OPEN

PRS GLOBAL OPEN

ORIGINAL ARTICLE
Cosmetic

Microneedle for Botulinum Toxin: A Randomized, Case-control, Single-blind Study to Assess Clinical Efficacy and Patient Satisfaction

Alberto Diaspro, MD, MSc*
Silvia Silenzi, PhD†

Background: Lateral canthal lines can be effectively treated with injections of botulinum toxin, whereas the aesthetic effects can vary due to factors such as injection depth, which is essential for achieving predictable clinical outcomes. Microneedles (MNs) have proven effective in intradermal skin rejuvenation procedures. However, a comparison of their performance with traditional needles is still lacking in the scientific literature to reliably evaluate their efficacy.
This study is therefore aimed to evaluate the clinical efficacy and patient satisfaction of botulinum toxin A injected intradermally using a standard needle versus an MN device.
Methods: Twenty recruited participants received a single injection of 10 Speywood Units (US, 0.05 mL) for each of the six standard points (total dose: 60 US) at T0 using both the control needle and the MN, randomly assigned.
Follow-up visits were scheduled at 30 (T1) and 90 (T2) days, along with patients and blind observer evaluation of the clinical improvement of periocular wrinkles.
Results: The measurement of wrinkle depth showed that MN injection had similar efficacy to a normal needle, whereas blind evaluation indicated better results for the MN. Patient subjective assessments of procedure pain/discomfort and likelihood of repeating the procedure also favored the MN.
Conclusions: This pilot study suggests that the MN device holds promise for optimizing the clinical results of botulinum toxin injections by controlling injection depth and enhancing patient acceptance and injection experience compared to standard needle injection. The absence of adverse events further supports the efficacy of MN for intradermal botulinum toxin use. *(Plast Reconstr Surg Glob Open 2025;13:e6610; doi: 10.1097/GOX.0000000000006610; Published online 17 March 2025.)*

INTRODUCTION

The periorbital area is a critical region in facial perception, where even subtle changes can greatly enhance facial aesthetics,[1] and is deemed the most susceptible to the signs of aging, as evidenced by the appearance of both static and dynamic crow's feet (CF).

Undoubtedly, repetitive facial expressions involving orbicularis oculi activation, coupled with its strong connection to the dermis and increased skin laxity, play a crucial role in determining their appearance[2]; however, they can be successfully treated with botulinum neurotoxin type A (BoNTA).

Its ability to alleviate CF was first described in 1993.[3] Since that time, it has been widely used in the cosmetic field to reduce wrinkles and rejuvenate the skin.[4]

BoNTA has been conventionally administered through local intramuscular or intradermal microdroplet injection, and in both cases, it has been proven effective in reversing facial wrinkles.[5] The aesthetic effect of its injection can vary and is primarily influenced by depth and dilution. The methods available in the literature to properly assess this effect are divided between clinical evaluation[6,7] and objective quantitative digital imaging and analysis.[8,9] Nowadays, proposals for new scales for wrinkle assessment

*From the *Medynet Centre, Turin, Italy; and †Microbiology Unit, University-Hospital Authority St. Orsola-Malpighi Polyclinic IRCCS, Bologna, Italy.*

Received for publication October 15, 2024; accepted January 22, 2025.

DOI: 10.1097/GOX.0000000000006610

Disclosure statements are at the end of this article, following the correspondence information.

Related Digital Media are available in the full-text version of the article on www.PRSGlobalOpen.com.

are increasingly being digitally validated[10] to overcome interindividual observation bias and ensure objective and reliable results.[11,12]

Microneedles have proven effective in the intradermal injection of drugs[13,14] and for skin rejuvenation procedures[15] including botulinum toxin therapy.[16] However, until now, they have not been compared with traditional needles to reliably evaluate their performances.

The aim of this study was therefore to objectively assess clinical efficacy and patient satisfaction regarding on-label diluted doses of BoNTA injected intradermally for CF treatment using a control needle versus a specific microneedle device.

Takeaways

Question: What is the difference in efficacy and patient satisfaction between microneedles and standard needles for crow's feet therapy with botulinum toxin injection?

Findings: The recorded results substantiate the efficacy and safety of microneedles for the treatment of crow's feet with intradermal botulinum toxin, demonstrating comparable efficacy to traditional methods, while offering a painless injection alternative that is highly valued by both patients and clinicians.

Meaning: The microneedle is a valid alternative to the traditional needle for improving patient comfort without losing therapeutic effectiveness.

MATERIALS AND METHODS

The study protocol adheres to the ethical guidelines outlined in the Declaration of Helsinki. Institutional review board/ethics committee ruled that approval was not required for this study. Patients selected to participate in this study were informed about the benefits, risks, and potential complications of the treatment before enrollment; all provided informed consent before participation.

Patient Selection and Study Design

Between December 1, 2021, and July 1, 2021, a total of 20 participants were enrolled from the senior author's patient pool for this study. All patients underwent treatment for periocular rejuvenation using Abo-botulinum toxin A (Azzalure; Ipsen S.p.a., Milan, Italy).

Women comprised 85% (n = 17) and men 15% (n = 3) of the sample. The median age of the patients was 44.2 (SD ± 13.4) years for women and 55 (SD ± 6) years for men. Demographic data are presented in Table 1.

