



UNIVERSITÀ DEL PIEMONTE ORIENTALE

Università del Piemonte Orientale “Amedeo Avogadro”
Novara

Doctoral Program in Medical Sciences and Biotechnology

XXX Cycle

Chair: Prof. Marisa Gariglio

**Clinical and instrumental evaluation of Botulinum Toxin type A
safety profile in post stroke spasticity rehabilitation treatment**

Doctoral Thesis

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Novara, March 26th 2018

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Abstract

Background

Post stroke spasticity (PSS) occurs approximately in 30% of stroke survivors. Spasticity varies from a subtle neurological sign to a gross increase in tone causing immobility of joints. PSS is associated with several complications, increasing care needs and utilisation of healthcare resources.

Botulinum toxin type A (BoNT-A) has been considered as an effective and safe treatment for focal spasticity in stroke survivors, with low prevalence of complications, reversibility of effect, and efficacy in reducing spastic hypertonia. Recent studies estimated that a significant percentage of patients affected by PSS could benefit from higher doses than those permitted by current country directives. However, at present time, there is no general consensus on the maximum dose of BoNT-A in terms of safety and clinical interchangeability among the three commercially approved products (abobotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA).

In light of these considerations, the aim of this thesis is to investigate the safety profile of BoNT-A high doses in the treatment of post stroke spasticity.

In our research activity we investigated the clinical effect of this treatment in severely affected patients, focusing on both clinical and instrumental assessment of systemic effects of BoNT-A.

Results

Although systemic BoNT-A toxicity is a rare event and as such not necessarily fatal, fear of systemic toxicity is still the most vigorous concern against application of increased BoNT-A doses.

Current evidence coming from published literature, considering both clinical and instrumental analysis of BoNT-A systemic diffusion, suggests that higher doses of BoNT-A are efficacious in reducing spasticity of the upper and lower limbs after stroke, with rare occurrence of mild adverse effect.

Conclusions

The evidence coming from published studies suggests that use of doses of BoNT-A higher than those reported in product labels could be considered as a safe therapeutic option to reduce multifocal or generalized post stroke spasticity in selected patients. The clinicians have to carefully define the clinical goal before starting with BoNT-A treatment, considering all the factors which could affect the safety profile of BoNT-A.

Further evidence is mandatory to confirm higher doses of BoNT-A as a safe and effective therapeutic option for the treatment of post stroke spasticity. In particular, it should be pointed out the potential role of higher doses of BoNT-A in order to improve the functional outcome of these patients.

Riassunto

Introduzione

La spasticità post ictus (PSS) è osservata in circa il 30% dei soggetti con esiti di stroke.

La presentazione può variare da un lieve incremento del tono muscolare ad una immobilizzazione di segmenti articolari, ed è causa di significative complicanze e di incremento di costi assistenziali e sanitari.

La tossina botulinica di tipo A (BoNT-A) è un trattamento sicuro ed efficace nel trattamento della PSS focale. Da studi recenti emerge che una significativa percentuale di pazienti potrebbe trarre benefici dall'utilizzo di dosi di BoNT-A superiori a quelle indicate in scheda tecnica. Tuttavia, non vi sono attualmente pareri unanimi in merito alla massima dose utilizzabile ed alla intercambiabilità fra le BoNT-A in commercio (abobotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA).

Obiettivo della tesi è la valutazione del profilo di sicurezza delle alte dosi di BoNT-A nel trattamento della PSS. Nella nostra attività di ricerca abbiamo analizzato l'effetto clinico di questo trattamento in pazienti affetti da PSS severa multifocale; particolare attenzione è stata rivolta alla valutazione clinica e strumentale degli effetti sistemici di BoNT-A.

Risultati

La tossicità sistemica è un evento raro e non sempre fatale, ma il timore della sua insorgenza è tuttora la maggiore criticità legata all'applicazione di alte dosi di BoNT-A.

Le attuali evidenze, che considerano sia una valutazione clinica che una valutazione strumentale della diffusione sistemica di BoNT-A, suggeriscono che alte dosi di BoNT-A sono efficaci nella riduzione della PSS all'arto superiore ed inferiore, con una bassa incidenza di effetti avversi.

Conclusioni

Le attuali evidenze suggeriscono che l'utilizzo di dosi di BoNT-A superiori a quelle indicate in scheda tecnica possono essere considerate come un'opzione terapeutica sicura ed efficace nel trattamento di PSS in pazienti selezionati. I clinici devono definire con accuratezza gli obiettivi terapeutici, considerando tutti i fattori che possono influenzare il profilo di sicurezza del farmaco. Ulteriori evidenze sono necessarie per confermare il profilo di efficacia e sicurezza delle alte dosi di BoNT-A nel trattamento della spasticità focale post ictus. Particolare attenzione deve essere rivolta a definire il ruolo delle alte dosi di BoNT-A nel miglioramento dell'outcome funzionale del paziente.

Introduction

Stroke-related disability is a significant health problem with relevant socioeconomic consequences for patients as well as society with long-lasting effects.

More than two-thirds of stroke survivors develop poststroke sequelae, including impaired motor function and spasticity. These impairments have a significant impact on a stroke survivor's daily life, such as eating, walking and self-care. In addition, these disabilities involve a significant burden on caregivers of these patients [Wissel et al, 2013].

Post stroke spasticity (PSS) occurs approximately in 30% of stroke survivors. Spasticity varies from a subtle neurological sign to a gross increase in tone causing immobility of joints. PSS is associated with several complications, increasing care needs and utilisation of healthcare resources [Lundstrom et al 2010], and carers of patients with spasticity are more likely to experience anxiety and depression [Denno et al, 2013].

Management of spasticity requires a balanced approach, weighing the benefits of treatment against the side effects.

Botulinum toxin type A (BoNT-A) has been considered as an effective and safe treatment for focal spasticity in stroke survivors, with low prevalence of complications, reversibility, and efficacy in reducing spastic hypertonia [Santamato et al, 2015], with the approval of the U.S. Food and Drug Administration and the European regulatory agencies for this indication. However, at present time, there is no general consensus on the maximum dose of BoNT-A in terms of safety and clinical interchangeability among the three commercially approved products (abobotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA).

A recent survey [Picelli A, Baricich A et al, 2017] suggested that there is a need to reconsider the maximum dose administered per single treatment in order to improve the clinical outcome of treated patients. In fact, the use of high doses of BoNT-A is an established practice that, moreover, addresses the very real need to improve the quality of life of patients with post-stroke spasticity [Baricich et al., 2015]. It has been reported that a high percentage of patients (up to about 62%) needs a combined BoNT-A treatment of upper and lower limb, while only a very low proportion (< 25% on average) requires treatment in the upper or lower limb alone in a single session; in addition, anecdotal, unpublished, 10- year follow-up observations showed that a tendency to increase BoNT-A doses over time was paralleled by a tendency of patients to be more satisfied [Picelli A, Baricich A et al, 2017]. However, the most important adverse effect of BoNT-A is the systemic diffusion of the toxin and it has been suggested [Lange et al, 1987] a potential relationship with BoNT-A dose, causing a possible increase of adverse events after injection.

Aim of the thesis

In light of these considerations, the aim of this thesis is to investigate the safety profile of BoNT-A high doses in the rehabilitation treatment of post stroke spasticity, focusing on both clinical and instrumental assessment of systemic effects of BoNT-A.

Presentation of data derived from published studies will be integrated with the findings obtained by our group.

Post stroke spasticity

The term spasticity as a clinical entity was proposed by J.W. Lance in the 1980s as “a motor disorder characterized by a velocity-dependent increase in tonic stretch reflex (muscle tone) with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex as one component of the upper motor neuron syndrome (UMNS)”. In clinical practice, spasticity describes a combination of symptoms and clinical signs after lesion formation in sensorimotor brain areas and tracts in the Central Nervous System (CNS), resulting from impaired reflex function; In addition, spasticity induces changes in rheological muscle properties like stiffness, fibrosis and atrophy [Dietz and Sinkjaer, 2007].

There is still no consensus for the definition of spasticity and this reflects the complexity and the diversity of the phenomena [Baricich et al, 2016; Picelli A, Vallies G et al, 2017]. This is especially true for post-stroke motor disorders, which can show a considerable variety of symptoms (e.g. clonus, dystonia, muscle weakness, abnormal reflex responses).

In general, the ‘upper motor neuron syndrome’ can be defined by the presence of positive and negative signs [Young, 1994]. Spasticity is part of the positive signs among other motor symptoms which occur after lesions in the descending corticospinal system such as spastic dystonia (muscle constriction in the absence of any voluntary movement), spastic co-contraction (contraction of both the agonist and antagonist muscles resulting from an abnormal pattern of commands in the descending supraspinal pathway), extensor or flexor spasms, clonus, exaggerated deep tendon reflexes and associated reaction [McComas, 1994; Sommerfeld et al, 1994]. On the other hand, negative signs are muscle weakness, loss of dexterity and fatigue.

Prevalence estimates of post stroke spasticity (PSS) were highly variable, ranging from 4% to 42.6%, with the prevalence of disabling spasticity ranging from 2% to 13%. Data on phases of the PSS continuum revealed evidence of PSS in 4% to 27% of those in the early time course (1–4 weeks post stroke), 19% to 26.7% of those in the postacute phase (1–3 months post stroke), and 17% to 42.6% of those in the chronic phase (>3 months post stroke) [Wissel et al, 2013].

In the upper limbs, the most frequently observed pattern is internal rotation and adduction of the shoulder coupled with flexion at the elbow, the wrist and the fingers; in the lower limbs, adduction and extension of the knee with equinovarus foot [Thibaut et al, 2013].

Spastic symptoms can induce pain, ankylosis, tendon retraction or muscle weakness in patients, which

can limit the success of rehabilitation. Spasticity can also affect quality of life and be highly detrimental to daily function [Duncan et al, 2005; Langhorne, 2011; Chae and Celnik, 2015]. However, there is currently a lack of specific guidelines for the stratification and individualization of rehabilitation programmes [Thibaut, 2013; Picelli A, Baricich A et al, 2017].

Therapeutic interventions include physical therapy, occupational therapy, self-rehabilitation, orthoses equipment and assistive devices, pharmacological treatment, orthopaedic surgery and neurosurgery [Thibaut et al, 2013; Deltombe et al, 2017].

Botulinum Toxin and post stroke spasticity

Botulinum Toxin type A (BoNT-A) administered by intramuscular injection, is the gold standard for the treatment of focal spasticity, with low prevalence of complications, reversibility, and efficacy in reducing spastic hypertonia with the approval of the U.S. Food and Drug Administration and the European regulatory agencies for this indication.

BoNT-A showed to increase patient's ability to actively mobilize their upper and lower limbs and improve their autonomy (e.g. self-care, walking) [Thibaut et al, 2013; Simpson et al, 2016].

Pharmacology and immunology of Botulinum Toxin

Botulinum neurotoxin (BoNT) is a microbial protein which exists in seven different serotypes, designated A through G. Although the individual serotypes are immunologically distinct, all members of the group present similar subunit structures, act on the same target organs, and produce similar functional outcomes [Lacy and Stevens, 1999; Johnson and Bradshaw, 2009]. Each molecule is typically released from bacteria as part of a noncovalent complex with other associated proteins. These auxiliary proteins do not play an active role in the therapeutic actions of the toxin, even if it was hypothesized a possible involvement in undesirable effects.

BoNT is an enzyme which acts in the cytosol of nerve endings: it cleaves three polypeptides governing exocytosis. Serotypes A and E cleave synaptosomal-associated protein (SNAP)-25, serotypes B, D, F, and G cleave vesicle-associated membrane protein (VAMP), and serotype C cleaves both syntaxin and SNAP-25 [Simpson, 2004; Humeau et al, 2000].

The block of acetylcholine release at neuro-muscular junctions is the mechanism involved in the therapeutic effect of BoNT to relieve dystonia, spasticity, and related disorders [Tassorelli et al, 2006].

BoNT showed additional therapeutic benefits, not necessarily related to neuromuscular transmission, including blockade of acetylcholine release at autonomic nerve endings and blockade of transmitter release at peripheral nerve endings that use other mediators.

In addition to peripheral effects of BoNT, indirect effects on CNS have been observed, probably resulting from changes in the normal balance of efferent and afferent signals. Interestingly, both the direct and indirect actions of the toxin are largely or completely reversible [Simpson et al, 2008].

At the present time, BoNT is commercially available in 2 serotypes, A and B. In United States, Food and Drug Administration approved four preparations of BoNT: onabotulinumtoxinA (Botox®, Allergan, Inc., United States), abobotulinumtoxinA (Dysport®, Ipsen, France), incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Germany), and rimabotulinumtoxinB (Myobloc/Neurobloc® (US WorldMeds/Solstice Neurosciences, United States). [Table 1]

Table 1: Botulinum toxins and FDA-approved indications (modified from Simpson et al, 2016)

BoNT preparation	Brand name (manufacturer)	FDA approved indications
OnabotulinumtoxinA	Botox (Allergan, Inc., Irvine, CA)	Blepharospasm, cervical dystonia, upper extremity spasticity, lower extremity spasticity, chronic migraine, treatment of urinary incontinence due to detrusor overactivity, axillary hyperhidrosis, strabismus
AbobotulinumtoxinA	Dysport (Ipsen Ltd., Paris, France)	Cervical dystonia, spasticity
IncobotulinumtoxinA	Xeomin (Merz Pharmaceuticals, Frankfurt, Germany)	Blepharospasm, cervical dystonia, upper extremity spasticity
RimabotulinumtoxinB	Myobloc/Neurobloc (US WorldMeds/Solstice Neurosciences, Louisville, KY)	Cervical dystonia

BoNT-A is approved for cervical dystonia and spasticity, whereas BoNT-B was approved for cervical dystonia only.

In nature, BoNT-A is synthesized as macromolecular protein complexes [Aoki and Guyer, 2001]. These protein complexes are referred to as progenitor toxins and consist of nontoxic accessory proteins (NAPs) bonded to the 150-kD active neurotoxin.

The BoNT-A progenitor toxins vary in molecular weight (300–900 kD) depending on the composition of NAPs and the manufacturing process [Dressler and Benecke, 2007]. The 150-kD neurotoxin must dissociate from NAPs in order to exert its pharmacologic effects. This dissociation occurs in physiologic pH conditions.

Although no clear differences in effectiveness between the various formulations were demonstrated, their comparability is still intensely debated, focusing on several issues such as potency, dose equivalence, immunogenicity, spread and systemic diffusion.

Potency

Although the various BoNT-A products differ in NAP composition, the 150-kD neurotoxin is the active part inhibiting acetylcholine release.

Since the toxin moiety is the same in all pharmaceutical preparations, differences in potency could depend of the amount of active toxin available. To become fully activated, the single chain 150-kD neurotoxin must be cleaved from the protein complex. All of the commercially available BoNT-A formulations are composed of the 150-kD neurotoxin with NAPs; the only exception is incobotulinumtoxinA, which contains only the 150-kD neurotoxin.

However, also the manufacturing process may affect the amount of active toxin; for instance, enzymes added to increase the percentage of cleaved active toxin may denature the neurotoxic protein itself.

BoNT-A formulations contain different percentages of inactive toxin which contribute to the overall protein load. For this reason, the potency is expressed in biological units. Potency is related to the quantity of toxin (in ng of protein content, *i.e.*, 150 kD neurotoxin including NAPs) required to achieve a median lethal dose (LD50) unit [Sesardic et al, 2003; McLellan et al, 1996]. However, many factors affect the mouse LD50 bioassay including mouse strain, sex, age, volume and route of injection, time of examination after injection, and delivery vehicle or reconstituting buffer. Moreover, the LD50 units of BoNT products are not standardized across manufacturers.

Due to the lack of LD50 bioassay harmonization, the unit potencies of BoNT formulations cannot easily be compared. For this reason, it is mandatory that physicians consider that, even if the active

molecule is botulinum neurotoxin type A, different forms of the complex can affect the therapeutic profiles. In fact, as previously described, there are several BoNT-A products on the market: onabotulinumtoxinA (ONA), incobotulinumtoxinA (INCO) and abobotulinumtoxinA (ABO) [Albanese, 2011].

However, despite the difficulties related to the biologic units, the most informative comparisons of BoNT-A products have been made in clinical studies.

Dose equivalence

Each BoNT-A formulation contains different amounts of the 150-kD toxin (and NAPs)/LD50 unit (Table 2). However, although there are some difficulties establishing the comparative potencies, the equivalence ratio of the dose should be established.

There are several reasons for identifying a conversion factor: medical (*i.e.*, patients may need to switch to another formulation) as well as economical (an incorrect conversion factor may negatively impact the real cost of treatment) [Chen and Dashtipour, 2013; Frevert, 2015].

Table 2: Botulinum toxin products and protein content/100 units (Adapted from Scaglione, 2016)

BoNT-A	150-kD protein content (ng)	Total protein content (150 kD and NAP) (ng)	Dose Equivalent Units
OnabotulinumtoxinA	0.73	5.00	1
IncobotulinumtoxinA	0.44	0.44	1
AbobotulinumtoxinA	0.65	0.87	2-3

NAP: nontoxic accessory proteins

INCO has been shown to be as effective as ONA with a comparable adverse event profile with a clinical conversion ratio of 1:1 or 1:1.2 [Benecke et al, 2005; Roggenkamper et al, 2006; Jost et al, 2005; Park et al, 2011; Zoons et al, 2012]. Clinical results are consistent with preclinical comparability data [Dressler and Benecke, 2007; Dressler et al, 2012]. Thus, both clinical and preclinical analyses have demonstrated a clinical conversion ratio between ONA and INCO very close to 1:1.

In contrast, the conversion ratio between ONA (or INCO, consequently) and ABO is highly debated. Even if the most commonly used conversion ratios are 1:3 or 1:4 [Aoki et al, 2006], they ranged from 1:1 [Wohlfarth et al, 2007] to as high as 1:11 [Marchetti et al, 2005]. This wide conversion ratio range

reflects real-life clinical practice; the treating physician determines the number of muscles to be treated and the empiric dose based on each patient's conditions, their clinical pattern, and treatment goals.

Although the various BoNT products differ in NAP composition, the toxins ultimately inhibit acetylcholine release. Since the active toxin content is established for each product, a conversion rate should be defined. More precise estimation of conversion ratios should also ensure the development of comparable clinical data on the efficacy and safety of currently available BoNT-A formulations since they have qualitatively and quantitatively similar clinical efficacies and side effects at equipotent doses.

A large number of studies have reported an ONA:ABO conversion factor of 1:3 with clinical equivalence [Marion et al, 1995; Whurr et al, 1995; Kollwe et al, 2010; Odergren et al, 1998; Shin et al, 2009]. Moreover, when the conversion factor is close to 1:3, ABO showed higher efficacy [Wohlfarth et al, 2008; Mohammadi et al, 2009; Rystedt et al, 2012], indicating that the conversion factor could be rather lower than equal to 1:3. Interestingly, studies where the conversion ratio was higher than 1:3 showed higher efficacy and longer duration of action of ABO compared to ONA, but with more adverse events, supposing an overdose of ABO determined by this conversion ratio [Sampaio et al, 1997; Nussgens et al, 1997; Ranoux et al, 2002; Bentivoglio et al, 2012].

These clinical data are consistent with preclinical data where a conversion ratio for ONA/ABO of 1:3 or lower has been found [Van den Berg and Lison, 1998; Rosales et al, 2006; Wohlfarth et al, 2009; Keren-Capelovitch et al, 2010; Brockmann et al, 2012; Kollwe et al, 2015; Rystedt et al, 2015; Yun et al, 2015; Hambleton and Pickett, 1994].

