© 2024 THE AUTHORS
Open access at https://www.minervamedica.it

European Journal of Physical and Rehabilitation Medicine 2024 August;60(4):567-80
DOI: 10.23736/S1973-9087.24.08029-8

SYSTEMATIC REVIEW

Noninvasive analysis of overactive muscle structure and elasticity after botulinum toxin type A injection: a systematic review and meta-analysis

Marine DEVIS [1] *, Frédéric LECOUVET [2, 3], Thierry LEJEUNE [1, 4], Gaëtan STOQUART [1, 4]

[1]Department of Health Sciences, Institute of Experimental and Clinical Research, Neuromusculoskeletal Lab (NMSK), Catholic University of Louvain, Brussels, Belgium; [2]Department of Health Sciences, Institute of Experimental and Clinical Research, IMAG lab, Catholic University of Louvain, Brussels, Belgium; [3]Department of Radiology, Saint-Luc University Clinic, Brussels, Belgium; [4]Department of Physical Medicine and Rehabilitation, Saint-Luc University Clinic, Brussels, Belgium

*Corresponding author: Marine Devis, Department of Health Sciences, Institute of Experimental and Clinical Research, Neuromusculoskeletal Lab (NMSK), Catholic University of Louvain, Brussels, Belgium. E-mail: marine.devis@uclouvain.be

This is an open access article distributed under the terms of the Creative Commons CC BY-NC-ND license which allows users to copy and distribute the manuscript, as long as this is not done for commercial purposes and further does not permit distribution of the manuscript if it is changed or edited in any way, and as long as the user gives appropriate credits to the original author(s) and the source (with a link to the formal publication through the relevant DOI) and provides a link to the license. Full details on the CC BY-NC-ND 4.0 are available at https://creativecommons.org/licenses/by-nc-nd/4.0/.

ABSTRACT

INTRODUCTION: Injections of botulinum toxin type A (BoNT-A) are the first-line treatment for spastic muscle overactivity (MO). Some authors observed that BoNT-A injections lead to changes in muscle structure and muscle elasticity that are probably not completely reversible. This possible effect is critical, as it could lead to negative impacts on the effectiveness of BoNT-A interventions. Our study aimed to evaluate the current literature regarding changes in muscle elasticity and structure after BoNT-A injection, by diagnostic imaging, in neurological populations with MO. Our second objective was to pool all articles published on this topic in order to provide a quantitative synthesis of the data.

EVIDENCE ACQUISITION: A systematic search was conducted between October 2021 and April 2023 using different databases in accordance with PRISMA guidelines. Two independent reviewers screened articles for inclusion, extracted data, and evaluated methodological quality of the studies. A meta-analysis was performed to compare muscle elasticity and structure before and after BoNT-A injections.

EVIDENCE SYNTHESIS: A sample of 34 studies was selected for qualitative review and 19 studies for quantitative review. Meta-analysis of pre-post studies demonstrated significant improvement with a medium effect size (standardized mean difference=0.74; 95% CI 0.46-1.02; P<0.001) of muscle elasticity assessed by ultrasound elastography (USE) 4 weeks after BoNT-A injection. No statistically significant difference was found for muscle thickness, pennation angle, and muscle echo-intensity assessed by magnetic resonance imaging and/or ultrasonography at short-term. On the other hand, normalized muscle volume decreased with a small effect size (standardized mean difference = -0.17; 95% CI -0.25 - -0.09; P<0.001) 6 months after BoNT-A injection.

CONCLUSIONS: Muscle elasticity measured by USE improves with a temporary effect at short-term following BoNT-A injections. Synthesis of studies that assesses muscle structure is hindered by methodological differences between studies. However, based on a small amount of data, normalized muscle volume seems to decrease at long-term after BoNT-A injections in children with CP suggesting that the timing of re-injection should be considered with caution in this population. Further work should focus on the long-term effect of repeated injections on muscle structure and elasticity in neurological populations.

(*Cite this article as:* Devis M, Lecouvet F, Lejeune T, Stoquart G. Noninvasive analysis of overactive muscle structure and elasticity after botulinum toxin type A injection: a systematic review and meta-analysis. Eur J Phys Rehabil Med 2024;60:567-80. DOI: 10.23736/S1973-9087.24.08029-8)

KEY WORDS: Muscle spasticity; Botulinum toxins; Muscular atrophy; Elasticity.

Introduction

Muscle overactivity (MO) is one of the consequences of upper motor neuron syndromes that can affect children and adults. MO was at first englobed by the term "spasticity", which was defined by Lance as "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 neuron syndrome"[1]. However, MO can present different clinical forms that are not covered by this definition. Consequently, in order to refine the taxonomy, Baude *et al.*[2] proposed a model where MO is a part of a larger neurological disorder and includes 3 components: spastic dystonia, spastic cocontraction and spasticity. Furthermore, a European consensus has developed a model to represent the hyper-resistance to passive stretch in patients with MO.[3] This model distinguishes 2 main components of hyper-resistance: a non-neural (tissue-related) component and a neural (central nervous system related) component.

Injections of botulinum toxin type A (BoNT-A) are the first-line therapeutic method to treat focal MO.[4, 5] BoNT-A inhibits the release of acetylcholine at neuromuscular junctions resulting in blocking neuromuscular transmission.[6-8] Therefore, BoNT-A injections act directly on the neural component of hyper-resistance.[3] Besides its effect on MO, BoNT-A injections also act on other body functions and structures according to the International Classification of Functioning, Disability and Health (ICF).[9] Indeed, the effects of a single injection of BoNT-A on overactive muscles have been largely studied and it has been well established that they lead to a reduction of hyper-resistance, as assessed by clinical scales.[10] However, these measurements have poor intra-/inter-rater reliability.[11, 12] So far, no gold standard assessment of hyper-resistance, whether clinical or instrumental, is available. Therefore, objective and reliable measures of the two components of muscle hyper-resistance should be implemented to determine the efficacy of BoNT-A injections over time. Muscle elasticity is a reflect of the non-neural component of hyper-resistance. Some authors have used ultrasound elastography (USE) to assess this elasticity in qualitative and quantitative ways. In recent years, USE has been used in the assessment of muscle elasticity, which is closely associated with clinical assessment of MO.[13] The two major techniques used in musculoskeletal elastography are strain sonoelastography and shear wave elastography (SWE). Strain elastography measures the deformation of a tissue when a manual compressive force is applied with the handheld US transducer. In SWE, an acoustic compressive radiation force is applied to tissues instead of external compression.

Furthermore, besides its effect on muscle elasticity, some authors have shown that repeated injections of BoNT-A are responsible for changes in muscle structure.[14] This possible effect is critical, as it could negatively impact the effectiveness of BoNT-A interventions. However, the effect of BoNT-A on muscle structure remains controversial. In animal models, authors observed a reduction of the muscle's cross-sectional area, a long-term muscle neurogenic atrophy (up to 1 year post-injection), a decreased fiber size, a decreased contractile material, an increase of the collagen proportion and of the intramuscular lipid accumulation after BoNT-A treatment.[15, 16] These findings are mainly based on anatomopathology sections studied with optic and electronic microscopy. In studies conducted to investigate the histopathologic changes in humans, there was a reduction of the muscle's cross-sectional area, a widespread neurogenic atrophy and a reduced size of type I and II fibers.[17, 18] The development of new sequences and softwares in Magnetic Resonance Imaging (MRI) and ultrasonography (US) opens up the field of non-invasive muscle structure assessment.

A first systematic review, performed by Mathevon *et al.* systematically reviewed the literature covering the muscle structure and stiffness assessment after BoNT-A injection, by means of imaging and histological evaluations.[19] Their review, published in 2015, included 21 articles and observed that "BoNT-A injections lead to structural changes in the muscle: lingering atrophy, with a remodeling of the muscles contractile proteins, which is probably not completely reversible; and changes in muscle elasticity". Thanks to improvements in the aforementioned new technologies and the increasing interest on the topic, many studies have been published since.

The primary objective of the present systematic review was to synthetize changes in muscle structure and elasticity after BoNT-A injection, as assessed by noninvasive imaging methods, in neurological populations presenting with MO. The secondary objective was to pool all selected articles in order to provide a quantitative synthesis of the effects of BoNT-A injections on muscle structure and muscle elasticity, as it has never been done before.

Evidence acquisition

The systematic review and meta-analysis protocol is registered with PROSPERO (CRD42022310902) and conducted in accordance with PRISMA guidelines.