Included patients were those older than 18 years of age at enrollment, who were either BoNTA treatment-naive or had prior treatment experience and were consistent in attending follow-up visits as required by the study. Exclusion criteria included a history of botulinum toxin or soft-tissue filler treatments within the past 12 months, individuals with preexisting neuromuscular or bleeding disorders, patients with active inflammation at the injection site, individuals with hypersensitivity to any components of the formulation, and patients taking medications that could potentiate the effects of botulinum toxin.

Table 1. Demographic Data

Sex	Patients, N (%)
Female	17 (85)
Male	3 (15)
Age (y)	
19–34	5 (25)
35–50	10 (50)
51–64	4 (20)
>65	1 (5)
Follow-up	
T1 (30 d)	14 (70)
T2 (90 d)	9 (45)

The split-face treatment was administered using two different needle types: the control needle, specifically the Invisible Needle 34G × 9 mm (TSK Laboratory Europe, Oisterwijk, the Netherlands), and the microneedle device, MicronJet600 (MJ600) 32.5G × 0.6 mm (NanoPass Technologies, Nes-Ziona, Israel). Each patient's face right and left sides were randomly assigned for treatment. The randomization process involved sealed envelopes numbered 1–20, indicating the allocation. Randomization was conducted using an online-generated random list (https://www.randomizer.org), and each patient selected a number from 1 to 20 from a closed urn.

Participants received a single treatment (T0), with follow-up visits scheduled at 30 (T1) and 90 (T2) days posttreatment. Photographs were taken using a standardized method at each visit. Both participants and the dermatologist assessing outcomes were blinded until all participants completed the protocol.

The study design was as follows:

T0:

- Photographic assessment;
- Injection of toxin (on-label dilution) in the CF area with both control needle and MJ600 (split-face, blinded for the patient);
- Questionnaire and visual analog scale (VAS) scale administered after the injection (Tables 2, 3).

T1 (30 d)–T2 (90 d):

- Photographic assessment;
- Subjective Global Aesthetic Improvement Scale administered (Table 4);
- External blind evaluation (Table 5).

Six drop-offs at T1 (n = 14) and 11 at T2 (n = 9) have been recorded.

Treatment

A total of 20 patients underwent treatment for the correction of mild to severe CF lines using abobotulinum toxin A. Each 125 US vial was reconstituted by adding 0.63 mL of sterile saline solution (0.9% sodium chloride).

Table 2. Subjective Blinded Patient Assessment Immediately After the Treatment on Probability to Repeat the Procedure

Grade	Needle, N (%)	MJ, N (%)
Very probable	10 (50)	11 (55)
Probable	7 (35)	8 (40)
Poorly probable	2 (10)	1 (5)
Not at all probable	1 (5)	0 (0)
Total	20 (100)	20 (100)

Table 3. Subjective Blinded Patient Assessment Immediately After the Treatment on Procedure Pain/Discomfort (VAS) Comparing the Devices

Grade	Needle, N (%)	MJ, N (%)
No pain	1 (5)	2 (10)
Mild pain	14 (70)	17 (85)
Moderate pain	5 (25)	1 (5)
Severe pain	0 (0)	0 (0)
Total	20 (100)	20 (100)

Several studies looked at the interpretability of VAS and reported nearly similar cutoff points for VAS, indicating that VAS ratings of 0–5 mm were very likely to be rated as no pain by patients, 6–44 mm were considered mild pain, 45–69 mm were considered moderate pain, and VAS ratings ≥70 mm were suggestive of severe pain.

For the injections, both a control needle and micron device were used. The injection method was identical for both devices and based on 3 injections performed laterally to the canthus, as indicated by the manufacturer. Injections proceeded in a medial-to-lateral direction to avoid the orbit, with the needle at a 30-degree angle. (**See Video 1 [online]**, which displays a microneedle for botulinum toxin delivery.) For the control needle, only the tip was inserted. A dose of 10 US (0.05 mL) was administered at each of the 6 standard points, resulting in a total dose of 60 US for both female and male patients.

Clinical Assessments

Images of the patients' faces were captured at each visit using a passive stereovision calibrated and stereoscopic digital camera, LifeViz Mini (Quantificare S.A., 06410 Biot, France), with all patients consenting to the reproduction of recognizable photographs. Photographs were taken with a full-size 1:1 ratio, with the Frankfurt horizontal plane parallel to the floor, while each patient looked straight ahead in a standing position, at a fixed distance.

The stereophotographic system comprises a customized Nikon D3400 digital reflex camera with a 24-megapixel resolution and a stereoscopic lens and the Dermapix software.

The camera captures 2 images simultaneously by taking a photograph from different angles in a single shot, resulting in a stereoimage. The full face is captured by acquiring multiple stereoimages from 4 viewpoints. The Dermapix software automatically integrates these stereoimages and produces a 3-dimensional (3D) reconstruction in the "fine analysis" mode, converting them into visualizations of 3D pictures with a resolution of up to 20 µm.

The 3D Track, built on 3D analysis technology, facilitates precise measurements of straight lines, curved lines, and enclosed surfaces, along with capturing data points such as volume, depth, roughness, and perimeter areas of the skin surface.

Following the 3D reconstruction, the skin surface obtained is analyzed using the 3D Track module to assess skin microroughness before (T0), 30 (T1), and 90 (T2) days after the botulinum injection. A reference surface is used to differentiate elevations (positive volumes) or depressions (negative volumes), which can then be identified and quantified. Measurements are then calculated on this selected surface, and differences are tracked over time (including parameters such as roughness, average height, average depth, and volume).