In conclusion, current data suggest that a conversion ratio ONA/ABO of 1:3—or even lower—is appropriate for treating spasticity, cervical dystonia, and blepharospasm or hemifacial spasm. A higher conversion ratio may lead to an excessive ABO dose (with the potential for an increased incidence of adverse events) or underdosing when switching ABO to ONA [Scaglione, 2016].

Immunogenicity

A possible reason for secondary treatment failure of any therapeutic protein is its neutralization [Kromminga and Schellekens, 2005]. Antibodies that block its pharmacological effects are termed neutralizing antibodies, addressed against the active toxin. In this case, the clinical effect may wane gradually, eventually leading to complete treatment failure.

In a study of 27 patients with complete treatment failure due to neutralizing antibodies, the majority (81%) of patients had previously experienced partial antibody-induced treatment failure [Dressler, 2002]. Most patients in this study developed treatment failure within 40 months of starting BoNT

treatment.

However, another study reported a high mean clinical benefit similar for ABO and ONA and <2% of the patients developed neutralizing antibodies [Mohammadi et al, 2009].

However, in more recent investigations, BoNT-A antibodies were not detected [Bakheit et al, 2012; Wissel et al, 2017].

The debate regarding immunogenicity includes the role of the non-toxic proteins, collectively referred to as complexing proteins or neurotoxin-associated proteins (NAPs). Under physiological pH conditions, the complexing proteins dissociate from the neurotoxin after constitution with saline and even before injection [Eisele et al, 2011; Benecke, 2012]. Complexing proteins are not expected to modify clinical outcomes, and specific antibodies generated against the complexing proteins are termed non-neutralizing and should not affect the secondary response. However, it has been argued that complexing proteins may increase the bacterial protein load and could potentially increase the immunogenic risk of neutralizing antibody formation [Kukreja et al, 2009]. Even if several studies have been conducted, there are no clear demonstrations that NAPs modify the immunogenicity of the active toxin [Atassi, 2004; Atassi, 2006; Bigalke, 2009]. However, these studies revealed that the toxoid complex is more immunogenic than the purified neurotoxin. This could be relevant considering that cross-reactivity may occur between the toxoid and toxin. However, despite the considerations mentioned above, the risk of immunogenicity of BoNT-A is very low in clinical practice as reported by a large data review [Jankovic et al, 2004; Wissel et al, 2017].

BoNT and systemic diffusion

BoNT ability to remain relatively localized at the site of injection is largely responsible for its remarkable safety profile. In general, spread and diffusion are supposed to underlie most of the local, distal, and systemic effects of BoNT [Ramirez-Castaneda et al, 2013].

BoNT spread (also called diffusion) describes the toxin's effect on areas away from the injection site. The potential risk for adverse effects due to toxin spread is described in the labeling for each BoNT-A product [Scaglione, 2016]. However, the mechanism of this phenomenon is not completely understood.

Spread to contiguous areas could increase the risk of adverse effects. For example, spread from injections in the cervical or craniofacial musculature may induce diplopia, dysarthria, or dysphagia, whereas injections in extremities could induce weakness in non-treated, close muscles.

Although uncommon, distant spread can occur, causing unintended neuromuscular blockade remote to the injection site. For example, systemic botulism symptoms such as dysphagia can occur when the toxin is injected at a distant site (e.g., lower extremities for spasticity). Even if generalized

weakness is a rare occurrence after BoNT injections, Bhatia and colleagues reported the case of three patients who developed generalized muscle weakness, drawing attention to the rare possibility of mild botulism after treatment with BoNT-A for dystonia [Bhatia et al, 1999]. Moreover, flu-like symptoms (another possible sign of distant spread or a systemic immune response) vary widely in different studies: 1.7% to 20% of patients treated with various preparations of BoNT-A, and 5% to 55% of patients receiving BoNT-B, reported mild to moderate flu-like symptoms [Baizabal-Carvallo et al, 2011].

In addition, although BoNT probably does not cross the blood–brain barrier [Truong et al, 2009], Hristova et al reported three cases of encephalitic clinical features that occurred shortly after treatment of focal dystonia with BoNT-A [Hristova et al, 2012].

It must be highlighted that any potential differences in toxin spread characteristics among the different BoNT would be clinically relevant.

Differences in the potential for contiguous spread among the BoNT products have been studied, but at the moment there is no clear evidence that differentiates the various products. It has been hypothesized that diffusion of neurotoxin into adjacent tissue is slower with the high molecular weight complex compared with the lower molecular weight or free neurotoxin [Dressler et al, 2012]. Therefore, theoretically, ONA with the highest complex size of 900 kD should be less diffusible, whereas INCO containing only the 150-kD neurotoxin (without NAPs) should be the most diffusible, with a higher rate of side effects related to toxin spread. However, this has not been demonstrated.

On the other hand, progenitor toxin size may be irrelevant with regard to toxin diffusion, because all BoNT progenitor complexes immediately dissociate following injection [Wagman and Bateman, 1953]; in addition, dissociation probably occurs in the vial on reconstitution with normal saline [Eisele et al, 2011]. This is consistent with data from an animal model, in which there were no significant differences in the field of effect among ABO, INCO, and ONA [Carli et al, 2009].

However, several factors other than the pharmaceutical preparation such as dose, dilution, injection technique, target site, location of injection within the muscle, level of muscle hyperactivity, depth of injection, and post-injection rehabilitation could influence the potential for spread [Roche et al, 2008; Pickett, 2009; Brodsky et al 2012; Baricich et al, 2015].

BoNT-A doses, spread and adverse events in Post stroke spasticity treatment

As reported above, in case of focal post stroke spasticity, BoNT-A injection is the gold standard therapy, with low prevalence of complications, reversibility, and efficacy in reducing spastic hypertonia [Simpson et al, 2016], with the approval of the U.S. Food and Drug Administration and the European regulatory agencies for this indication.

Current guidelines suggest the employment of a dose up to 600 units (U) of onabotulinumtoxinA (Botox®, Allergan, Inc., United States) and incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Germany) or up to 1500 U of abobotulinumtoxinA (Dysport®, Ipsen, France) per injection session to treat spasticity after stroke [Wissel et al, 2009]. However, in recent years, higher doses were used, especially in case of upper and lower limb severe spasticity. It is known that low doses of BoNT-A can be used to increase motor function in those patients affected by spasticity graded 1 or 2 as measured by Modified Ashworth Scale (MAS) [Bohannon and Smith, 1987], whereas in the case of severe spasticity elevated doses of BoNT-A may be useful to improve limb posture, to apply splinting, to consent hygiene, to increase passive articular range of motion, to walk and stand in patients with spastic equino-varus foot deformities, to improve joint range of motion and muscle extensibility or to reduce spasticity-related pain [Aoki, 2005]. However, many clinicians suggested that higher doses of BoNT-A may cause generalized, adverse effects [Hesse et al, 1995; Mancini et al, 2005; Varghese-Kroll and Elovic, 2009; Crowner et al, 2010; Thomas and Simpson, 2012]. In particular, Lange and colleagues (1987) hypothesized a possible relationship between BoNT-A dose and systemic effects, even if a clear relationship between dose and severity of symptoms was observed.

In light of these considerations, the assessment of patients treated with high doses of BoNT-A should include a systematic evaluation of the presence of undesired, adverse events. Moreover, in association with clinical assessment, a non-invasive, instrumental evaluation should be considered to detect also subclinical diffusion of BoNT-A, in order to better understand the pathophysiological mechanisms possibly involved in systemic side effects.

Clinical assessment

Hesse and colleagues [1995] published one of the first studies focused on high doses of BoNT-A. All patients of the group treated with 2000 U of AbobotulinumtoxinA (n=5) completed the study. Four weeks after injection they reported a muscle tone reduction, improved gait velocity, stride length, stance- and swing-symmetry without adverse effects, whereas a patient of the other group (treated with 1500 U) developed a bladder paresis, requiring catheterization for 14 days.

In a randomised, double-blind, dose-ranging study, Mancini and colleagues [2005] treated 45 patients with three different doses of OnabotulinumtoxinA, on the basis of suggestions in the literature. All the groups showed significant improvements after treatment. Group II (mean BoNT-A total dose: 322 U) and Group III (mean dose: 540 U) showed a greater and more prolonged response than Group I (mean dose: 167 U). However, Group III showed the highest rate of adverse effects 4 weeks post-treatment (prolonged weakness of the treated limb, flu-like syndrome and oedema of the injected leg). Varghese-Kroll and Elovic (2009) reported the first known case of repeated, contralateral weakness and fatigue after high-dose BoNT-A injection. A 53-yr-old woman developed contralateral weakness and fatigue, without autonomic symptoms, 2 weeks after receiving an injection with 800 units of onabotulinumtoxinA for management of poststroke spasticity. The patient reported resolution 4 weeks later. The patient experienced the same, contralateral symptoms more than a year later, after a 500 U injection, which took a similar length of time to resolve. Interestingly, three previous injections of onabotulinumtoxinA of 700 U, 500 U, and 600 U that were spaced three months apart were well tolerated.

In a case series, Crowner and colleagues (2010) described the adverse effect (difficulty getting on/off his bus) of a 16-year old male treated with 640 U of onabotulinumtoxinA into the left flexor carpi radialis, flexor carpi ulnaris, pronator teres, flexor digitorum superficialis, biceps, brachioradialis, and quadriceps muscles. These symptoms lasted only one month. Interestingly, also in this study the Authors reported a previous, well tolerated injection of 635–640 U of BoNT-A. After a re-injection of 650 U of onabotulinumtoxinA into the same muscles, the patient presented weakness in both upper and lower extremities, dysarthria, and increased falls and gait instability after the injection. Twelve weeks post-injection, he had continued difficulty ascending stairs but was no longer falling and had regained full strength in his upper extremities.

Thomas and Simpson also described [2012] contralateral weakness following repetitive onabotulinumtoxinA administrations in two patients affected by post-stroke spasticity.

In the first case report, a 43-year-old woman, treated for more than one year with 575–700 U of OnabotulinumtoxinA into the upper and lower limb muscles without adverse effects, developed after a re-injection of 700 U total dose, contralateral weakness in the shoulder girdle and distal arm; generalized weakness, bulbar, respiratory, sphincter, pain, sensory symptoms, or other systemic symptoms were not reported.

In the second case report, a 21-year-old woman with post-stroke spasticity and dystonia did not report adverse effects with total doses ranging from 550 to 700 U of onabotulinumtoxinA into the proximal upper limb muscles. However, after a new treatment in the same muscles with a total dose of 700 U, she reported weakness of her non-treated right arm, starting within days after the last injection. She

did not report 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. However, no adverse effects were described with 500 U of onabotulinumtoxinA, avoiding any muscles proximal to the elbow. In this case, the Authors hypothesized the development of contralateral limb weakness for diffusion of BoNT-A through tissue planes from proximal upper extremity muscles, across the midline, to contralateral muscles.

On the other hand, in recent years, many experienced clinicians investigated the effects of higher BoNT-A doses. In fact, as evidenced in expert consensus panel reviews [Wissel et al, 2009; Santamato et al, 2015] the recommended doses of BoNT-A in the product label reflect older clinical trials, whereas the clinical management of the patients could require higher doses in order to improve patients' clinical outcome and quality of life [Picelli A, Baricich A et al, 2017]. Interestingly, in a recent survey, Bensmail and colleagues [2014] estimated that 24.6% of the patients could benefit from higher doses than those permitted by current country directives.

In a prospective, non-randomized, open-label study, Santamato and colleagues [2013] described the safety and efficacy of higher doses (ranged from 750 to 840 U) of incobotulinumtoxinA in 25 subjects with upper and lower limb spasticity after stroke. The patients were treated under ultrasound guide in several muscles of the upper and lower limbs, reporting after 30 days of follow-up, a substantial improvement in functional disability, spasticity-related pain, and muscle tone. Only 16% of patients experienced treatment-emergent, mild adverse events (injection site pain, muscular weakness), resolved in a few days.

Intiso and colleagues [2014] reported the effectiveness of high doses (up to 840 U) of incobotulinumtoxinA to treat spasticity due to brain injury or cerebral palsy. A significant reduction of muscle hypertone and pain was observed, but global functionality and arm dexterity were unchanged. Three patients (13.6%) complained of adverse events: of these, 2 subjects had local side effects consisting of injection site hematoma and one subject complained of weakness and reduction of active motility of the injected arm lasting for 2 weeks. No generalized side effects were observed. Dressler and colleagues [2015] demonstrated that high doses of incobotulinumtoxinA (minimum 400 U and maximum 1200 U), injected into fifty-four patients suffering from spasticity of several etiologies, did not cause any generalized effects which could be attributed to BoNT therapy or complete secondary therapy failure. The Authors concluded that generalised weakness, being bedridden, feeling of residual urine and constipation were caused by the underlying tetra- or

paraparesis, blurred vision by presbyopia. Neurologic examination, serum chemistry and full blood count did not indicate any systemic adverse effects.

In a retrospective analysis [Baricich et al, 2015], we evaluated the efficacy and safety of high doses of onabotulinumtoxinA (from 600 to 800 units) in 26 patients affected by upper and/or lower limb post-stroke spasticity. They were assessed before, 30 and 90 days after treatment. We observed a significant muscle tone reduction and a significant functional improvement. No adverse events were reported.

In a recent study, Wissel and colleagues [2017] evaluated safety (primary objective) and efficacy of increasing doses (400 U up to 800 U) of incobotulinumtoxinA for patients with limb spasticity. In this prospective, single-arm, dose-titration study, patients (18-80 years) with spasticity due to cerebral causes, who were clinically deemed to require total doses of 800 U incobotulinumtoxinA, received 3 consecutive injection cycles with 400 U, 600 U, and 800 U incobotulinumtoxinA, respectively, each followed by 12-16 weeks' observation. In total, 155 patients were enrolled. IncobotulinumtoxinA dose escalation did not lead to an increased incidence of treatment-related AEs. No treatment-related serious AEs occurred. The Authors concluded that escalating incobotulinumtoxinA doses (400 U up to 800 U) did not compromise safety or tolerability, enabling treatment in a greater number of muscles/spasticity patterns with increased treatment efficacy.

However, the available evidence mainly referred to a single set of injections evaluating the efficacy and safety of BoNT-A. Interestingly, in a recent prospective, non-randomized, open-label study, Santamato and colleagues [2017] studied the safety of repeated higher doses of incobotulinumtoxinA in post-stroke upper and lower limb spasticity. Two years after the first set of injections, they evaluated in 20 stroke survivors with upper and lower limb spasticity the long-term safety of repeated high doses of incobotulinumtoxinA (up to 840 U) for a total of eight sets of injections. In a two-year follow-up, 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 without general adverse effects.

Instrumental assessment

As previously stated, the clinical manifestations of the systemic spread of BoNT-A, or remote effects, can be detected in various forms [Castaneda-Ramirez et al, 2013].

In the past years, several studies analyzed the subclinical impairment of endplate function in non-injected muscles by the use of neurophysiologic studies.

Jitter measurements have been suggested as instrumental assessment to demonstrate abnormal

neuromuscular transmission in muscles remote from the site of BoNT injections [Sanders, 2002]. A double-blind, placebo- controlled study of SFEMG changes in 42 patients assessed the efficacy of BoNT injections for cervical dystonia [Lange et al, 1991]. SFEMG was performed in a limb muscle before treatment and 2 weeks and 12 weeks after the injection of placebo or BoNT. Before and after treatment, the mean jitter was unchanged in the placebo group, whereas the mean jitter had a maximal increase after two weeks of BoNTA treatment and was still elevated after 12 weeks. The fiber density did not change in any patient during the study, and there were no remote clinical effects of BoNT. Girlanda and colleagues [1992] evaluated the distal effects of BoNT on neuromuscular transmission and on autonomic function in five patients who received BoNT-A injections for craniocervical dystonia and hemifacial spasm. Detection of increased neuromuscular jitter by single-fiber electromyography (SFEMG) on the extensor digitorum communis muscle and six tests of cardiovascular reflexes were performed. The Authors reported that BoNT-A injections induced an increase in mean jitter value above normal limits in all patients as well as an increase of fiber density recorded six weeks after the treatment. However, the Authors conducted this study with BoNT-A Oculinum, which is significantly different from ONA [Borodic et al, 1996]. Garner and colleagues [1993] analyzed repeated SFEMG in the extensor digitorum brevis muscle of eight patients who received a small dose of BoNT-A as therapy for focal dystonias in the head/neck region. They observed an increase of jitter and blocking in six of those patients. Fiber density of the extensor digitorum communis muscle on alternating sides showed a tendency to increase after BoNT-A injection [Garner et al 1993]

In addition to SFEMG, quantitative electromyography (EMG) has been described as a measure for distant effects of BoNT. A group of 27 patients with cervical dystonia was followed over an average of 31 months [Erdal et al, 1999]. They received repeated, unilateral BoNT-A injections of the sternocleidomastoid muscle (SCM), and quantitative EMG at rest and at maximal contraction were recorded. The study demonstrated no cumulative chemodenervation by repeated BoNT injections of the SCM measured by quantitative EMG. However, the contralateral, non-injected SCM showed significant reduction of quantitative EMG parameters, suggesting a functional weakening after long-term treatment.

Interestingly, since a frequent target of BoNT-A during botulism is the autonomic nervous system [Vita et al, 1987], several researchers have investigated autonomic function in patients with cervical dystonia receiving BoNT-A and BoNT- B, showing controversial evidence about the development of signs of subclinical diffusion.

In particular, heart rate variability (HRV), a simple and non-invasive electrocardiographic (ECG) derived measure, can provide detailed information about the control exerted by the autonomic nervous system (ANS) on cardiovascular activities including vagal and sympathetic components [Akselrod et al, 1981; Pomeranz et al, 1985; Kleiger et al, 1991; Tsuji et al, 1996; EuroAmerican Task Force, 1996]. Interest in these measures has recently increased in the light of predictive associations between reduced HRV and increased mortality after an acute myocardial infarction and between HRV and the incidence of coronary heart disease [Wichterle et al, 2004]. HRV has been categorised into high frequency (HF), low frequency (LF) and very low frequency (VLF) power ranges according to its frequency. HF is equivalent to the well-known respiratory sinus arrhythmia and is considered to represent vagal control of heart rate. LF is jointly contributed by both vagal and sympathetic nerves [Wichterle et al, 2004]. Because of its accessibility and non-invasiveness, frequency domain analysis of HRV has gained its popularity with broad clinical and research applications as a functional indicator of the ANS activity.

In previously published literature, there are few works investigating HRV modifications after BoNT-A injection, with contrasting results.

In a previously cited study, Girlanda et al [1992] observed in patients affected by cervical dystonia and hemifacial spasm significant differences in autonomic cardiac drive. As described above, this study was mainly an EMG study, conducted with BoNT-A Oculinum, which is significantly different from onabotulinumtoxinA [Borodic et al, 1996], and methods used to monitor the autonomic effects at cardiac level are not clearly described.

Nebe and colleagues [1996] showed that abobotulinumtoxinA did not modify significantly HRV in patients treated for cervical dystonia.

Meichsner et al [2005] evaluated the effect of BoNT-A on HRV in a quite large sample of patients affected by different diseases (cervical dystonia, spasticity and hyperhidrosis). They showed a reduction in the very low frequency domain and in the high frequencies in those treated with abobotulinumtoxin A, and a very marked reduction in the low frequencies in those treated with rimabotulinumtoxin B.