Data sources and search strategy

A systematic electronic search was conducted by two independent reviewers (MD and CR) between October 2021 and April 2023 using PubMed, Scopus, Embase, and the Cochrane Library databases. The search terms were developed for PubMed and adapted for the other databases

(Supplementary Digital Material 1: Supplementary Table I). In addition, the reference list of the publications selected was also manually screened to detect references not found during the initial electronic search. There were no restrictions on the publication date of the included papers. Published works until April 2023 were considered. No filter was used in terms of language. All the databases were limited to humans.

Eligibility criteria

The PICO method was used to assess eligibility criteria. Full-text articles were included if 1) Patient: studies were conducted in individuals with MO related to an upper motor neuron syndrome in upper and/or lower limb, no matter the age, the severity or neurological condition; 2) intervention: botulinum toxin injection; 3) comparison: individuals suffering from MO which didn't received BoNT-A injection or comparison before and after BoNT-A injection; 4) outcomes: muscle structure or muscle elasticity evaluation by noninvasive imaging methods, such as MRI, US, and elastography. Articles were excluded if there were other neurological diseases or health condition that could influence MO or the paretic limb function. Systematic reviews, meta-analyses, theses, letters, dissertations, guidelines, scientific congress abstracts were also excluded.

Study selection process

After removing duplicates, two independent researchers (MD, CR) screened titles and abstracts according to the selection criteria of this review to identify potentially eligible studies. Full texts were independently evaluated for inclusion criteria by the same two researchers. In case of disagreement, discussion was initiated until consensus was reached.

Data extraction and analysis

For each study, relevant data was extracted by two independent researchers (MD, CR) as follows: first author, publication year, study design, sample size, participant characteristics (neurological condition, gender, age), comparisons, measurement tool, main outcomes involving structural and muscle elasticity adaptations, injected/evaluated muscles, BoNT-A injection characteristics (molecule, doses, dilution), previous BoNT-A injections, and time of evaluation.

Methodological quality assessment

The methodological quality was evaluated by two independent researchers (MD, CR) using the modified Downs and Black assessment tool.[20] Higher scores indicate higher methodological quality. If there was any disagreement, the researchers discussed it to reach a consensus.

Statistical analysis

Meta-analyses were conducted across studies that made similar comparisons and used similar outcome measurements expressed in the same or different units. Case reports/studies were excluded because of their low proof of evidence. In addition, grouping required that there were at least 2 studies without excessive clinical heterogeneity based on intervention, participant characteristics, and study design. All analyses were conducted using Review Manager (version 5.4)[21] following the guidelines in the Cochrane Handbook for Systematic Reviews of Interventions.[22] Meta-analyses were displayed as forest plots. I^2 statistics was used to determine statistical heterogeneity among the studies.[23] Heterogeneity was considered to be acceptable for a $I^2<60\%$.[23] For all pre and post studies included in the meta-analyses, baseline and post-intervention means and standard deviations (SD) were extracted to perform the analyses. In case of mean and SD missing data, an estimation was calculated using the following formula proposed by Wan *et al.*[24] and the Cochrane handbook:[22] Mean=(q1+median+q3)/3 and SD=(q3-q1)/1.35 where q1 is first quartile and q3 third quartile. Where needed, standard error (SE) of mean differences was calculated using the following formula proposed by the Cochrane handbook: SE=(upper limit – mean difference)/1.96 or SE=SD/$\sqrt{n}$ where n is the sample size. A random effect model was used because of heterogeneity. Because different units (meter/second, kPa,…) were pooled together to determine the effect of BoNT-A injection before and after intervention on muscle elasticity, the effect size was reported as standardized mean difference with 95% CI. With regards to the muscle structure, mean difference or standardized mean difference were used to express the effect size depending on the units of the different outcome measures. No funnel plots were included in the review to visually explore publication bias due to an insufficient number of studies (<10) included in each meta-analysis.[25] Sensitivity analysis was conducted to verify results robustness in case of statistical heterogeneity or presence of outliers.

Evidence synthesis

Selection of the studies

A total of 1280 studies published until April 2023 were found in the databases (PubMed=344, Embase=773, Sco-

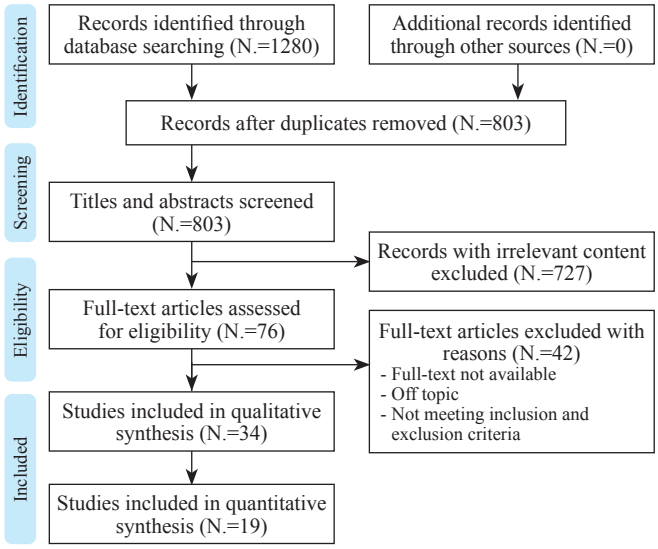


Figure 1.—Flow chart illustrating article screening and selection in accordance with PRISMA guidelines.

pus=163, Cochrane Library=0). After removing duplicates, the titles and abstracts of 803 articles were screened and from these, 727 were excluded for not fulfilling the eligibility criteria. Thereafter, the full text of 76 articles was read. A sample of 34 studies was selected for qualitative review. Of those 34 studies, 7 were published before 2015 and were already included in Mathevon's systematic review[19] and therefore not detailed in our systematic review. Finally, of those 34 studies included in our systematic review, 19 studies were selected for our meta-analyses in order to quantitatively synthetize the effects of BoNT-A injections on muscle structure and muscle elasticity (Figure 1).

Studies and patients characteristics

Included articles were mostly observational prospective longitudinal cohort studies (N.=19), case reports/series (N.=6), and randomized controlled trials (RCT) (N.=3)[26-28]

TABLE I.—*Modified Downs and Black.*[13, 26-58]

	Reporting	External validity	Biais (internal validity)	Confounding (internal validity)	Power	Total (/28)
Alexander *et al.*[29]	9	2	5	3	1	20
Aşkın *et al.*[30]	7	0	6	0	0	13
Bar-on *et al.*[31]	5	0	4	1	0	10
Battaglia *et al.*[32]	7	1	4	2	1	15
Bertan *et al.*[26]	9	1	6	5	0	21
Bilgici *et al.*[33]	8	0	6	0	0	14
Boyaci *et al.*[13]	9	1	5	4	0	19
Brandenburg *et al.*[34]	8	1	5	2	0	16
Campanella *et al.*[35]	9	0	6	1	0	16
Cosenza *et al.*[36]	7	0	4	1	0	12
Dağ *et al.*[37]	6	0	6	1	0	13
De Beukelaar *et al.*[38]	10	2	6	3	0	21
de Coulon *et al.*[39]	6	0	5	1	0	12
Furukuwa *et al.*[40]	6	0	4	1	0	11
Gao *et al.*[41]	7	1	5	3	0	16
Hasegawa *et al.*[42]	9	1	6	3	0	19
Kawano *et al.*[43]	7	1	5	2	0	15
Kwon *et al.*[44]	6	1	4	1	0	12
Kwon *et al.*[45]	8	0	5	1	0	14
Lee *et al.*[46]	7	0	5	0	0	12
O'Dell *et al.*[47]	6	0	4	0	0	10
Park *et al.*[48]	8	1	5	3	0	17
Peeters *et al.*[27]	9	1	6	4	0	20
Peeters *et al.*[49]	10	1	5	4	0	20
Picelli *et al.*[50]	9	1	6	4	1	21
Picelli *et al.*[28]	10	1	6	6	0	23
Picelli *et al.*[51]	7	1	5	2	0	15
Schless *et al.*[52]	8	1	4	4	0	17
Tok *et al.*[53]	8	1	5	3	0	17
Van Campenhout *et al.*[54]	8	1	6	2	0	17
Weide *et al.*[55]	6	0	4	1	0	11
Weidensteiner *et al.*[56]	6	0	4	1	0	11
Williams *et al.*[57]	9	1	5	3	0	18
Yi *et al.*[58]	7	0	5	0	0	12

(Supplementary Digital Material 2: Supplementary Table II).[13, 26-58] The quality of the reviewed articles based on the modified Downs and Black was variable but most part of the studies were of low (total score ≤14/28) (N.=13) to fair (total score between 15-19/28) (N.=14) quality (Table I).[13, 26-58] The main items not reported were those related to external validity, internal validity (confounding), and power.