Table 4. Blinded Evaluation of the Results According to Patient Satisfaction (sGAIS) at 30 and 90 Days After Neurotoxin Treatment

	T1 (30 d), %		T2 (90 d), %	
Grade	Needle	MJ	Needle	MJ
Very satisfactory	10 (71.4)	11 (78.6)	2 (22.2)	3 (33.3)
Satisfactory	3 (21.4)	3 (21.4)	6 (66.7)	5 (55.6)
Poorly satisfactory	1 (7.2)	0 (0)	1 (11.1)	1 (11.1)
Not at all satisfactory	0 (0)	0 (0)	0 (0)	0 (0)
Total	14		9	

sGAIS, subjective Global Aesthetic Improvement Scale.

Table 5. Evaluation of the Aesthetic Results Assessed by a Blind Physician (External Evaluator)

	T1 (30 d), %		T2 (90 d), %	
Grade	Needle	MJ	Needle	MJ
Very satisfactory	6 (42.8)	8 (57.2)	1 (11.1)	2 (22.2)
Satisfactory	7 (50)	6 (42.8)	5 (55.6)	5 (55.6)
Poorly satisfactory	1 (7.2)	0 (0)	3 (33.3)	2 (22.2)
Not at all satisfactory	0 (0)	0 (0)	0 (0)	0 (0)
Total	14		9	

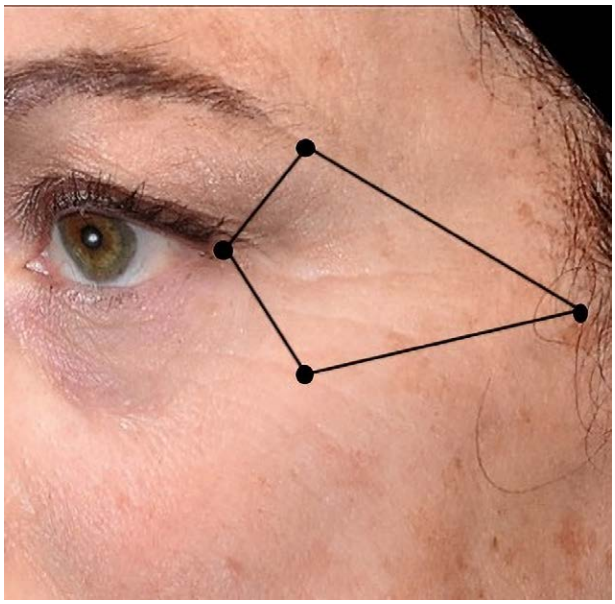

Fig. 1. Reference points for skin microroughness analysis.

These reference points were used to outline the area of the CF (Fig. 1):

- Excanthion (Exc);
- Zygion (Zyg);
- Eyebrow tail;
- A point inferiorly, specular to Exc-Zyg, perpendicular to the line connecting those 2 points.

The software facilitates the alignment of preoperative and postoperative images, enabling a comparison of various parameters. One such parameter is the maximum roughness Rz (also known as Rmax), which represents the difference between the highest and lowest values relative to the reference surface within the region delineated by the contour and quantifies the extent of surface irregularities (or roughness) in the specified area.

For self-assessment, blinded patients completed questionnaires regarding treatment efficacy for each side at 30 and 90 days posttreatment using the Global Aesthetic Improvement Scale, ranging from 0 (dissatisfied) to 4 (very satisfied).

Additionally, immediately after the treatment, blinded patients were asked about their likelihood to repeat the procedure using a 4-level probability scale ranging from 0 (not probable) to 4 (very probable), and to rate the pain/discomfort experienced during the treatment on the right and left sides using the VAS, which rates pain from none to mild, moderate, and severe.

Objective clinical assessment was conducted by a dermatologist, blinded to the study design and treatment, who compared photographs taken at 30 and 90 days posttreatment separately on each side of the face. The evaluations were graded into quartiles as follows: grade 1 = minimal to no improvement (0%–25%); grade 2 = moderate improvement (26%–50%); grade 3 = marked improvement (515–75%); and grade 4 = near total improvement (75%–100%).

Statistical Analysis

The data were analyzed using a generalized linear mixed model with normal distribution of the residuals and identity as a link function. (**See table, Supplemental Digital Content 1,** which displays the statistical analysis data, **http://links.lww.com/PRSGO/D906**.) Rz was considered the dependent variable, needle was considered a fixed factor, and time after the intervention was considered a repeated measure; the fixed factor was nested in the patients. The type I autoregressive model was selected for the covariances, and the degrees of freedom were calculated by the Kenward–Roger method.

RESULTS

The measurement of *wrinkle depth*, as indicated by the difference in Rz outcomes between the MJ600 and control needle observed at T1 and T2, revealed no statistically significant difference. This suggests that the MJ600 injection performs similarly to a control needle in terms of wrinkle depth reduction (Fig. 2).

The evaluation of the *aesthetic outcome* of periocular wrinkles treatment with botulinum toxin injection, based on patient subjective satisfaction, was nearly identical for both devices (Table 4). This similarity in patient satisfaction suggests that the toxin is effective once injected, and both devices provide similar skin penetration capabilities.

The results from patient assessments immediately after the treatment regarding the *likelihood to repeat the procedure* (MJ600 acceptance versus normal needle acceptance) showed that 95% of patients expressed willingness to repeat the procedure with the MJ600 needle, compared with 85% of patients injected with the control needle. Only 1 (5%) patient indicated that further treatment was unlikely following MJ600 injection, whereas 3 (15%) patients stated that they were unlikely to repeat the treatment after receiving the control needle. In both cases, the motivation was related to poor compliance with injection therapy, as reported by the abovementioned patients (Table 2).

