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FIELD: MEDICINE

BOTULINUM TOXIN INJECTION IN SPASTIC MUSCLE IN STROKE PATIENTS, GUIDED BY ULTRASOUND, ELASTOGRAPHIC PROCESSING, AND MUSCLE MAPPING

DOCTORAL THESIS ABSTRACT

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I. GENERAL PART

1. Post-Stroke Spasticity – General Considerations and Functional Implications

1.1. Definition and Clinical Relevance

Post-stroke spasticity is characterized by a complex etiopathogenic and clinical profile, requiring a broad and strategically structured diagnostic and therapeutic approach (1–5).

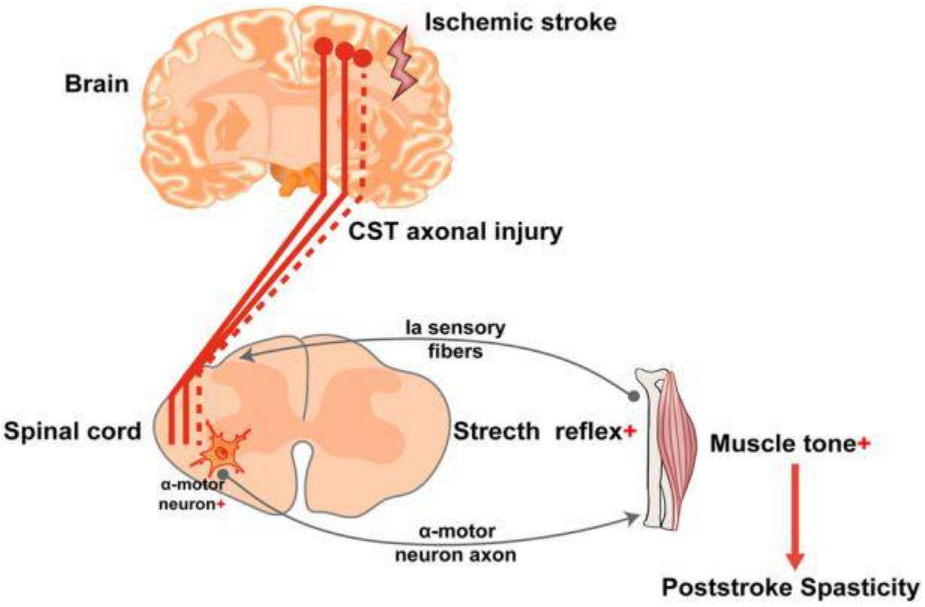
In 2023, the World Health Organization (WHO), through the World Stroke Organization (WSO), proposed a new definition of spasticity, based on the scientific contributions of Li et al. According to this definition, spasticity is a sensorimotor disorder caused by a lesion of the central nervous system and characterized by intermittent or sustained activation of muscles (6,7). Spasticity is part of a broader spectrum of neuromotor abnormalities, including hyperreflexia, involuntary cocontraction, spastic dystonia, synkinesis, and recruitment pattern changes (6–8).

Early and accurate recognition of spasticity is essential for initiating integrative, multimodal therapeutic strategies.

1.2. Pathophysiology of Post-Stroke Spasticity

Post-stroke spasticity is a complex pathophysiological process resulting from dysfunction of the central nervous system, particularly due to damage to descending inhibitory pathways, such as the corticospinal tract. This leads to the loss of cortical control over spinal reflexes and increased reflex excitability at the spinal level. The imbalance between alpha motoneuron excitability and the inhibitory control of spinal interneurons favors the emergence of exaggerated stretch reflexes and abnormal motor patterns, limiting functional movement. This reflects a neuronal reorganization process that is adaptive, but often maladaptive.

In patients with upper motor neuron syndrome (UMNS), muscular contractures significantly contribute to hypertonia (9–11), which includes both a reflex component (spasticity) and a non-reflex (intrinsic) component, independent of movement velocity. Clinically distinguishing between these components is challenging, especially in cases of fibrosis without muscle shortening (12,13). It is believed that muscle contracture facilitates the transmission of stretch forces to muscle spindles, thereby amplifying spasticity (8,14).



Pathophysiology of post stroke spasticity, assossiated with the corticospinal tract. Adapted after Wang et al., and *et al.*

1.3. Impact of Spasticity on the Upper Limb and Global Functionality

Post-stroke spasticity profoundly affects patient functionality, going beyond visible motor deficits. Upper limb impairment involves not only difficulties in performing voluntary active movements—such as grasping, manipulating objects, or completing daily tasks—but also compromises the passive function of the affected segment, which is essential for personal hygiene, dressing, or correct spatial positioning (15).

Over time, spasticity evolves through a combination of neurogenic components and muscular biomechanical changes, leading to stiffness, contractures, and joint deformities (16,17). Without treatment, these changes can become irreversible, significantly hindering rehabilitation participation. Evaluation is performed using various clinical and functional scales or robotic assessments (2,18), with the primary goal of preventing structural changes and maintaining optimal conditions for functional recovery (19–21).

2. Botulinum Toxin – Clinical Use in Post-Stroke Spasticity

2.1. Botulinum Toxin Between Toxicology and Therapy: Historical Evolution, Clinical Forms, and Classifications

Historical Overview and Characteristics of Botulinum Toxin

Botulinum toxin was first identified in 1895 by Emile van Ermengem, who associated *Clostridium botulinum* with a foodborne botulism outbreak (van Ermengem, 1897) (22,23). Earlier clinical descriptions were provided by Justinus Kerner (1817–1822), who anticipated the therapeutic potential of the toxin when administered in controlled doses (Kerner, 1822) (22,24). Later, in the 1940s–1950s, Dr. Edward Schantz isolated the toxin in the context of biological weapons research (Schantz, 1980) (22,24-26). The first medical applications were introduced by Dr. Alan Scott for the treatment of strabismus under the name Oculinum (Scott, 1980) (27), which later became known commercially as Botox® (22,24,27).

Microbiological Characteristics

C. botulinum is a Gram-positive, strictly anaerobic, spore-forming bacillus, commonly found in soil and animal environments (CDC, 2020) (28).

Classification and Toxin Types

There are seven main types of botulinum toxin (A–G), with an additional type (H) discovered in 2013, noted for its extreme toxicity (29-32). Types A, B, and E are most commonly implicated in human botulism (33,34). Type A toxin is the most widely used in medicine, acting by inhibiting acetylcholine release (Botox®, Dysport®). Type B (Myobloc®) is used less frequently, primarily in resistant dystonia cases (30).

Clinical Forms and Diagnosis

Botulism may occur in foodborne, wound, infant, iatrogenic, or inhalational forms. Diagnosis is based on toxin identification in biological samples and must be differentiated from other neuropathies (29).

2.2. Mechanism of Action of Botulinum Toxin

Botulinum toxin is a bacterial neurotoxin produced by *Clostridium botulinum*, with a specific inhibitory effect on neuromuscular transmission. Structurally, the toxin is a polypeptide chain of approximately 150 kDa that becomes biologically active through proteolytic cleavage.

This process yields two functional fragments: a heavy chain (100 kDa) and a light chain (50 kDa), connected by a disulfide bond (35,36). The heavy chain contains two functional domains: one responsible for intracellular translocation and another mediating binding to specific cholinergic receptors on the presynaptic nerve terminals. After binding to these receptors, the toxin enters the motor neuron through receptor-mediated endocytosis.

The translocation domain then facilitates the release of the light chain into the neuronal cytoplasm. This light chain acts as a zinc-dependent endopeptidase, catalytically targeting essential proteins of the SNARE complex (Soluble N-ethylmaleimide-sensitive factor Attachment protein REceptor). The SNARE complex—composed of SNAP-25 (Synaptosomal-associated protein 25), synaptobrevin (VAMP – Vesicle-associated membrane protein), and syntaxin—is involved in the fusion of synaptic vesicles with the presynaptic membrane and the release of acetylcholine into the synaptic cleft (30,36).

Depending on the botulinum toxin serotype (A–G), the specific target within the SNARE complex varies. For example, serotype A—the most commonly used clinically—cleaves SNAP-25, while serotype B targets synaptobrevin. By cleaving these proteins, the toxin blocks vesicle fusion and acetylcholine release, ultimately inhibiting nerve impulse transmission to muscle fibers.

The result is temporary muscular relaxation in the injected muscles, reducing pathological muscle tone—an essential aspect in the clinical management of spasticity. Although reversible, synaptic function recovery and reinnervation generally take 3 to 4 months, during which patients benefit from an optimal therapeutic window for active rehabilitation and neuromotor remodeling (17,19,25,26).

2.3. Indications, Dosage, and Administration Techniques in the Context of Post-Stroke Spasticity

Type A botulinum toxin (BoNT-A) is indicated for the treatment of focal spasticity following stroke, targeting both upper and lower limbs. This intervention is recommended for:

1. reducing increased muscle tone that interferes with function or hygiene;
2. preventing contractures and joint deformities;
3. serving as an adjunct in rehabilitation programs by facilitating mobility and functionality.

Tolerability and Adverse Effects

Botulinum toxin has an excellent safety profile when administered properly. The incidence of adverse reactions increases with cumulative doses or frequent injections, making it essential to rotate the targeted muscle groups.

Recommended Doses

BoNT-A dosage depends on spasticity severity, the number of muscles involved, and prior treatment response. According to clinical guidelines, standard doses of abobotulinum toxin (Dysport®) can reach up to 1500 units for both upper and lower limbs, injected into the target spastic muscles.

The total dose per session should not exceed 500 units to minimize adverse effects. The clinical effect of BoNT-A typically appears within 3–7 days post-injection, peaks at 4–6 weeks, and lasts approximately 12 weeks. Re-treatment may be considered at intervals of no less than 12 weeks, depending on the clinical response and therapeutic goals.

