Drugs (2017) 77:1413–1422
DOI 10.1007/s40265-017-0786-1

REVIEW ARTICLE

Benefits and Risks of Non-Approved Injection Regimens for Botulinum Toxins in Spasticity

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Published online: 19 July 2017

Abstract Spasticity with muscle paresis and loss of dexterity is a common feature of upper motor neuron syndrome due to injuries or the pyramidal tract in several neurological conditions. Botulinum toxin type A has been considered the gold standard treatment for spasticity and movement disorders, with efficacy, reversibility, and low prevalence of complications. During the last 30 years, thousands of studies of its use have been performed, but few guidelines are available. Therefore, there is great variability in both the doses and intervals of administration and the approaches taken by clinicians with considerable experience in spasticity and movement disorder treatment. In the present review article, we provide a short overview of the benefits and risks of non-approved injection regimens and doses for botulinum toxins, focusing on the treatment of post-stroke spasticity, where there is great interest in the potential for increasing the number of treatment/years and the dose of botulinum toxin treatment for subjects with upper and lower limb spasticity. However, many doubts exist regarding antibody development and possible adverse effects.

Key Points

Studies on high doses of botulinum toxin type A (BoNT-A) showed an important reduction of severe spasticity after stroke, but only for single injection and, to date, only one study describing long-term treatment has been performed.

The impact of antibodies (Abs) against BoNT-A could potentially increase with high doses, although Abs are also present with low-dose treatment.

Booster injections (a new treatment 2–3 weeks after the original injections) or a shorter interval between two cycles (1 month) should be avoided to reduce the risk of Ab formation.

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1 Introduction

In 1989, Das and Park studied the reduction in post-stroke spasticity using botulinum toxin type A (BoNT-A) [1]. Since then, there have been several studies showing the efficacy and safety of the drug in reducing spasticity due to brain and spine injury [2–6]. However, controversy also exists about the increase in motor function relative to the improvement in spasticity. BoNT-A has been clearly recommended as a first-choice treatment for focal upper and lower limb spasticity in several European consensus

Table 1 Recommended doses and approved indications of botulinum toxins type A (BoNT-A) in spasticity

BoNT-A	Recommended doses	Approved indications
OnabotulinumtoxinA (Botox®)	400 U for upper limb[a]	Spasticity after stroke in adult patients[a,b]
	300–400 U for ankle plantar-flexor[a]	Dynamic equinus foot deformity due to spasticity in ambulant pediatric cerebral palsy patients, two years of age or older[b]
	200–240 U for upper limb[b]	
	300 U for ankle plantar-flexor[b]	
	4–6 U/kg[b]	
AbobotulinumtoxinA (Dysport®)	1000 U for upper limb[a]	Spasticity by several etiologies[a,b] in adult patients
	1000 U for upper limb[b]	Dynamic equinus foot deformity in cerebral palsy[b]
	1500 U for lower limb[b]	Lower limb spasticity in pediatric patients 2 years of age and older[a]
	20–30 U/kg[b]	
	10–15 U/kg[a]	
IncobotulinumtoxinA (Xeomin®)	400 U for upper limb[a,b]	Spasticity after stroke[a,b]

U units

[a] For USA

[b] For Italy

statements and by the American Academy of Neurology [4, 5]. Current guidelines suggested doses of up to 600 units (U) of onabotulinumtoxinA (Botox®, Allergan, Inc., Irvine, CA, USA) or up to 1500 U of abobotulinumtoxinA (Dysport®, Ipsen, Slough, UK/Galderma, Paris, France) per injection session to treat spasticity after stroke [5] (Table 1). Several studies suggested that there was no difference in potency between onabotulinumtoxinA and incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) and supported a dose ratio of 1:1 for incobotulinumtoxinA and onabotulinumtoxinA [6, 7]. For this reason, for incobotulinumtoxinA clinicians also used the maximum dose of 600 U (Table 1). This is supported by many international consensus statements on doses, injection techniques, patient selection, and outcome measures [4, 5, 8]. Although BoNT-A was considered effective for reducing the effects of spasticity or movement disorder reduction [1–5, 8], it has been difficult to prove its effectiveness of the treatment, especially in terms of functional benefit [9].