Patient assessment regarding *procedure pain/discomfort* comparing the devices indicated that reported pain was absent to mild in 95% of patients injected with the MJ600 needle, compared with 75% among patients injected with the control needle. Only 1 (5%) patient reported a moderate inner pressure sensation after injection with the MJ600, whereas 5 (25%) patients reported moderate pain with a stinging sensation when injected with the control needle. (Table 3).

The external *blind evaluation* of aesthetic results regarding the improvement of periocular wrinkles through botulinum toxin treatment indicated slightly better outcomes for the MJ600 device both at T1 and T2. After 30-day posttreatment, the evaluation of "marked improvement" and "near total improvement" reached 100% and 92.8% for patients treated with the MJ600 needle and control needle, respectively. Moreover, the same trend was observed at 90 days posttreatment, where the aesthetic evaluation ranging from moderate to near total improvement was 77.8% and 66.7% for patients injected with the MJ600

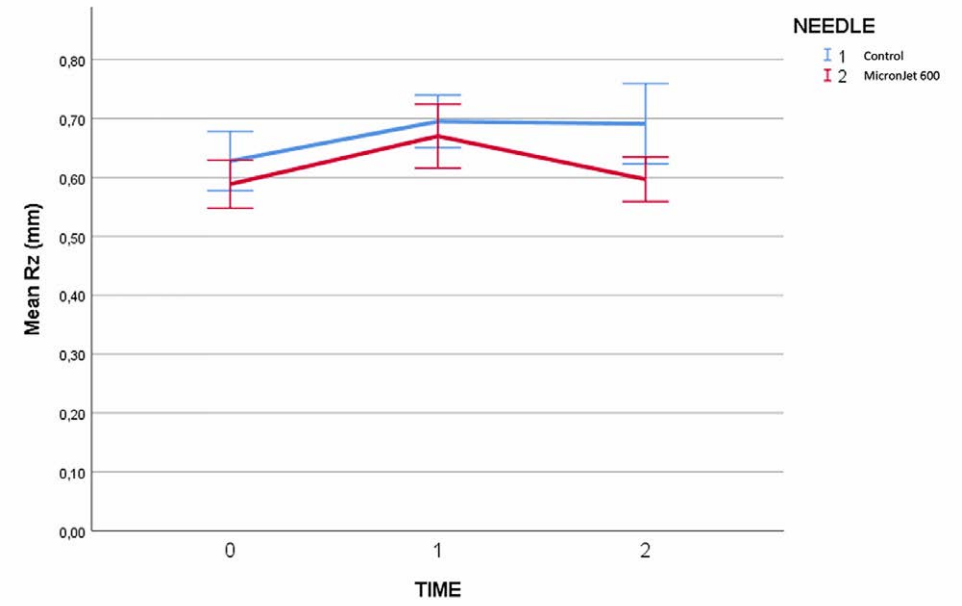

Fig. 2. Difference in Rz (maximum roughness) outcomes between the MJ600 and control needle observed at T1 (30 days) and T2 (90 days). Maximum roughness Rz (also known as Rmax), represents the difference between the highest and lowest values relative to the reference surface within the region delineated by the contour and quantifies the extent of surface irregularities (or roughness).

and control needle, respectively. The data are presented in Table 5.

Treated patients did not experience injection-point ecchymosis and did not report muscle weakness or facial asymmetries with either needle.

DISCUSSION

The stratum corneum is a formidable barrier that imposes significant limitations on therapeutics. The microneedle device, a minimally invasive hybrid between a hypodermic needle and a transdermal patch, has been developed to breach this barrier and facilitate the effective transportation of molecules across the skin. Although the first patent for microneedles was granted in 1976, it was not until 1998 that they were utilized as a drug delivery technique,[13,14] including in the aesthetic field.[15–19]

Among their clear investigated and proven advantages is the near-painless nature of their insertion and their minimal invasiveness, which potentially results in negligible penetration of microbial organisms through the skin. The most reported side effect experienced upon their use is transient, self-limiting erythema.[20]

The MJ600 is a microneedle-based device designed for intradermal delivery. It features 3 hollow micropyramids, each 600 µm long, with a specially designed inclined surface, protruding from a plastic hub. These micropyramids can be attached to any conventional syringe. This innovative design combines the mechanical robustness of a pyramid with an extremely sharp tip, sharper than standard needles. Additionally, it integrates an offset channel or bore that does not open at the tip but rather at the inclined surface (Fig. 3).

This setup enables the mechanical disconnection of features that have historically limited needle sharpness, size/gauge, or durability, all without compromising other important features.[21,22] It also prevents the potential for the needle bore opening to become clogged with tissue during microneedle skin insertion.

With a flow rate like that of a ~30–31G needle, despite having a diameter of less than 70 µm, the MJ600 received Food and Drug Administration clearance in 2010. Since then, it has been used for intradermal delivery to human subjects of various drug formulations, including vaccines (such as influenza, zoster, polio, and COVID-19), lidocaine, and insulin. Furthermore, it has been used for aesthetic formulations such as mesotherapy (Fig. 4). Reports suggest that its application is effective, accurate, and nearly painless.[23–27]

BoNTA temporarily inhibits the release of the neurotransmitter acetylcholine at the neuromuscular junction that leads to a temporary denervation and modulation of muscle activity.