3. Modern Tools for Guidance and Assessment of Botulinum Toxin Injection

Injection Techniques under Guidance

Botulinum toxin type A (BoNT-A) is administered via direct intramuscular injections into spastic muscles. To improve precision, guidance is recommended through:

1. **Electromyography (EMG)** – identifies active muscles and locates hyperactive targets.
2. **Electrical stimulation** – confirms needle placement within target muscle.
3. **Ultrasound/elastography (MSK-US / Elasto-US)** – provides real-time imaging of muscle structures and vasculature, reducing the risk of complications.

These guidance techniques enhance treatment efficacy and minimize side effects.

3.1. Ultrasound Guidance in Rehabilitation and Botulinum Toxin Injection

Ultrasound offers real-time visualization of relevant anatomical structures, increasing therapeutic accuracy—especially for deep or atrophied muscles. Two main needle insertion techniques are used:

- **In-plane**: visualizes the needle along its length.
- **Out-of-plane**: visualizes the needle in cross-section.

A further advantage of musculoskeletal ultrasound (MSK-US) is real-time measurement of muscle thickness and echogenicity, aiding precise dosage and tailored therapeutic planning (39,40).

3.2. Musculoskeletal Elastography – Principles, Applications, and Functional Correlations

Elastography is a non-invasive imaging technique that has significantly advanced over recent decades. It quantifies tissue stiffness and complements conventional ultrasound by providing biomechanical biomarkers for a range of chronic and acute conditions. In post-stroke spasticity, it assesses structural changes in soft tissues effectively.

Technically, elastography evaluates tissue deformation under controlled pressure. The **strain elastography** method yields color-coded, semi-quantitative maps of elasticity—but results depend on operator skill and compression control.

Clinical Applications:

- **Muscle stiffness estimation**: Clear differences seen between spastic and healthy muscles, with elastographic values correlating well with clinical instruments (41,42);

- **Treatment monitoring**: Enables objective tracking of reduced stiffness post-BoNT-A injection, reflecting therapeutic effect (41,43).
- **Treatment optimization**: Targets muscles with increased stiffness to guide dosage and injection locations (44).
- **Functional correlation**: Elevated stiffness in forearm flexors correlates with major functional limitations (e.g., dressing, feeding) (42–45).

Elastography thus supports improved understanding of spasticity and aids rehabilitation planning.

3.3. Muscle Mapping – History, Motor Endplate Localization, and Treatment Optimization

Historical Milestones

- **19th century**: Early anatomical and physiological observations of neuromuscular junctions (NMJ) via simple dissections and optical microscopy. Metal impregnation (gold, silver) revealed nerve endings and led to the concept of motor endplates (Doyère, de Quatrefages, Krause) (46-48).
- **20th century advances**: Electron microscopy confirmed the synaptic cleft and NMJ structure, reinforcing Cajal's neuron doctrine (49,50). Pharmacological and electrophysiological studies (1930–1970) identified acetylcholine as the synaptic mediator and clarified NMJ function (50-54).
- **Modern era**: Immunohistochemistry, fluorescent microscopy, patch-clamp, and labeled toxins (e.g., α-bungarotoxin) enabled molecular-level investigation of cholinergic receptors (nAChR), MuSK, and LRP4—key components in NMJ formation and maintenance (49,55,56). Electric fish models (Electrophorus, Torpedo) were essential for isolating nAChR (57). Techniques included anatomical dissections, metal impregnation, histological staining, electron microscopy, and advanced molecular imaging (49,58) (46,47) (59–61) (50–54) (62).

Endplate Localization: Advances and Clinical Applications

In recent decades, muscle mapping evolved significantly through the integration of advanced imaging methods like confocal microscopy, optical clearing, 3DISCO, and 3D digital reconstructions (50-54). Specific markers (e.g., α-BTX 647), combined with specialized software, allowed detailed mapping of motor endplates (MEP) and their correlation with nerve branching and motor units (52,54).

Animal and human studies using α-BTX 647 and verifying results through CMAP (50,63) reduction demonstrated MEP distribution and changes post-denervation. Tissue clearing and 3D reconstructions revealed MEP clustering patterns that disintegrate after denervation (54,63,64).

Practical Relevance for BoNT-A Injection

For example, mapping in the brachialis muscle suggests injections near the endplate zone located at 75% of the coracoid-to-medial epicondyle line, with medial and lateral approaches. EMG and ultrasound guidance account for individual anatomical variability (63,64).

Optimizing Treatment via Muscle Mapping and Imaging Guidance

Modern muscle mapping confirms that MEP clusters correspond to distinct motor units, enabling segmented therapeutic injections. Optimal BoNT-A injection zones are located in 3–4 regions along the muscle axis, ranging from 15 mm to 110 mm distal to the elbow crease. These include:

- **PLP** (proximal): for intrafascicular motor units.
- **DLP** (distal): for units extending to the tendon.

In practice, localization requires EMG or ultrasound guidance due to individual variation.

EMG and ultrasound are essential and complementary: EMG identifies motor unit electrical activity and endplate regions, while ultrasound visualizes muscle structure and nerve pathways, guiding precise injections. Elastography adds data on tissue stiffness, critical for assessing structural changes. Combined, these methods enhance treatment efficacy and safety.

II. PERSONAL CONTRIBUTIONS

4. Working Hypothesis and General Objectives

4.1. Working Hypothesis

The repeated administration of BoNT-A, integrated into a multimodal rehabilitation protocol, produces progressive and quantifiable functional improvements of the spastic upper limb post-stroke. The use of imaging methods such as ultrasound and elastography allows for the optimization of dosage, muscle selection, and monitoring of therapeutic effect.

4.2. Main General Objective

1. To establish a set of objective and reproducible criteria for the personalization of botulinum toxin treatment in post-stroke spasticity, based on an integrated analysis of clinical, functional, and paraclinical response.

4.3. Secondary General Objectives

1. To determine the parameters of clinical efficacy for focal botulinum toxin treatment at the level of the upper limb, through the functional evaluation of spastic muscle groups in the upper limb;
2. To determine the parameters of clinical efficacy for focal botulinum toxin treatment at the level of the upper limb, through the global functional assessment of the patient using standardized clinical scales;
3. To determine the parameters of paraclinical efficacy, through the use of imaging methods such as ultrasound and elastography, within various therapeutic strategies for botulinum toxin injection and to correlate these with the clinical and functional evolution of the patient.

5. General Research Methodology

5.1. Type and Structure of the Study

The present research was designed as a clinical, observational, longitudinal, and non-randomized study, conducted within an inpatient neurorehabilitation unit. The study unfolded in two complementary stages, encompassing two major components conducted in parallel over a 30-month period:

- a longitudinal, non-randomized clinical observational study involving a final sample of 47 patients (completers), each assessed over the course of three consecutive treatment cycles with botulinum toxin type A (BoNT-A), followed by a standardized medical rehabilitation program;
- a case series (n = 4) including patients at various stages of post-stroke chronicity (ranging from 2 months to 14 years post-incident), providing an in-depth qualitative insight into the therapeutic particularities and individual responses across the recovery continuum.

5.2. Participants and Inclusion Criteria

Participants were selected from an initial cohort of 253 patients evaluated in the neurorehabilitation clinic. A total of 68 patients who met the study criteria were included.

Inclusion criteria: written informed consent; age between 18 and 80 years; confirmed diagnosis of stroke at least two months prior; presence of spasticity in one or more segments (shoulder, elbow, wrist, fingers); availability of standardized assessments at T0 (pre-treatment) and T1 (28 ± 5 days post-treatment), including: motor control, spasticity and paresis angles, MAS score, muscle tone (MRC), active range of motion (ROM), GAS and Barthel scores, and perceived pain.

Exclusion criteria: history of recurrent stroke; intrathecal baclofen treatment; recent administration of BoNT-A (<3 months); prior adverse reactions to BoNT-A; severe cognitive deficits or aphasia; MAS score of 1 or 4; severe contractures, arthrodesis, or heterotopic ossification; refusal to participate.

Of the initial cohort, 185 patients were excluded. During the follow-up period, 21 patients were lost to follow-up. Final analysis included 52 patients who completed the first treatment cycle, 51 who completed the second, and 47 who completed all three therapeutic cycles.

5.3. Study Design

Although the total study duration was 30 calendar months, each patient followed an individual protocol lasting between 5 and 10 months.

Evaluation points for each treatment cycle were:

1. **T0** – initial assessment (upon admission, prior to BoNT-A injection and rehabilitation program);
2. **T1** – follow-up assessment (28 ± 5 days post-injection).

Treatment consisted of BoNT-A injections targeting spastic muscle groups of the affected upper limb and participation in a standardized rehabilitation program, including individualized therapeutic physical exercise, robotic therapy, virtual reality (VR) therapy, electrotherapy, and adjuvant methods such as massage, positioning, orthotic devices, and speech therapy.

The program was tailored to each patient's initial functional level, comorbidities, and exercise tolerance, aiming to maximize the effects of BoNT-A.

Primary objectives included:

1. reduction of muscle hypertonia;
2. improvement of voluntary motor control;
3. increase in active and passive range of motion;
4. reduction of pain and disability.

Each treatment cycle occurred every three months, with a total of three consecutive cycles documented per patient.

5.4. Evaluation and Treatment Procedures

Validated clinical and imaging tools were used for evaluating post-stroke spasticity. Segmental and global spasticity were assessed using the Tardieu Scale and the Modified Ashworth Scale (MAS), while voluntary motor control was evaluated using the Medical Research Council (MRC) scale. Global functional status was assessed via the Barthel Index and the Reintegration to Normal Living Index (RNLI), and segmental function via tone scores, goniometric ROM, ARAT, FM-UE, and the Chedoke McMaster scale. Pain intensity was measured using the Numeric Rating Scale (NRS), and functional goal achievement was assessed using the Goal Attainment Scale (GAS) (8,33,34).

BoNT-A (Dysport®) injections were administered under ultrasound and elastographic guidance, with personalized dosing based on spasticity severity, muscle mass, and treatment history, not exceeding 1500 U per session (68-71). Targeted muscles included groups responsible for shoulder adduction, elbow flexion, forearm pronation, and

wrist and finger flexion. Doses ranged from 50–200 U/mL depending on the goal (complete blockade vs. tone modulation). In cases of muscle fibrosis, injections were avoided in sclerotic zones to minimize the risk of uncontrolled diffusion. Each injection cycle was repeated approximately every 12 weeks, followed by four weeks of standardized active rehabilitation.

