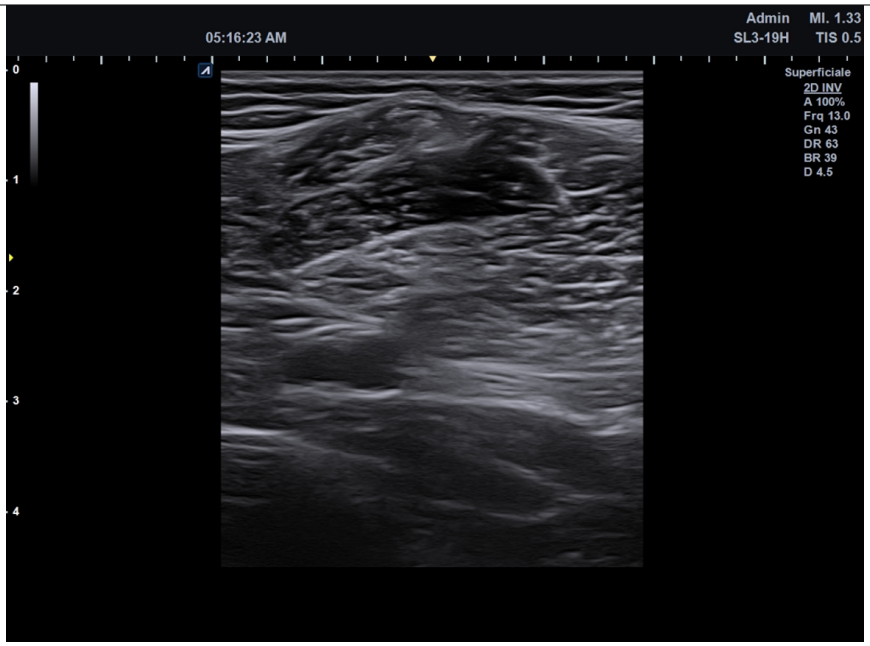

Fig. 1. Ultrasound transverse view of distal hamstrings.

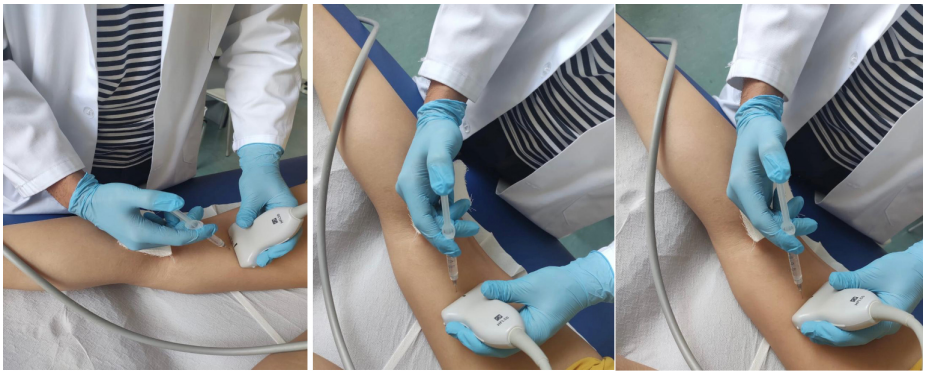

Fig. 2. Ultrasound guided hamstrings botulinum toxin type A injection.

MAS of hamstring muscles, Popliteal Angle [40,41], modified Popliteal Angle [42], Passive Knee Extension [43] and 10 Meters Walking Test (10MWT) [44]. All outcome measures were performed by a researcher experienced in spasticity assessment and by a second examiner experienced in pediatric physical medicine and rehabilitation, both with more than 10 years of experience.

Holt test, or standard PA test (also known as the Unilateral Popliteal Angle (PAU) test), was performed on patients in supine position, with the hip flexed at 90° and gradually extending the knee while the contralateral leg was lying on the examination table. PAU is defined as the angle between long axes of tibia and femur. The angle between the two ideal axes projected by the flexed thigh and by the contralateral limb was measured, both keeping contralateral limb extended (Popliteal Angle) and with the contralateral limb flexed (modified Popliteal Angle, mPA) [11,40–42]. The bilateral test (mPA) was introduced to avoid the posterior pelvic tilt that affects the observed hamstrings length, revealing true hamstring contractures [11].