The sample size ranged from 1 to 105 subjects. A total of 577 injected patients (666 limbs, 957 injected and evaluated muscles), 90 non-injected individuals with neurological condition presenting MO (98 limbs) and 111 healthy control subjects were included in the studies. The majority of the treated subjects were male (64%), and the mean age ranged from 2 to 66 years. Twenty-two studies assessed children with CP, 11 studies evaluated adults with chronic stroke, and 1 study included a child with spastic paresis of unknown origin.

Regarding comparisons, 9 studies included 1 control group or more and 9 studies compared the injected paretic muscles to the non-paretic contralateral muscles. Moreover, 16 studies did not include a control group.

The most used methods of analysis were US (N.=17), followed by USE (N.=15), MRI (N.=6) and dual-energy X-ray absorptiometry (N.=1).

The different evaluated outcomes were muscle elasticity (N.=15), muscle thickness (N.=8), muscle echo-intensity (N.=9), absolute and/or normalized muscle volume (N.=7), pennation angle (N.=5), muscle length and tendon length (N.=5), cross-sectional area (N.=4), fascicle length (N.=4), and muscle mass (N.=1).

Most of the studies evaluated their outcomes at short-term (2 weeks – 6 weeks) (N.=23) and mid-term (7 weeks – 12 weeks) (N.=14) after BoNT-A injection. Only 5 studies performed long-term evaluations (> 6 months). Furthermore, 3 studies[32, 36, 52] did not specify the time after BoNT-A injection.

The injected and evaluated muscles varied among studies. However, the most evaluated muscles were the gastrocnemius medialis in lower limb and the biceps brachialis in upper limb.

Muscle elasticity

Initially, the results of 14 pre-post studies that examined the effect of BoNT-A injection on muscle elasticity using SWE and/or strain USE were meant to be pooled in a meta-analysis. However, due to excessive statistical heterogeneity explained by the various measurement protocols and the poor methodological quality of some studies, 3 of them[33, 37, 48] were excluded from the meta-analysis after sensitivity analysis was performed (Supplementary Digital Material 3: Supplementary Figures 1-3). Moreover, 1 study was excluded because of missing data[45] and 1 additional study was excluded because of its design (case report).[40] Finally, 9 pre-post studies evaluating 11 different muscles were included. Five studies used strain USE and 5 studies made use of SWE. The method used did not seem to influence the results. However, due to different units of measurement, a standardized mean difference was used to pool the results of both methods instead of mean difference. Analyses were made for short-term (4 weeks) and mid-term (12 weeks) effect. At short-term, 5 studies evaluated lower limb muscles while 2 studies evaluated upper limb muscles. Results were comparable with an improvement of elasticity of the same magnitude. Heterogeneity was considered to be acceptable with I^2=37%. Moreover, 2 studies evaluated both upper and lower limb muscles at short-term and did not made any distinction between both in their results. The most evaluated muscles were the gastrocnemius in lower limb (7 of the 9 studies) and the biceps brachialis in upper limb (3 of the 9 studies). Related to the neurological condition and age, 4 studies evaluated children with CP and 5 studies evaluated adults suffering from chronic stroke. In summary, the analysis at short-term showed a statistically significant improvement of muscle elasticity with a medium effect size (standardized mean difference=0.74; 95% CI 0.46-1.02; P<0.000001) (Figure 2[13, 26, 28, 30, 34, 35, 41, 42, 51]). The analysis at mid-term included only gastrocnemius muscles of children with CP and showed no statistically significant within-group difference (Figure 2[13, 26, 28, 30, 34, 35, 41, 42, 51]). Furthermore, out of the 5 studies excluded from the meta-analysis, 4[33, 37, 45, 48] found a significant decrease (-23% to -56%) of USE values 4 to 12 weeks after treatment. In Furukawa's report of 2 cases[40] both patients showed improvement in muscle elasticity at 2, 4, and 8 weeks after BoNT-A injection. However, these two measures followed different patterns through week 12 of evaluation.

Muscle volume

Five studies mentioned measurement of muscle volume assessed by US (N.=4) or MRI (N.=1). The results of 2 pre-post studies[29, 57] that examined the effect of BoNT-A injection at short-term on muscle volume normalized to tibia length in children with CP were pooled in a meta-analysis. However, given the statistical heterogeneity (I^2=70%), this quantitative synthesis has not been kept in this paper, but is available as Supplementary Figures 1-3.

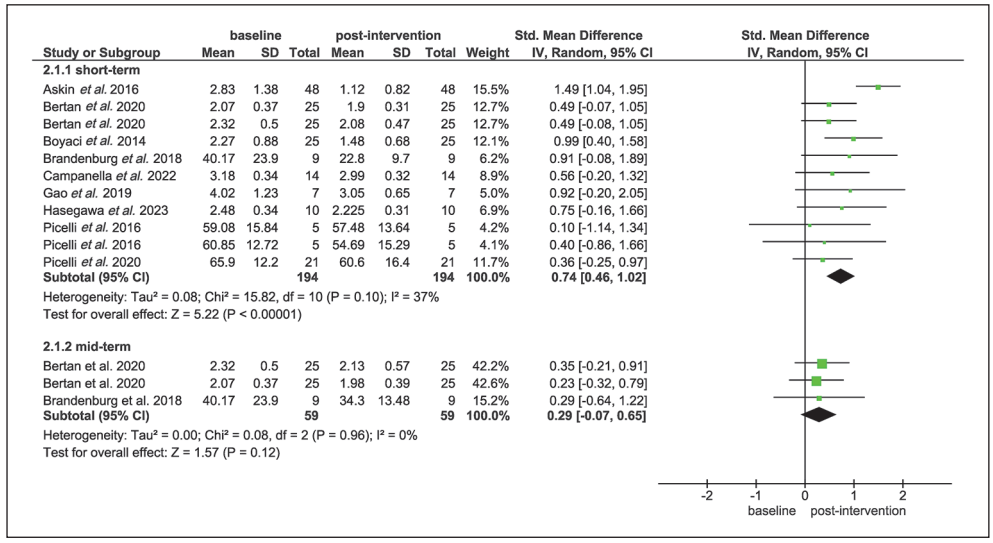


Figure 2.—Effect of BoNT-A injections on muscle elasticity.[13, 26, 28, 30, 34, 35, 41, 42, 51]

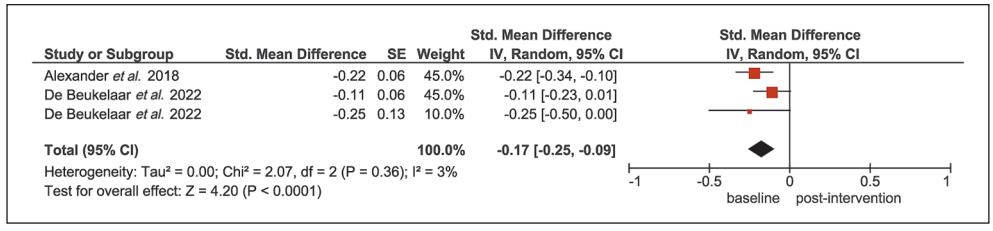


Figure 3.—Effect of BoNT-A injections on normalized muscle volume at long-term.[29, 38]

Both studies observed a significant decrease of normalized muscle volume at short- and/or mid-term.

The results of 2 pre-post studies that examined the long-term effects (24 weeks) of BoNT-A on muscle volume normalized to tibia length[29] or to the product of body mass and body length[38] in children with CP were pooled in a meta-analysis. A statistically significant decrease of normalized muscle volume, with a small effect size (standardized mean difference = -0.17; 95% CI -0.25 - -0.09; P<0.0001) was observed (Figure 3[29, 38]). In the study of De Beukelaar *et al.*[38] the amount of changes in normalized muscle volume assessed by US tended to be lower in BoNT-A patients who received a first BoNT-A injection (*i.e.* BoNTA-naive treated patients) compared to untreated spastic patients. However, no significant between-group difference was found between BoNT-A naive treated cohort and BoNT-A non-naive treated cohort.