The first BoNT-A treatment sometimes fails to produce an adequate response, but subsequent injections may give the desired clinical effect. A permanent non-response, in which both the first and subsequent treatments may be ineffective, is rarely seen and, in these patients, an immunological reaction is considered responsible. A primary non-response after the first BoNT-A application may occur in patients with a clinical subtype reducing sensitivity to botulinum toxin, whereas secondary treatment failure is more likely to be due to antibody (Ab) formation against therapeutic neurotoxin-protein evoked by "booster" injections (a new treatment 2–3 weeks after the original injections) or high doses administered [10]. Reduced or non-response is more likely to be due to [9]:

- inappropriate selection of muscles for treatment,
- inaccurate placement of the injections,
- spread over the targeted muscles,
- insufficient doses, inappropriate patient selection,
- a lack of a specific treatment goal or outcome measure,
- progression of the underlying disease,
- handling errors during drug storage or preparation.

Recently, increasing the number of treatments and dosage has been considered for upper and lower limb spasticity patients with less than adequate outcomes. However, there are many doubts regarding Ab development and possible adverse effects. International consensus statements, expert panel reviews, as well as clinician expertise, have also considered the efficacy of BoNT-A therapy for spasticity reduction [4, 5, 8]. In particular, several randomized controlled trials (RCTs) clearly demonstrated good efficacy with the doses currently used for abobotulinumtoxinA (500, 1000, and 1500 U) [11], onabotulinumtoxinA (200–240 U) [2], and incobotulinumtoxinA (maximum 400 U) [12]. Usually, the subjects submitted to BoNT-A injections repeated the treatment after few months. As the effect lasts 2–3 months, it is important to consider some the responsible factors for the length and efficacy of this treatment between two successive cycles of high-dose treatment. The aim of the present article is thus to review the current evidence of the benefits and risks of non-approved injection regimens and high doses for botulinum toxins in post-stroke spasticity treatment.

Table 2 Key and reviewed studies on higher doses onabotulinumtoxinA (Botox®) in the treatment of post-stroke adult spasticity

Patients	Doses (U)	Injection guide	Outcome measures	Clinical results/adverse effects	References
One hemiparetic patient	OnabotulinumtoxinA 500 U OnabotulinumtoxinA 600 U OnabotulinumtoxinA 700 U OnabotulinumtoxinA 800 U	Not indicated	MAS and MRC	Fatigue and contralateral weakness for 800 U, whereas 500 UI, 600 UI, and 700 U were well tolerated	[13]
One left hemiparetic patient	OnabotulinumtoxinA 640 U OnabotulinumtoxinA 650 U OnabotulinumtoxinA 700 U	Not indicated	RNS	Difficulty getting on/off the bus for 640 U and upper and lower weakness, dysarthria, increased falls and gait instability for 650 U	[14]
One right hemiparetic patient	OnabotulinumtoxinA 700 U	EMG and ES	MAS, MRC, RNS	Contralateral upper extremity weakness; no generalized weakness, bulbar, respiratory, sphincter symptoms The lower extremity was full-strength Previous treatment with 700 U was without adverse effects	[15]
One left hemiparetic patient		EMG and ES	MAS, MRC, RNS	Contralateral upper extremity weakness; no generalized weakness, diplopia, dysphagia, shortness of breath The lower extremity was full-strength. Previous treatment with 700 U and subsequent session with 600 UI caused recurrent weakness of un-injected proximal right upper extremity muscles	[15]
26 patients with upper and lower spasticity after stroke	OnabotulinumtoxinA 676.9 ± 86.3 U	US	MAS, DAS, GAE	Significant muscle tone reduction and clinical improvement with high doses of onabotulinumtoxinA, without any adverse events was observed.	[16]

DAS disability assessment scale, *EMG* electromyography, *ES* electrical stimulation, *GAE* Global Assessment of Efficacy, *MAS* Modified Ashworth Scale, *MRC* Medical Research Council, *RNS* repetitive nerve stimulation, *U* units, *US* ultrasound