5.5. Statistical Analysis

Data were analyzed using IBM SPSS Statistics v25, with a significance threshold set at $p < 0.05$ (72,73). Results were described using means and standard deviations or medians and interquartile ranges, depending on distribution (72,74).

Group comparisons employed the Kruskal-Wallis test and post-hoc Mann-Whitney U tests with Bonferroni correction (75,76). Correlations were assessed using Spearman or Pearson coefficients, depending on variable distribution (73,77). Predictive analysis involved simple and multiple linear regression models (74,76), and changes in scores were tested using the Wilcoxon signed-rank test (65,67,78).

The modified GAS-T score was used as the primary outcome variable, calculated per the standardized methodology including goal weighting and attainment levels. The entire statistical approach adhered to medical statistical standards for observational studies, ensuring external validity and controlling for confounding factors (77).

5.6. Ethical Considerations

The study was approved by the institutional Ethics Committee (INRMFB approval code 1/07.01.2019), in accordance with national and European regulations on human subject research, including the Declaration of Helsinki.

All participants provided written informed consent. No experimental procedures were conducted.

Here is the English academic translation of the section "III. Personal Contributions – Study 1", with references maintained:

III. PERSONAL CONTRIBUTIONS

6. Study 1 – Targeted Interventions in Post-Stroke Spasticity: Functional and Imaging Approaches

6.1. Working Hypothesis and Specific Objectives

6.1.1. Working Hypothesis

The application of an individualized therapeutic protocol—based on strategic selection of target muscle groups, the establishment of functional objectives using the Goal Attainment Scale (GAS), and the use of modern guidance tools such as muscular mapping and elastography—results in a significant reduction in spasticity and superior functional recovery of the affected upper limb post-stroke, compared to conventional approaches.

6.1.2. Specific Objectives

Main specific objective:

To identify the elbow flexor muscle groups that can be differentially targeted to increase the efficacy of botulinum toxin in reducing spasticity and improving motor control and overall motor performance at the elbow.

Secondary specific objectives:

1. To measure the elbow spasticity angle using the Tardieu Scale following administration of abobotulinumtoxin A (BoNT-A), in order to determine the optimal therapeutic strategy for targeting the elbow flexor muscles (BB, BR, Brachioradialis) — assessment performed after the first treatment cycle with botulinum toxin;
2. To measure the elbow paresis angle using the Tardieu Scale following administration of abobotulinumtoxin A (BoNT-A) — assessment performed after the first treatment cycle;
3. To determine active supination mobility using a goniometer and the Tardieu Scale following administration of abobotulinumtoxin A (BoNT-A) — assessment performed after the first treatment cycle.

6.2. Materials and Methods

6.2.1. Study Design

This observational clinical study was conducted at the National Institute for Rehabilitation, Physical Medicine and Balneoclimatology in Bucharest, between March 2021 and December 2023. The study followed a prospective, observational, and non-randomized design and aimed to assess the effectiveness of botulinum toxin type A

(BoNT-A) treatment on upper limb spasticity post-stroke, as well as to validate modern functional and imaging assessment tools (GAS, muscular mapping, elastography).

6.2.2. Participants

Patient selection followed the research methodology described in the previous chapter.

6.2.3. Evaluation and Procedure

The study utilized a comprehensive set of validated tools to evaluate the response of post-stroke spastic patients to botulinum toxin therapy. Individualized functional goals were set and quantified using the Goal Attainment Scale (GAS), with the GAS-T score as the primary outcome measure (8,33,34).

Spasticity was assessed via the Modified Ashworth Scale (MAS) and the Tardieu Scale, focusing on the elbow flexor muscles. Motor control was evaluated using the Medical Research Council (MRC) scale, and pain was measured using the Numeric Rating Scale (NRS).

Treatment involved ultrasound-guided administration of abobotulinumtoxin A (Dysport®, Ipsen, Paris), the only formulation available in the institution (84). Doses were adjusted based on the severity and anatomical distribution of spasticity, in accordance with international guidelines and the institutional clinical algorithm (84,85,170,171,172). All evaluations and injections were performed by a single examiner to ensure procedural consistency.

To enhance precision, muscular mapping guided by ultrasound and targeting the motor endplates was employed, optimizing toxin distribution and minimizing the risk of adverse effects (137,139,141,149,173).

Strain elastography was used to assess muscle stiffness (biceps brachii, flexor digitorum profundus, pronator teres), and reduction in stiffness was correlated with post-treatment reductions in muscle tone (105,114,115,118,120).

The post-injection program consisted of 10 consecutive days of standardized rehabilitation (31,55,84,174), including two daily sessions of active/passive physical therapy, robotic therapy, and electrotherapy. Treatment efficacy was evaluated based on changes in the spasticity angle as measured by the Tardieu Scale.

6.3. Results

Out of the initial total, 52 patients completed the treatment protocol for the first cycle and were assigned to three groups based on the muscles injected with BoNT-A.

The mean age ranged between 53.4 and 59.6 years, with the proportion of female participants varying between 20% and 50%, depending on the group. Most patients had ischemic stroke, which was predominant across all groups, although the frequency of hemorrhagic forms varied from 11.1% to 40% across groups.

The functional GAS-T score was significantly influenced by several factors: patients with prior BoNT-A injections recorded higher scores, while those requiring orthoses showed less improvement. Furthermore, an increase in the Functional Independence Measure (FIM) score at discharge predicted a significant improvement in the GAS-T score.

Linear regression analysis revealed that the number of previous injections and the increase in muscle strength, quantified through the Medical Research Council (MRC) score at proximal, intermediate, and distal levels, were important predictors for achieving functional goals. Multiple regression analysis confirmed two independent statistical predictors for the improvement in the GAS-T functional score: the total number of prior BoNT-A injections and the level of muscle strength at the shoulder girdle.

Additionally, significant correlations identified through Spearman and Pearson tests demonstrated direct associations between the GAS-T score and several clinical parameters: reduction in spasticity, pain reduction, prior BoNT-A injection experience, and level of motor control.

The only demographic variables serving as predictors of the GAS score were left hemibody involvement and a history of BoNT-A administration, both associated with greater functional improvement on the GAS-T score.

Statistical analysis showed that a higher number of prior BoNT-A injections, especially at the elbow level, was associated with better GAS-T scores, suggesting a cumulative efficacy of the treatment. At the wrist level, increased motor control was a significant predictor of GAS score improvement. Moreover, increased motor control, particularly in the proximal region, had a significant impact on the achievement of functional goals.

The final GAS-T score was positively correlated with the reduction in spasticity, pain, and improvement in motor control.

Additionally, a post-intervention reduction in pain was associated with superior functional response, reinforcing the therapeutic value of implementing the present study’s hypothesis.

Non-parametric correlation analysis (Spearman rho) revealed a statistically significant relationship between the improvement in overall upper limb motor control and

the achieved GAS-T score ($\rho = 0.591$, $p < 0.001$). This moderate-to-strong positive correlation indicates that patients who demonstrated greater improvements in active motor control also had a higher likelihood of achieving their individually set functional goals. The result supports the notion that active motor improvement directly and significantly contributes to the success of personalized post-stroke rehabilitation.

Table 6.15 confirms that patients with more severe impairment often required orthotic devices, which may partially explain the less favorable evolution and lower improvement in GAS scores observed in these patients.

Therapeutic Strategies for Elbow Spasticity

A statistically significant difference was identified between groups regarding the total dose of botulinum toxin type A (BoNT-A) administered to the elbow flexors ($p < 0.001$). Additionally, statistically significant differences were observed between groups in terms of spasticity angle, paresis angle, and Modified Ashworth Scale (MAS) scores for the elbow flexors and the pronator teres muscle, as shown in Table 6.1a. The brachialis plus brachioradialis group received the highest mean dose of BoNT-A, while the brachialis-only group required the lowest dose.

In the comparative analysis of intergroup changes (Kruskal-Wallis test), statistically significant differences were obtained, after Bonferroni correction, for: active supination angle at both baseline and follow-up, MAS score for pronator teres at follow-up, and change in MAS score for pronator teres.

Post hoc Mann-Whitney U tests with Bonferroni correction confirmed significant differences between group pairs regarding the dose of BoNT-A administered to the elbow flexors.

The Kruskal-Wallis test did not identify statistically significant differences between groups for the BoNT-A dose administered to the pronator teres muscle ($p = 0.103$). Pairwise group analysis using the Mann-Whitney U test with Bonferroni correction did not reach the threshold of statistical significance; however, a trend toward significance was noted between the biceps brachii group and the brachialis plus brachioradialis group ($p = 0.033$, adjusted value 0.0167 after correction), suggesting that patients in the biceps brachii group required higher doses for the pronator teres. The differences in mean ranks (27.5 for the biceps brachii group vs. 19 for the brachialis plus brachioradialis group) support this observation, indicating the need for further studies with larger samples to confirm the trend.

The Kruskal-Wallis analysis also revealed a statistically significant difference in the initial paresis angle between groups ($p = 0.027$), with higher values in the brachialis plus brachioradialis group, followed by the biceps brachii group, while the brachialis group exhibited the lowest values. The Mann-Whitney U test showed a statistically significant difference between the brachialis group and the brachialis plus brachioradialis group at baseline ($p = 0.007$).

Regarding the paresis angle at follow-up, although the overall analysis did not reveal statistically significant differences between groups, the order of mean ranks suggested the greatest improvement in the brachialis plus brachioradialis group, followed by the biceps brachii group, with the least improvement seen in the brachialis-only group.

These results indicate that the brachialis plus brachioradialis group achieved the best progress in reducing the paresis angle and improving motor control, followed by the biceps brachii group, whereas the brachialis group showed a more modest response.