One study could not be included in the meta-analysis because of missing data. The normalized muscle volume showed a significant reduction up to 16.2% at 8 weeks post BoNT-A injection.[49]

Furthermore, 2 studies were not included in the meta-analysis because of clinical heterogeneity. Schless *et al.* compared normalized muscle volume in untreated spastic patients and in spastic patients treated with BoNT-A (at least 3 injections). They showed that, more than 6 months after the intervention, the treated cohort had a smaller muscle volume. Furthermore, a higher number of previous injections was significantly associated with a lower normalized muscle volume.[52] On the contrary, Weide *et al.*[55] did not report any significant change in normalized muscle volume at mid- nor at long-term after BoNT-A injection in their case report.

Cross sectional-area

Results were very conflicting in the 4 studies that assessed cross sectional-area. No meta-analysis was made because of clinical heterogeneity. Cosenza *et al.*[36] reported no significant difference in cross-sectional area of BoNT-A treated rectus femoris compared to untreated spastic muscles. On the contrary, De Beukelaar *et al.*[38] observed a significant decrease (up to 17%) of normalized cross-sectional area of gastrocnemius muscle at long-term, while Weide *et al.*[55] showed that gastrocnemius cross sectional-area

normalized for body mass slightly increased at mid- and long-term. Furthermore, two studies[32, 38] didn't show any significant correlation between the number of previous BoNT-A injections in treated patients and cross-sectional area alteration over time.

Muscle mass

Only 1 study[46] evaluated the effect of BoNT-A injection on muscle mass. Lean body mass of the affected lower limb, assessed by dual-energy X-ray absorptiometry, decreased (-1.8%) without statistically significant difference at 4 weeks (P=0.316). However, 12 weeks after injection, lean body mass was significantly increased compared to preinjection (6.7%, P=0.003) and 4 weeks after injection (8.7%, P=0.0008).

Muscle thickness

Muscle thickness is defined as the longest distance between the superficial and the deep aponeuroses. Muscle thickness of the lower leg was investigated in 7 studies. The results of 5 pre-post studies[13, 43, 51, 53, 58] that examined the effect of BoNT-A injection on gastrocnemius muscle thickness in children with CP and adults suffering from chronic stroke were pooled in a meta-analysis. All 5 articles expressed muscle thickness in millimeter. Thus, results are comparable and the mean difference could be used to perform our meta-analysis. No statistically significant within-group difference was found (p>0.05) at short-term nor at mid-term (Figure 4[13, 43, 51, 53, 58]). Furthermore, Cosenza *et al.*[36] reported no significant difference in muscle thickness of BoNT-A treated muscles compared to untreated spastic muscles. Battaglia *et al.*[32] observed a significant smaller muscle thickness in BoNT-A treated limbs compared to unaffected limbs but without any significant correlation between BoNT-A injection and muscle thickness alteration over time.

Pennation angle

Pennation angle was investigated by 5 studies in the lower leg. The results of 3 pre-post studies that examined the effect of BoNT-A injection on pennation angle of 4 different gastrocnemius muscles in resting position at short-term were pooled in a meta-analysis (Figure 5[51, 53, 58]). No statistically significant within-group difference was found (P>0.05) at 4 weeks. Due to statistical heterogeneity (I^2=73%), meta-analysis at mid-term was not retained for the present paper (additional material). The 3 studies[43, 53, 58]

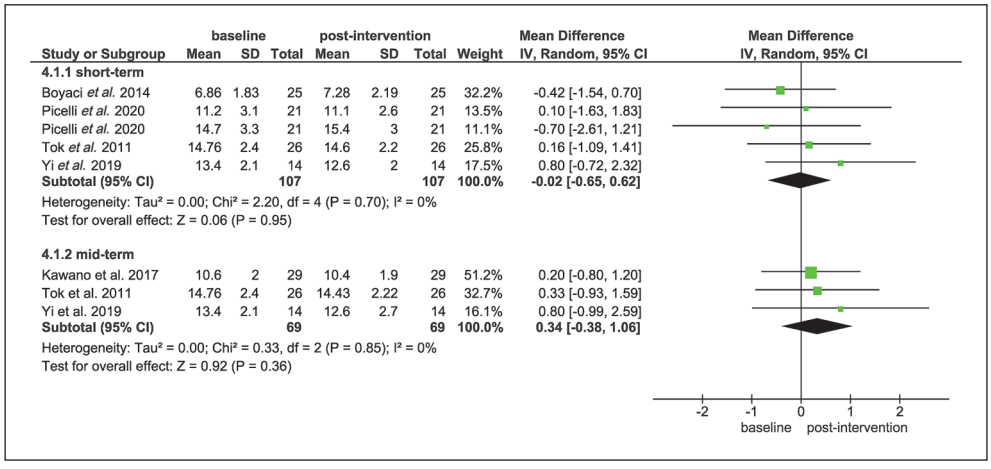


Figure 4.—Effect of BoNT-A injection on muscle thickness.[13, 43, 51, 53, 58]

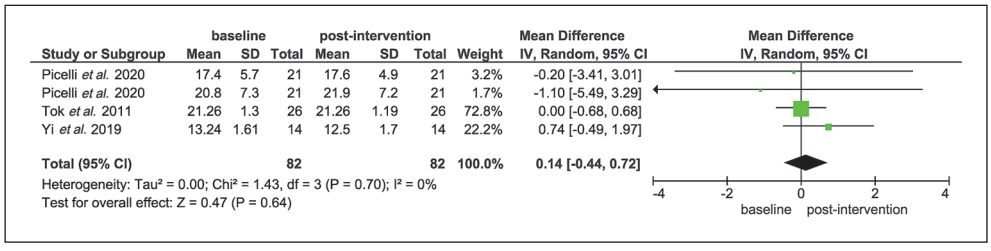


Figure 5.—Effect of BoNT-A injection on pennation angle at short-term.[51, 53, 58]

found a smaller pennation angle at mid-term depending on the ankle position. Furthermore, 1 non-included study[32] reported significantly smaller pennation angle in muscles of hemiparetic BoNT-A treated limb compared to the unaffected limb at rest, but without any significant correlation between the number of previous BoNT-A injections and pennation angle alteration over time.

Fascicle length

Four studies assessed fascicle length.[43, 53, 55, 58] No meta-analysis was made because of clinical heterogeneity. Results were contradictory in all studies with an increased or decreased fascicle length at short-, mid-, and/or long-term after BoNT-A injection in the gastrocnemius medialis, depending on the ankle position.

Muscle and tendon length

Four studies took muscle and tendon length in consideration. No meta-analysis was made because of clinical heterogeneity. Three studies showed an increase in muscle and/or tendon length in the gastrocnemius muscle at short-term[31, 59] and mid-term.[55] Furthermore, Weide *et al.*[55] observed a slightly decreased normalized muscle length at long-term while normalized tendon length had slightly increased. On the other hand, De Beukelaer *et al.*[38] did not observe any significant change in normalized muscle length and normalized muscle-tendon complex in the gastrocnemius muscle at long-term following BoNT-A injection.

Muscle echo-intensity

Nine of the included studies mentioned measurement of muscle echo-intensity after BoNT-A injection. The results of 4 pre-post studies[28, 35, 50, 51] evaluating muscle echo-intensity with the Heckmatt scale of 6 different muscles in children with CP and adults with chronic stroke were pooled in a meta-analysis. Five studies[32, 36, 38, 49, 52] were excluded due to excessive clinical heterogeneity. The analysis at short-term (4 weeks) showed no statistically significant within-group difference (Figure 6[28, 35, 50, 51]). In the same way, Peeters *et al.*[49] observed no statistically significant differences in muscle echo-intensity at mid-term (8 weeks). Furthermore, Cosenza *et al.*[36] showed no statistically significant differences in muscle echo-intensity between treated and untreated spastic cohorts. On the contrary, two studies[38, 52] found a significantly higher muscle echo-intensity in BoNT-A treated patients compared to untreated spastic patients. Furthermore, Schless *et al.*[52] observed a positive significant fair association between muscle echo-intensity and the number of previous BoNT-A interventions, while two studies[32, 38] didn't find any significant correlation with number of previous treatment cycles.