2 Literature Search Strategy

Clinical reports from the international literature published from December 1989 to March 2017 were reviewed, including randomized placebo-controlled, double-blind trials, case reports, and existing meta-analyses that demonstrated the use of higher doses of BoNT-A (i.e. a minimum dose of 600 U of onabotulinumtoxinA and incobotulinumtoxinA and 1800 U for abobotulinumtoxinA per injection session). Furthermore, these studies also provided the diagnostic criteria for the diagnoses of stroke and for the assessment of the upper and lower limb post-stroke spasticity, as well as giving safety data in terms of adverse events. The start date for these electronic searches were in December 1989 coinciding with the US Food and Drug Administration approval date for onabotulinumtoxinA use in treating strabismus, blepharospasm, and hemifacial spasm in patients aged >12 years. This narrative review was based upon searches of US National Library of Medicine databases using the following terms to identify the type of treatment (high doses of botulinum toxin or higher doses of botulinum toxin), relevant outcomes [spasticity and (stroke or post-stroke) or adverse effects or antibodies]. A search filter was developed to include only human studies and there was no language restriction on the search. The references of each study selected were screened to identify studies that were not included in the electronic search. Key textbooks were also searched in addition to the electronic database search. References mentioned in the textbooks were similarly reviewed. Tables 2, 3 and 4 show principal findings of the studies that met the study's eligibility criteria and were finally included in the overall review [13–24].

3 Management of Spasticity, Muscle Evaluation, and Injection Techniques

Spasticity has been defined as a form of muscle hypertonia caused by a velocity-dependent, hyper-active muscle stretch reflex [25]. BoNT-A reduces spasticity inhibiting

Table 3 Key and reviewed studies on higher doses of abobotulinumtoxinA (Dysport®) in the treatment of post-stroke adult spasticity

Patients	Doses (U)	Injection guide	Outcome measures	Clinical results/adverse effects	Reference
Six chronic hemiparetic patients with lower limb spasticity	AbobotulinumtoxinA 2000 U	EMG	AS, MRC, cycle parameters	Five patients of group treated with 2000 U of abobotulinumtoxinA safely completed the study reporting a muscle tone reduction, improving gait velocity, stride length, stance- and swing-symmetry 4 weeks after injection. Adverse effect in one subject: bladder paresis	[17]

AS Ashworth Scale, *EMG* electromyography, *MRC* Medical Research Council, *U* units

acetylcholine release at the neuromuscular junction with an effect lasting 3–4 months, and after this period, clinicians, if needed, may repeat the treatment. However, electromyographic examination of extensor brevis muscle compound muscle action potentials showed a prolonged effect also after 3 months [6] in patients injected and treated with adjunctive therapies, such as splinting, casting, taping, electrical stimulation compared to those treated with BoNT-A alone [26, 27].

Clinical and instrumental evaluations were used to distinguish spasticity from muscle hypertonia. As such, biomechanical changes in muscle and soft tissues sometimes contribute more to hypertonia than stretch hyperreflexia [10, 28]. In these situations, agents suppressing stretch reflexes would be unlikely to improve passive hypertonia or active movement. The presence of fibrosis, fat, or tendon retraction can modify the effect of BoNT-A and reduce its duration. Therefore, spasticity evaluation requires considerable experience to identify hyperactive muscles, co-contraction or spastic dystonia rather than treating a patient with fixed changes in muscles and soft tissues where a positive effect is unlikely.

Furthermore, it is difficult to predict the development of biomechanical changes in patients with long-term spasticity and in those not receiving mobilizing treatment (e.g. immobilization in shortened positions), who may develop intra-muscular modification. Surface electromyography allows an accurate injection of hyperactive muscles only. An ultrasound examination also demonstrates the presence of fat and fibrosis replacement, which can reduce the toxin's pharmacologic effect. Where there are no biomechanical changes, it is not necessary to repeat the BoNT-A injections into the same muscles after a few weeks, thus avoiding the risk of systemically diffusing BoNT-A and thus a botulism-like syndrome. Moreover, it is well demonstrated that the very long duration of effect of BoNT-A may result in the formation of temporary sprouts budding from the paralyzed nerve terminal in an attempt to restore some neuro-muscular transmission. Sprout formation appears to correlate with the clinical effect wearing off. Reinnervation of the parent terminal eventually occurs with dying back of the sprouts [29]. Repeating the injections after a few weeks could compromise this process leading to muscle atrophy. In fact, some studies in animal models suggested that skeletal muscle properties could not recover after BoNT-A injection due to a reduction in muscle mass and strength loss, as well as the percentage of contractile material [30–32].