Results Related to Supination, Active Extension, Spasticity, and Stroke Onset

Analysis of the active range of motion (aROM) during supination revealed a significant difference between groups. Specifically, the brachialis plus brachioradialis group demonstrated the greatest improvement in elbow spasticity, showing the strongest correlation between changes in spasticity angle and stroke onset.

The biceps brachii group exhibited the strongest negative correlation for the change in supination angle, indicating minimal improvement as the time since stroke increased. In contrast, the brachialis plus brachioradialis group showed the most significant improvement in active supination. Regarding changes in active extension and paresis angle, the brachialis group demonstrated the strongest correlation with improvement, although it started from the lowest initial paresis angle.

6.4. Discussion

The results of this study confirm the efficacy of botulinum toxin type A (BoNT-A) in facilitating functional recovery in patients with post-stroke spasticity when integrated into a personalized rehabilitation program. The Goal Attainment Scale (GAS) proved to be a sensitive and reliable tool for quantifying functional progress and differentiating therapeutic response according to individual patient characteristics.

One of the most consistent findings was the positive association between motor control and the GAS-T score, as demonstrated by significant correlations with MRC scores at the shoulder, elbow, and wrist levels. This supports the hypothesis that regaining muscle

strength—particularly proximally—facilitates active participation in functional tasks. Sequential motor recovery from proximal to distal appears to have a direct impact on the patient's ability to achieve rehabilitation goals.

Another noteworthy finding was the cumulative effect of repeated BoNT-A administrations. Statistical data showed that the total number of prior injections was significantly correlated with functional improvement, with each additional administration being associated with a progressive increase in GAS-T score. This phenomenon may be explained by a neuromuscular adaptation facilitated by repeated therapeutic exposure and supported by appropriate rehabilitation protocols.

Furthermore, multiple regression models identified independent predictors of functional progress, notably the increase in MRC values for the shoulder girdle musculature and the total number of previous BoNT-A administrations.

An apparently unexpected result was the negative correlation between post-injection orthosis/splint use and functional progress. However, this observation should be interpreted cautiously, as the need for orthoses is more likely an indirect marker of initial spasticity severity and functional limitations rather than a therapeutic barrier per se. This is supported in the present context by the statistically significant correlation between lower Barthel Index values and orthosis use.

Pain experienced in the affected limb was negatively correlated with the GAS-T score, emphasizing the importance of pain control in the rehabilitation process. Even in the absence of adjunct pharmacological treatment, BoNT-A demonstrated beneficial effects on muscular discomfort, confirming its indirect analgesic role through reduction of tone and abnormal muscle activity.

The rigorous statistical analysis, including linear and multiple regression models, provided a high level of internal and external validity, confirming the robustness of the results and the clinical relevance of the variables investigated. The significance of the coefficients obtained supports the use of GAS-T not only as an assessment tool but also as a guide for monitoring and adapting therapeutic interventions.

Forearm Position in the Evaluation of Elbow Flexor Spasticity

According to the literature (138,175–177), the contribution of elbow flexors varies with forearm position, with post-stroke spasticity being more severe in pronation and neutral positions. Therefore, in this study, assessments were performed with the forearm in pronation, taking into account the lack of dynamic EMG and the characteristics of type IV spasticity (28).

Here is the English academic translation of the provided text, with references maintained:

Target Muscle Selection for Elbow Spasticity

The selection of target muscles for botulinum toxin type A (BoNT-A) treatment in focal post-stroke spasticity, particularly in type IV spasticity patterns (28), remains a topic of ongoing debate in the literature without a clear therapeutic consensus (60,178,179). Although the biceps brachii has historically been the most frequently targeted muscle in clinical practice—due to its anatomical accessibility and low risk of adjacent structure injury (25,138,176,177,180)—recent data suggest that the brachialis (138) and brachioradialis (181,182) may be more effective choices, either individually or in combination. From a biomechanical perspective, the brachialis appears to require lower doses of BoNT-A for comparable efficacy relative to the biceps brachii (Table 6.17), and its combination with the brachioradialis may provide additional benefits, particularly in clinical scenarios involving abnormal postures or increased tone in the intermediate flexor and pronator muscles of the affected upper limb (176,181,182).

In the present study, the brachialis plus brachioradialis group exhibited a high degree of spasticity at baseline (T0), which explained the need for a higher total dose of BoNT-A. Genet et al. (138) argue that the brachialis is the primary elbow flexor and that combining it with the brachioradialis offers no additional advantage. However, the data from this study indicate that this combination yielded superior clinical-functional outcomes, possibly due to the brachioradialis' contribution to pronation-related functional movements (176,181,182). This finding underscores the importance of tailoring muscle selection based on the clinical spasticity pattern.

Primary and Secondary Outcomes: Key Conclusions and Implications of Elbow Spasticity

The analysis of primary and secondary outcomes demonstrated significant improvements within each group; however, between-group differences after Bonferroni correction were limited to a few key parameters. A strong correlation was observed between active range of motion (ROM) and the angle of paresis both at baseline and at follow-up, contradicting findings from a previous study which claimed that arm function does not improve following injection (54). In our study, active ROM did increase, but it cannot be definitively stated that overall elbow functionality improved, which constitutes a limitation of the present research. Furthermore, although previous literature (54) suggested

that the MAS score improves regardless of the muscle injected, our data revealed meaningful differences related to specific muscle selection.

The brachialis plus brachioradialis group received the highest BoNT-A dose and achieved the greatest improvements in supination, requiring a lower dose for the pronator teres muscle compared to the biceps brachii group, which required the highest dosage (183). Nevertheless, treatment strategies aimed at reducing total dose—for example, injecting only the brachialis—may be useful for avoiding the maximum recommended dosage and optimizing toxin resource management (183). Regarding active ROM, although statistical significance was not reached, the rank order indicated the largest gains in the brachialis plus brachioradialis group, followed by the biceps brachii group, suggesting a more substantial role of the brachioradialis muscle in controlling active movement and mitigating spasticity.

Additional Significant Findings Related to Elbow Spasticity

The additional analysis revealed significant differences between groups regarding active supination range of motion (ROM) at both baseline and follow-up, as well as MAS scores for the pronator teres at follow-up and the change in MAS scores for this muscle. The brachialis plus brachioradialis group showed the greatest improvement in active supination (Table 6.17, Figure 6.6). This result may be explained by anatomical features described in the literature: the biceps brachii, being a biarticular muscle dependent on scapular stability, plays a limited role in pure elbow flexion among hemiplegic patients (53,138), whereas the brachialis and brachioradialis, with their pennate structure and direct action at the elbow joint, contribute more effectively to flexion (138).

Moreover, a trend toward statistical significance was observed for the BoNT-A dose administered to the pronator teres, with the highest doses recorded in the biceps brachii group and the lowest in the brachialis plus brachioradialis group. This may reflect current clinical practices that favor certain muscle selections, potentially compromising the supinator function of the biceps brachii and leading to increased spasticity in the pronator teres, thereby requiring higher doses. However, the lack of data regarding previous BoNT-A treatments and prior injection sites represents a limitation of this study.

Impact of Stroke Chronicity on Primary and Secondary Outcome Parameters

Spearman correlation analysis revealed significant associations between time since stroke onset and several clinical parameters, supporting the hypothesis that the spasticity profile and treatment response differ depending on the target muscle and stroke chronicity. The brachialis plus brachioradialis group showed the strongest association with

improvements in passive extension at maximum speed (V3), indicating the highest efficacy in reducing elbow spasticity. Conversely, the biceps brachii group demonstrated the greatest effectiveness for passive extension at low speed (V1), which may reflect this muscle's adaptation to spasticity, its role in muscular coordination, and its eccentric and multiarticular properties, making it more resilient during passive movements—particularly in the chronic phase of post-stroke recovery.

With respect to supination, the biceps brachii group exhibited the strongest negative correlation between time since stroke and change in supination angle, indicating reduced improvement over time. In contrast, the brachialis plus brachioradialis group achieved the best results in improving active supination. Additionally, the brachialis-only group showed the strongest correlation for improvements in active extension and the angle of paresis, despite starting with the lowest baseline paresis angle—thus confirming its effectiveness in this domain.

6.5. Partial Conclusions

The study confirms the efficacy of botulinum toxin type A (BoNT-A) in the treatment of post-stroke spasticity, demonstrating clear benefits on upper limb functionality and on achieving personalized therapeutic goals, as assessed by the GAS-T score.

Improvement in motor control—particularly at the proximal level of the shoulder girdle—and a greater number of prior BoNT-A administrations were significantly correlated with functional progress. This cumulative effect suggests that repeated injectable therapy, when combined with a comprehensive medical rehabilitation program, leads to superior motor and functional recovery.

The use of orthoses was associated with more modest functional progress, likely reflecting more severe initial clinical impairment in those cases. Additionally, pain reduction contributed to achieving functional goals, reinforcing its relevance in rehabilitation outcomes.

The brachialis plus brachioradialis group demonstrated the most visible functional improvements, highlighting the importance of muscle selection and personalized dosing. Although the biceps brachii is frequently selected due to its anatomical accessibility and the simplicity of injection procedures, targeting the brachialis—alone or in combination with the brachioradialis—may offer superior advantages, requiring lower doses of BoNT-

A and optimizing functional outcomes, particularly in patients presenting with pronounced flexion and pronation patterns.

These findings support the premise that muscle selection should be customized based on the clinical spasticity pattern, with the brachialis and brachioradialis combination proving to be the most effective strategy in advanced spasticity. This approach had a positive impact on motor control and functional movements of the affected upper limb.

The analysis of primary and secondary outcomes revealed significant improvements across all groups, with a strong correlation between active range of motion (ROM) and the angle of paresis at both baseline and follow-up. These results contradict some earlier findings in the literature that reported no functional gains post-injection (54), and emphasize the importance of target muscle selection, as relevant differences were observed based on the specific muscles injected.