Other MRI-related outcomes

Three studies[39, 47, 56] reported hyperintensity on T_2w scans and increased T_2 from 6 weeks up to 34 months. Weidensteiner *et al.*[56] also observed a trend to an increase of fat fraction in the hyperintense region of interest at 12 weeks but not at 6 weeks post-BoNT-A injection. Furthermore, Weidensteiner *et al.*[56] observed an increased fractional anisotropy and an unchanged apparent diffusion coefficient in hyperintense regions of interest.

Discussion

The present study reviewed muscle elasticity and muscle structure changes as reported in the literature after BoNT-A injection in overactive muscles measured by non-invasive imaging methods in humans with neurological conditions. For the first time, a quantitative analysis was performed on this topic. Overall, this systematic review and meta-analysis found, at short-term, a significant improvement of muscle elasticity assessed by USE 4 weeks after BoNT-A injection.

Furthermore, no statistically significant difference was found for muscle structure (muscle thickness, pennation angle, and echo-intensity) at short-term. At long term, however, normalized muscle volume decreased.

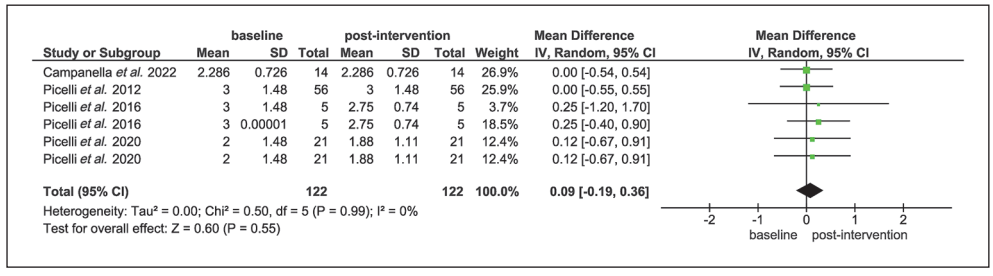


Figure 6.—Effect of BoNT-A injections on muscle echo-intensity at short-term.[28, 35, 50, 51]

Muscle elasticity

According to Mathevon, muscle stiffness, as any viscoelastic tissue, depends on its intrinsic structure and contractile status at rest.[19] In our systematic review, 14 studies have addressed muscle elasticity measured by USE. In a systematic review and meta-analysis of Miller *et al.*,[60] the reliability of USE was shown to be moderate in evaluating *in vivo* muscle elasticity in neurological populations. It also showed good convergent validity with relevant clinical assessments, and good divergent validity in discriminating tissue changes within and between groups. The same authors[60] showed a linear relationship between USE values and clinical assessment of "spasticity". Our results are in the same line as 6 authors reported faster shear-wave velocities when MAS/TS score is greater. However, no standardized measurement protocol exists regarding which method (type of USE, control of the exerted pressure, number of regions of interest/measurements, probe position, etc.) may be optimal for assessing muscle elasticity among patients with neurological conditions. Based on our quantitative data analyses, we can assume that muscle elasticity measured by USE improves with a temporary effect at short term following BoNT-A injections. These results are in line with Mathevon's systematic review,[19] which included 4 studies that analyzed viscoelastic properties of spastic muscles with USE. These studies already showed an improvement in muscle elasticity at short-term after a single BoNT-A injection. These results raise questions, as the reasons explaining this improvement remain unclear. Indeed, the impaired resistance to passive stretch in central neurological disorders, named hyper-resistance, can have neural and non-neural components.[3] By decreasing MO, BoNT-A is supposed to act on the neural component of hyper-resistance. However, BoNT-A seems to also act on the non-neural component of hyper-resistance as this reduction of elasticity is a priori linked to tissue-related properties. Bollens *et al.* also showed a decrease of the neural- and non-neural component as measured by a mechanical oscillatory device 2 and 6 months following BoNT-A injection.[61] In the same line, Bar-on *et al.*[31] observed a decrease of both the neural (-96%) and non-neural (-92%) components of ankle joint hyper-resistance, as measured by an instrumented spasticity assessment 2 weeks post BoNT-A injection.

Muscle structure

Seventeen studies reported outcome parameters related to muscle structure: muscle volume, cross-sectional area, muscle mass, muscle thickness, muscle and tendon length, fascicle length, pennation angle, and echo-intensity.

Muscle atrophy

Muscle atrophy is defined as the loss of muscle tissue, and can include decreased muscle volume, muscle thickness and cross-sectional area. Muscle atrophy is critical, as it could affect the muscle's functional ability and decrease the effectiveness of future BoNT-A injection. Regarding muscle volume, the quantitative data analysis provides some arguments for a decreased normalized muscle volume at long-term after BoNT-A injection, even if these results should be taken with caution given the small sample size. These results are in line with the numerous studies reporting muscle atrophy in humans with MO undergoing BoNT-A injections. Among others, a decrease of muscle volume had already been reported in the systematic review of Mathevon *et al.*,[19] as 3 studies[17, 54, 57] included in their review observed moderate muscle atrophy (from 4 to 30%), assessed by MRI, and lasting from 5 weeks to 1 year after a single BoNT-A injection. Data from animal studies in Mathevon's systematic review are in the same line and report post-BoNT-A injection muscle atrophy (from 30% to 60%), starting from 2 weeks post-injection.[62] Higher doses of BoNT-A injections increased and prolonged muscle atrophy.[15, 63, 64] The recovery was complete by 12 weeks post-injection in 1 study,[64] while 2 other studies reported an incomplete recovery of 89%[63] and 96%[62] up to 1 year later. Furthermore, Fortuna *et al.*[16] observed that repeated injections major this atrophy and increase its duration in animals. However, our qualitative data analysis does not allow us to draw any conclusion in humans about this cumulative effect, as results were contradictory in 2 studies.[38, 52]

Regarding muscle thickness, no significant change can be identified in the 5 studies included in the meta-analysis at short- and mid-term.[13, 43, 51, 53, 58] No conclusions can be drawn about the effects of BoNT-A on muscle thickness at long-term. Results at short-term were conflicting in the review by Mathevon *et al.*[19]

Regarding cross-sectional area, results were also very conflicting in the 4 studies.

Thus, while muscle atrophy has been evidenced, its magnitude, duration, and reversible nature remain unclear. This lack of consensus may result from multiple factors related to the heterogeneity between studies, including measurement techniques, normalization, assessed muscles, neurological condition, ambulation status, BoNT-A dosage, and previous BoNT-A exposure. These factors were

not studied or not described in our included studies, making interpretation difficult.

Pennation angle

Pennation angle indicates the position of muscle fascicles in relation to the aponeurosis and is an important parameter related to musculoskeletal function. Based on measurements of pennation angle, estimations of the orientation of fascicles can be made.[65] In pennate muscles, such as the gastrocnemius, muscle fascicles are obliquely arranged.[43] A decrease in pennation angle indicates an increase in fascicle length, which indicates that the muscle fiber has elongated, leading to an improvement in muscle extensibility.[43] Pennation angle was investigated in 5 studies on the lower leg. Based on our quantitative data analysis, there are some arguments for an unchanged pennation angle in resting position at short-term after BoNT-A injection. However, those results should be taken with caution due to the small sample size. Furthermore, based on our qualitative synthesis, we can assume that pennation angle decreases in resting angle position at mid-term following BoNT-A injections. Statistical heterogeneity of 73% in the mid-term meta-analysis could be explained by the different timing of assessment in the 3 included studies. Indeed, 2 articles reported a greater reduction in pennation angle 12 weeks after BoNT-A injection[43, 58] then at 8 weeks.[53] No studies evaluated the effects of BoNT-A injections on pennation angle at long-term.