A short-term power spectral analysis of heart rate and systolic blood pressure variability, high-frequency and low-frequency oscillations of heart rate variability, low frequency/high frequency ratio, and baroreflex sensitivity were measured in 12 patients with cervical dystonia before and 2 to 4 weeks after onabotulinumtoxinA injection and were compared with normative data [Tiple et al, 2008]. Their data demonstrated a dose-dependent effect on neuromuscular transmission in distal muscles, possibly owing to hematogenous spread of BoNT-A through the bloodstream. Overall, these results suggest that the effect of locally injected, intramuscular BoNT-A on autonomic cardiovascular

innervation cumulates over time. None of these findings, however, were noticed by the patient, nor were they clinically relevant.

However, it must be pointed out that in cervical dystonia doses are largely inferior to those utilized in spasticity.

Personal contributions

In the last years, our research group deeply investigated the possible effect on autonomic nervous system of high doses of BoNT-A.

In a case control study [Invernizzi et al, 2015], we evaluated the changes in autonomic heart drive induced by high doses (higher than 600 units) of IncobotulinumtoxinA injection in patients affected by post stroke spasticity. Moreover, we considered the treatment safety by monitoring adverse events. Each patient underwent an ECG recording before injection and 10 days after treatment. Linear and non-linear HRV measures were derived from ECGs with a dedicated software. None of the variable considered showed statistically significant changes after BoNT-A injection.

More recently, in order to confirm these results, we evaluated changes in HRV induced by high doses (>600 U) of IncobotulinumtoxinA or OnabotulinumtoxinA [Baricich et al, 2017]. We recruited patients affected by post stroke spasticity in a single blind, randomized controlled crossover study. In the first part of the study, patients in the first group were injected with incobotulinumtoxinA while patients in the second group with onabotulinumtoxinA; after 6 months, a crossover intervention was performed. All patients were blinded to BoNT-A type, and performed an ECG registration in the 24 h before injection (t0) and 10 days after treatment (t1), both in the first and in the second part of the study. Functional status was also evaluated. In this study HRV analysis showed no significant changes after each BoNT-A injection in both groups at any evaluation time. Moreover, no statistically significant differences were found regarding each variable between the two groups.

Related articles

- Invernizzi M, Carda S, Molinari C, Stagno D, Cisari C, Baricich A. Heart Rate Variability (HRV) modifications in adult hemiplegic patients after botulinum toxin type A (NT-201) injection. *Eur J Phys Rehabil Med.* 2015 Aug;51(4):353-9. PubMed PMID: 25051207.
- Baricich A, Grana E, Carda S, Santamato A, Cisari C, Invernizzi M. High doses of onabotulinumtoxinA in post-stroke spasticity: a retrospective analysis. *J Neural Transm (Vienna).* 2015 Sep;122(9):1283-7. doi: 10.1007/s00702-015-1384-6. PubMed PMID: 25724294.
- Baricich A, Picelli A, Molteni F, Guanziroli E, Santamato A. Post-stroke spasticity as a condition: a new perspective on patient evaluation. *Funct Neurol.* 2016 Jul-Sep;31(3):179-80. PubMed PMID: 27678212; PubMed Central PMCID:PMC5115233.
- Baricich A, Grana E, Carda S, Santamato A, Molinari C, Cisari C, Invernizzi M. Heart Rate Variability modifications induced by high doses of incobotulinumtoxinA and onabotulinumtoxinA in hemiplegic chronic stroke patients: A single blind randomized controlled, crossover pilot study. *Toxicon.* 2017 Nov;138:145-150. doi:10.1016/j.toxicon.2017.08.027.

Heart Rate Variability (HRV) Modifications in adult hemiplegic patients after botulinum toxin type A (NT-201) injection

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Eur J Phys Rehabil Med. 2015 Aug;51(4):353-9. PubMed PMID: 25051207.

Abstract

Background. The most important adverse effect of BoNT-A is the systemic diffusion of the toxin. There is some evidence that the administration of high doses can increase the risk of systemic diffusion and the development of clinically evident adverse effects, however an international consensus does not exist about its maximum dose.

Aim. The aim of this study was to evaluate changes in autonomic heart drive induced by high doses (higher than 600 units) of incobotulinumtoxinA injection in spastic stroke patients. Moreover, the treatment safety by monitoring adverse events occurrence was assessed.

Design. Case control study.

Population. Eleven stroke survivors with spastic hemiplegia.

Methods. Patients were treated with intramuscular focal injections of IncobotulinumtoxinA (NT 201; Xeomin[®], Merz Pharmaceuticals GmbH, Frankfurt, Germany). Doses were below 12 units/Kg. Each patient underwent an ECG recording before injection and 10 days after treatment. Linear and non-linear Heart Rate variability (HRV) measures were derived from ECGs with a dedicated software.

Results. None of the variable considered showed statistically significant changes after BoNT-A injection.

Conclusion. The use of incobotulinumtoxinA in adult patients at doses up to 12 units/kg seems to be safe regarding autonomic heart drive.

Clinical Rehabilitation Impact. The use of IncobotulinumtoxinA up to 600 units could be a safe therapeutic option in spastic hemiplegic stroke survivors.

Key words: Botulinum toxins, Type A, Stroke; Muscle spasticity; Heart rate.

Introduction

Botulinum toxin type A (BoNT-A) has been utilized from more than twenty years to treat focal spasticity in different pathologies such as stroke, traumatic brain injury, cerebral palsy and multiple sclerosis. In Europe and in the USA BoNT-A is available with three established drug names: abobotulinumtoxinA, incobotulinumtoxinA and onabotulinumtoxinA. Even if these three products are not interchangeable, the suggested dose ratio is 1:1 for ona- and incobotulinumtoxinA,¹⁻³ while for the conversion ratio between ona- and incobotulinumtoxinA or abobotulinumtoxinA the reported ratio ranges are from 1:3⁴ to 1:4⁵ or 1:5.^{6, 7} At present time, despite it has been clinically utilized for several years, a clear international consensus does not exist about recommended and maximum BoNT-A dose. Current guidelines suggest maximal doses ranging from 360 to 400 units of onabotulinumtoxinA and 1000 units of abobotulinumtoxinA.⁸ However, in some countries, the use of up to 600 units of onabotulinumtoxinA or incobotulinumtoxinA is admitted,⁹ and the safe administration of even higher doses of incobotulinumtoxinA in the adult patient if medically indicated is reported as well.^{10, 11}

BoNT-A has shown to be effective,¹² safe¹³ and well tolerated,^{14, 15} but some adverse effects can occur, and the most important is the local and systemic diffusion of the toxin.¹⁶ There is some evidence that the administration of higher doses can increase the risk of systemic diffusion and the development of clinically evident adverse effects¹⁷ that can resemble, to a lesser extent, those seen during botulism. Since a frequent target of BoNT-A during botulism is the autonomic nervous system,¹⁸ several researchers have investigated autonomic function in patients with cervical dystonia receiving BoNT-A and BoNT- B, showing controversial evidence about the development of signs of subclinical diffusion.¹⁹⁻²³ However, in cervical dystonia doses are largely inferior to those utilized in spasticity, and, to our knowledge, nobody at present time has investigated the possible effect on

autonomic nervous system of high doses of BoNT-A.

Heart rate variability (HRV), a simple and non-invasive electrocardiographic (ECG) derived measure, can provide detailed information about the control exerted by the autonomic nervous system (ANS) on cardiovascular activities including vagal and sympathetic components.²⁴⁻²⁸ Interest in these measures has recently increased in the light of predictive associations between reduced HRV and increased mortality after an acute myocardial infarction and between HRV and the incidence of coronary heart disease.²⁹ HRV has been categorized into high frequency (HF), low frequency (LF), and very low frequency (VLF) power ranges according to its frequency. HF is equivalent to the well-known respiratory sinus arrhythmia and is considered to represent vagal control of heart rate. LF is jointly contributed by both vagal and sympathetic nerves.²⁹ Because of its accessibility and non-invasiveness, frequency domain analysis of HRV has gained its popularity with broad clinical and research applications as a functional indicator of the ANS activity.

The aim of this study was to evaluate changes in HRV induced by high doses (higher than 600 units) of incobotulinumtoxinA injection in spastic stroke patients. Moreover, we assessed the treatment safety by monitoring adverse events occurrence.

Materials and methods

We recruited 11 stroke survivors with spastic hemiplegia (5 male and 6 female) aged from 44 to 72 years at the Rehabilitation Unit of the Maggiore Hospital in Novara. Demographic data are resumed in Table 1.

The inclusion criteria were: 1) focal spasticity graded ≥ 2 on Modified Ashworth Scale at upper and lower limb muscles, requiring at least 600 incobotulinumtoxinA units; 2) hemiplegia after ischemic or hemorrhagic stroke documented by CT scan and/ or available case history; 3) age >18 years. Exclusion criteria were: 1) heart failure with NYHA ≥ 3 ; 2) previous diagnosis of cardiac arrhythmia; 3) concomitant use of beta-blockers; 4) pace-maker implant; 5) presence of fixed contractures at BoNT-A target muscles.

TABLE I.—*Demographical Data of patients enrolled*

	mean (SD)	median (IQR)	IC95
n° patients	11		
Age [years]	59.55 (12.86)	62 [47-68]	50.9-68.19
Sex (M/F)	5/6	-	
Weight (kg)	71.55 (5.988)	71 [68-73]	67.59-75.5
BMI	25.81 (1.76)	25.06 [24.45-26.53]	23.96-26.33
type of stroke (ischaemic/haemorrhagic)	8/3	-	
BoNT-A Total dose (units)	677 (69.3)	700 [600-700]	630.7-723.8
Dose per Kg (units/Kg)	8.7 (1.18)	9.3 [8.63-9.58]	8.52-9.49
Time from stroke [days]	318.7 (192.7)	256 [138-485]	189.2-448.2
Barthel Index	79.14 (14.57)	80 [70-95]	69.76-89.33
Functional Ambulation Categories	4.2 (1.01)	4.5 [3-5]	3.29-4.95
Motricity Index Lower Limb	54.82 (11.4)	61 [48-62]	47.15-62.48
Motricity Index Upper Limb	39.45 (11.71)	40 [30-51]	31.59-47.32

After enrollment patients were injected with IncobotulinumtoxinA (NT 201; Xeomin[®], Merz Pharmaceuticals GmbH, Frankfurt, Germany) with a dilution of 100 units/2 mL of 0.9 % sterile saline). All patients received doses below 12 units/Kg. The study protocol consisted in two ECG recordings of 30 minutes each, the first one has been performed in the 24h before incobotulinumtoxinA injection (Baseline) and the second one 10 days after the treatment. Each ECG recording was performed in a quiet room with a constant temperature of 24° C with an analogical ECG recorder. Participants were instructed to avoid a heavy meal, to abstain from smoking, caffeine beverages and alcohol and to avoid physical activity for at least two hours prior to ECG measurement. All examinations were performed from 11 to 15 h to limit circadian influences on cardiac rhythm. Treatment safety was assessed by monitoring adverse events occurrence of any degree. Lastly, the following functional measures were recorded at baseline: Barthel Index,³⁰ Motricity Index (MI) for upper and lower limb,³¹ Functional ambulation category (FAC).³²

Data obtained from analogic ECG were processed with an A/D converter (micro 1401 CED[®] Cambridge Electronic Design, Cambridge, UK) and recorded on a PC by means of a data acquisition system (Spike2 v.5, CED) with a sampling rate of 3000 Hz. Guidelines were followed for time recordings, sampling rate and HRV analysis of electrocardiograms.²⁷ Artifacts and noise regions were removed and in case of premature beats they have been manually corrected. Only recordings that contained <1% of premature beats were considered.

HRV outcome measures

ECG's have been analyzed by means of a software called "Kubios HRV analysis", the evolution of the software "HRV analysis" originally created by Niskanen *et al.*,³³ which can perform a wide range of measurements of HRV.

HRV indexes can be classified in two main categories: linear and non-linear variables.³⁴

Linear variables

The so called time-domain variables simply calculate the intervals and standard deviations between each consecutive RR interval and are the easiest and simplest among all the HRV variables.

1. The Standard Deviation of RR intervals (SDNN) reflects the overall (both short-term and long-term) variation within the RR interval series, whereas the standard deviation of successive RR interval differences (SDSD), can be used as a measure of the short-term variability. These measurements of short-term variation estimate high frequency variations in heart rate and thus are highly correlated.
2. Geometrical Indexes (TINN and RR tri-index) express overall HRV measured over 24 h and are more influenced by the lower than by the higher frequencies. The major disadvantage is the need for a reasonable number of NN intervals to construct the geometric pattern. In practice, recordings of at least 20 min (but preferably 24 h) should be used to ensure the correct performance of the geometric methods.^{27, 33}

The other category in this group are frequency-domain variables based on spectral analysis which provide direct information about the vagal and sympathetic activity driven to the heart. The power ranges commonly suggested by guidelines and used in this study are the following: very low frequency (VLF, <0.04 Hz), low frequency (LF, ranging from 0.04 to 0.15 Hz) and high frequency (HF, ranging from 0.15 to 0.4 Hz).²⁷ Frequency Domain variables are rather complex compared to time domain, however they are commonly and extensively used for both clinical (Holter recordings) and research purposes.²⁷

Non-linear Variables

In the non-linear group are included variables based on complex mathematical fractal algorithms, which are able to investigate the deep correlations between ANS, Central nervous system, hemodynamic and cardiac electrophysiology.³⁴ However, whereas these algorithms have been demonstrated as powerful tools in describing and predicting the behavior of complex systems in different science fields, at present time their use in large cohorts of patients has not been performed, so the evidence of their reliability in the medicine is scarce. Notwithstanding, since they are not affected by non-stationarity, as it happens for linear HRV indexes, they are a promising technique in light of the vision of physiological processes in the human body as the result of a complex interaction between multiple systems. Among these variables we used the one provided by the HRV software: the Poincaré plot, Approximate Entropy (ApEn), Sample Entropy (SampEn), the Detrended fluctuation analysis (DFA) with short term and long term fluctuation slope (α_1 and α_2), correlation dimension (D2), and lastly the recurrence plot (RP) with the following variables: mean line length (Lmean), Maximum line length (Lmax), recurrence rate (REC), determinism (DET) and Shannon

Entropy (ShanEn). A more detailed description is available in the Annex I.

Statistical analysis

Statistical analysis was performed using the GraphPad 4 package, version 4.0 (GraphPad Software, Inc., San Diego, CA, USA). Due to the small sample size we supposed a non-gaussian distribution of variables. Thus, differences between single-variable measurements in each group were evaluated with Wilcoxon's signed-rank test. A type I error level of 0.05 was chosen. In order to obtain comparable and univocal data, for frequency domain variables only the power ranges expressed in normalized units were statistically analysed.²⁷

Results

Demographic data of patients enrolled, mean doses of incobotulinumtoxinA used and injection sites are resumed in Table I. Details of injected muscles and single doses of incobotulinumtoxinA used for each patient are resumed in Table II. As shown in Table III and Table IV, none of the variable considered for time, frequency domain and non linear domain showed statistically significant changes after BoNT-A injection. Moreover, none of the patients enrolled in the study experienced adverse event after injection.

Discussion

Our data show that high doses of incobotulinumtoxin A do not influence the autonomic drive directed to the heart in stroke survivors with spasticity. Moreover, no clinical adverse events of any kind occurred in anyone of our patients.

The absence of relevant effect on autonomic drive directed to the heart may have a clinical relevance when deciding to treat with high doses of BoNT-A stroke survivors affected by cardiovascular comorbidities, in which it is known that a reduction in HRV is able to increase the incidence of cardiovascular events²⁹.

Moreover, even in patients without known cardiac diseases, our results can limit the potential concerns about the use of incobotulinumtoxinA at a dosage greater than 600 units.¹⁷ Actually, dose limitations often influence the clinician's decision about which muscles should be injected. There are few previous works investigating HRV modifications after BoNT-A injection with contrasting results. Wissel *et al.* showed that BoNT-A did not modify significantly HRV;²¹ however, this study was performed on patients with cervical dystonia treated with abobotulinumtoxin A at lower dosages than those utilized in our study. In a more recent paper, Tiple *et al.*²³ showed mild, subclinical abnormalities in autonomic cardiovascular regulation after treatment with onabotulinumtoxin A in

patients with cervical dystonia. However, this study was conducted with a different toxin and at doses far lower than in our study. Meichsner *et al.* ³⁵ evaluated the effect of BoNT-A on HRV in a quite large sample of patients affected by different diseases (cervical dystonia, spasticity and hyperhidrosis). They showed a reduction in the very low frequency domain and in the high frequencies in those treated with abobotulinumtoxin A, and a very marked reduction in the low frequencies in those treated with rimabotulinumtoxin B. Again, it should be noted that the used doses were much lower than in our study. Lastly, Girlanda *et al.* ¹⁹ found in patients affected by cervical dystonia and hemifacial spasm significant differences in autonomic cardiac drive. This study was mainly an EMG study, conducted with Oculinum, which is significantly different from onabotulinumtoxinA,³⁶ and methods used to monitor the autonomic effects at cardiac level are not clearly described.

These effects, even if inconsistent, can be explained by the diffusion of the toxin far from the injection side, via the blood circulation or via the retrograde transport and transcytosis to the central nervous system.³⁷ These mechanisms could explain the reduction in vagal control of the heart,³⁸ that has been observed also in botulism.^{18, 39, 40} It should be remembered that there are some evidence that, in both animals ⁴¹ and humans, ^{42, 43} BoNT-A can spread far from the injection site. However, the clinical relevance of these phenomena has never been clarified and they probably remain observations without consequences for patients.

Conclusions

In conclusion, our work, even if it has been carried out on a relatively small sample, confirms that the use of incobotulinumtoxin A in adult patients at doses up to 12 units/kg seems to be safe regarding autonomic heart drive.

References

1. Jost WH, Kohl A, Brinkmann S, Comes G. Efficacy and tolerability of a botulinum toxin type A free of complexing proteins (NT 201) compared with commercially available botulinum toxin type A (BOTOX) in healthy volunteers. *J Neural Transm* 2005;112:905-13.
2. Benecke R, Jost WH, Kanovsky P, Ruzicka E, Comes G, Grafe S. A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. *Neurology* 2005;64:1949-51.
3. Dressler D, Mander G, Fink K. Measuring the potency labelling of onabotulinumtoxinA (Botox[®]) and incobotulinumtoxinA (Xeomin[®]) in an LD50 assay. *J Neural Transm* 2012;119:13- 5.
4. Ranoux D, Gury C, Fondarai J, Mas JL, Zuber M. Respective potencies of Botox and Dysport: a double blind, randomised, crossover study in cervical dystonia. *J Neurol Neurosurg Psychiatry* 2002;72:459-62.
5. Sampaio C, Ferreira JJ, Simoes F, Rosas MJ, Magalhães M, Correia AP *et al.* DYSBOT: a single-blind,

randomized parallel study to determine whether any differences can be detected in the efficacy and tolerability of two formulations of botulinum toxin type A--Dysport and Botox--assuming a ratio of 4:1. *Mov Disord* 1997;12:1013-8.

6. Summary of Product Characteristics Dysport 500 U. [Internet]. Available from: <http://www.medicines.org.uk/emc/medicine/870/SPC/Dysport+300+units%2c+Dysport+500+units/> [cited on 2013, November].

7. Summary of Product Characteristics Xeomin 100 U. [Internet]. Available from: <http://www.medicines.org.uk/emc/medicine/20666/SPC/Xeomin+100+Units/> [cited on 2013, November].

8. Physicians RCo. Spasticity in adults: management using botulinum toxin. National Guidelines. RCP 2009.

9. Wissel J, Kempf F. Botulinum toxin in clinical neurology. *Fortschr Neurol Psychiatr* 2012;80:167-78; quiz 179.

10. Dressler D, Saberi FA. Botulinum toxin: from drug to poison. *Fortschr Neurol Psychiatr* 2009;77 Suppl 1:S49-54.

11. Santamato A, Panza F, Ranieri M, Frisardi V, Micello MF, Filoni S *et al*. Efficacy and safety of higher doses of botulinum toxin type A NT 201 free from complexing proteins in the upper and lower limb spasticity after stroke. *J Neural Transm* 2012;120:469-76.