Instrumental guidance is recommended as standard practice for BoNT-A injections. A wide range of injection techniques has been described and injection site localization methods included electrical stimulation with surface or needle motor point localization, electromyographic-guided technique localizing the area of most active motor unit activity, ultrasound guide, and muscle localization by palpation or surface anatomic landmarks [5, 33–35]. Usually, manual needle placement based on approximate anatomic locations of muscles and palpation was considered an acceptable technique for large, superficial muscles, but not for small, slender, or deep-seated muscles. In those last cases, instrumental guidance has been recommended as a necessary tool. This is supported by a consensus of experts on the use of BoNT-A, who described correct needle placement by instrumental guidance in the targeted muscles, especially for the modulation of muscle hypertonia when it is necessary to avoid the sprouting of the toxin in case of residual dexterity of hyperactive muscles (particularly for hand spastic-dystonia) [5, 33–35]. Several studies suggested that different BoNT-A injection techniques showed a spasticity reduction with improvement of different clinical outcome measures [36–38]. More recently, ultrasound guidance has been shown to be accurate and, while training is required, it is a simple and acceptable method of muscle location. Moreover, it is the only technique that allows the injector to observe toxin administration, identify fat and fibrosis, vessels and the depth of the target muscle. It can thus minimize the spread of toxin outside the targeted muscle belly, and, thus, potentially improve clinical outcomes. It could be superior to electromyographic guidance, but the latter observes electrically silent structures.

Table 4 Key and reviewed studies on higher doses of incobotulinumtoxinA (Xeomin®) in the treatment of post-stroke adult spasticity

Patients	Doses (U)	Injection guide	Outcome measures	Clinical results/adverse effects	References
11 stroke survivors with spastic hemiplegia. Heart rate variability measures derived from ECGs	IncobotulinumtoxinA up to 600 U	US	MAS, DAS, GAE, HRV	The use of incobotulinumtoxinA in adult patients at doses up to 12 units/kg seems to be safe regarding autonomic heart drive	[22]
14 patients with hemiparesis	IncobotulinumtoxinA up to 840 U	US	MAS, MRC, VAS, FAT, GOS, BS	Pain and spasticity reduction, global functionality and arm dexterity unchanged. Two patients had local side effects (injection site hematoma) and one subject complained of muscular weakness and reduction of active motility of the injected arm lasting 2 weeks	[20]
20 patients with upper and lower limb spasticity	IncobotulinumtoxinA up to 840 U	US	AS, DAS, GATR	Disability and spasticity reduction; good safety without general adverse effects	[24]
25 patients with upper and lower limb spasticity	IncobotulinumtoxinA up to 840 U	US	AS, DAS, VAS, GATR	Disability, pain and spasticity reduction; one patient reported injection site pain, four patients experienced muscular weakness	[19]
36 patients with hemispasticity	IncobotulinumtoxinA 476.5 ± 168.3 U	Not indicated	Not indicated	No patients experienced systemic adverse effects, neither motor nor autonomic	[18]
54 patients (15 with hemispasticity, 13 with arm spasticity, 12 with tetra-spasticity, 9 with para-spasticity and 5 with leg spasticity)	IncobotulinumtoxinA up to 1200 U	EMG and US	STQ, NE, LS	No patients showed signs of motor or autonomic dysfunction, distant from the target muscles and attributable to the toxin LS did not show any remarkable abnormalities for serum chemistry	[21]
155 patients (18–80 years) with spasticity due to cerebral causes	IncobotulinumtoxinA up to 800 U	Not indicated	AS, R, GAS, FEV_1, HAD, LS, GATR	IncobotulinumtoxinA dose escalation did not lead to an increased incidence of treatment-related adverse events. No treatment-related serious adverse events occurred. The most frequent adverse events overall were falls (7.7%), nasopharyngitis, arthralgia, and diarrhea (6.5% each). Five patients (3.2%) discontinued due to adverse events. No patient developed secondary non-responsiveness due to neutralizing antibodies	[23]