Fascicle length

Fascicle length is the primary determinant of muscle excursion because it represents the number of sarcomeres working in series.[60] This outcome was investigated by 3 studies on the lower leg. Based on our qualitative data analysis, we were unable to draw conclusions about the effects of BoNT-A injection on fascicle length. Indeed, the fascicle length is dependent of the ankle position and the 3 studies measured fascicle length at a different ankle positions.[43, 55, 58] Moreover, there was a lack of normalization to account for natural muscle/skeletal growth, as only one study[55] investigated normalized fascicle length. Since skeletal growth is found to be altered in spastic CP children compared to typically developing children, normalization of muscle outcomes to skeletal growth should be done.[38]

Muscle echo-intensity

Increased echo-intensity on US is supposed to reflect increased muscle fibrosis and intramuscular fat content.[50] Picelli *et al.*[50] reported that patients with higher spastic muscle echo-intensity have a reduced response to BoNT-A and thus hypothesized that the toxin dosage needs to be increased in order to obtain a significant reduction of spasticity. On the other hand, it has been shown that repeated BoNT-A injections decrease the percentage of contractile material, and that muscle fibers are primarly replaced by fibroadipose tissue.[16] This leads to a vicious circle which has a possible critical effect, as it could lead to negative impacts on the effectiveness of BoNT-A interventions. In the present review, 9 of the included studies mentioned measurements of muscle echo-intensity after BoNT-A injection. Some articles used a computer-aided gray scale analysis, others used the Heckmatt Scale as an outcome measure for muscle echo-intensity. Six studies, including 3 papers written by the same author, showed no statistically significant difference at short-term[28, 35, 50, 51] nor between treated and untreated hemiparetic limbs.[32, 36] Thus, based on our literature search and data analysis, there are some arguments for an unchanged muscle echo-intensity after a single BoNT-A injection but no conclusion can be drawn about the effects of previous BoNT-A exposure. Based on 1 study,[50] Mathevon *et al.*[19] reached the same conclusion in their systematic review.

Perspectives

The results of the present study underline the effects of BoNT-A on muscle elasticity and structure. To date, MRI is considered the most precise and reliable, non-invasive and quantitative method, for measuring regional skeletal muscle mass.[66] Besides morphological changes, new MRI sequences and softwares are now able to assess other muscle characteristics. The most promising ones are muscle composition and BoNT-A diffusion. First, fatty infiltration could allow monitoring the extent and severity of changes in muscle composition after BoNT-A injection. Second, the use of MRI is also a promising approach to study the movement of BoNT-A within human muscles.[47] Based on animal studies, BoNT-A diffusion can occur up to a distance of 4.5-5cm from the injection site and even into adjacent muscles.[16, 54, 56, 67] The precise displacement of BoNT-A could be influenced by different factors, like modality of injection or dilution. Indeed, some studies[68, 69] demonstrated that higher doses and/or dilutions result in wider BoNT-A muscle distribution. Determining BoNT-A diffusion would improve the understanding of its action and could help to optimize injection technique. In the study of Weidensteiner *et al.*[56] and O'Dell *et al.*[47] T_2w images and T_2 maps showed hyperintensity but only in parts of the

muscles indicating changes at or near the site of BoNT-A injection. Beyond visual analysis, quantitative biomarkers, in particular T2 relaxation times, average apparent diffusion coefficients, and fractional anisotropy can be derived from tailored MRI sequences and followed up over time to monitor changes induced by the treatment. In the study of Weidensteiner *et al.*[56] fractional anisotropy increased while apparent diffusion coefficient did not change in hyperintense regions of interest, indicating a BoNT-A induced increase in extracellular space and a simultaneous decrease of muscle fiber diameter. In summary, MRI is a promising tool to evaluate the effect of BoNT-A injections on muscle properties. However, further studies are needed on this topic to draw firmer conclusions. There is still place for carefully designed long-term studies using multiparametric MRI studies in neurological patients with MO undergoing BoNt-A injections.

Finally, the long-term effect of BoNT-A on muscle structure remains a matter of debate. Clinically, BoNT-A has a plateau of its peak effect on MO reduction by approximately 1 month post-injection and its pharmacological effect washes out by approximately 3 months. Therefore, injections are repeated every 3-6 months in clinical routine. The lack of recovery of muscle properties following a BoNT-A injection might reduce the effects of the following injections, and the cumulative effect of injections could even be worse. Thus, it is important to understand the long-term impact of BoNT-A beyond this period and to understand the effects of repeated injections on muscle structure and elasticity as the denervation effects of BoNT-A may be still present in the injected muscle.

Limitations of the study

Limitations of the studies reviewed

The quality of the reviewed articles was variable but most studies had low (N.=13) to fair (N.=14) quality and only 7 studies had good quality. In half of the studies, the cohorts had received an unspecified number of previous BoNT-A interventions, precluding the study of the effect of previous injections or even the natural evolution of the spastic pathology. Moreover, only one study evaluated the effects of repeated BoNT-A injections, despite those injections being repeated every 3-6 months in clinical practice. Furthermore, all studies but one included patients with CP or stroke. Thus, we cannot draw any conclusion regarding other neurological conditions (TBI, SCI, etc.). Another limitation is the use of complementary treatments. Although adjunct treatments are now considered as standard of care, it is difficult to attribute the measured effects solely to the BoNT-A injections. For example, extracorporeal shock wave therapy could have both a neurological effect and a non-neural rheological effect while casting could have an additional non-neural rheological effect. Moreover, rehabilitation (strengthening) could counteract the muscle atrophy effect of BoNT-A injections.

Limitations of our systematic review and meta-analysis

There are some limitations to consider. First, we included randomized and nonrandomized clinical trials (including pre and post studies) because there were few RCTs on this topic. Indeed, BoNT-A for MO management is now best practice care; therefore, RCTs are not ethically appropriate. The number of RCTs was very small (N.=3), therefore a pre-post design was the only option. Furthermore, except for the meta-analysis on muscle elasticity, forest-plots included a small number of subjects and/or studies. Therefore, in their current forms, our statistical analyses might lack some power to detect a significant difference. In addition, protocols were highly variable between the different studies, which can explain the small meta-analyses and the heterogeneous and sometimes contradictory results. Furthermore, due to the small meta-analyses, no subgroup analysis was made at short-term for children and adults nor for upper and lower limb muscles. However, the studies included in our systematic review lead us to believe that BoNT-A injections induce the same effects on muscle elasticity and muscle structure in children and in adults or in upper and lower limb muscles at short-term. Indeed, Furukawa *et al.*[40] showed equivalent improvements in muscle elasticity in both the biceps brachii and medial gastrocnemius at four weeks after BoNT-A injection. Furthermore, Picelli *et al.*[28] did not find any significant difference in muscle echo-intensity in both upper and lower limb muscles at short-term. Moreover, all studies included in this systematic review showed statistically significant improvements in muscle elasticity at short-term both in children and in adults. In addition, all studies included in this systematic review showed no statistically significant difference in muscle echo-intensity and pennation angle at short-term both in children and in adults.

Conclusions

Our study synthetized the current literature regarding changes in muscle structure and elasticity after BoNT-A injection, by diagnostic imaging, in individuals with neurological conditions presenting MO. This review demonstrates that muscle elasticity measured by USE improves

temporarily at short-term following BoNT-A injections. Synthesis of existing clinical studies that include post-treatment assessment of muscle structure is hindered by methodological differences between studies. However, muscle thickness, pennation angle and muscle echo-intensity do not seem to be modified at short-term. On the other hand it appears that, based on a small amount of data, normalized muscle volume decreases at long-term following BoNT-A injections in children with CP suggesting that the timing of re-injection should be considered with caution in this population. No conclusions on other outcome parameters related to muscle structure can be formulated due to a cruel lack of studies investigating the effects of BoNT-A injections on muscle structure at long-term. US and MRI seem to be promising tools to evaluate the effects of BoNT-A injections on muscle structure and elasticity. Further work should focus on the long-term effects of repeated injections on muscle structure and elasticity in neurological populations.

References

1. Lance JW. The control of muscle tone, reflexes, and movement: Robert Wartenberg Lecture. Neurology 1980;30:1303–13.

2. Baude M, Nielsen JB, Gracies JM. The neurophysiology of deforming spastic paresis: A revised taxonomy. Ann Phys Rehabil Med 2019;62:426–30.

3. van den Noort JC, Bar-On L, Aertbeliën E, Bonikowski M, Braendvik SM, Broström EW, *et al.* European consensus on the concepts and measurement of the pathophysiological neuromuscular responses to passive muscle stretch. Eur J Neurol 2017;24:981–e38.

4. Simpson DM, Hallett M, Ashman EJ, Comella CL, Green MW, Gronseth GS, *et al.* Practice guideline update summary: Botulinum neurotoxin for the treatment of blepharospasm, cervical dystonia, adult spasticity, and headache: Report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 2016;86:1818–26.

5. Heinen F, Desloovere K, Schroeder AS, Berweck S, Borggraefe I, van Campenhout A, *et al.* The updated European Consensus 2009 on the use of Botulinum toxin for children with cerebral palsy. Eur J Paediatr Neurol 2010;14:45–66.