12. Brashear A, Gordon MF, Elovic E, Kassicieh VD, Marciniak C, Do M *et al*. Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after a stroke. *N Engl J Med* 2002;347:395-400.

13. Naumann M, Jankovic J. Safety of botulinum toxin type A: a systematic review and meta-analysis. *Curr Med Res Opin* 2004;20:981-90.

14. Bakheit AM, Pittock S, Moore AP, Wurker M, Otto S, Erbguth F *et al*. A randomized, double-blind, placebo-controlled study of the efficacy and safety of botulinum toxin type A in upper limb spasticity in patients with stroke. *Eur J Neurol* 2001;8:559-65.

15. Simpson DM, Alexander DN, O'Brien CF, Tagliati M, Aswad AS, Leon JM *et al*. Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial. *Neurology* 1996;46:1306-10.

16. Sheean G, Lannin NA, Turner-Stokes L, Rawicki B, Snow BJ. Botulinum toxin assessment, intervention and after-care for upper limb hypertonicity in adults: international consensus statement. *Eur J Neurol* 2010;17 Suppl 2:74-93.

17. Varghese-Kroll E, Elovic EP. Contralateral weakness and fatigue after high-dose botulinum toxin injection for management of poststroke spasticity. *Am J Phys Med Rehabil* 2009;88:495-9.

18. Vita G, Girlanda P, Puglisi RM, Marabello L, Messina C. Cardiovascular-reflex testing and single-fiber electromyography in botulism. A longitudinal study. *Arch Neurol* 1987;44:202-6.

19. Girlanda P, Vita G, Nicolosi C, Milone S, Messina C. Botulinum toxin therapy: distant effects on neuromuscular transmission and autonomic nervous system. *J Neurol Neurosurg Psychiatry* 1992;55:844-5.

20. Claus D, Druschky A, Erbguth F. Botulinum toxin: influence on respiratory heart rate variation. *Mov Disord* 1995;10:574-9.

21. Nebe A, Schelosky L, Wissel J, Ebersbach G, Scholz U, Poewe W. No effects on heart-rate variability

- and cardiovascular reflex tests after botulinum toxin treatment of cervical dystonia. *Mov Disord* 1996;11:337-9.
22. Tintner R, Gross R, Winzer UF, Smalky KA, Jankovic J. Autonomic function after botulinum toxin type A or B: a double-blind, randomized trial. *Neurology* 2005;65:765-7.
23. Tiple D, Strano S, Colosimo C, Fabbrini G, Calcagnini G, Prencipe M *et al.* Autonomic cardiovascular function and baroreflex sensitivity in patients with cervical dystonia receiving treatment with botulinum toxin type A. *J Neurol* 2008;255:843-7.
24. Kleiger RE, Bigger JT, Bosner MS, Chung MK, Cook JR, Rolnitzky LM *et al.* Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol* 1991;68:626-30.
25. Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D *et al.* Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985;248:H151-3.
26. Tsuji H, Larson MG, Venditti FJ Jr., Manders ES, Evans JC, Feldman CL *et al.* Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 1996;94:2850-55.
27. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996;93:1043-65.
28. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;213:220-22.
29. Wichterle D, Simek J, La Rovere MT, Schwartz PJ, Camm AJ, Malik M. Prevalent low-frequency oscillation of heart rate: novel predictor of mortality after myocardial infarction. *Circulation* 2004;110:1183-90.
30. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel Index. *Md State Med J* 1965;14:61-5.
31. Demeurisse G, Demol O, Robaye E. Motor evaluation in vascular hemiplegia. *Eur Neurol* 1980;19:382-9.
32. Holden MK, Gill KM, Magliozzi MR, Nathan J, Piehl-Baker L. Clinical gait assessment in the neurologically impaired. Reliability and meaningfulness. *Phys Ther* 1984;64:35-40.
33. Niskanen JP, Tarvainen MP, Ranta-Aho PO, Karjalainen PA. Software for advanced HRV analysis. *Comput Methods Programs Biomed* 2004;76:73-81.
34. Buccelletti F, Bocci MG, Gilardi E, Fiore V, Calcinaro S, Fragnoli C *et al.* Linear and Nonlinear Heart Rate Variability Indexes in Clinical Practice. *Computational and Mathematical Methods in Medicine* 2012;2012:1-5.
35. Meichsner M, Reichel G. Effect of botulinum toxin A and B on vegetative cardiac innervation. *Fortschr Neurol Psychiatr* 2005;73:409-14.
36. Borodic G, Johnson E, Goodnough M, Schantz E. Botulinum toxin therapy, immunologic resistance, and problems with available materials. *Neurology* 1996;46:26-9.
37. Caleo M, Antonucci F, Restani L, Mazzocchio R. A reappraisal of the central effects of botulinum neurotoxin type A: by what mechanism? *J Neurochem* 2009;109:15-24.
38. Shahani BT, Day TJ, Cross D, Khalil N, Kneebone CS. RR interval variation and the sympathetic skin

response in the assessment of autonomic function in peripheral neuropathy. *Arch Neurol* 1990;47:659-64.

39. Chen JT, Chen CC, Lin KP, Wang SJ, Wu ZA, Liao KK. Botulism: heart rate variation, sympathetic skin responses, and plasma norepinephrine. *Can J Neurol Sci* 1999;26:123-6.

40. Topakian R, Heibl C, Stieglbauer K, Dreer B, Nagl M, Kno ach P *et al.* Quantitative autonomic testing in the management of botulism. *J Neurol* 2009;256:803-9.

41. Rosales RL, Bigalke H, Dressler D. Pharmacology of botulinum toxin: differences between type A preparations. *Eur J Neurol* 2006;13 Suppl 1:2-10.

42. Lange DJ, Rubin M, Greene PE, Kang UJ, Moskowitz CB, Brin MF *et al.* Distant effects of locally injected botulinum toxin: a double-blind study of single ber EMG changes. *Muscle Nerve* 1991;14:672-5.

43. Garner CG, Straube A, Witt TN, Gasser T, Oertel WH. Time course of distant effects of local injections of botulinum toxin. *Mov Disord* 1993;8:33-7

High doses of onabotulinumtoxinA in post-stroke spasticity: a retrospective analysis

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J Neural Transm (Vienna). 2015 Sep;122(9):1283-7. doi: 10.1007/s00702-015-1384-6. PubMed PMID: 25724294

Abstract

We retrospectively evaluated the efficacy and safety of high doses of onabotulinumtoxinA (from 600 to 800 units) in 26 patients affected by upper and/or lower limb post-stroke spasticity. They were assessed before, 30 and 90 days after treatment. We observed a significant muscle tone reduction and a significant functional improvement (assessed with the Disability Assessment Scale). No adverse events were reported. In our retrospective analysis the treatment with high doses of onabotulinumtoxinA showed to be effective and safe.

Keywords: Stroke Spasticity Botulinum toxin type A OnabotulinumtoxinA Higher doses

Introduction

Post-stroke spasticity (PSS) has been described as a relevant clinical problem in stroke survivors, as it can impair manual dexterity, mobility and balance, with a negative impact on independence (Martin et al. 2014).

OnabotulinumtoxinA has been proposed as a part of effective integrated treatment programme for the management of PSS (Brashear et al. 2002a, b; Wissel et al. 2009; Baker and Pereira 2013).

Clinical experience showed a good safety profile (Ghasemi et al. 2013) both in the short- (Naumann and Jankovic 2004) and in the long-term use (Naumann et al. 2006).

The optimal dose for onabotulinumtoxinA is determined by the patient’s characteristics and by the treatment’s goal but there is not a general consensus on maximum dose. Francisco (2004) suggested a dose up to 400–600 units (U) per session, whereas Wissel et al. (2009) remarked that it should not

exceed 600 U. However, in clinical practice doses as high as 800 U are used by some practitioners, even if safety and efficacy of routine use of doses higher than 500 U still await further evidence (Francisco 2004).

The aim of our study was to retrospectively evaluate the efficacy and safety profile of higher doses of onabotulinumtoxinA (up to 800 U) in patients affected by upper and/or lower limb PSS.

Materials and methods

Patients

We retrospectively analysed data from 119 patients affected by upper and/or lower limb PSS who referred to the Physical and Rehabilitative Medicine Unit of University Hospital “Maggiore della Carita” in Novara (Italy) between July 2012 and April 2014.

The inclusion criteria were: spasticity due to an ischemic or hemorrhagic stroke; time from stroke at least 6 months; total dose required of onabotulinumtoxinA \geq 600 U; age $>$ 18 years. The exclusion criteria were: previous treatment with Botulinum Toxin Type A (BoNT-A) in the last 4 months; spasticity due to any other cause; presence of other concomitant neurological or neuromuscular diseases; dementia; concomitant therapy with myorelaxants (oral or intrathecal baclofen, benzodiazepines, tizanidine); previous treatment of PSS with phenol, alcohol injection or local surgery; presence of fixed contractures or muscular fibrosis at ultrasound evaluation that could have negatively influenced the treatment with onabotulinumtoxinA.

26 patients who fulfilled the inclusion criteria were included in this study; 93 patients were excluded due to treatment with other BoNT-A formulations (abobotulinumtoxinA, incobotulinumtoxinA) or doses of onabotulinumtoxinA \leq 600 U.

Each patient and/or caregiver gave his/her written consent before the treatment.

Assessment

The same physician evaluated all the patients before treatment and 1 and 3 months after injections, as performed in clinical routine. Before treatment the patients, together with the treating physician, chose their primary therapeutic target between the four domains of the Disability Assessment Scale (DAS), i.e. dressing, limb position, pain and hygiene (Brashear et al. 2002a, b). At baseline and 30 and 90 days after treatment the functional impairment of the upper limb was evaluated with DAS (a four-point scale from 0 = no disability to 3 = severe disability), whereas changes in muscle tone were assessed with Modified Ashworth Scale (MAS) (a five-point scale from 0 = no increase in tone, to 4 = affected parts rigid in flexion or extension) (Brashear et al. 2002a, b; Bohannon and Smith 1987). To evaluate the efficacy of the treatment, investigators, patients and their caregivers were asked to rate the patients' overall treatment tolerability (Global Assessment of Efficacy, GAE) in a four-

point scale (from 1 = very good to 4 = poor) after a postinjection period of 30 and 90 days (Kanovsky et al. 2011).

In addition, a clinical examination was performed to evaluate the safety of the treatment and the presence of adverse events, which were assessed at each visit using a semi-quantitative scale (0, no adverse effects; 4, serious adverse effects) (Mancini et al. 2005).

Treatment

OnabotulinumtoxinA (Botox[®], Allergan Inc., Irvine CA) was administered in 2 mL of 0.9 % dilution saline; the injections were performed under ultrasonographic guide by the same investigator. The clinicians planned target muscles, doses and number of injection sites for each muscle depending on spastic hypertonia grade and muscle size. After onabotulinumtoxinA injection, all patients participated in a 10 day-rehabilitation programme (electrical stimulation and stretching of injected muscles, strengthening exercise, gait training if applicable).

Statistical analysis

Since data were not normally distributed, according to Shapiro–Wilk test (data not shown), within-group comparisons were made using the Friedman test for repeated measures. In addition, Dunn’s Multiple Comparison Test was performed to evaluate differences between single variable measurements (t1 vs t0, t2 vs t0 and t2 vs t1).

For statistical purpose, a MAS score “1” was considered as 1, a MAS score “1+” as 2, and so on until 5 (Biering- Sørensen et al. 2006). An alpha error level of 0.05 was chosen.

Statistical analysis was performed using GraphPad Prism 1.4 for Macintosh OS 10.6.

Results

The demographical and clinical characteristics of the 26 patients studied are represented in Table 1. Considering all the patients, 23 of them received the treatment at both upper and lower limb, whereas 3 patients were treated at lower limb only. 14 patients (53.8 %) were naive to treatment with onabotulinumtoxinA (8 previously treated with other BoNT-A formulations, 6 naive to any BoNT-A formulation for spasticity).

Muscles treated and relative doses are shown in Table 1.

Table 1 Patients' demographical and clinical characteristics

Patients (n)	26
Total dose of onabotulinumtoxinA ≥ 700 U (n)	13
Age (years) mean \pm SD	54.7 \pm 11.6
Gender	
Female % (n)	50 (13)
Male % (n)	50 (13)
Time from stroke (months) mean \pm SD	50 \pm 48.8
Type of stroke	
Ischemic % (n)	57.7 (15)
Hemorrhagic % (n)	42.3 (11)
Type of hemiparesis	
Right % (n)	73.1 (19)
Left % (n)	26.9 (7)
Total dose of BoNT-A (U) mean \pm SD	676.9 \pm 86.3
Dose of BoNT-A pro kg (U) mean \pm SD	9.6 \pm 1.4
Total dose elbow/shoulder (U) mean \pm SD	148.5 \pm 58.6
Pectoralis major (U) mean \pm SD	41.1 \pm 11.7
Biceps brachii (U) mean \pm SD	61.6 \pm 16.2
Brachialis (U) mean \pm SD	58.9 \pm 15.7
Brachioradialis (U) mean \pm SD	35 \pm 22.6
Total dose wrist/finger (U) mean \pm SD	165.2 \pm 79.2
Flexor ulnaris carpi (U) mean \pm SD	42.9 \pm 14.7
Flexor radialis carpi (U) mean \pm SD	45.4 \pm 16.3
Flexor superficialis digitorum (U) mean \pm SD	43.6 \pm 22.4
Flexor profundus digitorum (U) mean \pm SD	39.1 \pm 16.6
Flexor longus pollicis (U) mean \pm SD	23.4 \pm 11.1
Flexor brevis pollicis (U) mean \pm SD	21 \pm 7.4
Adductor pollicis (U) mean \pm SD	20 \pm 0
Total dose thigh (U) mean \pm SD	75.6 \pm 21.3
Rectus femoris (U) mean \pm SD	67.5 \pm 17.1
Biceps femoris (U) mean \pm SD	100 \pm 0
Adductor longus/brevis/magnus (U) mean \pm SD	100 \pm 0
Total dose leg (U) mean \pm SD	404.4 \pm 112.4
Gastrocnemius medialis (U) mean \pm SD	92 \pm 17.3
Gastrocnemius lateralis (U) mean \pm SD	92 \pm 17.3
Soleus (U) mean \pm SD	89.2 \pm 19.8
Flexor hallucis longus (U) mean \pm SD	41.6 \pm 10.1
Flexor digitorum longus (U) mean \pm SD	48.3 \pm 11.2
Tibialis posterior (U) mean \pm SD	72.9 \pm 22
Tibialis anterior (U) mean \pm SD	37.9 \pm 9.9
Extensor hallucis longus (U) mean \pm SD	40 \pm 32.9
Muscles treated (n) mean \pm SD	11.6 \pm 2.3

Data are presented as mean \pm standard deviation (SD) or percentage

Concerning the GAE, both 30 days (t1) and 90 days (t2) after injection patients, caregivers and clinicians rated the efficacy of treatment as “good” or “very good”, except in one case where it was evaluated as “moderate” by clinicians. The complete results of GAE are represented in Table 2, together with the results of clinical evaluations with MAS and the principal target in DAS at baseline (t0), t1 and t2.

Spasticity after injections showed a significant reduction ($p < 0.0001$) considering MAS results at elbow/shoulder, wrist/finger, thigh and leg. We observed a significant reduction in muscle tone in all muscle groups both at t1 vs t0 and t2 vs t0, whereas no significant difference was seen at t2 vs t1 (Table 2). As primary therapeutic target in DAS evaluation, 18 patients (69.2 %) chose limb position, 4 patients (15.4 %) dressing, 3 patients (11.5 %) hygiene and 1 patient (3.9 %) chose pain. Notably,

a significant improvement in DAS principal target score has been observed at t1 vs t0 ($p < 0.001$) and t2 vs t0 ($p < 0.05$).

No adverse events were reported in patients' group (mean score 0).

Table 2 MAS, DAS and GAE evaluation at baseline (t0), 30 days (t1) and 90 days (t2)

	t0 (n = 26)	t1 (n = 26)	t2 (n = 26)
MAS elbow/shoulder			
Mean \pm SD	3.5 \pm 1	1.5 \pm 0.5*	2 \pm 0.8 [‡]
95 % CI	3.1–3.9	1.3–1.7	1.7–2.4
MAS wrist/finger			
Mean \pm SD	3.6 \pm 0.7	1.4 \pm 0.5*	2.1 \pm 0.7 [‡]
95 % CI	3.3–3.9	1.2–1.6	1.8–2.4
MAS thigh			
Mean \pm SD	2.4 \pm 0.7	0.9 \pm 0.4*	1.3 \pm 0.5 [^]
95 % CI	1.8–3	0.6–1.2	0.9–1.6
MAS leg			
Mean \pm SD	3.7 \pm 0.7	1.5 \pm 0.6*	2.1 \pm 0.7 [‡]
95 % CI	3.4–4	1.2–1.7	1.8–2.4
DAS principal target			
Mean \pm SD	2.3 \pm 0.5	1.5 \pm 0.6*	1.8 \pm 0.7 [^]
95 % CI	2.1–2.5	1.2–1.7	1.5–2
GAE patients			
Very good % (n)	–	65.4 (17)	61.5 (16)
Good % (n)	–	34.6 (9)	38.5 (10)
GAE caregivers			
Very good % (n)	–	57.7 (15)	69.2 (18)
Good % (n)	–	42.3 (11)	30.8 (8)
GAE clinicians			
Very good % (n)	–	69.2 (18)	69.2 (18)
Good % (n)	–	30.8 (8)	26.9 (7)
Moderate % (n)	–	0 (0)	3.9 (1)

Data are presented as mean \pm standard deviation (SD) or percentage

* $p < 0.001$ t1 vs t0

[‡] $p < 0.001$ t2 vs t0

[^] $p < 0.05$ t2 vs t0

Discussion

In our study, we observed a significant muscle tone reduction and clinical improvement with high doses of onabotulinumtoxinA, without any adverse events.

In recently published literature, the efficacy and safety of higher doses of incobotulinumtoxinA in PSS treatment has been described: Santamato et al. (2013) reported no adverse events in 25 patients with upper and lower limb PSS, evaluated 30 and 90 days after injections with doses up to 840 U; moreover, Invernizzi et al. (2014) evaluated changes in autonomic heart drive potentially induced by doses greater than 600 U, without meaningful alterations in linear and non linear Heart Rate Variability measures in 11 stroke survivors.

On the other hand, the current recommended dose of onabotulinumtoxinA is 400 U per session (Brin 1997) and, even if clinical experience suggests a maximum dose of 600 U (Francisco 2004; Wissel et al. 2009), there is no evidence of safety for doses greater than 500 U except for paediatric patients (Francisco 2004; Goldstein 2006).

Interestingly, Mancini et al. (2005) reported minor adverse effects (generalised weakness, weakness

of the treated limb, flu-like syndrome and oedema; mean score 1.2) 4 weeks after administration of onabotulinumtoxinA in lower limb PSS, with a mean dose of 540 U. In addition, also Varghese-Kroll and Elovic (2009) presented a case report about contralateral weakness and fatigue after repeated high doses (800 and 500 U) of onabotulinumtoxinA for PSS.

In our study, the mean total dose of onabotulinumtoxinA was 676.9 ± 86.3 U, but we did not report any adverse event. A possible explanation might be the use of ultrasound to identify target muscles; in fact, as reported by Henzel et al. (2010), ultrasound localization may improve accuracy of needle placement, avoiding injection into vascular structures and reducing the potential risk of systemic diffusion of BoNT-A. Moreover, this technique can improve clinical outcome both in upper and lower limb PSS (Picelli et al. 2014; Santamato et al. 2014).