Its potential dose- and site-related toxicity concerns necessitate its administration through multiple painful intramuscular or intradermal injections to achieve the desired clinical results. This approach helps to avoid temporary disfigurement or functional impairment that may occur if the toxin inadvertently diffuses into adjacent muscles.[5,28,29]

Several attempts have been made to reduce patient discomfort.[30–33] Indeed, pain related to conventional injection is a significant factor contributing to poor patient compliance with therapy. Thus, several studies have been conducted to determine the level of pain associated with microneedle insertion into the skin.[34–36] The moderate

Fig. 3. MJ600 device.

pain sensation previously reported following the insertion of a hollow microneedle device is likely attributable to the extrusion of the liquid solution, which causes tissue expansion due to the excess volume, rather than the insertion of the microneedle itself.[37] Indeed, in the present study, an injection volume of less than 0.1 mL per injection was used, and 95% of the study population reported only mild to moderate discomfort.

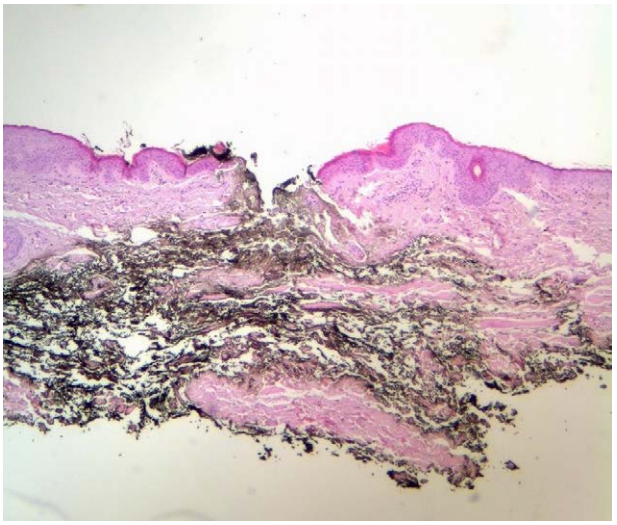

Fig. 4. MicronJet 600 injection of 0.2 mL HA 5 mg/mL + 0.2 mL stain in eyelid skin, Ex vivo samples not obtained from patients included in the study population. Magnification 4 × 10; epithelium thickness 100 µm.

Since 2015, the injection of microdroplets of diluted BoNTA into the skin and the immediate subdermal plane has been conceived and carried out to reduce fine lines without compromising the action of mimic muscles.[38–42]

The periorbital region is particularly susceptible to aging among facial areas, often affected lateral canthal lines: the efficacy of BoNTA in alleviating them was initially firstly described in the late nineties,[43] and since then, it has been extensively utilized in the cosmetic field to reduce wrinkles and rejuvenate the skin.[44,45]

The use of hollow microneedle devices for BoNTA injection has been advocated since the very first year of their development[13] as a less invasive alternative.[46] Research trends also focus on rejuvenating the periocular area but primarily utilizing patches[18,47,48] and novel botulinum toxin topical gels.[49,50]

As aging progresses, the skin texture is mainly characterized by wrinkles, which develop gradually due to atrophy of the various skin layers and to dermal elastosis, as well as those related to facial expressions,[51] evolving from fine lines to wrinkles. Indeed, this microrelief of the skin, barely visible to the naked eye, can only be effectively studied through highly resolving technologies such 2D imagery systems[52,53] and 3D high-resolution devices with dedicated software. These tools enable a robust and precise characterization of the skin texture and can assess the efficacy of the injection of botulinum toxin.[54]

Measurements of facial signs, particularly with regard to the antiaging effects on wrinkles, are essential for both researchers and consumers, as they provide the most effective demonstration of the efficacy of proposed therapies, thereby supporting their claims. Furthermore, the quantitative instrumental evaluation of intradermal botulinum toxin injections to reduce the signs of facial aging and enhance skin quality has been documented in the literature,[12,55,56] especially in relation to CF.[8,9]

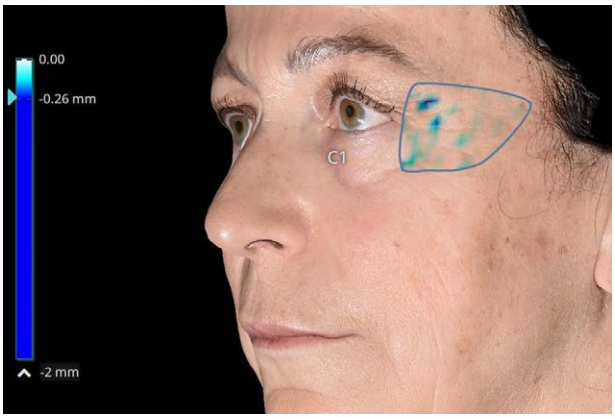

Fig. 5. Photographic assessment and Rz colorimetric scale at T0.

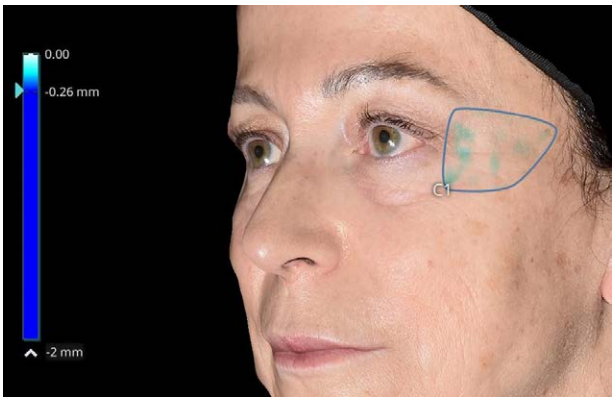

Fig. 6. Photographic assessment and Rz colorimetric scale at T1 (30 days).