6. Delgado MR, Hirtz D, Aisen M, Ashwal S, Fehlings DL, McLaughlin J, *et al.*; Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Practice parameter: pharmacologic treatment of spasticity in children and adolescents with cerebral palsy (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2010;74:336–43.

7. Dobkin BH, Landau WM, Sahrmann S, Thomas Thach W, Simpson DM, Gracies JM, *et al.* Assessment: botulinum neurotoxin for the treatment of spasticity (an evidence-based review). Neurology 2009;73:736–8, author reply 737–8.

8. Baskaran P, Thyagarajan B. Acute and chronic effects of botulinum neurotoxin a on the mammalian neuromuscular junction. Muscle Nerve 2014;50:206–15.

9. World Health Organization. International Classification of Functioning, Disability and Health: ICF. Geneva: World Health Organization. 2001.

10. Novak I, McIntyre S, Morgan C, Campbell L, Dark L, Morton N, *et al.* A systematic review of interventions for children with cerebral palsy: state of the evidence. Dev Med Child Neurol 2013;55:885–910.

11. Aloraini SM, Gäverth J, Yeung E, MacKay-Lyons M. Assessment of spasticity after stroke using clinical measures: a systematic review. Disabil Rehabil 2015;37:2313–23.

12. Fleuren JF, Voerman GE, Erren-Wolters CV, Snoek GJ, Rietman JS, Hermens HJ, *et al.* Stop using the Ashworth Scale for the assessment of spasticity. J Neurol Neurosurg Psychiatry 2010;81:46–52.

13. Boyaci A, Tutoglu A, Boyaci N, Koca I, Calik M, Sakalar A, *et al.* Changes in spastic muscle stiffness after botulinum toxin A injections as part of rehabilitation therapy in patients with spastic cerebral palsy. NeuroRehabilitation 2014;35:123–9.

14. Minamoto VB, Suzuki KP, Bremner SN, Lieber RL, Ward SR. Dramatic changes in muscle contractile and structural properties after 2 botulinum toxin injections. Muscle Nerve 2015;52:649–57.

15. Choi WH, Song CW, Kim YB, Ha CS, Yang GH, Woo HD, *et al.* Skeletal muscle atrophy induced by intramuscular repeated dose of botulinum toxin type A in rats. Drug Chem Toxicol 2007;30:217–27.

16. Fortuna R, Vaz MA, Youssef AR, Longino D, Herzog W. Changes in contractile properties of muscles receiving repeat injections of botulinum toxin (Botox). J Biomech 2011;44:39–44.

17. Schroeder AS, Ertl-Wagner B, Britsch S, Schröder JM, Nikolin S, Weis J, *et al.* Muscle biopsy substantiates long-term MRI alterations one year after a single dose of botulinum toxin injected into the lateral gastrocnemius muscle of healthy volunteers. Mov Disord 2009;24:1494–503.

18. Valentine J, Stannage K, Fabian V, Ellis K, Reid S, Pitcher C, *et al.* Muscle histopathology in children with spastic cerebral palsy receiving botulinum toxin type A. Muscle Nerve 2016;53:407–14.

19. Mathevon L, Michel F, Decavel P, Fernandez B, Parratte B, Calmels P. Muscle structure and stiffness assessment after botulinum toxin type A injection. A systematic review. Ann Phys Rehabil Med 2015;58:343–50.

20. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998;52:377–84.

21. Manager R. (RevMan) [computer program]. Version 5.4. The Cochrane Collaboration, 2020.

22. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page M, *et al.* Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022). Cochrane, 2022. Available from www.training.cochrane.org/handbook

23. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.

24. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. BMC Med Res Methodol 2014;14:135.

25. Higgins JP, Thompson SG, Chandler J, Cumpston M, Li T, Page M, *et al.* Chapter 13: Assessing risk of bias due to missing results in a synthesis. Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane, 2019. Available from www.training.cochrane.org/handbook. In.

26. Bertan H, Oncu J, Vanli E, Alptekin K, Sahillioglu A, Kuran B, *et al.* Use of Shear Wave Elastography for Quantitative Assessment of Muscle Stiffness After Botulinum Toxin Injection in Children With Cerebral Palsy. J Ultrasound Med 2020;39:2327–37.

27. Peeters N, Van Campenhout A, Hanssen B, Cenni F, Schless SH, Van den Broeck C, *et al.* Joint and Muscle Assessments of the Separate Effects of Botulinum NeuroToxin-A and Lower-Leg Casting in Children With Cerebral Palsy. Front Neurol 2020;11:210.

28. Picelli A, La Marchina E, Gajofatto F, Pontillo A, Vangelista A, Filippini R, *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;20:160–4.

29. Alexander C, Elliott C, Valentine J, Stannage K, Bear N, Donnelly CJ, *et al.* Muscle volume alterations after first botulinum neurotoxin A treatment in children with cerebral palsy: a 6-month prospective cohort study. Dev Med Child Neurol 2018;60:1165–71.

30. Aşkın A, Kalaycı ÖT, Bayram KB, Tosun A, Demirdal ÜS, Atar E, *et al.* Strain sonoelastographic evaluation of biceps muscle intrinsic stiffness after botulinum toxin-A injection. Top Stroke Rehabil 2017;24:12–7.

31. Bar-On L, Van Campenhout A, Schless S, Hansen B, Papageorgiou E, Goudriaan M, *et al.* Clinical Case: the effect of Botulinum Toxin-A and casting on the neural and non-neural components of ankle hyper-resistance, medial gastrocnemius muscle morphology and gait in a child with spastic cerebral palsy. Gait Posture 2017;57:100–1.

32. Battaglia M, Cosenza L, Scotti L, Bertoni M, Polverelli M, Loro A, *et al.* Triceps Surae Muscle Characteristics in Spastic Hemiparetic Stroke Survivors Treated with Botulinum Toxin Type A: Clinical Implications from Ultrasonographic Evaluation. Toxins (Basel) 2021;13:889.

33. Bilgici MC, Bekci T, Ulus Y, Ozyurek H, Aydin OF, Tomak L, *et al.* Quantitative assessment of muscular stiffness in children with cerebral palsy using acoustic radiation force impulse (ARFI) ultrasound elastography. J Med Ultrason 2018;45:295–300.

34. Brandenburg JE, Eby SF, Song P, Bamlet WR, Sieck GC, An KN. Quantifying Effect of Onabotulinum Toxin A on Passive Muscle Stiffness in Children with Cerebral Palsy Using Ultrasound Shear Wave Elastography. Am J Phys Med Rehabil 2018;97:500–6.

35. Campanella W, Corazza A, Puce L, Privitera L, Pedrini R, Mori L, *et al.* Shear wave elastography combined with electromyography to assess the effect of botulinum toxin on spastic dystonia following stroke: A pilot study. Front Neurol 2022;13:980746.

36. Cosenza L, Picelli A, Azzolina D, Minetto MA, Invernizzi M, Bertoni M, *et al.* Rectus Femoris Characteristics in Post Stroke Spasticity: Clinical Implications from Ultrasonographic Evaluation. Toxins (Basel) 2020;12:490.

37. Dağ N, Cerit MN, Şendur HN, Zinnuroğlu M, Muşmal BN, Cindil E, *et al.* The utility of shear wave elastography in the evaluation of muscle stiffness in patients with cerebral palsy after botulinum toxin A injection. J Med Ultrason 2020;47:609–15.

38. De Beukelaer N, Bar-On L, Hanssen B, Peeters N, Prinsen S, Ortibus E, *et al.* Muscle Characteristics in Pediatric Hereditary Spastic Paraplegia vs. Bilateral Spastic Cerebral Palsy: An Exploratory Study. Front Neurol 2021;12:635032.

39. de Coulon G, Canavese F, Armand S, Bonnefoy-Mazure A, Merlini L. MRI changes in calf muscles of two children with cerebral palsy following Botulinum Toxin Type A injections: a preliminary report. J Pediatr Orthop B 2022;31:e81–4.

40. Furukawa T, Masakado Y. Changes in Sonoelastography After Using Botulinum Toxin Type A for the Treatment of the Patients with Post-stroke Spasticity: report of 2 Cases. Tokai J Exp Clin Med 2021;46:83–8.

41. Gao J, Rubin JM, Chen J, O'Dell M. Ultrasound Elastography to Assess Botulinum Toxin A Treatment for Post-stroke Spasticity: A Feasibility Study. Ultrasound Med Biol 2019;45:1094–102.