To our knowledge, this is the first study showing the safety and the efficacy of PSS treatment with doses of onabotulinumtoxinA up to 800 U, higher than those typically used in clinical practice for PSS.

Nevertheless, we have to take into account that our paper suffers for the limitations of a retrospective study, as selection bias and observer bias. Besides that, the sample size is relatively small.

Further research is required to better identify the optimal dose of onabotulinumtoxinA to optimize clinical outcome and safety profile.

References

- Baker JA, Pereira G (2013) The efficacy of botulinum toxin A for spasticity and pain in adults: a systematic review and meta-analysis using the Grades of Recommendation, Assessment, Development and Evaluation approach. *Clin Rehabil* 27:1084–1096
- Biering-Sørensen F, Nielsen JB, Klinge K (2006) Spasticity-assessment: a review. *Spinal Cord* 44:708–722
- Bohannon RW, Smith MB (1987) Interrater reliability of a modified Ashworth Scale of muscle spasticity. *Phys Ther* 67:206–207
- Brashear A, Gordon MF, Elovic E, Kassicieh VD, Marciniak C, Do M, Lee CH, Jenkins S, Turkel C (2002a) Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after a stroke. *N Engl J Med* 347:395–400
- Brashear A, Zafonte R, Corcoran M, Galvez-Jimenez N, Gracies JM, Gordon MF, McAfee A, Ruffing K, Thompson B, Williams M, Lee CH, Turkel C (2002b) Inter- and intrarater reliability of the Ashworth Scale and the Disability Assessment Scale in patients with upper-limb poststroke spasticity. *Arch Phys Med Rehabil* 83:1349–1354
- Brin MF (1997) Dosing, administration, and a treatment algorithm for use of botulinum toxin A for adult-onset spasticity. Spasticity Study Group. *Muscle Nerve Suppl* 6:S208–S220
- Francisco GE (2004) Botulinum toxin: dosing and dilution. *Am J Phys Med Rehabil* 83:S30–S37

- Ghasemi M, Salari M, Khorvash F, Shaygannejad V (2013) A literature review on the efficacy and safety of botulinum toxin: an injection in post-stroke spasticity. *Int J Prev Med* 4:S147– S158
- Goldstein EM (2006) Safety of high-dose botulinum toxin type A therapy for the treatment of pediatric spasticity. *J Child Neurol* 21:189–192
- Henzel MK, Munin MC, Niyonkuru C, Skidmore ER, Weber DJ, Zafonte RD (2010) Comparison of surface and ultrasound localization to identify forearm flexor muscles for botulinum toxin injections. *PM R* 2:642–646
- Invernizzi M, Carda S, Molinari C, Stagno D, Cisari C, Baricich A (2014) Heart rate variability (HRV) modifications in adult hemiplegic patients after botulinum toxin type a (NT-201) injection. *Eur J Phys Rehabil Med* (Epub ahead of print)
- Kanovsky P, Slawek J, Denes Z, Platz T, Comes G, Grafe S, Pulte I (2011) Efficacy and safety of treatment with incobotulinum toxin A (botulinum neurotoxin type A free from complexing proteins; NT 201) in post-stroke upper limb spasticity. *J Rehabil Med* 43:486–492
- Mancini F, Sandrini G, Moglia A, Nappi G, Pacchetti C (2005) A randomised, double-blind, dose-ranging study to evaluate efficacy and safety of three doses of botulinum toxin type A (Botox) for the treatment of spastic foot. *Neurol Sci* 26:26–31
- Martin A, Abogunrin S, Kurth H, Dinet J (2014) Epidemiological, humanistic, and economic burden of illness of lower limb spasticity in adults: a systematic review. *Neuropsychiatr Dis Treat* 10:111–122
- Naumann M, Jankovic J (2004) Safety of botulinum toxin type A: a systematic review and meta-analysis. *Curr Med Res Opin* 20:981–990
- Naumann M, Albanese A, Heinen F, Molenaers G, Relja M (2006) Safety and efficacy of botulinum toxin type A following long- term use. *Eur J Neurol* 13(Suppl 4):35–40
- Picelli A, Lobba D, Midiri A, Prandi P, Melotti C, Baldessarelli S, Smania N (2014) Botulinum toxin injection into the forearm muscles for wrist and fingers spastic overactivity in adults with chronic stroke: a randomized controlled trial comparing three injection techniques. *Clin Rehabil* 28:232–242
- Santamato A, Panza F, Ranieri M, Frisardi V, Micello MF, Filoni S, Fortunato F, Intiso D, Basciani M, Logroscino G, Fiore P (2013) Efficacy and safety of higher doses of botulinum toxin type A NT 201 free from complexing proteins in the upper and lower limb spasticity after stroke. *J Neural Transm* 120:469–476
- Santamato A, Micello MF, Panza F, Fortunato F, Baricich A, Cisari C, Pilotto A, Logroscino G, Fiore P, Ranieri M (2014) Can botulinum toxin type A injection technique influence the clinical outcome of patients with post-stroke upper limb spasticity? A randomized controlled trial comparing manual needle placement and ultrasound-guided injection techniques. *J Neurol Sci* 347:39–43

Post-stroke spasticity as a condition: a new perspective on patient evaluation.

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Funct Neurol. 2016 Jul-Sep;31(3):179-80. PubMed PMID: 27678212

Dear Sir,

Stroke is a major cause of long-term disability. Post-stroke spasticity (PSS) has been described as a velocity-dependent increase in muscle tone with exaggerated tendon jerks, resulting from hyperexcitability of the stretch reflex and presenting as intermittent/sustained involuntary muscle activation (Pandyan et al., 2005). In clinical practice, accurate quantitative measures of spasticity can be difficult to obtain in a single examination: indeed, PSS can be modified in different conditions, e.g. static conditions as opposed to dynamic situations, such as walking. In addition, the impact of PSS on subjective sensations and activities of daily living (ADL) can be hard to describe. Furthermore, in order to optimize treatment procedures in patients with PSS, assessment of patient-reported outcomes and perceptions should be reported, given that sensorimotor alterations due to PSS may influence “interoception”, i.e. the sense of the physiological condition of the entire body (Craig, 2002; Franceschini et al., 2014).

In order to improve understanding of these components of PSS, we studied 116 adults affected by first-ever unilateral stroke (more than 3 months from onset) with spasticity (less than 3 months from the last botulinum toxin treatment) in the affected arm (41 right hemiparesis and 75 left hemiparesis), graded ≥ 1 on the Modified Ashworth Scale (MAS). Spasticity was measured with the MAS in the affected shoulder, elbow, wrist and fingers, and associated reactions of the affected arm were

recorded during the sit-to-stand movement and during walking. Also the following variables were evaluated in the affected arm: Motricity Index (MI) sub-items for the upper limb; active range of motion of the shoulder, elbow, wrist and finger (percentage differences from normal values); self-assessment of functioning of the arm during ADL, as rated on a visual analog scale (0 no use; 100 normal use); Disability Assessment Scale; and self-estimation of pain, heaviness and rigidity in the shoulder, elbow, wrist and finger joints according to the Numerical Rating Scale (NRS) (0 no symptom; 100 worst symptom). Afterwards, we performed a principal component analysis (PCA), which is a variable reduction procedure, in order to obtain a smaller number of principal components (artificial variables), and also because the PCA would account for the variance in the observed data, while retaining most of the information from the sample.

On the basis of the PCA results, we defined three classes based on the main unpleasant sensations reported by each patient (heaviness, rigidity and pain) as follows: Class I (29 patients; 12 right hemiparesis and 17 left hemiparesis; mean age 63.4 years; mean time from stroke onset 58.2 months) corresponded to a higher level of proximal (shoulder) heaviness and a low level of pain (NRS 23.3 and 12.7, respectively); Class II (29 patients; 11 right hemiparesis and 18 left hemiparesis; mean age 60.7 years; mean time from stroke onset 74.1 months) corresponded to the highest level of rigidity (NRS 59.5, 70.7, 77.8 and 76.4 at the shoulder, elbow, wrist and fingers, respectively) and pain (NRS 17.1, 7.9, 11.7 and 13.1 at the shoulder, elbow, wrist and fingers, respectively); Class III (58 patients; 18 right hemiparesis and 40 left hemiparesis; mean age 63.2 years; mean time from stroke onset 70.9 months) corresponded to a lower level of heaviness (NRS 12.8, 2.7, 1.3 and 1.1 at the shoulder, elbow, wrist and fingers, respectively), the intermediate level of rigidity (NRS 21.5, 27.8, 21 and 18.1 at the shoulder, elbow, wrist and fingers, respectively), a greater level of functional ability and a low level of proximal (shoulder) pain (NRS 13.3).

According to the non-parametric Kruskal-Wallis test (alpha level for significance $p < 0.05$), no significant differences were found between Classes I, II and III in the MAS (shoulder adductors, elbow flexors, wrist and finger flexors) and MI (shoulder, elbow and pinch grip) scores. On the basis of this finding, we suggest that unpleasant sensations of pain, heaviness and rigidity may relate not only to muscle tone (as measured by the MAS), but also to altered proprioceptive and body ownership information, as well as to the individual's self-estimated ability to achieve functional goals. This is in keeping with previous findings about the impact of PSS on limitations in ADL, wellbeing and life satisfaction, which may not be indicated by quantitative scores but are demonstrated by patient-reported outcome measures (Sunnerhagen and Francisco, 2013). Indeed, PSS also has an afferent, sensory component, which might be related to some differences in the sensations described by patients (Craig, 2002; Franceschini et al., 2014). It is well known that proprioceptive afferent

information coming from mechanoreceptors in joints, muscles, tendons and stretch-sensitive receptors in the skin, together with efferent motor signals, can play a key role in postural schema understood as dynamic representations of body posture. Moreover, the sense of body ownership, too, is presumably developed using sensory information, as recently described by Walsh and colleagues (2011), who demonstrated that non-tactile proprioceptive cues might contribute to this sense.

Another possible explanation for the current observations could be related to problems occurring in patient-provider communication and the role that this communication plays in PSS rehabilitation within the context of patient-centered health care, which addresses illness from a holistic perspective (Sunnerhagen and Francisco, 2013). Furthermore, treatment goals should be patient-centered and the rehabilitation program should be tailored to the needs of each patient, identifying what they describe as limitations and trying to focus on possible correlations between these and PSS.

In conclusion, our patients with PSS described different patterns of sensations even without showing significant differences in their MAS and MI scores. We suggest that PSS might be considered not only as a modification of muscle tone, but also as a clinical condition that is specific to the single patient, and has a significant impact on his/her sensations and self-estimated autonomy in ADL. Future studies are needed to further investigate these issues.

References

- Craig AD (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat Rev Neurosci* 2002;3:655- 666.
- Franceschini M, Iocco M, Molteni F, et al. (2014). Management of stroke patients submitted to botulinum toxin type A therapy: a Delphi survey of an Italian expert panel of specialist injectors. *Eur J Phys Rehabil Med* 50:525-533.
- Pandyan AD, Gregoric M, Barnes MP, et al (2005). Spasticity: clinical perceptions, neurological realities and meaningful measurement. *Disabil Rehabil* 27:2-6.
- Sunnerhagen KS, Francisco GE (2013). Enhancing patient-provider communication for long-term post-stroke spasticity management. *Acta Neurol Scand* 128:305-310.
- Walsh LD, Moseley GL, Taylor JL, et al (2011). Proprioceptive signals contribute to the sense of body ownership. *J Physiol* 589:3009- 3021.

Appendix: Philosophical Botulinum Toxin Club

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Heart Rate Variability modifications induced by high doses of incobotulinumtoxinA and onabotulinumtoxinA in hemiplegic chronic stroke patients: a single blind randomized controlled, crossover pilot study

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Toxicon. 2017 Nov;138:145-150. doi:10.1016/j.toxicon.2017.08.027.

Abstract

Background: Botulinum toxin type A is a valid and safe treatment for focal spasticity, with documented effects on both sympathetic and parasympathetic systems. Heart rate variability can provide detailed information about the control of the autonomic nervous system on cardiovascular activities. Previous studies in literature showed no significant changes in Heart Rate Variability with doses >600 U of incobotulinumtoxinA in chronic post stroke spastic patients; however, at present time, these results have not been confirmed with doses >600 U of onabotulinumtoxinA.

Aim: To evaluate changes in Heart Rate Variability induced by high doses (>600 U) of incobotulinumtoxinA or onabotulinumtoxinA in spastic stroke patients over a 1-year period.

Design: single blind randomized controlled crossover study design.

Setting: Rehabilitation Unit of the University Hospital in Novara.

Population: 10 stroke survivors with spastic hemiplegia (Modified Ashworth Scale ≥ 2) were recruited and randomly divided in two groups (A and B).

Methods: In the first part of the study, patients in Group A were injected with incobotulinumtoxinA

while patients in Group B with onabotulinumtoxinA; after 6 months, a crossover intervention was performed. All patients were blinded to Botulinum toxin type A type, and performed an ECG registration in the 24 h before injection (t0) and 10 days after treatment (t1), both in the first and in the second part of the study. Functional status was evaluated with Barthel Index, Motricity Index and Functional Ambulation Category scores.

Results: Heart Rate Variability analysis showed no significant changes after each Botulinum toxin type A injection in both groups at any evaluation time. Moreover, no statistically significant differences were found regarding each variable between the two groups.

Conclusions: Our data show that high doses (>600 U) of incobotulinumtoxinA and onabotulinumtoxinA do not influence the cardiovascular activity of the autonomic nervous system in chronic hemiplegic spastic stroke survivors.

1. Introduction

Botulinum toxin type A (BoNT-A) has been considered as an effective and safe treatment for focal spasticity in stroke survivors (Baker and Pereira, 2013). BoNT-A can directly modify the heart function acting on the sympathetic (through the preganglionic sympathetic innervation) and parasympathetic systems (through the vagal nerve) (Girlanda et al., 1992), and at present time there is no general consensus on the maximum dose of BoNT-A in terms of safety and clinical interchangeability among the three commercially approved products (abobotulinumtoxinA, onabotulinumtoxinA, incobotulinumtoxinA). In clinical practice the maximum dose admitted per session is up to 400-600 units (U) both for onabotulinumtoxinA (Francisco, 2004; Brin, 1997) and for incobotulinumtoxinA (Wissel and Kempf, 2012). However, two recent open-label studies suggested the safety of doses up to 840 U of incobotulinumtoxinA (Santamato et al., 2013) and of doses up to 800 U of onabotulinumtoxinA (Baricich et al., 2015) for spasticity treatment in stroke survivors.

Heart Rate Variability (HRV) is a simple and non-invasive electrocardiographic derived measure useful to monitor the control of the autonomic nervous system (ANS) on cardiovascular activities including vagal and sympathetic ones (Task Force, 1996; Akselrod et al., 1981). In consideration of its accessibility and low invasiveness, HRV measurement represents a functional indicator of the ANS activity, being a valid method to study the potential impact of high doses of BoNT-A on the autonomic drive directed to the heart in stroke survivors.

Recently no relevant changes have been showed in the autonomic heart drive measured by HRV with

doses >600 U of incobotulinumtoxinA in patients with chronic spasticity (Invernizzi et al., 2015). However, at present time, instrumental data about safety of high doses (>600 U) of onabotulinumtoxinA in chronic stroke survivors are lacking. To our knowledge, only Dressler evaluated in a crossover study the safety of high doses of incobotulinumtoxinA (mean dose 450.5 ± 177.1 U) in patients affected by spasticity and previously treated with onabotulinumtoxinA at same doses, showing no differences between the two products in terms of efficacy and adverse events occurrence, evaluated clinically, but without any functional or instrumental indicator (Dressler, 2009). Similarly, Baricich et al. evaluated the efficacy and safety in terms of adverse events of high doses of onabotulinumtoxinA (up to 800 U with a mean of 676.9 ± 86.3 U) in stroke survivors through a clinical evaluation (Baricich et al., 2015). Lastly, Lee et al. compared the efficacy and safety of incobotulinumtoxinA with onabotulinumtoxinA in treating periorbicular rhytides and masseteric hypertrophy, showing no differences between the two molecules (Lee et al., 2014); however, the maximum dose used was far lower compared to those normally prescribed to treat post stroke spasticity.

In light of these considerations, the aim of this study was to evaluate changes in HRV induced by high doses (>600 U) of incobotulinumtoxinA or onabotulinumtoxinA in spastic stroke patients over a 1-year period using a crossover study design. Moreover, we assessed the treatment safety by monitoring adverse events occurrence.

2. Materials and methods

2.1. Patients

This work was a randomized, single blind, controlled crossover study. A total of 10 stroke survivors with spastic hemiplegia were consecutively enrolled at the Rehabilitation Unit of the University Hospital in Novara from September 2014 to January 2015. The inclusion criteria were: I) focal spasticity graded ≥ 2 on Modified Ashworth Scale (Bohannon and Smith, 1987) at upper and lower limb muscles, requiring at least 600 incobotulinumtoxinA units; II) hemiplegia after ischaemic or haemorrhagic stroke documented by CT scan and/or available case history; III) age > 18 years. Exclusion criteria were: I) heart failure with NYHA ≥ 3 ; II) previous diagnosis of cardiac arrhythmia; III) concomitant use of beta-blockers; IV) pace-maker implant; V) presence of fixed contractures at BoNT-A target muscles.

2.2. Study design

After the enrollment, patients were allocated to one of the two treatment arms by the use of a randomization scheme generated by software. Patients were then divided into two groups (A and B)

and entered the first part of the study. In this first part of the study, patients in group A were injected with incobotulinumtoxinA (NT 201; Xeomin[®], Merz Pharmaceuticals GmbH, Frankfurt, Germany) with a dilution of 100 units/2 mL of 0.9% sterile saline, while group B patients were injected with onabotulinumtoxinA (Botox[®], Allergan, Irvine, CA, USA), 100 MU in 2 ml of 0.9% sterile saline. Patients in both groups were blinded to BoNT-A treatment type and received doses below 12 units/Kg.

After 6 months, the second part of the study started, and each group received the crossover intervention: group A patients were treated with onabotulinumtoxinA (Botox[®], Allergan, Irvine, CA, USA, 100 MU in 2 ml 0.9%NaCl/H₂O), whereas group B patients were treated with incobotulinumtoxinA (NT 201; Xeomin[®], Merz Pharmaceuticals GmbH, Frankfurt, Germany with a dilution of 100 units/2 mL of 0.9% sterile saline). The same target muscles and doses of the first part of the study were maintained. Study design is described in Fig. 1.

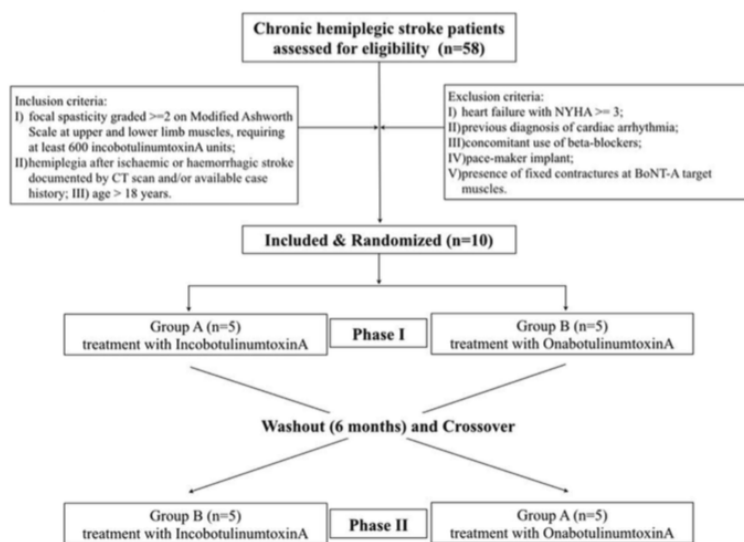


Fig. 1. Study design.