AS Ashworth Scale, *BS* Barthel Scale, *DAS* disability assessment scale, *ECG* electrocardiogram, *EMG* electromyography, *FAT* Frenchay Arm Test, FEV_1 forced expiratory volume in one second, *GAE* global assessment of efficacy, *GAS* goal attainment scale, *GATR* global assessment of treatment response, *GOS* Glasgow Outcome Scale, *HAD* hemidiaphragm assay, *HRV* heart rate variability, *LS* laboratory screening, *MAS* Modified Ashworth Scale, *MRC* Medical Research Council, *NE* neurological questionnaire, *R* Repas, *VAS* visual analogue scale, *STQ* systemic toxicity questionnaire, *U* units, *US* ultrasound

4 Approved Spasticity Indications and Dosages for Botulinum Toxin Type A Formulations

There several licensed indications for the commercial BoNT-A preparations in post-stroke spasticity. In Europe, onabotulinumtoxinA can be used for wrist, fingers and ankle spasticity, incobotulinumtoxinA for upper limb spasticity, and abobotulinumtoxinA for upper and lower limb spasticity irrespective of the origin (Table 1). The licensed doses are different for the several marked formulations, as well as the approved indication in Europe and the USA (Table 1). In the USA, for abobotulinumtoxinA, the maximum dose is 1000 U for upper-limb spasticity [39], whereas the approved dose of onabotulinumtoxinA is 400 U maximum for upper-limb spasticity and 300–400 U for ankle plantar-flexor spasticity [40]. In the USA, incobotulinumtoxinA can be injected at the maximum dose of 400 U into subjects affected by upper-limb spasticity,

without any indication for lower limb [41]. OnabotulinumtoxinA and abobotulinumtoxinA are licensed in Italy also for dynamic equinus foot deformity due to spasticity in ambulant pediatric cerebral palsy patients aged ≥2 years, abobotulinumtoxinA is licensed in the USA for lower-limb spasticity in pediatric patients aged ≥2 years, while incobotulinumtoxinA has no indication in pediatric subjects (Table 1).

A consensus of clinicians reviewed evidence from the available clinical guidelines in 2009 on BoNT-A treatment in regular clinical practice [5], confirming the use of up to 600 U for onabotulinumtoxinA or 1500 U for abobotulinumtoxinA per injection session to treat spasticity after stroke. At that time, incobotulinumtoxinA was not included, as it was not licensed for the indication across most European countries [5]. Moreover, high doses refer to total dose injected in the same session. This usually reflects treating a larger number of muscles and, therefore, the dose into each muscle does not change, confirming previous data [42]. In fact, usually, the single dose chosen is per muscle, so it can be smaller in small muscles and bigger in large muscles, with inter-injection intervals of less than three months to reduce the risk of Ab formation [42].

5 Non-Approved Regimens for Botulinum Toxin Type A

Recently, there has been a trend to inject BoNT-A in higher doses and at shorter intervals with the aim of improving outcomes. This applies, particularly, to non-functional patients seeking to improve limb posture and hygiene, apply splinting and increase passive joint range of motion. Maintaining these outcomes through shorter intervals would result in better quality of life. This is supported by the evidence from the studies selected in this review [13–24] (Tables 2, 3, 4) and from a previous systematic review [43] that suggested that higher doses of BoNT-A and short-interval therapy [44] appeared to be both effective and safe in reducing upper- and lower-limb spasticity after stroke.

5.1 OnabotulinumtoxinA (Botox®)

Table 2 shows reviewed studies on higher doses of onabotulinumtoxinA in the treatment of post-stroke adult spasticity [13–16]. In 2009, a first study reported contralateral weakness in a 53-year-old woman with post-stroke spasticity following a fourth upper and lower limb injection of 800 U of onabotulinumtoxinA despite good tolerance from her three previous injections of 700, 500, and 600 U onabotulinumtoxinA [13]. Crowner and colleagues described a case series and literature review of systematic weakness after BoNT-A injection. This included weakness (difficulty getting on/off his bus) in a 16-year-old male with a history of right middle cerebral artery aneurysm rupture at age of 10 years, who had been treated with 640 U of onabotulinumtoxinA [14]. This adverse effect lasted only one month, but he had received the same dosage previously, which was well tolerated. After a re-injection of 650 U of onabotulinumtoxinA, he presented with weakness in both upper and lower extremities, dysarthria resulting in increased falls and gait instability. Twelve weeks post-injection, he had continued difficulty climbing stairs, but was no longer falling and had regained full strength in his upper extremities [14].