Furthermore, the strategy involving the brachialis and brachioradialis combination proved to be the most effective in improving active supination, while also requiring a lower dose for the pronator teres compared to the biceps brachii group, which necessitated the highest dose (183). This underlines the potential of treatment strategies that aim to minimize the total BoNT-A dose (e.g., injecting brachialis only), which can help avoid exceeding maximum recommended doses and better manage toxin resources (183).

With respect to active ROM, although statistical significance was not achieved, the ranking of mean scores indicated the greatest improvements in the brachialis plus brachioradialis group, followed by the biceps brachii group, suggesting a greater role for the brachioradialis in active motion control and spasticity management.

Additional study findings confirmed significant group differences in active supination and MAS scores for the pronator teres, with the most notable improvements recorded in the brachialis plus brachioradialis group. These results underscore the anatomical and functional advantages of these muscles in controlling elbow flexion and supination, compared to the biceps brachii, whose contribution is limited in hemiplegic patients due to its reliance on scapular stability.

Moreover, the trend toward higher BoNT-A doses required for the pronator teres in the biceps brachii group suggests that initial target muscle selection may indirectly influence the spasticity of other muscle groups.

The analysis confirmed that stroke onset significantly influences treatment response and spasticity profile, with outcomes varying by target muscle and stroke chronicity. The brachialis plus brachioradialis group proved most effective in reducing elbow spasticity,

showing the best improvements in high-velocity passive extension and active supination. In contrast, the biceps brachii group responded better to slow passive extension—likely due to this muscle's adaptive role in spasticity and its structural characteristics. The brachialis-only group demonstrated the highest efficiency in improving active extension and the angle of paresis, thus confirming its important role in motor control among patients with post-stroke spasticity.

7. Study 2 – Longitudinal Clinical Aspects in the Treatment of Post-Stroke Spasticity

7.1. Working Hypothesis and Specific Objectives

This section presents the results of the clinical research conducted between March 2021 and December 2023 within the Neurorehabilitation Department of the National Institute of Recovery, Physical Medicine and Balneoclimatology in Bucharest. The study monitored the functional evolution of patients with post-stroke spasticity across three consecutive treatment cycles, each spaced three months apart.

This second stage of the research represents both a comprehensive longitudinal analysis of the prospective, observational, non-randomized, and non-interventional clinical study—focused on the clinical-functional progression of patients with post-stroke spasticity throughout three consecutive treatment cycles—and a longitudinal analysis of a case series involving four patients. The aim of this dual approach was to allow for an in-depth, individualized perspective on the complex clinical presentation, variable functional evolution, and response to personalized treatment in this multifaceted medical condition, represented by the post-stroke spasticity patient.

In accordance with the research methodology outlined in the previous chapter, assessments were performed before and after each treatment cycle and included a comprehensive set of globally validated and widely used clinical tools: the Modified Ashworth Scale (MAS) and the Tardieu Scale for muscle tone, the Medical Research Council Scale (MRC) for muscle strength, and passive and active range of motion measurements (pROM/aROM) via goniometry, as well as pain assessment using the Numeric Rating Scale (NRS).

The clinical-functional status of the patients was evaluated using the aforementioned methods, in order to establish individualized functional goals. In parallel, paraclinical evolution was monitored with an increased level of objectivity using imaging investigations, specifically musculoskeletal ultrasound and elastography.

Each patient underwent three consecutive treatment sessions combining BoNT-A administration at optimally personalized intervals—based on international guidelines, pharmaceutical recommendations, and individual functional evolution. The data collected allowed for both individual and group-level comparative analysis of progress across treatment cycles.

With regard to the case series methodology, additional clinical instruments were incorporated—such as the Action Research Arm Test (ARAT), Chedoke-McMaster Stroke Assessment (CMSA), CAHAI (Chedoke Arm and Hand Activity Inventory), the Walking Index, and the Fugl-Meyer Assessment (FMA and FM-UE), as well as the Reintegration to Normal Living Index (RNLI)—tailored to each patient based on their specific clinical presentation and functional rehabilitation objectives.

The core structure of the non-experimental therapeutic intervention followed the same principles established by the study and consisted, for both the cohort and the case series, of targeted BoNT-A administration to selected spastic muscles of the affected upper limb, followed by a standardized medical rehabilitation protocol initiated after focal toxin administration.

7.1.a. Working Hypothesis and Specific Objectives for the Case Series

Working Hypothesis

The administration of botulinum toxin type A (BoNT-A), when integrated into a personalized assessment and treatment strategy adapted to the stage of post-stroke recovery, leads to superior functional improvements when applied early, compared to later stages of spasticity.

Specific Objectives

- **Main Specific Objective**:

To evaluate the stepwise clinical-functional outcomes of BoNT-A treatment in patients with post-stroke spasticity at different stages of evolution, using validated functional scales, with the goal of substantiating a personalized, stage-dependent intervention protocol.

- **Secondary Specific Objectives**:

1. To illustrate functional evolution through detailed description of four clinical cases, selected for their exemplar and descriptive value, representing distinct clinical scenarios of post-stroke upper limb spasticity at different recovery stages—acute, subacute, chronic, and late chronic;
2. To determine the correlation between the timing of BoNT-A administration and the magnitude of functional gain achieved, as expressed by changes in clinical-functional scale scores;
3. To identify therapeutic response differences based on the initial distribution and severity of spasticity, in the context of individualized BoNT-A treatment.

7.1.b. Working Hypothesis and Specific Objectives for the Observational Clinical Study

Working Hypothesis

Systematic administration of BoNT-A, integrated into a personalized rehabilitation program, produces significant medium-term improvements in motor function, muscle tone, and the achievement of individualized functional goals.

Specific Objectives

- **Main Specific Objective**:

To monitor, both clinically and paraclinically, the global and segmental functional evolution of the upper limb in a cohort of 52 patients treated through three successive cycles of BoNT-A combined with rehabilitation, with evaluations conducted after the second and third treatment cycles.

- **Secondary Specific Objectives**:

To track the progression of global and segmental upper limb functionality after each of the three treatment and rehabilitation cycles involving BoNT-A;

To analyze the correlation between the post-stroke recovery stage and the therapeutic response observed following the second and third treatment cycles.

7.2. Materials and Methods

7.2.a. Case Series

7.2.a.1. Patient Selection

Patients were selected using a convenience sampling method from the patient population of the neurorehabilitation department, with a confirmed diagnosis of stroke based on imaging, and clinically evident spasticity.

The selection process, as well as the inclusion and exclusion criteria, are detailed in the previous chapter and were applied identically to those used for participants in the clinical study, in accordance with the established methodology.

The cases included in the analysis were deliberately chosen to represent a broad spectrum of post-stroke recovery stages—acute, subacute, chronic, and late chronic—as well as varied levels of spasticity and different degrees of initial motor control. This heterogeneity allowed for a detailed comparative analysis of clinical trajectories and therapeutic responses, depending on each patient's individual context.

Case Study 1 – Shoulder Pain and Functional Limitation

A 66-year-old male with chronic left spastic hemiparesis following a right capsulo-lenticular hemorrhagic stroke (June 2019) presented with significant motor impairment (MRC 2/5 proximal, 1/5 distal), high spasticity (MAS 2–3), and neuropathic/mechanical pain, predominantly in the shoulder and elbow. Ultrasound confirmed Achilles tendon retraction and periarticular calcifications. Orthoses were required for limb positioning and gait.

Three BoNT-A injection sessions (December 2021–July 2022) targeted spastic upper and lower limb muscles, with doses up to 300 U for biceps brachii and 200 U for soleus. Despite repeated treatment, functional improvements were limited, likely due to chronicity, joint complications, and severe baseline impairment.

Case Study 2 – Elbow Flexor and Pronator Spasticity

A 56-year-old female in the late chronic phase post-ACA aneurysm rupture (2011) presented with right-sided spastic hemiparesis and partially resolved expressive aphasia. Despite consistent rehabilitation, she had not received previous BoNT-A treatment.

Clinical evaluation showed good proximal motor control (MRC 4/5) and moderate distal function (MRC 3/5) in the upper limb, with MAS grade 2 spasticity in elbow, wrist, and finger flexors. No pain or tendon retractions were noted. Lower limb motor function was preserved (MRC 5/5 proximally; 3/5 distally), with mild to moderate spasticity (MAS 1–2).

BoNT-A was administered across upper and lower limb flexor groups, with initial doses up to 150 U in FDS and 100 U in biceps brachii. A second session, four months later, used slightly reduced dosages targeting the same muscles. Initial treatment was well-tolerated and marked the beginning of pharmacologic management for this patient's spasticity.

Case Study 3 – Clenched Fist and Hand Dysfunction

This case involves a 56-year-old male in the acute phase of stroke recovery following a right MCA ischemic stroke in August 2020, which resulted in left-sided spastic hemiparesis. Functional impairment and loss of independence contributed to a depressive episode. A wrist-hand orthosis was prescribed for proper limb positioning.

Clinical examination showed relatively preserved upper limb motor control (MRC 4-/5 proximally; 3/5 distally), with moderate-to-severe spasticity (MAS 2–3) in elbow, wrist, and finger flexors. Lower limb function was rated 4/5 across all segments, with mild plantar flexor spasticity (MAS 1+), and no pain or contractures.

The first BoNT-A injection occurred 25 days post-stroke, targeting upper limb flexors with doses up to 100 U in biceps brachii, FDS, and pronator teres. A second session, five months later, included dosage adjustments with continued focus on the same muscle groups. Early intervention aimed to prevent fixed deformities and facilitate functional use of the hand.

Case Study 4 – Combined Spasticity of Elbow Flexors and Extensors

This case describes a 37-year-old female in the subacute phase of recovery following a left-sided spastic hemiparesis due to a hemorrhagic stroke in June 2021. Initially dependent for transfers and non-ambulatory, she showed marked clinical improvement by February 2022, achieving independent transfers and ambulation with a tripod cane and ankle-foot orthosis.

Clinical evaluation revealed upper limb weakness (MRC 3/5 proximally, 2/5 intermediately, 1/5 distally) and significant spasticity—MAS 2–3 in triceps brachii and MAS 2 in elbow, wrist, and finger flexors. Neuropathic pain was reported in the proximal upper limb. Lower limb function was comparatively preserved (MRC 4-/5 to 4/5), with mild plantar flexor spasticity (MAS 1+) and no contractures.