Patients were blinded to BoNT-A treatment type during the whole study period. Each patient signed an informed consent to the treatment and the evaluations of this study. The local committee of our Hospital approved our protocol, which was conducted according to the principles of the Declaration of Helsinki.

2.3. Assessment

All patients in both groups performed a total of four ECG registrations of 30 min each. In the first part of the study, patients performed the ECG registration in the 24 h before BoNT-A injection (baseline, t₀) and 10 days after the treatment (t₁); similarly, in the second, crossover, part of the study,

they performed the ECG registration 24 h before the new BoNT-A injection and 10 days after the treatment. Each ECG recording was performed as described elsewhere (Invernizzi et al., 2015).

Treatment safety was assessed by monitoring adverse events occurrence of any degree.

Lastly, the following functional measures were recorded at baseline: Barthel Index (Shah et al., 1989), Motricity Index (MI) for upper and lower limbs (Collin and Wade, 1990), Functional ambulation category (FAC) (Collen et al., 1990).

Data obtained from analogic ECG were processed with an A/D converter (micro 1401 CED[©] Cambridge Electronic Design, Cambridge, UK) and recorded on a PC by means of a data acquisition system (Spike2 v.5, CED) with a sampling rate of 3000 Hz. Guidelines were followed for time recordings, sampling rate and HRV analysis of electrocardiograms (Task Force, 1996). Artifacts and noise regions were removed, and, in case of premature beats, they have been manually corrected. Only the recordings that contained <1% of premature beats were considered.

ECG's have been analysed by means of software called "Kubios HRV analysis", originally created by Niskanen et al. (2004). In this study we considered both linear and non-linear HRV indexes (Buccelletti et al., 2012). A detailed description of all linear and non-linear HRV indexes used in this study can be found in the supplementary material of Invernizzi et al. (2015).

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad 6 package, version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). The patients were randomly assigned to one of the treatment arms using a randomization scheme generated by software with a 1:1 allocation and without blocks. Due to the small sample size, we supposed a non-gaussian distribution of variables.

Differences between each variable in each group have been evaluated with Friedman's analysis of variance (ANOVA) and Dunn post hoc comparison was used to identify significant differences between mean values. Differences between single variables in different groups were evaluated with the Mann-Whitney U-test. A type I error (alpha) level of 0.05 was chosen.

3. Results

Demographic data of the enrolled patients, dose/kg and mean overall doses of onabotulinumtoxinA and incobotulinumtoxinA utilised are resumed in Table 1. As shown in Tables 2 and 3, none of the variables considered for time, frequency and non-linear domains showed any statistically significant changes after each BoNT- A injection in both Groups A and B at any evaluation time. Moreover, no statistically significant differences were found regarding each variable considered between the two groups. Lastly, one patient in each group experienced mild, self-limiting adverse events after the

treatment (pain at the injection site). In each case, adverse events were related to the injection procedure and spontaneously resolved without any intervention 15 min after treatment.

Table 1
Patients' demographical and clinical characteristics. Data are presented as mean \pm standard deviation (SD), median and IQR.

	Total	Group A	Group B
Patients (n)	10	5	5
Age (years)	69 \pm 10.5 71 (60.25–76.5)	66 \pm 14.2 61 (54–80.5)	72 \pm 5.1 73 (67–76.5)
Sex			
Male (n)	7	3	4
Female (n)	3	2	1
Time from stroke (months)	116.8 \pm 65.20 87 (73.75–161.8)	126.8 \pm 84.95 91.5 (73–215.8)	106.8 \pm 49.34 87 (75.75–157.5)
Type of stroke			
Ischemic (n)	5	3	2
Hemorrhagic (n)	5	2	3
Weight (kg)	70.3 \pm 10.36 68 (59–83)	73.8 \pm 8.42 70 (63–78)	71.8 \pm 9.46 71 (62.5–81.5)
BMI	24.37 \pm 1.26 24.81 (23.88–26.16)	25.46 \pm 1.38 25.24 (24.48–26.37)	25.19 \pm 1.84 25.02 (24.32–26.06)
Total dose of BoNT-A (units)	665 \pm 81.82 625 (600–725)	660 \pm 89.44 600 (600–750)	670 \pm 83.67 650 (600–750)
Dose pro kg (units)	9.63 \pm 0.66 9.51 (8.56–10.19)	9.48 \pm 0.57 9.38 (8.66–10.12)	9.72 \pm 0.61 9.55 (8.46–10.27)
Functional Ambulation Category	4.2 \pm 0.45 4 (4–5)	4.2 \pm 0.45 4 (4–5)	4.2 \pm 0.45 4 (4–5)
Barthel Index	69 \pm 6.58 70 (65–80)	67 \pm 5.7 65 (65–75)	71 \pm 7.42 70 (70–80)
Motricity Index upper limb	47 \pm 3.74 47 (47–52)	46.2 \pm 3.03 47 (47–49)	47.8 \pm 4.59 47 (47–52)
Motricity Index lower limb	58 \pm 2.58 58.5 (57–60)	57.2 \pm 3.27 57 (57–60)	58.8 \pm 1.64 60 (57–60)

Table 2
Results of linear variables of the two groups in the first part of the study and after the crossover procedure, before and after BoNT-A treatment. Mean RR: mean value of RR intervals; Mean HR: mean value of heart rate; RMSSD: root mean square of successive differences; NN50: number of successive intervals differing more than 50 ms; pNN50: relative amount of successive intervals differing more than 50 ms; RR tri index: HRV triangular index; TINN: baseline width of the RR histogram; AR LF: autoregressive modeling based method Low Frequency; AR HF: autoregressive modeling based method High Frequency; AR LF/HF: autoregressive modeling based method Low/High frequency ratio; data are represented as mean and (SD).

	First part				Crossover			
	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)
Mean RR (ms)	807 (79.75)	832 (160.9)	865 (101.5)	885 (108.7)	826 (84.12)	813 (73.2)	859 (88.2)	863 (75.44)
Mean HR (1/min)	75.11 (7.83)	74.18 (13.16)	70.18 (8.15)	69.02 (9.6)	77.3 (6.4)	73.9 (7.4)	72.8 (6.9)	74.5 (8.12)
RMSSD (ms)	29.88 (8.38)	41.15 (18.78)	26.05 (8.78)	41.5 (35.37)	31.45 (7.25)	36.15 (8.42)	33.6 (9.42)	39.7 (7.56)
NN50 (count)	24.5 (14.55)	40.75 (11.59)	22.25 (18.14)	46.1 (24.5)	21.6 (15.29)	32.35 (11.75)	19.8 (13.26)	39.1 (18.01)
pNN50 (%)	3.75 (2.121)	6.03 (2.51)	3.43 (3.3)	6.63 (3.95)	4.8 (2.2)	5.66 (2.48)	4.34 (2.33)	6.63 (2.87)
RR tri index	7.1 (2.66)	7.27 (2.38)	7.16 (0.92)	9.96 (1.39)	8.3 (2.94)	7.87 (2.56)	7.69 (3.47)	6.85 (2.94)
TINN (ms)	241.3 (62.23)	233.8 (38.16)	192.5 (92.42)	242.5 (140.3)	211.6 (58.22)	219.6 (60.73)	187.44 (61.3)	196.2 (54.89)
AR LF (n.u.)	56.4 (29.85)	42.6 (23.87)	56.03 (18.83)	55.35 (19.22)	51.6 (23.13)	50.62 (19.84)	53.4 (20.02)	55.35 (22.66)
AR HF (n.u.)	44.13 (19.27)	57.03 (23.77)	43.85 (18.75)	44.5 (19.2)	48.23 (17.5)	48.84 (15.83)	46.6 (19.43)	44.5 (16.58)
AR LF/HF	2.54 (2.3)	1.22 (1.13)	1.67 (1.3)	1.88 (1.05)	1.91 (1.2)	1.48 (1.07)	1.63 (0.93)	1.83 (1.1)

p < 0.05, Post injection Group A vs Group A, first part of the study.
p < 0.05, Post injection Group B vs Group B, first part of the study.
p < 0.05, Post injection Group B vs Post injection Group A, first part of the study.
p < 0.05, Post injection Group A vs Group A, after cross-over procedure.
p < 0.05, Post injection Group B vs Group B, after cross-over procedure.
p < 0.05, Post injection Group B vs Post injection Group A, after cross-over procedure.

Table 3

Results of non-linear variables of the two groups in the first part of the study and after the crossover procedure, before and after BoNT-A treatment. Lmean: Recurrence plot analysis - mean line length; Lmax: Recurrence plot analysis - maximum line length; REC: Recurrence plot analysis - recurrence rate; DET: determinism of the time series; ShanEn: Shannon entropy; ApEn: Approximate Entropy; SampEn: Sample Entropy; DFAa1: Detrended fluctuation analysis - short-term fluctuations; DFAa2 Detrended fluctuation analysis - long-term fluctuations; D2: Correlation dimension; SD1: standard deviation of the Poincaré plot perpendicular to line of identity; SD2: standard deviation of the Poincaré plot along the line of identity; data are represented as mean and (SD).

	First part				Crossover			
	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)	Group A Mean (SD)	Group B Mean (SD)	Post Group A Mean (SD)	Post Group B Mean (SD)
Lmean (beats)	19.62 (10.13)	20.69 (11.26)	14.23 (4.8)	17.83 (6.42)	16.54 (9.42)	21.29 (12.45)	14.87 (8.45)	18.42 (10.48)
Lmax (beats)	180.2 (172.5)	243.1 (142.5)	210.3 (131.8)	237.5 (122.3)	226 (168.2)	218.3 (130.3)	238.36 (137.2)	215.44 (119.76)
REC (%)	38.58 (15.43)	48.17 (10.42)	42.94 (11.36)	44.55 (11.85)	36.1 (18.7)	42.34 (9.66)	43.75 (12.45)	41.15 (13.72)
DET (%)	103.2 (4.9)	98.85 (1.13)	98.77 (2.04)	99.08 (3.13)	144 (8.3)	84.5 (7.47)	106.48 (20.42)	101.38 (18.81)
ShanEn	3.43 (0.67)	3.5 (0.33)	3.4 (0.37)	3.53 (0.36)	3.56 (0.41)	3.3 (0.68)	3.41 (0.74)	3.23 (0.45)
ApEn	1.09 (0.32)	1.01 (0.37)	1.11 (0.19)	0.93 (0.31)	1.02 (0.27)	0.97 (0.19)	1.04 (0.21)	1.02 (0.3)
SampEn	1.84 (0.33)	1.63 (0.31)	1.3 (0.25)	0.99 (0.42)	1.7 (0.42)	1.42 (0.26)	1.34 (0.53)	1.59 (0.61)
DFA:a1	1.132 (0.41)	0.7 (0.38)	0.99 (0.25)	1.02 (0.28)	0.89 (0.33)	0.84 (0.22)	1.043 (0.46)	0.93 (0.29)
DFA:a2	0.933 (0.19)	0.89 (0.3)	0.95 (0.12)	0.98 (0.15)	0.96 (0.26)	0.91 (0.33)	0.94 (0.29)	0.95 (0.41)
D2	0.73 (0.28)	0.58 (0.31)	0.55 (0.1)	0.83 (0.28)	0.64 (0.18)	0.66 (0.27)	0.73 (0.15)	0.71 (0.38)
SD1 (ms)	15.74 (11.51)	29.13 (13.34)	18.43 (6.25)	29.38 (15.02)	17.7 (10.63)	19.32 (9.49)	19.12 (12.36)	18.12 (14.72)
SD2 (ms)	39.16 (18.32)	44.03 (18.47)	39.9 (5.2)	54.60 (29.24)	38.89 (20.71)	45.42 (23.52)	39.07 (25.13)	42.60 (30.26)

p < 0.05, Post injection Group A vs Group A, first part of the study.

p < 0.05, Post injection Group B vs Group B, first part of the study.

p < 0.05, Post injection Group B vs Post injection Group A, first part of the study.

p < 0.05, Post injection Group A vs Group A, after cross-over procedure.

p < 0.05, Post injection Group B vs Group B, after cross-over procedure.

p < 0.05, Post injection Group B vs Post injection Group A, after cross-over procedure.

4. Discussion

The results of this study show that high doses (>600 U) of incobotulinumtoxinA and onabotulinumtoxinA do not influence the autonomic drive directed to the heart in chronic hemiplegic spastic stroke survivors. These results are also confirmed if the two BoNT-A formulations are interchanged in a crossover design suggesting the potential absence of cumulative effects. Moreover, both the treatment with incobotulinumtoxinA and the treatment with onabotulinumtoxinA showed only one mild self-limiting adverse event in each group, related to the injective procedure and spontaneously resolved.

The results obtained in this study not only confirm the safety of incobotulinumtoxinA from a cardiovascular point of view, but also, for the first time, produce data about the autonomic heart modifications in stroke survivors with spasticity treated with high doses (>600 U) of onabotulinumtoxinA.

In 2009, Dressler in a crossover prospective study converted patients previously treated with onabotulinumtoxinA to incobotulinumtoxinA and repeatedly injected them for 3 years, monitoring clinical efficacy and adverse events occurrence. The author did not find any difference in the efficacy, treatment duration and adverse events occurrence after changing the two drug formulations, suggesting a clinical equivalence of the two BoNT-A formulations (Dressler, 2009). Our results are somehow comparable with those obtained by Dressler, considering also the fact that all outcome measures related to safety in that study were obtained with an anamnestic evaluation only, without any instrumental objective measure. More recently, Mehnert et al. showed similar results observing onabotulinumtoxinA effects on cardiac function after intradetrusor injection, showing no HRV modifications after treatment (Mehnert et al., 2016). The absence of a relevant effect on the autonomic

drive directed to the heart may have a clinical relevance in the treatment decision with high doses of BoNT-A in stroke survivors affected by cardiovascular comorbidities, in which a reduction in HRV is known to be able to increase the incidence of cardiovascular events (Task Force, 1996). Moreover, even in patients without known cardiac diseases, our results can limit the potential concerns about the use of BoNT-A at a dosage greater than 600 units.

As underlined, only few studies in literature investigated the possible autonomic cardiovascular modifications induced by onabotulinumtoxinA, and with doses far lower than those utilised in our work. Lastly, the fact that these two drugs at the same dose in the same patients at different times induce the same modifications on HRV can in part explain the clinical results regarding efficacy and safety found by Dressler (2009). However, due to the low sample size, our data should be taken cautiously and for this reason further experience with high dose use of BoNT-A is needed.

As a last consideration, our data suggest also, in line with the results obtained by Dressler, that the treatment with high doses of BoNT-A (both onabotulinumtoxinA and incobotulinumtoxinA) does not seem to produce any cumulative effect on HRV modifications and adverse events occurrence. Even though the overall study period involved only two BoNT-A injections, these data are consistent with previous studies of safety in multiple spasticity treatment performed with BoNT-A and give further elements about overall BoNT-A safety at high doses (>600 U) in hemiplegic stroke survivors (Santamato et al., 2013; Baricich et al., 2015).

This study has several limitations: the first is the low sample size; the second is the overall study duration (1 year) that hinders the possibility to evaluate long-term modifications induced by multiple BoNT-A injections.

5. Conclusions

In conclusion, this study confirms the safety of incobotulinumtoxinA at doses up to 12 units/kg regarding the autonomic heart drive modifications and adverse events occurrence in adult stroke hemiplegic spastic patients. Moreover, for the first time in literature, this study shows the same results in terms of safety of onabotulinumtoxinA. Lastly, multiple administrations of BoNT-A at high doses in adult hemiplegic patients do not seem to induce any cumulative effect. However, further experience with high doses of BoNT-A is needed in order to confirm these results and, in particular, for the clinical use of these two BoNT-A formulations in patients with cardiovascular comorbidities.

References

Akselrod, S., Gordon, D., Ubel, F.A., Shannon, D.C., Berger, A.C., Cohen, R.J., 1981. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat- to-beat cardiovascular control. *Science* 213 (4504), 220-222.

Baker, J.A., Pereira, G., 2013. The efficacy of Botulinum Toxin A for spasticity and pain in adults: a systematic review

- and meta-analysis using the Grades of Recommendation, Assessment, Development and Evaluation approach. *Clin. Rehabil.* 27 (12), 1084-1096. <http://dx.doi.org/10.1177/0269215513491274>.
- Baricich, A., Grana, E., Carda, S., et al., 2015. High doses of onabotulinumtoxinA in post-stroke spasticity: a retrospective analysis. *J. Neural Transm. (Vienna)* 122 (9), 1283-1287. <http://dx.doi.org/10.1007/s00702-015-1384-6>.
- Bohannon, R.W., Smith, M.B., 1987. Interrater reliability of a modified Ashworth Scale of muscle spasticity. *Phys. Ther.* 67 (2), 206-207.
- Brin, M.F., 1997. Dosing, administration, and a treatment algorithm for use of botulinum toxin A for adult-onset spasticity. Spasticity Study Group. *Muscle Nerve Suppl.* 6, S208-S220.
- Buccelletti, F., Bocci, M.G., Gilardi, E., et al., 2012. Linear and nonlinear heart rate variability indexes in clinical practice. *Comput. Math. Methods Med.* 2012, 219080. <http://dx.doi.org/10.1155/2012/219080>.
- Collen, F.M., Wade, D.T., Bradshaw, C.M., 1990. Mobility after stroke: reliability of measures of impairment and disability. *Int. Disabil. Stud.* 2 (1), 6-9.
- Collin, C., Wade, D., 1990. Assessing motor impairment after stroke: a pilot reliability study. *J. Neurol. Neurosurg. Psychiatry* 53 (7), 576-579.
- Dressler, D., 2009. Routine use of Xeomin in patients previously treated with Botox: long term results. *Eur. J. Neurol.* 16 (Suppl. 2), 2-5. <http://dx.doi.org/10.1111/j.1468-1331.2009.02877.x>.
- Francisco, G.E., 2004. Botulinum toxin: dosing and dilution. *Am. J. Phys. Med. Rehabil.* 83 (10 Suppl. 1), S30-S37.
- Girlanda, P., Vita, G., Nicolosi, C., et al., 1992. Botulinum toxin therapy: distant effects on neuromuscular transmission and autonomic nervous system. *J. Neurol. Neurosurg. Psychiatry* 55 (9), 844-845.
- Invernizzi, M., Carda, S., Molinari, C., et al., 2015. Heart rate variability (HRV) modifications in adult hemiplegic patients after botulinum toxin type a (NT- 201) injection. *Eur. J. Phys. Rehabil. Med.* 51 (4), 353-359.
- Lee, J.H., Park, J.H., Lee, S.K., et al., 2014. Efficacy and safety of incobotulinum toxin A in periocular rhytides and masseteric hypertrophy: side-by-side comparison with onabotulinum toxin A. *J. Dermatol. Treat.* 25 (4), 326-330. <http://dx.doi.org/10.3109/09546634.2013.769041>.
- Mehnert, U., de Kort, L.M., Wollner, J., et al., 2016. Effects of onabotulinumtoxinA on cardiac function following intradetrusor injections. *Exp. Neurol.* 285 (Pt B), 167e172. <http://dx.doi.org/10.1016/j.expneurol.2016.06.022>.
- Niskanen, J.P., Tarvainen, M.P., Ranta-Aho, P.O., Karjalainen, P.A., 2004. Software for advanced HRV analysis. *Comput. Methods Programs Biomed.* 76 (1), 73e81.
- Santamato, A., Panza, F., Ranieri, M., et al., 2013. Efficacy and safety of higher doses of botulinum toxin type A NT 201 free from complexing proteins in the upper and lower limb spasticity after stroke. *J. Neural Transm. (Vienna)* 120 (3), 469-476. <http://dx.doi.org/10.1007/s00702-012-0892-x>.
- Shah, S., Vanclay, F., Cooper, B., 1989. Improving the sensitivity of the Barthel Index for stroke rehabilitation. *J. Clin. Epidemiol.* 42 (8), 703-709.
- Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93 (5), 1043-1065.
- Wissel, J., Kempf, F., 2012. Botulinum toxin in clinical neurology. quiz 179 *Fortschr Neurol. Psychiatr.* 80 (3), 167e178. <http://dx.doi.org/10.1055/s-0031-1299095> (Article in German).