Thomas and Simpson also described the contralateral weakness following BoNT-A for post-stroke spasticity in two patients treated with repeated onabotulinumtoxinA injections [15]. In the first case report, a 43-year-old hemorrhagic stroke woman with a spastic right hemiparesis, treated safely for more than one year with 575–700 U of onabotulinumtoxinA into the upper and lower limb muscles, then went on to be injected with a total dose of 700 U. Contralateral weakness in the shoulder girdle and distal arm was noted without generalized, bulbar, respiratory, or sphincter weakness, pain, sensory symptoms, or other systemic symptoms [15]. In the second case report, a 21-year-old woman with post-stroke spasticity and dystonia tolerated total doses of 550–700 U of onabotulinumtoxinA well into the proximal upper limb muscles, but after a further BoNT-A treatment with a total dose of 700 U in the same muscles, she reported weakness of her non-treated right arm starting within days after the injection. She denied neck pain, radiating symptoms to the right upper extremity, sensory disturbances, diplopia, dysphagia, or shortness of breath. The same symptoms were reported after the injection of 600 U, but no adverse effects were reported after 500 U when any muscles proximal to the elbow were not injected. The authors explained the development of contralateral limb weakness by toxin diffusion through tissue planes from proximal upper extremity muscles across the midline, to the contralateral muscles [15]. Finally, in a recent retrospective analysis, Baricich and colleagues evaluated the efficacy and safety of higher doses of onabotulinumtoxinA (from 600 to 800 units) before, 30, and 90 days after treatment in 26 patients affected by upper- and/or lower-limb post-stroke spasticity [16]. The authors observed significant muscle tone reduction and functional improvement. No adverse events were reported [16].

5.2 AbobotulinumtoxinA (Dysport®)

We found only one study addressing the impact of higher doses of abobotulinumtoxinA in post-stroke spasticity

(Table 3) [17]. This was one of the first studies of higher doses of BoNT-A, in which Hesse and colleagues demonstrated the efficacy of electrical stimulation after the injection of BoNT-A into spastic lower-limb muscles of ischemic stroke patients [17]. A group of 5 subjects treated with 2000 U of abobotulinumtoxinA did not report severe adverse effects, while the first patient of another group developed a bladder paresis, requiring catheterization for 14 days, so the remaining 4 patients were treated with 1500 U of abobotulinumtoxinA. All patients of group treated with 2000 U of abobotulinumtoxinA completed the study reporting a muscle tone reduction, improving gait velocity, stride length, stance- and swing-symmetry 4 weeks after injection without any adverse effects [17].

5.3 IncobotulinumtoxinA (Xeomin®)

Table 4 shows reviewed studies on higher doses of incobotulinumtoxinA (up to a maximum of 600 U) in the treatment of adult post-stroke spasticity [18–24]. As incobotulinumtoxinA has no accessory complexing proteins, the therapeutic effect is said to be mediated by the purified neurotoxin itself, maintaining an elevated specific biological activity. This could be related to a lower risk of general side effects and immunogenicity, but this effect had not yet been demonstrated. Many clinicians started to use more than the 400 U recommended by the European incobotulinumtoxinA product label, so exceeding the established 600 U limit of BoNT-A. This resulted in several studies describing higher doses of incobotulinumtoxinA (>600 U) for the treatment of severe upper- and lower-limb spasticity without important and persistent adverse effects [18–24] (Table 4). In particular, a recent Phase III, non-randomized, single arm, multicenter trial of incobotulinumtoxinA (TOWER) (ClinicalTrials.gov Identifier: NCT01603459) investigated the efficacy and safety in a dose-titration study in 155 subjects receiving total body doses of 800 U of the neurotoxin in upper- and lower-limb spasticity of cerebral causes [23] (Table 4). In this trial, incobotulinumtoxinA dose escalation did not lead to an increased incidence of treatment-related adverse events, with only five patients (3.2%) discontinued and without development of secondary non-responsiveness due to neutralizing Abs (NAbs) against the neurotoxin component of the BoNT-A drug [23]. Finally, in a very recent prospective, non-randomized, open-label study, the long-term safety of repeated high doses of incobotulinumtoxinA (up to 840 U) was evaluated in 20 patients with post-stroke spasticity affecting the upper and lower limb two years after the first set of injections, for a total of eight sets of injections [24]. Patients continued to report an improvement in their clinical outcomes for reducing spasticity and disability for elbow, wrist, fingers and ankle flexor muscles, as measured 30 days after the last set of injections (eighth set) compared to the baseline [19]. Therefore, in this study, repeated high doses of incobotulinumtoxinA, administered for eight sets of injections, appeared to be safe in patients with upper- and lower-limb spasticity after stroke [24].