MAS is a 6-point ordinal scale that qualifies the resistance of the muscle to passive movement (from no increase in muscle tone to stiffness in extension or flexion). However, it has several limitations because it is operator-dependent, with poor inter-rater reliability and little sensitivity [45]. To perform the assessment,

Table 1
Baseline characteristics

Age (mean ± SD), year	9.91 ± 3.59
Sex (male/female), n (%)	5 (38.5%)/8 (62.5%)
Cerebral palsy type (spastic hemiplegia/spastic diplegia), n (%)	3 (23.1%)/10 (76.9%)

starting from 90° hip flexion, hamstrings muscles were stretched through a knee extension in supine position, starting from a maximally flexed knee position. Firstly, each muscle was tested twice by a researcher experienced in spasticity assessment; then a second examiner experienced in pediatric physical medicine and rehabilitation likewise tested each muscle twice. The examiners agreed on each evaluation performed.

Passive knee extension measurement was manually taken using a goniometer [43].

Finally, the 10MWT, performed as functional outcome test, to measure the walking speed (seconds) over 10 meters [44,46], was conducted in a corridor with two marks on the floor delimiting the two ends of a 10-meter walkway. The subjects had to walk a distance of 10 meters at their fastest speed, following verbal instructions and commands provided before and during the exam.

All patients were evaluated at time T0 (baseline), immediately before infiltrative treatment, and subsequently at time T1, three weeks after the first evaluation. Patients' evaluation after three weeks from treatment allows to observe botulinum toxin maximum effect, generally obtained about two-three weeks after injections.

2.4. Statistical analysis

The statistical analysis of the collected data was performed using the statistical package R 3.5.2 (R foundation, Vienna, Austria). Categorical variables were reported using frequency and percentages; continuous variables were reported as means and standard deviations.

Assuming a non-normal distribution of the sample, a non-parametric test was performed; then, the Wilcoxon signed-rank test was used to calculate the difference between values obtained at T0 and at T1, for a p-value < 0.05.

For statistical purposes, for MAS evaluation, a score of 1 was 1, while a score of 1+ was considered equal to 2 and so on up to a score equal to 4 which was considered equal to 5.

3. Results

Thirteen CP patients (5 male, 8 female; mean age of 9.91 ± 3.59 years) with crouch gait correlated to hamstring spasticity were enrolled in this study: three patients had a spastic hemiplegia pattern, ten had a spastic diplegia pattern (see Table 1 for further details).

Table 2
Differences in outcome measures after botulinum toxin injection

	T0	T1	P value
MAS	2.4 ± 0.6	1.7 ± 0.6	< 0.01
PA	$-51.7° \pm 11.0°$	$-41.3° \pm 7.0°$	< 0.001
MPA	$-39.5° \pm 11.0°$	$-32.9° \pm 10.4°$	< 0.001
EXT	$-14.0° \pm 8.7°$	$-4.0° \pm 4.2°$	< 0.05
10MWT (s)	14.3 ± 4.6	12.6 ± 4.8	< 0.05

Abbreviations: MAS: Modified Ashworth scale; PA: Popliteal Angle; MPA: Modified Popliteal Angle; EXT: Passive Knee Extension; 10MWT: 10 Meters Walking Test.

Baseline clinical evaluation (T0) showed an average MAS value of the hamstrings of 2.4 ± 0.6, Popliteal Angle (PA) $-51.7° \pm 11.0°$, Modified Popliteal Angle (mPA) $-39.5° \pm 11°$, Passive Knee Extension (PKE) $-14.0° \pm 8.7°$, and 10MWT 14.3 ± 4.6 seconds (s).