She received two BoNT-A sessions targeting the spastic upper limb. The first, two months post-stroke, involved multiple flexor and extensor muscles; the second, five months later, focused on key flexor/extensor groups with reduced dosages. Notable improvements were observed in posture, functional independence, and dynamic balance.

7.3. Results

7.3.1. Results of the Case Series

7.3.1.a. Case 1 (Chronic stage): modest functional progress

The patient exhibited modest functional progress: the Barthel Index increased from 65 to 75, the Berg Balance Scale from 14 to 24, the FM-UE score from 11 to 15, and the RNLI from 45 to 59. The ARAT score remained 0 throughout the monitoring period. Neuropathic pain improved only after the third BoNT-A injection, indicating a delayed therapeutic response.

7.3.1.b. Case 2 (Late chronic stage): gradual favorable response

The patient showed a slow to moderate functional improvement: the Barthel Index increased from 85 to 95, the Berg Balance Scale from 45 to 49, ARAT from 35 to 45, FM-UE from 33 to 48, and RNLI from 76 to 82. The patient did not report any pain initially and no post-injection adverse effects were noted.

7.3.1.c. Case 3 (Acute stage): significant functional improvements

The patient demonstrated significant functional improvements: the Barthel Index increased from 80 to 100, the Berg Balance Scale from 46 to 54, ARAT from 42 to 51, RNLI from 74 to 102, and the Walking Index from 92 to 96. CMSA showed progress in arm function (5/7 → 6/7), hand function (3/7 → 5/7), leg function (5/7 → 6/7), and both lower limb function and gross motor function reached the maximum score (7/7).

7.3.1.d. Case 4 (Subacute stage): significant favorable changes

The patient recorded significant favorable changes: the Barthel Index increased from 55 to 95, the Berg Balance Scale from 7 to 50, and RNLI from 40 to 84. CMSA indicated progress in arm function (4/7 → 6/7), hand function (2/7 → 5/7), leg function (5/7 → 6/7), with both lower limb and gross motor function reaching the maximum score (7/7). Pain resolved completely within three months post-injection, and both mobility and balance improved significantly.

The treatment response was better in the acute and subacute stages, with notable progress in motor function, independence, and reintegration, whereas in the chronic stage the results were more modest, particularly in terms of distal motor control, social reintegration, and pain relief.

Tabel 7.1. Summary of the Functional Outcomes of the Patients in the Clinical Case Series.

Caz	Stadiul AVC	Indicele Barthel (Inițial → Final)	Scala Berg (Inițial → Final)	ARAT (Inițial → Final)	FM-UE (Inițial → Final)	CMA – CAHAI (Inițial → Final)	RNLI (Inițial → Final)	Reducere a durerii	Diferența medie de doză BoNT-A/mușchi
Caz 1	**Cronic**	65 → 75	14 → 24	0	11 → 15	N/A	45 → 59	După a treia injectare	i1→i2 ↓~50 U/m i2→i3 ↓~20 U/m
Caz 2	**Cronic tardiv**	85 → 95	45 → 49	35 → 45	33 → 48	N/A	76 → 82	Fără durere inițială	i1→i2 ↓~30 U/m i2→i3 ↓~20 U/m
Caz 3	**Acut**	80 → 100	46 → 54	42 → 51	N/A	28/35 → 31/35	74 → 102	Da	i1→i2 ↓~20 U/m i2→i3 ↓~20 U/m
Caz 4	**Subacut**	55 → 95	7 → 50	N/A	N/A	23/35 → 30/35	40 → 84	Completă în 3 luni	i1→i2 ↓~50 U/m i2→i3 ↓~20 U/m

7.3.2. Results of the Observational Study

The observational study demonstrated significant functional improvements following three consecutive treatment cycles with BoNT-A. Elbow spasticity decreased markedly during the first two cycles (~ -28°), with a smaller reduction in the third cycle (~ -18°). Elbow motor control progressively improved (+11°, +13°), with a smaller gain in the third cycle (+7°). Wrist spasticity gradually decreased between cycles, while active control increased in the first two cycles (+8.5°, +10.8°), with a maintained improvement thereafter (+5.7°). Passive and active shoulder mobility showed consistent increases throughout the treatment period. The results indicate greater efficiency during the first two cycles and functional stabilization in the third cycle.

➢ Post-hoc Analysis Findings:

Post-hoc analysis revealed statistically significant differences in elbow paresis angle between cycles 1–3 and 2–3 ($p < 0.001$). Significant improvements were observed in elbow and wrist spasticity between cycles 2–3 and 1–3. The wrist paresis angle did not show significant changes, while shoulder pROM demonstrated significant differences between cycles 1–3 and 2–3.

▪ Summary of Analyses of Key Clinical Variables Across Treatment Cycles

The summary analysis of key clinical variables using the Friedman test showed statistically significant differences between cycles for the elbow spasticity angle ($\chi^2 = 43.20$, $p < 0.000000001$), elbow paresis angle ($\chi^2 = 42.77$, $p < 0.000000001$), shoulder pROM ($\chi^2 = 29.30$, $p < 0.000001$), and wrist spasticity angle ($\chi^2 = 6.83$, $p \approx 0.033$). No significant differences were found for the wrist paresis angle ($\chi^2 = 2.08$, $p \approx 0.35$). Pairwise comparisons and correlations (Wilcoxon, Spearman) confirmed progressive improvements, particularly at the elbow and shoulder. Significant improvements were observed for the elbow spasticity angle in all comparisons, and for the elbow paresis angle between cycles 2–3 and 1–3 ($p < 0.001$). At the wrist, modest but significant improvements in spasticity were noted between cycles 2–3 and 1–3, without significant changes in paresis. Shoulder pROM became significant in comparisons with cycle 3.

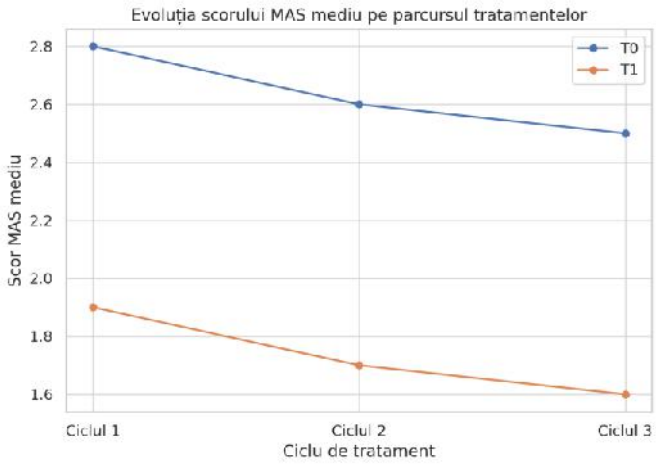


Figura 7.1. The evolution of mean MAS (composite) values at T0 and T1 across the three treatment cycles.

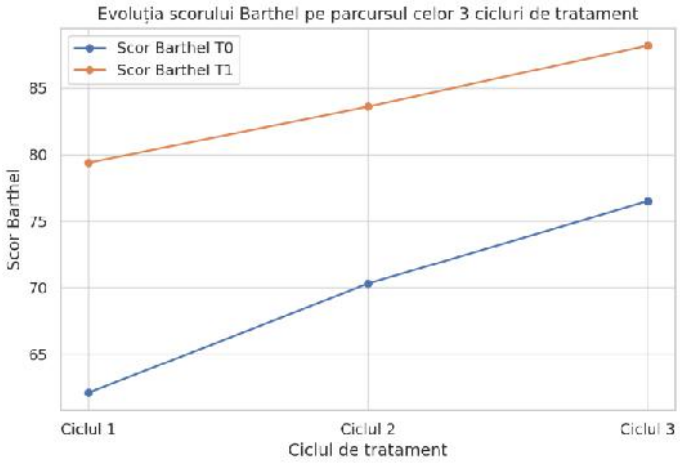


Figura 7.2. The evolution of the Barthel index at T0 and T1 for each treatment cycle.

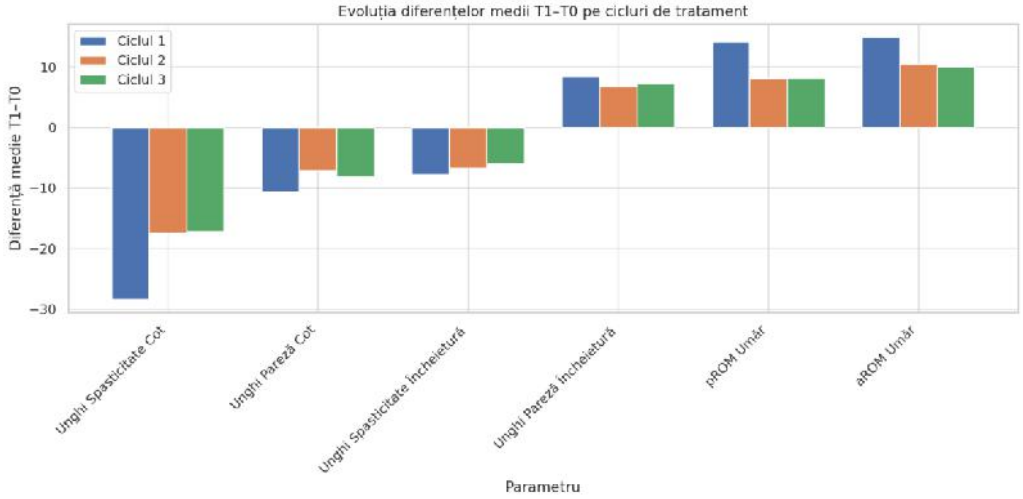


Figura 7.3. The evolution of the main parameters across the three treatment cycles, based on the mean differences at timepoints T1 and T0.