6 Potential Risks of Non-Approved Regimens for Botulinum Toxin Type A

6.1 Adverse Effects

BoNT-A treatment has been shown to be well tolerated without severe adverse effects, if correctly injected and it is possible to differentiate between localized and generalized side effects. Localized effects comprised those directly associated with the injection site such as hematoma, bruising, swelling and pain lasting a few days with resolution without any complications. The generalized adverse events were related to spread of toxin distant from the site of injection, such as a botulism-like syndrome with dysphagia, breathing or speech difficulty. Very rarely, a severe allergic reaction can result in death, even at low doses [45]. Generalized weakness was one of the most frequent treatment-related adverse events with higher doses of BoNT-A [43], but it can also occur for licensed doses of BoNT-A [46].

Therefore, clinicians have recommended care in frequent re-injecting doses of more than 600 U of abobotulinumtoxinA or incobotulinumtoxinA. Reducing the potential for systemic side effects may occur, if clinicians stick to dosage intervals of four months or greater and care in diluting the toxin. In fact, high doses and/or volume of toxin may saturate local cholinergic nerve terminals, spreading (diffusing) into nearby tissues or the blood supply. In particular, special care should be given to high dose and high dilution (large) volumes injected into proximal upper extremity muscles, as these may cross the midline, risking contralateral weakness, as described by Thomas and Simpson [15]. As stated above, accurate injections using instrumental guidance (i.e. electrical stimulation or ultrasonography) to identify muscles correctly may reduce the spread of the toxins to the nearby tissues and the risk of adverse effects. Although high doses of BoNT-A look to be safe and effective for severe spasticity reduction, further studies are needed to exclude the actual risk of systemic effects and to see the effect of Ab formation after multiple injections [22, 43]. The few studies showing the safety and efficacy of high doses of BoNT-A considered one treatment administration only, so several sets of high-dose treatments should be given to exclude, with greater certainty, Ab formation associated with higher dosages.

6.2 Antibody Development

In clinical practice, the choice of dose and frequency of injections have been designed to minimize Ab formation with treatment intervals restricted to around 12 weeks, in order to reduce the risk of NAb formation against BoNT-A. This risk was directly related to a single dose and inversely related to the inter-injection interval. In addition, it has been suggested that the reactivity of the actual patient's immune reaction may influence the formation of NAbs [47]. The immunological quality of the BoNT-A formulations used has also been recognized as a risk factor [48]. For this reason, incobotulinumtoxinA, containing only the pure neurotoxin through a manufacturing process that separates it from complexing proteins, could be related to a lower risk of immunogenicity, but this effect is not yet demonstrated [49]. NAbs directed against the core neurotoxin can interfere with pharmacological activity, potentially leading to loss of clinical efficacy and thus, partially or completely, reducing its therapeutic effect. Ab-induced failure of therapy usually developed within the first 2–3 years of BoNT-A treatment [50].