After us-guided injection of hamstring muscles with Incobotulinum Toxin A, hamstrings spasticity decrease at time T1 was statistically significant, with an average MAS 1.7 ± 0.6 ($p < 0.01$). Coherently, also other evaluations performed at T1, as the Popliteal Angle ($41.3° \pm 7.0°$; p-value < 0.001), Modified Popliteal Angle ($-32.9° \pm 10.4°$; $p < 0.001$) and Passive Knee Extension ($-4.0° \pm 4.2°$; $p < 0.05$), confirmed positive effects after treatment. Likewise, 10MWT reported a statistically significant improvement with an average value of 12.6 ± 4.8 seconds ($p < 0.05$) (see Table 2 for further details).

None of the treated patients reported any adverse event attributable to Incobotulinum Toxin A injection.

4. Discussion

Several studies in the literature cover the efficacy and safety of Onabotulinum Toxin A and Abobotulinum Toxin A for CP spasticity treatment, while at present there are few studies regarding safety and efficacy profile of Incobotulinum Toxin A. Recently, Carraro et al. published a double-blind randomized controlled trial, aimed to evaluate the Incobotulinum toxin A (Xeomin®) safety for muscle spasticity management in CP patients: Incobotulinum Toxin A has been shown

to be safe (safety profile similar to Onabotulinum A toxin) and effective for triceps surae muscle spasticity treatment [31].

Kurenkov et al. [47] evaluated safety, clinical and neurophysiological efficacy of Incobotulinum toxin A (Xeomin®) in CP affected children with spastic equine and equino-varus foot deformity: efficacy was assessed based on clinical characteristics (Modified Ashworth Scale and Range of Motion) and electromyographic data, with three months follow-up. Results showed Incobotulinum toxin A safety and efficacy of gastrocnemius muscle spasticity treatment in CP patients.

According to the present study, hamstring muscles injection with Incobotulinum toxin A resulted in a statistically significant reduction of spasticity at three weeks follow-up. This treatment also resulted in a statistically significant improvement in popliteal angle and modified popliteal angle, passive knee extension and 10MWT. None of the treated patients reported the occurrence of any side effect related to treatment with botulinum toxin type A.

Early intervention is essential for patients with spastic forms of CP, to delay progressive gait deterioration and the appearance of osteoarticular deformities and pain, among the leading causes of musculoskeletal disability. In this context, intramuscular injection of botulinum toxin type A is currently considered the gold standard for the focal treatment of spasticity [16,21,25,48]; furthermore, botulinum toxin type A administration has been recently introduced even in the comprehensive rehabilitative management of neuropathic pain [49,50].

Dosage protocol was defined considering child's weight and muscles size, in compliance with international indications. Toxin effects appear within 15–20 days from administration, but they often may be already present after few days. The degree of toxin dilution with physiological solution increases both the earliness of action and the extent of diffusion. Study results confirm the evidence towards the reduction of spasticity after three weeks from BoNT-A administration [27,33,45,46], in presence of dynamic contractures interfering with function, in absence of fixed contractures. The main indications for BoNT-A treatment of lower limb spasticity are to avoid impeding of gross motor function, pain and to improve care, hygiene, posture and tolerance of other treatments [21].

Furthermore, there is a share of patients in whom early botulinum toxin treatment associated with well-conducted clinical rehabilitation treatment is able to modify the course of the disease [6,15,45,46]. The average of botulinum toxin injection treatment duration is 3–6 months, but effects may be sometimes longer. Administration is repeatable over time, in the same or in different sites. However, a reduced efficacy may be possible after 3–4 inoculations. The antibody development, recorded in 5–10% of cases, is not in close correlation with such reduction of efficacy; moreover, recent studies suggest that current BoNT-A preparations present low immunogenicity, that could allow more frequent injections [49,50].

As a proof-of-concept study, the present study will be extended in future to further analyze the Incobotulinum toxin A injections' effects, with follow-ups at three and six months from treatment start.

Physiotherapy treatment assumes a crucial relevance after botulinum toxin injections. It should be set in an intensive regimen to increase functional acquisitions. Inclusion and cooperation within a multidisciplinary rehabilitation team is essential, as the functional objectives, and any conceivable side effect, should be shared and discussed by all the professionals involved in the CP affected patient rehabilitation and management [16,21].