Discussion

Current evidence coming from published literature suggests that higher doses of BoNT-A are efficacious in reducing spasticity of the upper and lower limbs after stroke, with rare occurrence of mild adverse effect.

Although systemic BoNT-A toxicity is a rare event and as such not necessarily fatal, fear of systemic toxicity is still the most vigorous concern against application of increased BoNT-A doses.

Previous neurophysiological studies demonstrated that neuromuscular transmission could be temporally abnormal also in muscles distant from the target muscles: however, these findings were not related to any clinical dysfunction. In addition, cumulative data suggest that high doses (>600 U) of incobotulinumtoxinA and onabotulinumtoxinA do not influence the cardiovascular activity of the autonomic nervous system in chronic hemiplegic spastic stroke survivors, showing a satisfactory safety profile. On the other hand, at present time, there is no evidence with respect to doses of abobotulinumtoxinA higher than 1500, even if Hesse and colleagues [1995] reported good tolerability after administration of 2000 U.

Moreover, as described in previous studies, it must be pointed out that generalized weakness can occur also for recommended doses of BoNT-A [Bakheit et al, 1997].

A possible explanation is that local and systemic diffusion of BoNT could depend on several factors: injection technique, volume, dilution, needle size, hematogenous transport and other physical factors. For example, the use of ultrasonography to identify target muscles may improve accuracy of needle placement, avoiding injection into vascular structures and reducing the potential risk of systemic diffusion of BoNT-A [Henzel et al, 2010]. Moreover, this technique can improve clinical outcome both in upper and lower limb PSS [Picelli et al, 2014; Santamato et al, 2014].

Another relevant issue related to the risk of side effects regards the dilution of BONT-A.

Current guidelines on adult dosing of BoNT-A recommend a maximum of 1 ml per site, except in selected situations [Mayer and Simpson, 2010]. This raises the possibility that higher doses or volumes of BoNT-A could saturate local cholinergic nerve terminals, allowing unbound toxin to spread to the adjacent structures or the blood stream. In particular, attention is due in case of high doses of BoNT-A and/or high volumes injected into the proximal muscles across the midline in order to avoid the risk of contralateral weakness, as reported by Thomas and Simpson [2012].

In addition, it should be pointed out that, despite the reduction of severe spasticity, there is limited

evidence that treating patients with elevated BoNT-A doses in the upper and lower limbs is related to significant functional improvement. However, this observation could be related to several possibilities.

First of all, it is well known that in the case of severe spasticity the improvement in active performance is sometimes difficult to acquire; however, in many neurological conditions high doses should be considered in order to obtain a reduction of muscle tone (especially in the adductor and calf muscles) with significant improvement in hygiene, gait, and balance.

In addition, these observations are reflected in the paucity of studies able to identify the true correlation between spasticity and disability [Ada et al, 1998. Pradon et al, 2013]. On the other hand, different studies demonstrated the efficacy of spasticity treatment in passive and active functional improvement of patients [Ward et al, 2014].

However, in a study examining disability in post stroke spasticity patients, treatment of spasticity that was only moderately severe according to the Ashworth Scale resulted in notable improvements in performing activities of daily living, as indicated by a patient-assessed composite disability scale [Bhakta BB et al, 2000]. In light of these considerations, a Delphi Panel consensus [Zorowitz et al, 2017] recently proposed a screening tool in order to evaluate the spasticity in need of treatment.

However, the impact of spasticity on a subject might be characterized by the degree of limitation in performing functional tasks and by the impact on well-being and life satisfaction, which may not be indicated by objective score results but are demonstrated by patient-reported outcome measures [Sunnerhagen and Francisco, 2013; Baricich et al, 2016].

A possible explanation of these controversial observations could be related to problems occurring in patient-provider communication, and its role in post-stroke spasticity rehabilitation.

In a context of patient-centered health care, it could address the holistic experience of illness from patient's perspective [Sunnerhagen and Francisco, 2013; Leach et al, 2010].

In addition, it should be considered that PSS also has an afferent, sensory component, which might be related to some differences in the sensations described by patients [Baricich et al, 2016]. In fact, it is well known that proprioceptive afferent information coming from mechanoreceptors in joints, muscles, tendons and stretch-sensitive receptors in the skin, together with efferent motor signals, can play a key role in postural schema understood as dynamic representations of body posture. Moreover, the sense of body ownership, too, is presumably developed using sensory information, as recently described by Walsh and colleagues [2011] who demonstrated that non-tactile proprioceptive cues might contribute to this sense.

On these bases, we hypothesized a patient-reported screening tool able to identify the functional impact of spasticity after stroke. This tool is potentially fit to be used in routine clinical practice in

order to improve an early detection of functionally relevant spasticity and ameliorate patients' care. An expert panel identified a 15-items questionnaire which investigates the clinical impact of spasticity in activities of daily living (ADL), named SPasticity Questionnaire in Real life (SPQR) (see Appendix). The score ranges from 0 (no clinical impact) to 45 (very significant impact in the whole panel of ADL). The tool is designed on the basis of the Rasch measurement model, a probabilistic mathematic modelling technique, used to assess the psychometric properties of outcome measures; it examines wider attributes, analyzing the item's performances in terms of relevance, usefulness for measuring the underlying construct, redundancy and appropriateness [Tesio, 2003]. We preliminarily tested SPQR in a population of chronic stroke survivors affected by spasticity, according to the recommended guidelines (unpublished data). SPQR showed an excellent internal consistency (Cronbach's alpha 0.95), and good reproducibility and validity [Pearson's coefficient 0.76 $p < 0.05$; Kappa coefficient 0.76 (95% CI 0.61-0.91); Intraclass Correlation Coefficient 0.89 (95% CI 0.71- 0.96)]. In addition, at T2 SPQR showed a significant score variation for upper and lower limb functional improvement, according to the changes in muscle tone evaluated by Modified Ashworth Scale. To the best of our knowledge, this is the first tool specifically designed to evaluate the functional implications of spasticity and the clinical impact of its treatment on patients' outcome.

Conclusions and future perspectives

The evidence coming from published studies suggests that use of doses of BoNT-A higher than those reported in product labels could be considered as a safe therapeutic option to reduce multifocal or generalized post stroke spasticity in selected patients.

However, it must be pointed out that the clinicians have to carefully define the clinical goal before starting with BoNT-A treatment. In addition, based on current evidence, they must consider all the factors which could affect the safety profile of BoNT-A, such as injection technique, dose and dilution.

Further evidence is mandatory to confirm higher doses of BoNT-A as a safe and effective therapeutic option for the treatment of post stroke spasticity.

In particular, it should be pointed out the potential role of higher doses of BoNT-A in order to improve the functional outcome of patients affected by PSS. As previously stated, it should be noted that the impact of PSS on limitations in ADL, wellbeing and life satisfaction may not be indicated by quantitative scores but could be demonstrated by patient-reported outcome measures such as SPQR questionnaire.

In light of these considerations, further research is also required in order to confirm the validity and reliability of these tools.

Bibliography

- Ada L, Vattanasilp W, O'Dwyer NJ, Crosbie J. Does spasticity contribute to walking dysfunction after stroke? *J Neurol Neurosurg Psychiatry*. 1998;64(5):628-35
- Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*. 1981;213:220-22.
- Albanese A. Terminology for preparations of botulinum neurotoxins: what a difference a name makes. *JAMA* 2011;305:89–90.
- Aoki KR. Review of a proposed mechanism for the antinociceptive action of Abotulinum toxin type A. *NeuroToxicology* 2005;26:785–93.
- Aoki KR Guyer B. Botulinum toxin type A and other botulinum toxin serotypes: A comparative review of biochemical and pharmacological actions. *Eur. J. Neurol*. 2001; 8 (Suppl. 5): 21–29.
- Aoki, KR, Ranoux, D, Wissel J. Using translational medicine to understand clinical differences between botulinum toxin formulations. *Eur. J. Neurol*. 2006; 13 (Suppl. 4): 10–19.
- Atassi MZ. Basic immunological aspects of botulinum toxin therapy. *Mov Disord*. 2004: 19 (Suppl. 8), S68–S84.
- Atassi MZ. On the enhancement of anti-neurotoxin antibody production by subcomponents HA1 and HA3b of Clostridium botulinum type B 16S toxin haemagglutinin. *Microbiology* 2006, 152, 1891–1895.
- Baizabal-Carvalho JF, Jankovic J, Pappert E. Flu-like symptoms following botulinum toxin therapy. *Toxicon* 2011;58:1–7.
- Bakheit AM, Ward CD, McLellan DL. Generalised botulism-like syndrome after intramuscular injections of botulinum toxin type A: a report of two cases. *J Neurol Neurosurg Psychiatry* 1997;62:198.
- Bakheit AM, Liptrot A, Newton R, Pickett AM. The effect of total cumulative dose, number of treatment cycles, interval between injections, and length of treatment on the frequency of occurrence of antibodies to botulinum toxin type A in the treatment of muscle spasticity. *Int J Rehabil Res* 2012;35:36–9.
- Baricich A, Grana E, Carda S, Santamato A, Cisari C, Invernizzi M. High doses of onabotulinumtoxinA in post-stroke spasticity: a retrospective analysis. *J Neural Transm (Vienna)*. 2015 Sep;122(9):1283-7. doi: 10.1007/s00702-015-1384-6
- Baricich A, Grana E, Carda S, Santamato A, Molinari C, Cisari C, Invernizzi M. Heart Rate Variability modifications induced by high doses of incobotulinumtoxinA and onabotulinumtoxinA in hemiplegic chronic stroke patients: A single blind randomized controlled, crossover pilot study. *Toxicon*. 2017 Nov;138:145-150. doi:10.1016/j.toxicon.2017.08.027.
- Baricich A, Picelli A, Molteni F, Guanziroli E, Santamato A. Post-stroke spasticity as a condition: a new perspective on patient evaluation. *Funct Neurol*. 2016 Jul-Sep;31(3):179-80.
- Benecke R. Clinical relevance of botulinum toxin immunogenicity. *Biodrugs* 2012; 26: e1–e9.
- Benecke R, Jost, WH, Kanovsky P, Ruzicka E, Comes G, Grafe S. A new botulinum toxin type A free of complexing proteins for treatment of cervical dystonia. *Neurology* 2005; 64: 1949–1951.
- Bensmail D, Hanschmann A, Wissel J. Satisfaction with botulinum toxin treatment in post-stroke spasticity: results from two cross-sectional surveys (patients and physicians). *J Med Econ*. 2014 Sep;17(9):618-25. doi: 10.3111/13696998.2014.925462.
- Bentivoglio AR, Ialongo T, Bove F, de Nigris F, Fasano A. Retrospective evaluation of the dose equivalence of Botox and Dysport in the management of blepharospasm and hemifacial spasm: A novel paradigm for a never ending story. *Neurol. Sci*. 2012; 33: 261–267.
- Bhakta BB, Cozens JA, Chamberlain MA, Bamford JM. Impact of botulinum toxin type A on disability and carer burden due to arm spasticity after stroke: a randomised double blind placebo controlled trial. *J Neurol Neurosurg Psychiatry* 2000;69:217–21
- Bhatia KP, Munchau A, Thompson PD, et al. Generalised muscular weakness after botulinum toxin injections for dystonia: a report of three cases. *J Neurol Neurosurg Psychiatry* 1999;67:90–93.
- Bigalke H. Properties of pharmaceutical products of botulinum neurotoxins. In *Botulinum Toxin: Therapeutic Clinical Practice and Science*; Jankovic, J., Albanese, A., Atassi, M.Z., Dolly, J.O., Hallett, M., Mayer, N.H., Eds.; Saunders Elsevier: Philadelphia, PA, USA, 2009; 389–397.

- Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther* 1987;67:206–7.
- Borodic G, Johnson E, Goodnough M, Schantz E. Botulinum toxin therapy, immunologic resistance, and problems with available materials. *Neurology* 1996;46:26-9.
- Brockmann K, Schweitzer K, Beck, G, Wächter, T. Comparison of different preparations of botulinumtoxinA in the treatment of cervical dystonia. *Neurol. Asia* 2012;17: 115–119.
- Brodsky MA, Swope DM, Grimes D. Diffusion of botulinum toxins. *Tremor Other Hyperkinet. Mov.* 2012, 2, 1346. Available online: <http://tremorjournal.org/article/view/85>
- Carli, L, Montecucco C, Rossetto O. Assay of diffusion of different botulinum neurotoxin type A formulations injected in the mouse leg. *Muscle Nerve* 2009;40:374–380.
- Chae J, Celnik PA. Stroke Rehabilitation. *Phys Med Rehabil Clin N Am.* 2015 Nov;26(4):xv-xvi. doi: 10.1016/j.pmr.2015.08.013.
- Chen, JJ, Dashtipour K. Abo-, Inco-, Ona-, and Rima-Botulinum toxins in clinical therapy: A primer. *Pharmacotherapy* 2013; 33: 304–318.
- Crouner BE, Torres-Russotto D, Carter AR, Racette BA. Systemic weakness after therapeutic injections of botulinum toxin a: a case series and review of the literature. *Clin Neuropharmacol* 2010;33:243–7
- Deltombe T, Wautier D, De Cloedt P, Fostier M, Gustin T. Assessment and treatment of spastic equinovarus foot after stroke: Guidance from the Mont-Godinne interdisciplinary group. *J Rehabil Med.* 2017 Jun 28;49(6):461-468. doi: 10.2340/16501977-2226. PubMed PMID: 28451697
- Denno MS, Gillard PJ, Graham GD, DiBonaventura MD, Goren A, Varon SF, Zorowitz R. Anxiety and depression associated with caregiver burden in caregivers of stroke survivors with spasticity. *Arch Phys Med Rehabil.* 2013 Sep;94(9):1731-6. doi: 10.1016/j.apmr.2013.03.014
- Dietz V, Sinkjaer T. Spastic movement disorder: Impaired reflex function and altered muscle mechanics. *Lancet Neurology* 2007;6: 725–733.
- Dressler D, Adib Saberi F, Kollwe K, Schrader C. Safety aspects of incobotulinumtoxinA high-dose therapy. *J Neural Transm.* 2015 Feb;122(2):327-33. doi: 10.1007/s00702-014-1252-9
- Dressler D. Clinical features of antibody-induced complete secondary failure of botulinum toxin therapy. *Eur. Neurol.* 2002, 48, 26–29.
- Dressler D, Benecke R. Pharmacology of therapeutic botulinum toxin preparations. *Disabil. Rehabil.* 2007, 29, 1761–1768.
- Dressler D, Mander G, Fink, K. Measuring the potency labelling of onabotulinumtoxinA (Botox[®]) and incobotulinumtoxinA (Xeomin[®]) in an LD50 assay. *J. Neural Transm.* 2012; 119: 13–15.
- Duncan PW, Zorowitz R, Bates B, Choi JY, Glasberg JJ, Graham GD, Katz RC, Lamberty K, Reker D. Management of adult stroke rehabilitation care: A clinical practice guideline. *Stroke* 2005;36: e100–143
- Dysport 300 units, Dysport 500 units—Summary of Product. Available online: <https://www.medicines.org.uk/emc/medicine/870/SPC/Dysport+300+units,+Dysport+500+units/> .
- Eisele KH, Fink K, Vey M, Taylor HV. Studies on the dissociation of botulinum neurotoxin type A complexes. *Toxicon* 2011, 57, 555–565.
- Erdal J, Ostergaard L, Fuglsang-Frederiksen A, et al. Long-term botulinum toxin treatment of cervical dystonia-EMG changes in injected and noninjected muscles. *Clin Neurophysiol* 1999;110: 1650–1654.
- Frevert, J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. *Drugs R & D* 2015; 15: 1–9.
- Full prescribing information Xeomin. Available online: <http://www.xeomin.com/consumers/pdf/xeomin-full-prescribing-information.pdf>
- Garner CG, Straube A, Witt TN, Gasser T, Oertel WH. Time course of distant effects of local injections of botulinum toxin. *Mov Disord* 1993;8:33–37.
- Girlanda P, Vita G, Nicolosi C, Milone S, Messina C. Botulinum toxin therapy: distant effects on neuromuscular transmission and autonomic nervous system. *J Neurol Neurosurg Psychiatry* 1992; 55:844–845.