42. Hasegawa Y, Niimi M, Hara T, Sakurai Y, Soshi S, Udaka J, *et al.* Shear Wave Velocity to Evaluate the Effect of Botulinum Toxin on Post-Stroke Spasticity of the Lower Limb. Toxins (Basel) 2022;15:14.

43. Kawano A, Yanagizono T, Kadouchi I, Umezaki T, Chosa E. Ultrasonographic evaluation of changes in the muscle architecture of the gastrocnemius with botulinum toxin treatment for lower extremity spasticity in children with cerebral palsy. J Orthop Sci 2018;23:389–93.

44. Kwon DR, Park GY, Lee SU, Chung I. Spastic cerebral palsy in children: dynamic sonoelastographic findings of medial gastrocnemius. Radiology 2012;263:794–801.

45. Kwon DR, Kwon DG. Botulinum Toxin a Injection Combined with Radial Extracorporeal Shock Wave Therapy in Children with Spastic Cerebral Palsy: Shear Wave Sonoelastographic Findings in the Medial Gastrocnemius Muscle, Preliminary Study. Children (Basel) 2021;8:1059.

46. Lee D, Kim J, Oh JY, Han MH, Kim DY, Kang JH, *et al.* Changes in Muscle Mass after Botulinum Toxin Injection in Children with Spastic Hemiplegic Cerebral Palsy. Toxins (Basel) 2021;13:278.

47. O'Dell MW, Villanueva M, Creelman C, Telhan G, Nestor J, Hentel KD, *et al.* Detection of Botulinum Toxin Muscle Effect in Humans Using Magnetic Resonance Imaging: A Qualitative Case Series. PM R 2017;9:1225–35.

48. Park GY, Kwon DR. Sonoelastographic evaluation of medial gastrocnemius muscles intrinsic stiffness after rehabilitation therapy with botulinum toxin a injection in spastic cerebral palsy. Arch Phys Med Rehabil 2012;93:2085–9.

49. Peeters N, Papageorgiou E, Hanssen B, De Beukelaer N, Staut L, Degelaen M, *et al.* The Short-Term Impact of Botulinum Neurotoxin-A on Muscle Morphology and Gait in Children with Spastic Cerebral Palsy. Toxins (Basel) 2022;14:676.

50. Picelli A, Bonetti P, Fontana C, Barausse M, Dambruoso F, Gajofatto F, *et al.* Is spastic muscle echo intensity related to the response to botulinum toxin type A in patients with stroke? A cohort study. Arch Phys Med Rehabil 2012;93:1253–8.

51. Picelli A, Filippetti M, Melotti C, Guerrazzi F, Modenese A, Smania N. Does Botulinum Toxin Treatment Affect the Ultrasonographic Characteristics of Post-Stroke Spastic Equinus? A Retrospective Pilot Study. Toxins (Basel) 2020;12:797.

52. Schless SH, Cenni F, Bar-On L, Hanssen B, Kalkman B, O'brien T, *et al.* Medial gastrocnemius volume and echo-intensity after botulinum neurotoxin A interventions in children with spastic cerebral palsy. Dev Med Child Neurol 2019;61:783–90.

53. Tok F, Ozçakar L, Safaz I, Alaca R. Effects of botulinum toxin-A on the muscle architecture of stroke patients: the first ultrasonographic study. J Rehabil Med 2011;43:1016–9.

54. Van Campenhout A, Verhaegen A, Pans S, Molenaers G. Botulinum toxin type A injections in the psoas muscle of children with cerebral palsy: muscle atrophy after motor end plate-targeted injections. Res Dev Disabil 2013;34:1052–8.

55. Weide G, Sloot L, Oudenhoven L, Jaspers RT, Harlaar J, Buizer A, *et al.* Comprehensive evaluation of gait, spasticity, and muscle morphology: A case report of a child with spastic paresis treated with Botulinum NeuroToxin-A, serial casting, and physiotherapy. Clin Case Rep 2019;7:1637–46.

56. Weidensteiner C, Madoerin P, Deligianni X, Haas T, Bieri O, Akinci D'Antonoli T, *et al.* Quantification and Monitoring of the Effect of Botulinum Toxin A on Paretic Calf Muscles of Children With Cerebral Palsy With MRI: A Preliminary Study. Front Neurol 2021;12:630435.

57. Williams SA, Reid S, Elliott C, Shipman P, Valentine J. Muscle volume alterations in spastic muscles immediately following botulinum toxin type-A treatment in children with cerebral palsy. Dev Med Child Neurol 2013;55:813–20.

58. Yi YG, Jang DH, Lee D, Oh JY, Han MH. Botulinum Toxin Injection in Children with Hemiplegic Cerebral Palsy: Correction of Growth through Comparison of Treated and Unaffected Limbs. Toxins (Basel) 2019;11:688.

59. Peeters N, Hanssen B, De Beukelaer N, Dewit T, Weide G, Dan B, *et al.* Morphological alterations between spasticity subtypes in lower limb muscles of children with spastic cerebral palsy. Gait Posture 2020;81:267–8.

60. Miller T, Ying M, Sau Lan Tsang C, Huang M, Pang MY. Reliability and Validity of Ultrasound Elastography for Evaluating Muscle Stiffness in Neurological Populations: A Systematic Review and Meta-Analysis. Phys Ther 2021;101:pzaa188.

61. Bollens B, Gustin T, Stoquart G, Detrembleur C, Lejeune T, Deltombe T. A randomized controlled trial of selective neurotomy versus botulinum toxin for spastic equinovarus foot after stroke. Neurorehabil Neural Repair 2013;27:695–703.

62. Ma J, Elsaidi GA, Smith TL, Walker FO, Tan KH, Martin E, *et al.* Time course of recovery of juvenile skeletal muscle after botulinum toxin A injection: an animal model study. Am J Phys Med Rehabil 2004;83:774–80, quiz 781–3.

63. Tsai FC, Hsieh MS, Chou CM. Comparison between neurectomy and botulinum toxin A injection for denervated skeletal muscle. J Neurotrauma 2010;27:1509–16.

64. Stone AV, Ma J, Callahan MF, Smith BP, Garrett JP, Smith TL, *et al.* Dose- and volume dependent-response to intramuscular injection of botulinum neurotoxin-A optimizes muscle force decrement in mice. J Orthop Res 2011;29:1764–70.

65. Zhou Y, Li JZ, Zhou G, Zheng YP. Dynamic measurement of pennation angle of gastrocnemius muscles during contractions based on ultrasound imaging. Biomed Eng Online 2012;11:63.

66. Huber FA, Del Grande F, Rizzo S, Guglielmi G, Guggenberger R. MRI in the assessment of adipose tissues and muscle composition: how to use it. Quant Imaging Med Surg 2020;10:1636–49.

67. Yaraskavitch M, Leonard T, Herzog W. Botox produces functional weakness in non-injected muscles adjacent to the target muscle. J Biomech 2008;41:897–902.

68. Borodic GE, Ferrante R, Pearce LB, Smith K. Histologic assessment of dose-related diffusion and muscle fiber response after therapeutic botulinum A toxin injections. Mov Disord 1994;9:31–9.

69. Shaari CM, George E, Wu BL, Biller HF, Sanders I. Quantifying the spread of botulinum toxin through muscle fascia. Laryngoscope 1991;101:960–4.

Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Funding
This research was supported by the "Fonds Spécial de la Recherche" (UCLouvain) and the "Fund for Research Training in Industry and Agriculture" (FRIA).

Authors' contributions
Conception and design, analysis and interpretation of Data:: Marine Devis, Gaëtan Stoquart; acquisition of data: Marine Devis, Charles Rabet; drafting the article: Marine Devis; revising it for intellectual content: Gaëtan Stoquart, Thierry Lejeune, Frédéric Lecouvet; final approval of the completed article: Marine Devis, Gaëtan Stoquart, Thierry Lejeune, Frédéric Lecouvet. All authors read and approved the final version of the manuscript.

Acknowledgements
We would like to thank Charles Rabet who participated in screening articles for inclusion, extracting data, and evaluating methodological quality of the studies.

History
Article first published online: July 3, 2024. - Manuscript accepted: June 10, 2024. - Manuscript revised: March 13, 2024. - Manuscript received: May 8, 2023.

Supplementary data
For supplementary materials, please see the HTML version of this article at www.minervamedica.it