Meta-analytic findings coming from 8525 patients (1170 with spasticity) showed that the frequency of NAbs was 5.9% for spasticity and the prevalence of NAbs was lower (3.5%) among clinically responding patients and higher (53.5%) in patients with secondary non-responsiveness [51]. However, data are missing on the NAbs prevalence in adult patients submitted to long-term re-injection for spasticity, although there are studies showing Ab detection after several sets of injection [52, 53]. This lack of findings was due to the difficulty to demonstrate Ab formation. For this aim, extensor digitorum brevis muscle test or frontalis muscle test are less sensitive than mouse diaphragm assay, but easier to use and less expensive. Therefore, there are changing conditions responsible for Ab formation; however, a correct administration of BoNT-A can reduce the risk of their development even if the results of long-term trials are required using the mouse diaphragm assay, which represents a highly sensitive and specific quantitative test for NAbs. A study described the employment of BoNT-A therapy with inter-injection intervals of less than 12 weeks (69.0 ± 8.1 days) in 30 patients treated with incobotulinumtoxinA in a dose of 259 ± 159 U with different dystonias [44]. None of these patients showed signs of Ab-induced therapy failure or increased treatment-related adverse effects, suggesting that short-interval therapy may be safe even if other studies with other BoNT-A drugs are needed to confirm safety of short inter-injection intervals [44]. Another recent study described a short inter-injection interval (one month after the first set of BoNT-A treatment) in 7 of 11 subjects previous treated with incobotulinumtoxinA, who still had disability related to spasticity interfering with normal activities (after the 30 days). The patients reported a clinical improvement measured with Disability Assessment Scale, Modified Ashworth Scale and Global Assessment Scale. However, in this study, there was no control group and follow-up, so it was impossible to exclude the risk of side effects and antibody-formation after repeated short-interval treatments [54].

7 Potential Benefits of Non-Approved Regimens for Botulinum Toxin Type A

Short-interval therapy of BoNT-A (one or two months after the injection) could be used to treat a clinical scenario early in the rehabilitation process, i.e. muscles not previously injected, especially in case of spastic dystonia, in which the pattern can change frequently, in order to balance the agonist and antagonist muscles to reach the treatment goal. Moreover, an additional treatment could be useful to reduce the impairment and increase the active movement time, if present, using a lower dose to minimize the risk of adverse effects due to a cumulative increment. Another possible reason to use short-interval BoNT-A therapy is to reduce disability related to spasticity and restore function [54]. For higher BoNT-A doses, in cases of severe spasticity without movements of the affected limb, it is possible to increase the doses of the toxin, increasing the number of muscles injected, to obtain other common goals of treatment, relieving pain and spasms, reducing rigidity or unpleasant sensations, improving care and hygiene, preventing contractures, improving dressing and mobility, and allowing wearing of splints and orthoses. In addition, some studies have shown that incobotulinumtoxinA [12] and abobotulinumtoxinA [55] may have an effect up to 20 weeks with standard doses in upper-limb spasticity; only one, to date, has described a two-year long-term treatment with higher incobotulinumtoxinA doses, reporting good signals of safety without general adverse effects [24].

8 Conclusions

Increasing the number of treatments and dosages of BoNT-A for patients with upper- and lower-limb spasticity has benefits and risks. Studies on high neurotoxin doses showed reductions in spasticity, but only for single injections and, to date, only a single study describing long-term treatment has been performed [24]. Higher neurotoxin doses may be used also for upper- and lower-limb pain reduction or to avoid muscle shortening. If patients need treatment for severe upper- and lower-limb post-stroke spasticity, it is necessary to know that higher BoNT-A doses are safe. In fact, BoNT-A treatment may reduce spasticity, but may also decrease the motor control of

injected muscles and high doses could spread to near muscles, so reducing the residual motor function of the patients. Secondary non-responsiveness due to NAb formation is seen over several cycles of treatment, whereas general weakness can occur also after one treatment. All treatments should have a clear aim, but repeating it after one or two months should be considered only in certain circumstances, such as for early functional preservation. Early BoNT-A has the potential to saturate cholinergic terminals, risking diffusion and possible fibrosis at a later date. BoNT-A treatment requires expertise to ensure accurate injection and, thereby, reduce the possibility of diffusion to adjacent muscles. Further systematic reviews and meta-analyses are required to provide data on long-term treatment and the safety of high dosages of BoNT-A and booster injections in post-stroke spasticity.

Acknowledgements We wish to thank Professor Anthony B. Ward for translation and preparation of the English text.

Compliance with Ethical Standards

Funding Dr. Santamato and Dr. Panza reported no funding for the present paper.

Conflicts of interest Dr. Santamato and Dr. Panza reported no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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