Hence, to assess the efficacy of intradermal botulinum toxin injection using the MJ600 microneedle, we opted for a quantitative analysis previously documented in the literature. This was coupled with a blind evaluation of the results by the authors and a self-assessment score conducted by the patients themselves.

The recorded results confirm the efficacy of microneedles for treating CF with intradermal botulinum toxin (Figs. 5–7), thereby providing an opportunity for painless injection, which is appreciated by both patients and clinicians.

The recorded advantages for clinicians using microneedles include better control of injection depth, preventing the drug from being injected too deeply, especially in the periocular area where the muscle is located just beneath the skin. This also optimizes drug efficacy, as the injected dose is fully available at the injection site, ensuring maximum effectiveness. Additionally, microneedles provide safety for less experienced clinicians by helping to prevent incorrect, overly deep injections, particularly at the most caudal point, where a deep injection could allow the drug to diffuse into the underlying zygomatic muscles, potentially altering facial expressions. For patients, the advantages include greater tolerance of the therapy due to the absence of sharp pain during the injection and a reduced

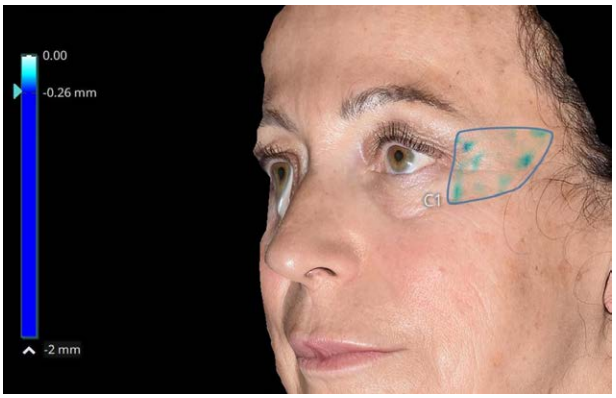

Fig. 7. Photographic assessment and Rz colorimetric scale at T2 (90 days).

long-term risk, as microneedles decrease the likelihood of injection fatigue.

These findings are supported by the recorded safety profile with absence of any adverse events, such as muscle weakness or facial asymmetry related to incorrect injection depth or uncontrolled spread of the drug's effect.

Further investigations in different facial areas could validate the presented findings, highlighting the use of microneedles as the gold standard for intradermal botulinum toxin therapy.

Indeed, this pilot study has some limitations, such as the relatively small number of subjects in the study population and the drop-off rate at T2, which must be properly considered along with the follow-up duration that allows for the evaluation of a repeated injection regimen, hopefully in a larger sample size.

Another limitation of this pilot study is the learning curve of the injector with the microneedle device.[38,39] However, in a recent postmarketing survey, The MicronJet600 was reported to be "used well" by users after 1–2 injections in the majority (80%) of users, and 95% were satisfied with the device (Nanopass Survey, 2022).

Another limitation is the flow resistance caused by dense dermal tissue compressed around the microneedle tip during insertion. Indeed, the periocular skin, along with the eyelid, comprises the thinnest skin area of the face. The study has not investigated other frequently treated areas like the glabella or the forehead, which typically have thicker skin.[57]

Finally, the statistical validation was performed solely on the analytical, objective data available through the software analysis system. Conducting statistical validation on patient-reported data seemed of limited interest, especially given the small sample size and beyond the percentages already presented in Table 4. A small tendency toward postoperative pain reduction and higher classes of satisfaction seems to emerge in patients treated with the device. However, a formal statistical analysis of these outcomes of the experiment could not be performed because of the limited sampling size. Therefore, these findings need to be confirmed and validated in further study aimed at improving the number of patients included.

CONCLUSIONS

The presented results demonstrate, both clinically and instrumentally, that the MJ600 can be effectively used to treat CF through BoNTA injection, with optimized depth and improved patient comfort.

To further validate the findings of this pilot investigation, larger study populations and randomized double-blind studies conducted in other facial areas would be necessary.

Alberto Diaspro, MD, MSc
Facial Plastic Surgery Department
Medynet Centre
via A. Lamarmora 80
10128, Turin, Italy
E-mail: info@albertodiaspro.com

DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

PATIENT CONSENT

Patients provided written consent for the use of their images.