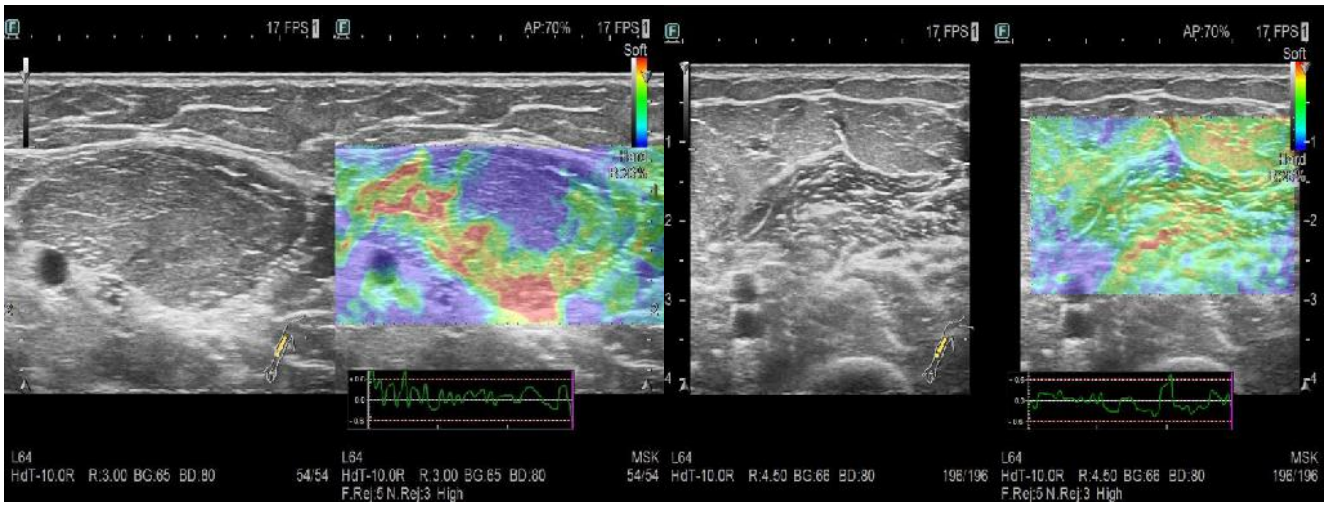


Figura 7.4. Elastograms of spastic muscles: spastic pronator teres (left) and biceps brachii (right) (from personal archive).

▪ **Elastographic Analysis – Evolution Over Three Treatment Cycles**

Elastographic analysis showed a significant reduction in muscle stiffness after each of the three treatment cycles with BoNT-A ($p < 0.01$), with consistent decreases observed in the biceps, pronator teres, subscapularis, and flexor digitorum superficialis (FDS). Moderate correlations were noted between the strain index and the BoNT-A dose, and for the FDS, with time since stroke onset. Overall, no direct correlations were found between the strain index and MAS. The biceps, pronator teres, and subscapularis muscles exhibited correlations between the strain index and MAS ($\rho = 0.49–0.62$) and the administered dose ($r = 0.45–0.85$), with each muscle showing a significant post-treatment reduction in stiffness ($r > 0.93$, $p < 0.001$). For the FDS, stiffness correlated more strongly with stroke chronicity, suggesting a link to late-stage fibrotic changes, whereas in the other muscles, current muscle tone was more relevant than stroke chronicity.

7.4.a. Individual Trends (Case Series)

In cases of severe spasticity, BoNT-A plays a crucial role in preserving functionality by reducing muscle hyperactivity and preventing secondary musculoskeletal complications, such as tendon contractures or periarticular heterotopic ossifications (1,27,77). These peripheral effects are enhanced through concurrent stretching programs and orthotic support, which improve proprioceptive feedback and joint alignment, facilitating functional recovery.

For such complex clinical profiles, dominated by marked spasticity, validated subscales like the Fugl-Meyer Assessment (FMA, FM-UE) and ARAT enable comprehensive assessment of motor and sensory function, passive mobility, and pain.

In patients with moderate or mild spasticity and partially preserved motor control, individualized BoNT-A therapy has led to meaningful functional gains (19,51). These were supported by progressively reduced dosages administered at increasing intervals beyond three months, indicating a favorable response and ongoing motor plasticity (213,214). In such contexts, clinical tools such as CMSA, CAHAI, the Walking Index, FMA, and FM-UE offer sensitive and detailed evaluation of motor coordination and task execution.

Treatment efficacy closely depended on the clinical team's ability to align therapeutic goals with appropriate functional assessment tools, integrating clinical data into reintervention decisions (161,195). The results underscore the need to tailor muscle selection, dosing, injection frequency, and outcome measures not only to clinical severity but also to the recovery potential specific to each post-stroke phase (185).

The observations highlight the importance of stage-specific, individualized strategies based on recovery status and degree of motor impairment. Beyond its peripheral effects, BoNT-A is increasingly recognized for central neuromodulatory mechanisms. By reducing abnormal tone and suppressing involuntary motor activity, BoNT-A alters afferent proprioceptive input to the CNS, interrupting maladaptive sensorimotor loops. This neurophysiological shift fosters cortical reorganization and recalibration of motor pathways. Additionally, reduced excitability in descending tracts—especially the reticulospinal tract—may support attenuation of pathological synergies and promote more selective, physiologically appropriate movements (215–217). These mechanisms are particularly relevant during early post-stroke phases, when neural plasticity is heightened.

Nonetheless, even in chronic stages, BoNT-A remains effective in supporting motor relearning and functional stabilization, particularly when integrated into task-oriented rehabilitation programs. Functional neuroimaging supports this concept, showing increased activation in cortical areas related to motor planning and sensory integration post-BoNT-A administration (3,218).

Moreover, the therapeutic effects of BoNT-A extend beyond motor function. It has shown efficacy in managing post-stroke neuropathic pain, particularly central post-stroke pain (CPSP), which often proves resistant to standard pharmacologic treatment (216,217). CPSP typically results from ischemic or hemorrhagic damage to the brainstem, thalamus, or cortex and is characterized by chronic pain and sensory abnormalities.

Experimental data suggest that BoNT-A, administered peripherally, can undergo retrograde axonal transport and modulate central synaptic activity. A double-blind study (218) demonstrated that patients receiving 500 units of BoNT-A for upper limb spasticity reported substantial, sustained reductions in pain, measured via VAS, with effects lasting up to six months. These findings support the hypothesis that BoNT-A may also function as a centrally acting analgesic, in addition to its spasticity-reducing role.

7.4.b.3. Summary of the Three Treatment Cycles

The three treatment cycles demonstrated a progressive and cumulative therapeutic effect of BoNT-A administration, with consistent improvements in neuromuscular parameters, particularly at the elbow and shoulder. Wrist spasticity responded favorably to treatment, while distal paresis remained less responsive.

7.4.b.4. Evolution of the Barthel Score and Average MAS

The evolution of the Barthel Index indicated a significant increase in functional independence, confirming the benefits of an integrated approach combining rehabilitation and BoNT-A injections on patients' daily activities. Additionally, analysis of the average MAS score revealed a progressive decrease in spasticity over the course of the treatment cycles, reflecting a sustained therapeutic response and the cumulative efficacy of the interventions.

7.4.b.5. Post Hoc Analysis – Pairwise Comparisons

The post hoc analysis highlighted significant functional improvements between treatment cycles, especially at the elbow, where spasticity and paresis angles showed marked improvements over time. At the wrist, spasticity responded favorably to treatment, while recovery of active motor control was slower.

7.4.b.6. Extended Analysis – Evolution of Strain Elastography Index (T0 vs. T1)

The analysis of the elastographic strain index (SI) demonstrated a significant and consistent reduction in muscle stiffness after each of the three BoNT-A treatment cycles ($p < 0.01$), confirming the cumulative efficacy of the intervention. The greatest percentage reductions were observed in the biceps brachii, followed by the flexor digitorum superficialis and the pronator teres.

The muscle-group analysis revealed significant correlations between the strain index and clinical parameters, especially the MAS score and the administered BoNT-A dose. Biceps brachii, pronator teres, and subscapularis showed consistent correlations between strain index and MAS score ($\rho = 0.49–0.62$), as well as with the administered dose ($r =$

0.45–0.85), supporting the relevance of elastographic assessment in treatment planning. Each muscle demonstrated a significant post-treatment reduction in stiffness ($r > 0.93$, $p < 0.001$).

In the case of the flexor digitorum superficialis, stiffness correlated more strongly with stroke chronicity than with the MAS score, suggesting a potential role in the development of late-stage fibrosis. The absence of a correlation between strain index and chronicity in the other muscles suggests that the current functional tone is more relevant than post-stroke duration in predicting treatment response.

Elastographic analysis indicated a significant, progressive, and sustained reduction in muscle stiffness throughout the three BoNT-A treatment cycles, confirming the cumulative efficacy of the intervention. Therapeutic effectiveness was maintained over time, and elastography proved to be a valuable tool for objectively monitoring treatment response. The elastographic findings were consistent with clinical improvements observed in MAS, aROM, and pROM measures, and the BoNT-A dose frequently correlated with the strain index. Stroke chronicity had limited influence on muscle stiffness.

7.4.b.7. Management of Missing Data and Patient Dropout

The management of missing data reflected the practical challenges of conducting long-term studies in neurorehabilitation, primarily due to patient dropout during the course of treatment. Statistical analyses employed pairwise deletion to maximize the use of available data, and the number of valid observations was reported for each analysis, ensuring transparency and rigor in interpreting the results.

Strain elastography confirmed the value of the strain index (SI) as an objective parameter for assessing muscle stiffness and monitoring the efficacy of BoNT-A therapy. The significant reduction in SI indicated a decrease in tissue rigidity, and the correlations with dosage and stroke chronicity suggest the utility of this imaging method as a complementary tool in optimizing treatment planning.