- Goldstein EM. Safety of high-dose botulinum toxin type A therapy for the treatment of pediatric spasticity. *J Child Neurol.* 2006 Mar;21(3):189-92. PubMed PMID: 16901418.
- Hambleton P, Pickett AM. Potency equivalence of botulinum toxin preparations. *J. R. Soc. Med.* 1994, 87, 719.
- Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996;93:1043-65.
- Henzel MK, Munin MC, Niyonkuru C, Skidmore ER, Weber DJ, Zafonte RD. Comparison of surface and ultrasound localization to identify forearm flexor muscles for botulinum toxin injections. *PM R* 2010; 2:642–646
- Hesse S, Jahnke MT, Luecke D, Mauritz KH. Short-term electrical stimulation enhances the effectiveness of Botulinum toxin in the treatment of lower limb spasticity in hemiparetic patients. *Neurosci Lett* 1995;201:37–40.
- Highlights of prescribing information. Available online: http://www.allergan.com/assets/pdf/botox_pi.pdf
- Hristova AH, Joseph LN, Sathe SA, Wade JB. Severe nervous system complications after botulinum type A therapy: three case reports with reviews of FDA-reported nervous system adverse effects. *PM R* 2012;4:613–623.
- Humeau Y, Doussau F, Grant NJ, Poulain B. How botulinum and tetanus neurotoxins block neurotransmitter release. *Biochimie.* 2000;82:427–446.
- Intiso D, Simone V, Di Rienzo F, Iarossi A, Paziienza L, Santamato A, et al. High doses of a new botulinum toxin type A (NT-201) in adult patients with severe spasticity following brain injury and cerebral palsy. *NeuroRehabilitation* 2014;34:515–22.
- Invernizzi M, Carda S, Molinari C, Stagno D, Cisari C, Baricich A. Heart Rate Variability (HRV) modifications in adult hemiplegic patients after botulinum toxin type A (NT-201) injection. *Eur J Phys Rehabil Med.* 2015 Aug;51(4):353-9.
- Jankovic, J.; Esquenazi, A.; Fehlings, D.; Freitag, F.; Lang, A.; Naumann, M. Evidence-based review of patient-reported outcomes with botulinum toxin type A. *Clin. Neuropharmacol.* 2004, 27, 234–244.
- Johnson EA, Bradshaw M. Clostridium botulinum and its neurotoxins: a metabolic and cellular perspective. *Toxicon* 2001;39:1703–1722.
- Jost WH, Kohl A, Brinkmann S, Comes G. Efficacy and tolerability of a botulinum toxin type A free of complexing proteins (NT 201) compared with commercially available botulinum toxin type A (BOTOX) in healthy volunteers. *J. Neural Transm.* 2005, 112, 905–913.
- Keren-Capelovitch T, Jarus T, Fattal-Valevski A. Upper extremity function and occupational performance in children with spastic cerebral palsy following lower extremity botulinum toxin injections. *J. Child Neurol.* 2010, 25, 694–700.
- Kleiger RE, Bigger JT, Bosner MS, Chung MK, Cook JR, Roln- itzky LM *et al.* Stability over time of variables measuring heart rate variability in normal subjects. *Am J Cardiol* 1991;68:626-30.
- Kollewe K, Mohammadi B, Dengler R, Dressler D. Hemifacial spasm and reinnervation synkinesias: Long-term treatment with either Botox or Dysport. *J. Neural Transm.* 2010, 177, 759–763.
- Kollewe K, Mohammadi B, Köhler S, Pickenbrock H, Dengler R, Dressler D. Blepharospasm: Long-term treatment with either Botox[®], Xeomin[®] or Dysport[®]. *J. Neural Transm.* 2015, 122, 427–431.
- Kromminga A, Schellekens H. Antibodies against erythropoietin and other protein based therapeutics: An overview. *Ann. N.Y. Acad. Sci. USA* 2005, 1050, 257–265.
- Kukreja R, Chang TW, Cai S, Lindo P, Riding S, Zhou Y, Ravichandran E, Singh BR. Immunological characterization of the subunits of type A botulinum neurotoxin and different components of its associated proteins. *Toxicon.* 2009; 53: 616–624.
- Lacy DB, Stevens RC. Sequence homology and structural analysis of the clostridial neurotoxins. *J Mol Biol* 1999;291:1091–1104.
- Lance J. Spasticity: disorders motor control. In: Feldman RG, Young RP, Koella WP editors. *Symposium synopsis.* Miami, FL: Year Book Medical Publishers; 1980.
- Lange DJ, Brin MF, Warner CL, Fahn S, Lovelace RE. Distant effects of local injection of botulinum toxin. *Muscle Nerve.* 1987 Jul-Aug;10(6):552-5. Erratum in: *Muscle Nerve* 1988 May;11(5):520

- Lange DJ, Rubin M, Greene PE, et al. Distant effects of locally injected botulinum toxin: a double-blind study of single fiber EMG changes. *Muscle Nerve* 1991;14:672–675.
- Leach E, Cornwell P, Fleming J, Haines T. Patient centered goal-setting in a subacute rehabilitation setting. *Disabil Rehabil* 2010;32:159–72
- Lundström E, Smits A, Borg J, Terént A. Four-fold increase in direct costs of stroke survivors with spasticity compared with stroke survivors without spasticity: the first year after the event. *Stroke*. 2010 Feb;41(2):319-24. doi:10.1161/STROKEAHA.109.558619
- Mancini F, Sandrini G, Moglia A, Nappi G, Pacchetti C. A randomised, double-blind, dose-ranging study to evaluate efficacy and safety of three doses of botulinum toxin type A (Botox) for the treatment of spastic foot. *Neurol Sci*. 2005 Apr;26(1):26-31. PubMed PMID: 15877184
- Marchetti A, Magar R, Findley L, Larsen JP, Pirtosek Z, Ruzicka E, Jech R, Sławek J, Ahmed F. Retrospective evaluation of the dose of Dysport and BOTOX in the management of cervical dystonia and blepharospasm: The REAL DOSE study. *Mov. Disord*. 2005, 20, 937–944.
- Marion MH, Sheehy M, Sangla S, Soulayrol S. Dose standardisation of botulinum toxin. *J. Neurol. Neurosurgery Psychiatry* .1995;59:102–103.
- Mayer NH, Simpson DM, editors. *Spasticity: etiology, evaluation, management, and the role of botulinum toxin type A*. 3rd ed. New York: We Move; 2005.
- McComas AJ. Human neuromuscular adaptations that accompany changes in activity. *Medicine & Science in Sports & Exercise*. 1994; 26:1498–1509.
- McLellan K, Das RE, Ekong TA, Sesardic D. Therapeutic botulinum type A toxin: Factors affecting potency. *Toxicon*. 1996; 34: 975–985.
- Meichsner M, Reichel G. Effect of botulinum toxin a and B on vegetative cardiac innervation. *Fortschr Neurol Psychiatr*. 2005;73: 409–414.
- Mohammadi B, Buhr N, Bigalke H, Krampfl K, Dengler R, Kollwe K. A long-term follow up of botulinum toxin A in cervical dystonia. *Neurol Res*. 2009; 31: 463–466.
- Nebe A, Schelosky L, Wissel J, Ebersbach G, Scholz U, Poewe W. No effects on heart-rate variability and cardiovascular reflex tests after botulinum toxin treatment of cervical dystonia. *Mov Disord*. 1996;11:337-9.
- Nussgens Z. Comparison of two botulinum toxin preparations in the treatment of essential blepharospasm. *Arch. Clin. Exp. Ophthalmol*. 1997; 235:197–199.
- Odergren T.; Hjaltason H, Kaakkola S, Solders G, Hanko J, Fehling C, Marttila RJ, Lundh H, Gedin S, Westergren I et al. A double-blind, randomised, parallel group study to investigate the dose equivalence of Dysport and Botox in the treatment of cervical dystonia. *J. Neurol. Neurosurgery Psychiatry* 1998, 64, 6–12.
- Park J, Lee MS, Harrison AR. Profile of Xeomin (incobotulinumtoxinA) for the treatment of blepharospasm. *Clin. Ophtalmol*. 2011, 5, 725–732.
- Picelli A, Lobba D, Midiri A, Prandi P, Melotti C, Baldessarelli S, Smania N. Botulinum toxin injection into the forearm muscles for wrist and fingers spastic overactivity in adults with chronic stroke: a randomized controlled trial comparing three injection techniques. *Clin Rehabil* 2014; 28:232–242
- Picelli A, Baricich A, Cisari C, Paolucci S, Smania N, Sandrini G. The Italian real-life post-stroke spasticity survey: unmet needs in the management of spasticity with botulinum toxin type A. *Funct Neurol*. 2017 Apr/Jun;32(2):89-96.
- Picelli A, Vallies G, Chemello E, Castellazzi P, Brugnera A, Gandolfi M, Baricich A, Cisari C, Santamato A, Saltuari L, Waldner A, Smania N. Is spasticity always the same? An observational study comparing the features of spastic equinus foot in patients with chronic stroke and multiple sclerosis. *J Neurol Sci*. 2017 Sep 15;380:132-136. doi: 10.1016/j.jns.2017.07.026.
- Pickett A. Dysport: Pharmacological properties and factors that influence toxin action. *Toxicon* 2009, 54, 683–689.
- Poewe W. Respective potencies of Botox and Dysport: A double blind, randomised, crossover study incervical dystonia. *J. Neurol. Neurosurgery Psychiatry* 2002, 72.
- Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D *et al*. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985;248:H151-3.

- Pradon D, Roche N, Enette L, Zory R. Relationship between lower limb muscle strength and 6-minute walk test performance in stroke patients. *J Rehabil Med.* 2013 Jan;45(1):105-8. doi: 10.2340/16501977-1059. PubMed PMID: 23095981
- Ramirez-Castaneda J, Jankovic J, Comella C, Dashtipour K, Fernandez HH, Mari Z. Diffusion, spread, and migration of botulinum toxin. *Mov Disord.* 2013 Nov;28(13):1775-83. doi: 10.1002/mds.25582.
- Ranoux D, Gury C, Fondarai J, Mas, JL, Zuber M. Respective potencies of Botox and Dysport: A double-blind, randomised, crossover study in cervical dystonia. *J. Neurol. Neurosurgery Psychiatry* 2002, 72, 459–462.
- Roche N, Schnitzler A, Genet FF, Durand MC, Bensmail, D. Undesirable distant effects following botulinum toxin type A injection. *Clin. Neuropharmacol.* 2008, 31, 272–280. [
- Roggenkamper P, Jost, WH, Bihari K, Comes G, Grafe S, NT 201 blepharospasm study team. Efficacy and safety of a new botulinum toxin type A free of complexing proteins in the treatment of blepharospasm. *J. Neural Transm.* 2006, 113, 303–312.
- Rosales RL, Bigalke H, Dressler D. Pharmacology of botulinum toxin: Differences between type A preparations. *Eur J Neurol.* 2006, 13 (Suppl. 1), 2–10.
- Rystedt A, Nyholm D, Naver H. Clinical experience of dose conversion ratios between toxin products in the treatment of cervical dystonia. *Clin. Neuropharmacol.* 2012, 35, 278–282.
- Rystedt A, Zetterberg L, Burman J, Nyholm D, Johansson A. A comparison of Botox 100 U/mL and Dysport 100 U/mL using dose conversion ratio 1:3 and 1:1.7 in the treatment of cervical dystonia: A double-Blind, randomized, crossover trial. *Clin. Neuropharmacol.* 2015, 38, 170–176.
- Sampaio C, Costa J, Ferreira JJ. Clinical comparability of marketed formulations of botulinum toxin. *Mov. Disord.* 2004, 19 (Suppl. 19), S129–S136.
- Sampaio C, Ferreira JJ, Simões F, Rosas MJ, Magalhães M, Correia AP, Bastos-Lima A, Martins R, Castro-Caldas A. Dysport: A single-blind, randomized parallel study to determine whether any differences can be detected in the efficacy and tolerability of two formulations of botulinum toxin type A—Dysport and Botox, assuming a ratio of 4:1. *Mov. Disord.* 1997, 12, 1013–1018.
- Sanders DB. Clinical impact of single-fiber electromyography. *Muscle Nerve* 2002;(suppl 11):S15–S20.
- Santamato A, Micello MF, Ranieri M, Valeno G, Albano A, Baricich A, Cisari C, Intiso D, Pilotto A, Logroscino G, Panza F. Employment of higher doses of botulinum toxin type A to reduce spasticity after stroke. *J Neurol Sci.* 2015 Mar 15;350(1-2):1-6. doi: 10.1016/j.jns.2015.01.033.
- Santamato A, Micello MF, Panza F, Fortunato F, Baricich A, Cisari C, Pilotto A, Logroscino G, Fiore P, Ranieri M. Can botulinum toxin type A injection technique influence the clinical outcome of patients with post-stroke upper limb spasticity? A randomized controlled trial comparing manual needle placement and ultrasound-guided injection techniques. *J Neurol Sci* 2014;347:39–43
- Santamato A, Panza F, Intiso D, Baricich A, Picelli A, Smania N, Fortunato F, Seripa D, Fiore P, Ranieri M. Long-term safety of repeated high doses of incobotulinumtoxinA injections for the treatment of upper and lower limb spasticity after stroke. *J Neurol Sci.* 2017 Jul 15;378:182-186. doi:10.1016/j.jns.2017.04.052.
- Santamato A, Panza F, Ranieri M, Frisardi V, Micello MF, Filoni S, et al. Efficacy and safety of higher doses of botulinum toxin type A NT 201 free from complexing proteins in the upper and lower limb spasticity after stroke. *J Neural Transm* 2013; 120:469–76.
- Sesardic D, Leung T, Gaines-Das R. Role for standards in assays of botulinum toxins: International collaborative study of three preparations of botulinum type A toxin. *Biologicals* 2003, 31, 265–276.
- Shin JH, Jeon C, Woo KI, Kim YD. Clinical comparability of Dysport and Botox in essential blepharospasm. *J. Korean Ophthalmol. Soc.* 2009, 50, 331–335.
- Simpson DM, Hallett M, Ashman EJ, Comella CL, Green MW, Gronseth GS, Armstrong MJ, Gloss D, Potrebic S, Jankovic J, Karp BP, Naumann M, So YT, Yablon SA. 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 May 10;86(19):1818-26. doi: 10.1212/WNL.0000000000002560.
- Simpson LL. Identification of the major steps in botulinum toxin action. *Annu Rev Pharmacol Toxicol* 2004; 44:167–193.

- Sommerfeld DK, Eek EU, Svensson AK, Holmqvist LW, von Arbin MH. Spasticity after stroke: Its occurrence and association with motor impairments and activity limitations. *Stroke* 2004;35: 134–139.
- Sunnerhagen KS, Francisco GE. Enhancing patient–provider communication for long-term post-stroke spasticity management. *Acta Neurol Scand* 2013; 128: 305–310
- Tassorelli C, Mancini F, Balloni L, Pacchetti C, Sandrini G, Nappi G, Martignoni E. Botulinum toxin and neuromotor rehabilitation: An integrated approach to idiopathic cervical dystonia. *Mov Disord.* 2006 Dec;21(12):2240-3
- Tesio L. Measuring behaviours and perceptions: Rasch analysis as a tool for rehabilitation. *J Rehabil Med* 2003;35:105-15
- Thibaut A, Chatelle C, Ziegler E, Bruno MA, Laureys S, Gosseries O. Spasticity after stroke: physiology, assessment and treatment. *Brain Inj.* 2013;27(10):1093-105. doi: 10.3109/02699052.2013.804202.
- Thomas AM, Simpson DM. Contralateral weakness following botulinum toxin for poststroke spasticity. *Muscle Nerve* 2012;46:443–8.
- Tiple D, Strano S, Colosimo C, et al. Autonomic cardiovascular function and baroreflex sensitivity in patients with cervical dystonia receiving treatment with botulinum toxin type A. *J Neurol* 2008;255:843–847.
- Truong D, Dressler D, Hallett M, editors. *Manual of Botulinum Toxin Therapy*. Cambridge, United Kingdom: Cambridge University Press; 2009.
- Tsuji H, Larson MG, Venditti FJ Jr., Manders ES, Evans JC, Feldman CL *et al.* Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation* 1996;94:2850-55.
- Van den Bergh PYK, Lison DF. Dose standardization of botulinum toxin. *Adv. Neurol.* 1998, 78, 231–235.
- Varghese-Kroll E, Elovic EP. Contralateral weakness and fatigue after high-dose botulinum toxin injection for management of poststroke spasticity. *Am J Phys Med Rehabil* 2009;88:495–9.
- Vita G, Girlanda P, Puglisi RM, Marabello L, Messina C. Cardiovascular reflex testing and single-fiber electromyography in botulism. A longitudinal study. *Arch Neurol* 1987;44:202-6.
- Wagman J, Bateman JB. Botulinum type A toxin: Properties of toxic dissociation product. *Arch. Biochem. Biophys.* 1953, 46, 375–383.
- Walsh LD, Moseley GL, Taylor JL, et al. Proprioceptive signals contribute to the sense of body ownership. *J Physiol.* 2011;589:3009- 3021.
- Ward AB, Wissel J, Borg J, Ertzgaard P, Herrmann C, Kulkarni J, Lindgren K, Reuter I, Sakel M, Säterö P, Sharma S, Wein T, Wright N, Fulford-Smith A. Functional goal achievement in post-stroke spasticity patients: The BOTOX[®] Economic Spasticity Trial (BEST). *J Rehabil Med.* 2014 Jun;46(6):504-13. doi: 10.2340/16501977-1817
- Whurr R, Brookes G, Barnes C. Comparison of dosage effects between the American and British botulinum toxin A product in the treatment of spasmodic dysphonia. *Mov. Disord.* 1995, 10.
- Wichterle D, Simek J, La Rovere MT, Schwartz PJ, Camm AJ, Malik M. Prevalent low-frequency oscillation of heart rate: novel predictor of mortality after myocardial infarction. *Circulation* 2004;110:1183-90.
- Wissel J, Bensmail D, Ferreira JJ, Molteni F, Satkunam L, Moraleda S, Rekan T, McGuire J, Scheschonka A, Flatau-Baqué B, Simon O, Rochford ET, Dressler D, Simpson DM; TOWER study investigators. Safety and efficacy of incobotulinumtoxinA doses up to 800 U in limb spasticity: The TOWER study. *Neurology.* 2017 Apr 4;88(14):1321-1328. doi: 10.1212/WNL.0000000000003789.
- Wissel J, Manack A, Brainin M. Toward an epidemiology of post stroke spasticity. *Neurology* 2013;80 (Suppl 2):S13–S19
- Wissel J, Ward AB, Ertzgaard P, Bensmail D, Hecht MJ, Lejeune TM, Schnider P, Altavista MC, Cavazza S, Deltombe T et al. European consensus table on the use of botulinum toxin type A in adult spasticity. *J Rehabil Med* 2009;41:13–25.
- Wohlfarth K, Goschel H, Frevert J, Dengler R, Bigalke H. Botulinum A toxins: Units versus units. *Arch. Pharmacol.* 1997, 355, 335–340.
- Wohlfarth K, Schwandt I, Wegner F, Jürgens T, Gelbrich G, Wagner A, Bogdahn U, Schult Mattler W.

- Biological activity of two botulinum toxin type A complexes (Dysport[®] and Botox[®]) in volunteers: A double-blind, randomized, dose-ranging study. *J. Neurol.* 2008, 255, 1932–1939, Erratum in *J. Neurol.* 2009, 256, 1201.
- Wohlfarth K, Sycha T, Ranoux D, Naver H, Caird D. Dose equivalence of two commercial preparations of botulinum neurotoxin type A: Time for a reassessment? *Curr. Med. Res. Opin.* 2009, 25, 1573–1584.
 - Yablon SA, Brashear A, Gordon MF, Elovic EP, Turkel CC, Daggett S et al. Formation of neutralizing antibodies in patients receiving botulinum toxin type A for treatment of post stroke spasticity: a pooled-data analysis of three clinical trials. *Clin Ther* 2007; 29:683–90.
 - Young RR. Spasticity: A review. *Neurology* 1994;44(S9):S12–S20.
 - Yun JY, Kim JW, Kim HT, Chung SJ, Kim JM, Cho JW, Lee JY, Lee HN, You S, Oh E et al. Dysport and Botox at a ratio of 2.5:1 units in cervical dystonia: A double-blind, randomized study. *Mov. Disord.* 2015, 30, 206–213.
 - Zoons E, Dijkgraaf MGW, Dijk JM, van Schaik IN, Tijssen MA. Botulinum toxin as treatment for focal dystonia: A systematic review of the pharmaco-therapeutic and pharmaco-economic value. *J. Neurol.* 2012, 259, 2519–2526.
 - Zorowitz RD, Wein TH, Dunning K, Deltombe T, Olver JH, Davé SJ, Dimyan MA, Kelemen J, Pagan FL, Evans CJ, Gillard PJ, Kissela BM. A Screening Tool to Identify Spasticity in Need of Treatment. *Am J Phys Med Rehabil.* 2017;96(5):315-320. doi: 10.1097/PHM.0000000000000605

Appendix

SPQR – SPasticity Questionnaire in Real Life

Istruzioni: il seguente questionario riguarda le Sue capacità di eseguire alcune azioni con la parte del corpo colpita dall'ictus. Risponda a **ogni domanda** selezionando una delle quattro voci.

La spasticità è la rigidità dei muscoli dovuta all'ictus e non la debolezza dei muscoli. Nell'ultima settimana quanto la sua spasticità ha influenzato le seguenti attività quotidiane?

	Sede	Item	Per nulla 0	Poco 1	Abbastanza 2	Molto 3
1	AA	Uscire dal letto	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2	AA	Vestirsi al mattino	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3	AA	Mettersi le scarpe	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4	AA	Sedersi sulla sedia/carrozzina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5	AA	Alzarsi dalla sedia/carrozzina	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6	AI	Camminare dentro casa	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7	AI	Camminare fuori casa	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8	AI	Fare le scale	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9	AS	Afferrare un oggetto	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10	AS	Prepararsi un pasto	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11	AS	Utilizzare entrambe le mani per mangiare	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12	AS	Lavarsi braccio/mano paralizzata	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
13	AA	Lavarsi tutto (doccia o bagno)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
14	AA	Svestirsi la sera	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
15	AI	Andare al letto	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Risultati			AS _____	AI _____	Globale ____ / 45	