REFERENCES

1. Diaspro A, Calvisi L, Sito G. Hyaluronic acid gel Injection for the treatment of tear trough deformity: a multicenter, observational, single-blind study. *Aesthetic Plast Surg*. 2022;46:1860–1867.
2. Ching JA, Ford JM, Decker SJ. Aging of the adult bony orbit. *J Craniofac Surg*. 2020;31:1082–1085.
3. Blitzer A, Brin MF, Keen MS, et al. Botulinum toxin for the treatment of hyperfunctional lines of the face. *Arch Otolaryngol Head Neck Surg*. 1993;119:1018–1022.
4. Carruthers J, Fournier N, Kerscher M, et al. The convergence of medicine and neurotoxins: a focus on botulinum toxin type A and its application in aesthetic medicine—a global, evidence-based botulinum toxin consensus education initiative: part II: incorporating botulinum toxin into aesthetic clinical practice. *Dermatol Surg*. 2013;39:510–525.
5. Imhof M, Kuhne U. Introduction of the microdroplet technique with incobotulinumtoxin A for the treatment of crow's feet. *J Clin Aesthet Dermatol*. 2013;6:40–44.
6. Carruthers A, Carruthers J, Hardas B, et al. A validated grading scale for crow's feet. *Dermatol Surg*. 2008;34:S173–S178.
7. Kane MA, Blitzer A, Brandt FS, et al. Development and validation of a new clinically-meaningful rating scale for measuring lateral canthal line severity. *Aesthet Surg J*. 2012;32:275–285.
8. Cavallini M. Preliminary report on an objective, fast, and reproducible method to measure the effectiveness of botulinum toxin type A. *Aesthet Surg J*. 2015;35:715–720.
9. Cavallini M, Papagni M, Gazzola R. An objective method to assess the improvements of skin texture roughness after botulinum toxin type a treatment of crow's feet. *Skin Res Technol*. 2019;25:54–59.
10. Pavicic T, Pooth R, Prinz V, et al. Validated 5-point photonumeric scales for the assessment of the periorbital region. *J Cosmet Dermatol*. 2022;21:158–166.
11. Heckmann M, Schön-Hupka G. Quantification of the efficacy of botulinum toxin type A by digital image analysis. *J Am Acad Dermatol*. 2001;45:508–514.
12. Diaspro A, Calvisi L, Manzoni V, et al. Microbotulinum: a quantitative evaluation of aesthetic skin improvement in 62 patients. *Plast Reconstr Surg*. 2020;146:987–994.
13. Fukamizu H, Fujiwara M, Kim T, et al. Development of a three-microneedle device for hypodermic drug delivery and clinical application. *Plast Reconstr Surg*. 2012;130:451–455.
14. Xu J, Xu D, Xuan X, et al. Advances of microneedles in biomedical applications. *Molecules*. 2021;26:5912.
15. McCrudden MT, McAlister E, Courtenay AJ, et al. Microneedle applications in improving skin appearance. *Exp Dermatol*. 2015;24:561–566.
16. Fan X, Long J, Quan X, et al. Safety and efficacy evaluation of botulinum toxin an injection by FITTER® microneedle to treat medial lower eyelid wrinkles. *J Cosmet Dermatol*. 2024;23:1253–1258.
17. Lee KJ, Jeong SS, Roh DH, et al. A practical guide to the development of microneedle systems—in clinical trials or on the market. *Int J Pharm*. 2020;573:118778.
18. Choi SY, Kwon HJ, Ahn GR, et al. Hyaluronic acid microneedle patch for the improvement of crow's feet wrinkles. *Dermatol Ther*. 2017;30:e1–e6.
19. Massufero Vergilio M, Caradoc Birchall J, Lauro Lima L, et al. Drug delivery systems based in microneedles for dermatological diseases and aesthetic enhancement. *Curr Med Chem*. 2023;31:3473–3487.
20. Sabri AH, Kim Y, Marlow M, et al. Intradermal and transdermal drug delivery using microneedles—fabrication, performance evaluation and application to lymphatic delivery. *Adv Drug Deliv Rev*. 2020;153:195–215.
21. Levin Y, Kochba E, Hung I, et al. Intradermal vaccination using the novel microneedle device MicronJet600: past, present, and future. *Hum Vaccin Immunother*. 2015;11:991–997.
22. Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. *Vaccine*. 2009;27:454–459.
23. Tuan-Mahmood TM, McCrudden MT, Torrisi BM, et al. Microneedles for intradermal and transdermal drug delivery. *Eur J Pharm Sci*. 2013;50:623–637.
24. Pu QS, Luttge R, Gardeniers HJ, et al. Comparison of capillary zone electrophoresis performance of powder-blasted and hydrogen fluoride-etched microchannels in glass. *Electrophoresis*. 2003;24:162–171.
25. Sjödahl J, Melin J, Griss P, et al. Characterization of micromachined hollow tips for two-dimensional nanoelectrospray mass spectrometry. *Rapid Commun Mass Spectrom*. 2003;17:337–341.
26. Dell'Avanzato R, Gualdi A, Vescovi E, et al. Facial rejuvenation using a microneedle-based device with a revitalizing solution and free hyaluronic acid. *J Cosmet Dermatol*. 2021;20:3701–3703.
27. Boca A, Fanian F, Smit R, et al. Evaluation of the performance and safety of a new micro-needle technology in comparison with the classic needle on the antiaging effects of a biorevitalizing solution: a randomized split face/neck study. *J Cosmet Dermatol*. 2024;23:3974–3985.
28. Cao Y, Yang JP, Zhu XG, et al. A comparative *in vivo* study on three treatment approaches to applying topical botulinum toxin A for crow's feet. *Biomed Res Int*. 2018;2018:6235742.
29. Jia Z, Lu H, Yang X, et al. Adverse events of botulinum toxin type A in facial rejuvenation: a systematic review and meta-analysis. *Aesthetic Plast Surg*. 2016;40:769–777.
30. Jung GS, Kim HS. A novel technique to reduce pain from intradermal injection of botulinum toxin type A. *Plast Reconstr Surg Glob Open*. 2021;9:e3417.
31. Shi LL, Sargen MR, Chen SC, et al. Effective local anesthesia for onabotulinumtoxin A injections to treat hyperhidrosis associated with traumatic amputation. *Dermatol Online J*. 2016;22:13030/qt38b203d0.
32. Ishikawa K, Fukamizu H, Takiguchi T, et al. Application of a three-microneedle device for the delivery of local anesthetics. *Patient Prefer Adherence*. 2015;9:585–588.