For the aim of this study, the rehabilitation protocol foresaw that patients have continued their rehabilitation program, with a frequency of three times a week, with a treatment time ranging from 30 to 60 minutes per session according to the patient's participation. Physiotherapy included hamstring lengthening, antigravity muscles strengthening, core stability exercise to improve balance and proprioception and walking training with adaptations when necessary. One of the most important targets in spasticity treatment is to fix individualized age and developmentally appropriate goals. PA and mPA measurement were performed through a non-invasive evaluation for the patients, and it was useful in monitoring the treatment outcome after the rehabilitation program.

Some researchers that evaluated PA and hamstrings spasticity changes during childhood in walking children with spastic bilateral CP reported a correlation between age and PA increase [13], which is an index of a greater muscle retraction.

Reducing hamstring muscles spasticity by ultrasound-guided Incobotulinum toxin A injection treatment could lead to functional improvement of gait pattern, prevent hamstring muscles retraction and facilitate orthotic management and physiotherapy treatment. Botulinum toxin injections provide the opportunity to optimize surgical timing, delay or even reduce the number of orthopedic surgeries. This treatment option plays a key role considering that surgery to improve

gait should be postponed until gait maturation, avoiding a higher risk of failure, relapse and less predictable results due to child abilities immaturity, as well as to differential growth patterns between muscles, tendons, and bones in the younger child [17]. Furthermore, it should be considered that in these patients, particularly during growth spurts, an unbalance between muscle growth and bone growth is observed: bone growth does not correspond to equal growth of longitudinal musculotendinous unit, which can undergo to a further muscle retraction [17,45].

BTX-A injections, combined with physiotherapy and orthotic management, synergically act to improve range of motion, consequently minimizing energy expenditure and improving proprioception, selective motor control and strength, resulting in a higher functional performance and enhancing patients' quality of life [16].

Our study presents some strengths, including not only the evaluation of the safety and efficacy of botulinum toxin treatment in association with physiotherapy but also the evaluation of the usefulness and efficacy of PA as a simple and useful method to assess the extent of biomechanical dysfunction of the patient.

This study allows to provide further support to the evidence currently available in literature, but it is not free from limitations: first, there is an absence of a control group, which will be the natural prosecution of the present study, as a RCT and a with a longer follow-up; second, integration of the collected data, completed by a kinematic gait analysis, would be desirable; lastly, longer follow-up evaluations (i.e., 6 months or 1 year) might be useful to assess the longer effect of the intervention in combination with the multidisciplinary approach.

5. Conclusion

Botulinum toxin A infiltrative treatment in children with CP represents a major therapeutic intervention but should never be considered as a stand-alone treatment. A clinical and rehabilitative approach to spastic movement disorders associated with CP must consider all the available options of conservative and surgical strategies and requires an interdisciplinary, multi-modal team intervention.

In this proof-of-concept study, infiltrative treatment with Incobotulinum Toxin A has been proven to be safe and effective for hamstring muscles spasticity management in the selected sample of patients with CP and crouch gait. The treatment of hamstrings muscles spasticity with Incobotulinum toxin A could have a rationale in reducing spasticity and improving knee extension for the Unilateral Popliteal Angle and modified Popliteal Angle, in order to prevent or delay the appearance of muscle retractions, which, as mentioned above, could lead to skeletal deformities, requiring surgical correction. Furthermore, this treatment, especially if associated with physiotherapy, could be useful to optimize surgical timing, delay or even avoid surgery. Further studies with larger samples and longer follow-up are warranted to furtherly assess and to consolidate the findings of this study.

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None to report.

Author contributions

Conceptualization: MM, AdS, and AB; Methodology: MM and AB; Investigation: MM, PR, FA; Formal analysis: PR, AVB, and GP; Data curation: MM, FA, and AB; Writing-original draft preparation, MM and AdS; Writing-review and editing: MP and AB; Visualization: PR, FA, AVB, GP, TP, JB; Supervision: MM, AdS, and AB. All the authors read and approved the final version of the manuscript.

Ethics statement

The study was approved by the Institutional Review Board of Sapienza University of Rome, Italy. The study was performed according to the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki. All study participants were fully informed about all experimental procedures and signed a written informed consent form prior to participation.

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Conflict of interest

The authors have no conflicts of interests to declare.

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