7.5. Partial Conclusions

7.5.a. Case Series

Botulinum toxin treatment, when tailored to the evolutionary stage, demonstrated efficacy across all phases of post-stroke recovery, with maximum benefits observed during the acute and subacute stages. In these phases, BoNT-A significantly contributed to the early reduction of spasticity, the prevention of contractures, and the stimulation of motor

recovery. In the chronic phase, its role was essential in maintaining functionality and preventing musculoskeletal complications.

The results of the four analyzed cases confirmed the benefits of a personalized approach: patients in early stages achieved notable functional progress, while those in chronic stages showed moderate but clinically relevant improvements. Validated evaluation tools (Barthel Index, FM-UE, ARAT, RNLI, etc.) were crucial in guiding the interventions.

BoNT-A proves effective throughout the entire course of post-stroke rehabilitation, provided it is integrated into a multidisciplinary program and managed according to the recovery stage.

7.5.b. Extended Longitudinal Observational Study

The study confirmed the efficacy of botulinum toxin type A (BoNT-A) treatment in reducing post-stroke spasticity, with progressive and sustained effects over the course of three treatment cycles. The results showed that the most consistent improvements were obtained during the acute and subacute stages of stroke, when neuroplasticity is most active.

Strain elastography proved to be a valuable tool for the objective evaluation of muscle stiffness, including in deep muscle groups. The Strain Index showed significant correlations with BoNT-A dose and muscular response, supporting its use in guiding treatment. Unlike subjective clinical scores, elastography provided quantitative data useful for the personalization of intervention.

A clear correlation was observed between the reduction of spasticity and the increase in active motor function, particularly at the elbow and shoulder joints, indicating the conversion of excessive tone into functional mobility. The integration of BoNT-A treatment into multidisciplinary programs, together with imaging-based monitoring, supports the development of effective and personalized protocols for post-stroke rehabilitation.

Strain elastography confirmed a significant reduction in muscle stiffness after treatment, indicating the biological efficacy of botulinum toxin type A (BoNT-A). The Strain Index correlated significantly with the MAS score and the BoNT-A dose in the case of the biceps brachii, pronator teres, and subscapularis muscles, but not with stroke chronicity. For the flexor digitorum superficialis, stiffness correlated with both dose and chronicity, but not with MAS. The treatment response was significant in all analyzed

muscles ($r > 0.93$), supporting the utility of elastography as a complementary objective tool for the evaluation and personalization of post-stroke spasticity treatment.

7. General Conclusions and Personal Contributions

8.1. General Conclusions

The methodology supported a personalized and integrative approach to the treatment of post-stroke spasticity, through:

- Patient staging (acute to late chronic);
- Assessment of global and segmental functionality (clinical scales and imaging scores);
- Integration of patient-reported outcomes (GAS, RNLI);
- Use of strain elastography and muscle mapping as guiding tools for BoNT-A dosing and optimal localization of injection sites;
- Absence of a control group justified on ethical and clinical grounds;
- Longitudinal design, in which each patient functioned as their own control in subsequent treatment cycles.

The present research pursued an integrated clinical and paraclinical approach to the treatment of upper limb post-stroke spasticity with BoNT-A. Through longitudinal monitoring of patients over three consecutive therapeutic cycles and the integration of objective imaging evaluation methods (strain elastography), the study has highlighted several scientifically and clinically relevant conclusions:

1. Repeated administration of BoNT-A induces a significant and progressive reduction in muscle spasticity, with cumulative effects observed from one cycle to the next.
2. The highest efficacy of intervention was observed in the subacute post-stroke phase, where the treatment response is supported by a greater degree of neural plasticity.
3. The Strain Index proved to be a sensitive, reproducible, and non-invasive marker of post-treatment mechanical muscular changes, significantly correlating with the administered BoNT-A dose and functional clinical scores.
4. A direct correlation was confirmed between the reduction in muscle tone (spasticity) and the improvement in active motor control, supporting the interdependence between reflex tone and voluntary movement.
5. Deep muscles (such as subscapularis and flexor digitorum profundus) respond effectively to BoNT-A and can be successfully evaluated using elastography, supporting the extension of this method's use in neuromotor rehabilitation.

6. The previous number of injections and the difference in FIM between discharge and admission demonstrated a significant positive association with the improvement in GAS-T scores, supporting the hypothesis of a cumulative treatment effect and the benefits of sequential interventions. Conversely, the use of post-injection adjuncts such as orthoses correlated with a lower improvement in GAS-T scores; this may reflect not only the influence of therapeutic strategy but also the fact that these patients presented with a more severe initial functional deficit, necessitating additional support measures.

7. Brachialis, as the primary elbow flexor, represents a more efficient injection target, requiring lower doses of BoNT-A to achieve a therapeutic effect similar to that of the biceps brachii. The combination of brachialis and brachioradialis injections may provide additional benefits, especially in patients with severe spasticity characterized by marked flexion and pronation.

8. The study data suggest that an injection strategy targeting the brachialis muscle, with or without brachioradialis, may offer an optimal balance between clinical efficacy and minimization of total botulinum toxin dose, contributing to increased safety and more efficient use of therapeutic resources.

9. The brachialis–brachioradialis combination yields additional functional benefits, particularly through improvements in supination and active movement.

10. The brachialis plus brachioradialis group recorded the greatest increase in active supination compared to biceps brachii.

11. Furthermore, a tendency toward differing BoNT-A dose requirements was observed for the pronator teres muscle, with the highest doses in the biceps brachii group and the lowest in the brachialis plus brachioradialis group, possibly reflecting the impact of muscle selection on the functional balance between supination and pronation.

12. The correlation analysis between the time since stroke onset and clinical parameters revealed a muscle-specific differential response, with superior efficacy observed for the brachialis plus brachioradialis combination in reducing spasticity and improving active supination. The brachialis group showed the best results for active extension, while the biceps brachii group demonstrated limited progress in supination relative to the increasing time since stroke onset.

13. The results indicate that the inclusion of imaging and functional evaluations in therapeutic algorithms can lead to superior treatment personalization, optimizing both target muscle selection and BoNT-A dosing.

14. BoNT-A treatment, accompanied by an active rehabilitation program, enables the exploitation of a favorable therapeutic window, during which the patient may benefit from optimized neuromotor training.

15. The findings indicate significant improvement in motor function, particularly during the acute and subacute stages, where neuroplasticity is most active.

16. In chronic stages, BoNT-A contributes to maintaining functionality and preventing musculoskeletal complications.

17. Positive correlations between reduced spasticity and increased active motor control support the utility of BoNT-A in facilitating motor relearning.

18. The integration of objective evaluation methods, particularly strain elastography, provided valuable quantitative data on muscle stiffness, complementing traditional clinical assessments. The Strain Index correlated with BoNT-A dosage and enabled more precise selection of target muscles, thereby supporting personalized therapy.

19. The importance of functionally guided treatment, using adapted cyclic protocols supported by multidisciplinary rehabilitation, was emphasized throughout the research.

20. The results support the use of BoNT-A as an integral component of multimodal therapeutic strategies, in which imaging evaluation, accurate dosing, and individualized planning play essential roles in optimizing post-stroke rehabilitation.

21. BoNT-A proved effective in reducing spasticity and supporting functional recovery of the upper limb after stroke, with benefits documented both clinically and biomechanically. Its efficacy was demonstrated in observational clinical studies and case series, covering all evolutionary stages—from the acute to the chronic phase.

These conclusions support the applicative value of an integrated protocol and argue for the inclusion of elastography as a standardized monitoring tool in clinical practice.

8.1. Personal Contributions

1. **Development of an Integrated Evaluation Protocol**

I developed and implemented a combined clinical protocol that integrates standardized functional assessment (MAS, Tardieu, CMSA, Barthel, ARAT, FM-UE, etc.) with

biomechanical imaging assessment (strain elastography) for the quantification of muscle hypertonia in post-stroke upper limb spasticity. This approach enabled a more accurate characterization of the muscular profile and individual therapeutic response.

2. **Longitudinal Elastographic Monitoring of Muscle Groups in Post-Stroke Spasticity: Foundations for an Imaging-Based Evaluation Prototype in Successive Rehabilitation Cycles within an Individualized Medical Rehabilitation Program**

I originally employed strain elastography in a longitudinal study over the course of three successive treatment cycles with BoNT-A, demonstrating its value in the objective monitoring of tissue response evolution, including in deep or clinically hard-to-access muscles.

3. **Correlation of Biomechanical Data with BoNT-A Dose and Stroke Chronicity**

I investigated the relationships between the Strain Index, clinical scores, BoNT-A dose, and time since stroke onset, identifying distinct muscular response patterns. These findings offer an objective basis for personalizing dosage according to the actual stiffness of the muscle.

4. **Differentiated Documentation of Therapeutic Response According to the Stage of Post-Stroke Recovery**

Through a series of four clinical case studies, I demonstrated differences in treatment response depending on the stage of recovery (acute, subacute, chronic), the degree of motor impairment, and residual functional potential, thereby supporting the importance of a differentiated, phased, and patient-centered management approach.
The results served as the foundation for a model of multimodal therapeutic intervention in which BoNT-A is correlated with functional objectives, imaging guidance, rehabilitation programs, and cyclic planning, with potential for broad clinical applicability.

5. **Integration of Anatomical Mapping and Elastography in Guiding Botulinum Toxin Injections**

In this study, botulinum toxin injections were initially performed based on anatomical mapping, using target points validated through anatomical dissection, Sihler staining, and EMG data. Beginning with the second injection cycle, the strategy was optimized by integrating strain elastography into the guidance process.
Muscle regions that displayed lower stiffness on elastographic imaging (color-coded as

yellow, orange, or red), indicative of muscle tissue with biomechanical properties more favorable to toxin response, were preferentially selected.

6. **By Combining Anatomical Mapping with Real-Time Biomechanical Evaluation Provided by Elastography, This Strategy Can Be Considered an Original Contribution with the Potential to Optimize Treatment Efficiency and Reduce Botulinum Toxin Consumption in Spasticity Management**

Furthermore, the elastographic changes observed post-injection correlated well with the evolution of clinical scores (e.g., MAS, Tardieu), offering an additional objective indicator of therapeutic efficacy.

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