33. Alam M, Geisler A, Sadhwani D, et al. Effect of needle size on pain perception in patients treated with botulinum toxin type A injections: a randomized clinical trial. *JAMA Dermatol.* 2015;151:1194–1199.
34. Azzopardi EA, Whitaker IS. Acute pain management during facial injection of botulinum toxin: an evidence-based systematic review. *Plast Reconstr Surg.* 2010;125:162e–164e.
35. Paracka L, Kollewe K, Wegner F, et al. Strategies to decrease injection site pain in botulinum toxin therapy. *J Neural Transm (Vienna).* 2017;124:1213–1216.
36. Chorney SR, Villwock JA, Suryadevara AC. Vibration versus ice to reduce cosmetic botulinum toxin injection pain-a randomized controlled trial. *Ear Nose Throat J.* 2019;98:351–355.
37. Gupta J, Park SS, Bondy B, et al. Infusion pressure and pain during microneedle injection into skin of human subjects. *Biomaterials.* 2011;32:6823–6831.
38. Liew S. Discussion: microbotox of the lower face and neck: evolution of a personal technique and its clinical effects. *Plast Reconstr Surg.* 2015;136:101S–103S.
39. Wu WTL. Microbotox of the lower face and neck: evolution of a personal technique and its clinical effects. *Plast Reconstr Surg.* 2015;136:92S–100S.
40. Steinsapir KD, Rootman D, Wulc A, et al. Cosmetic microdroplet botulinum toxin A forehead lift: a new treatment paradigm. *Ophthalmic Plast Reconstr Surg.* 2015;31:263–268.
41. Kapoor R, Shome D, Jain V, et al. Facial rejuvenation after intradermal botulinum toxin: is it really the botulinum toxin or is it the pricks? *Dermatol Surg.* 2010;36:2098–2105.
42. Fabi SG, Park JY, Goldie K, et al. Microtoxin for improving pore size, skin laxity, sebum control, and scars: a roundtable on integrating intradermal botulinum toxin type A microdoses into clinical practice. *Aesthet Surg J.* 2023;43:1015–1024.
43. Ascher B, Klap P, Marion MH, et al. Botulinum toxin in the treatment of frontoglabellar and periorbital wrinkles. An initial study. *Ann Chir Plast Esthet.* 1995;40:67–76.
44. Cavallini M, Papagni M, Augelli F, et al. Heterogeneous crow's feet line patterns and customized botulinum toxin rejuvenating treatment. *J Cosmet Dermatol.* 2022;21:4294–4300.
45. Piao JZ, Oh W, Choi YJ, et al. Ultrasonographic analyses of crow's feet and novel guideline for botulinum toxin injection. *J Cosmet Dermatol.* 2022;21:3787–3793.
46. Torrisi BM, Zarnitsyn V, Prausnitz MR, et al. Pocketed microneedles for rapid delivery of a liquid-state botulinum toxin A formulation into human skin. *J Control Release.* 2013;165:146–152.
47. Hong JY, Ko EJ, Choi SY, et al. Efficacy and safety of a novel, soluble microneedle patch for the improvement of facial wrinkle. *J Cosmet Dermatol.* 2018;17:235–241.
48. Kang G, Tu TNT, Kim S, et al. Adenosine-loaded dissolving microneedle patches to improve skin wrinkles, dermal density, elasticity and hydration. *Int J Cosmet Sci.* 2018;40:199–206.
49. Glogau R, Blitzer A, Brandt F, et al. Results of a randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of a botulinum toxin type A topical gel for the treatment of moderate-to-severe lateral canthal lines. *J Drugs Dermatol.* 2012;11:38–45.
50. Brandt F, O'Connell C, Cazzaniga A, et al. Efficacy and safety evaluation of a novel botulinum toxin topical gel for the treatment of moderate to severe lateral canthal lines. *Dermatol Surg.* 2010;36:2111–2118.
51. Nkengne A, Bertin C. Aging and facial changes-documenting clinical signs, part 1: clinical changes of the aging face. *Skinmed.* 2012;10:284–289.
52. Colomb L, Flament F, Wagle A, et al. In vivo evaluation of some biophysical parameters of the facial skin of Indian women. Part I: variability with age and geographical locations. *Int J Cosmet Sci.* 2018;40:50–57.
53. Colomb L, Flament F, Wagle A, et al. In vivo evaluation of some biophysical parameters of the facial skin of Indian subjects living in Mumbai. Part II: Variability with age and gender. *Int J Cosmet Sci.* 2018;40:141–147.
54. Levy JL, Servant JJ, Jouve E. Botulinum toxin A: a 9-month clinical and 3D in vivo profilometric crow's feet wrinkle formation study. *J Cosmet Laser Ther.* 2004;6:16–20.
55. Sapra P, Demay S, Sapra S, et al. A single-blind, split-face, randomized, pilot study comparing the effects of intradermal and intramuscular injection of two commercially available botulinum toxin A formulas to reduce signs of facial aging. *J Clin Aesthet Dermatol.* 2017;10:34–44.
56. Awaida CJ, Jabbour SF, Rayess YA, et al. Evaluation of the microbotox technique: an algorithmic approach for lower face and neck rejuvenation and a crossover clinical trial. *Plast Reconstr Surg.* 2018;142:640–649.
57. Wang PM, Cornwell M, Hill J, et al. Precise microinjection into skin using hollow microneedles. *J Invest Dermatol.* 2006;126:1080–1087.