




IncobotulinumtoxinA for the Treatment of Glabella and Forehead Dynamic Lines: A Real-Life Longitudinal Case Series

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Background: There is substantial interpersonal variation in the patterns of muscular contraction that substantiates the use of personalized points of application and dosages in clinical practice to achieve optimal results. Nevertheless, there has been no real-life therapeutic series with botulinum toxin for aesthetic treatment of the face in which the subjects were systematically followed to assess its long-term benefit.

Purpose: To assess the performance and length of the treatment of glabellar and forehead lines with IncobotulinumtoxinA in a real-life setting.

Patients and Methods: We enrolled 20 adults with indications for the treatment of upper facial dynamic lines (glabella and forehead) with botulinum toxin. The protocols of injection points were personalized by the injectors. The participants were photographed under maximum facial contraction before the application (D0) and after 15, 90, 120, and 180 days. The photos were randomly assessed by two blinded experienced raters to consensually grade the dynamic lines according to the Merz Aesthetics Scales (MAS). Efficacy was defined as the reduction in the MAS score.

Results: At D15, 18 (90%; 95% CI: 80%–100%) participants reached the zero score, or a 2-point reduction on the MAS score from the forehead and 16 (80%; 95% CI: 65–90%) reached that reduction for the glabella. These values from D90 were 14 (70%; 95% CI: 55–85%) for both sites. At D120, these values were 11 (55%; 95% CI: 35–75%) and 8 (40%; 95% CI: 25–55%) for the forehead and glabella. At D180, 10 (50%; 95% CI: 30–70%) participants presented a MAS score for forehead or glabella dynamic lines lower than the score assessed at D0.

Conclusion: As much as 70% of the patients sustained a reduction of scores after 120 days of the treatment for dynamic glabellar and forehead lines. Half of the patients evidenced prolonged benefit at 180 days.

Keywords: incobotulinumtoxinA, botulinum toxin, injection technique, ONE21, dynamic wrinkles, upper face, Xeomin

Introduction

Facial aging is a complex process that involves genetic, behavioral, and environmental factors. It comprises the loss of volume in fat compartments, sagging as well as skin dyschromia, alteration in texture, radiance, patterns of facial mimic, and dynamic wrinkles, which result from repeated muscle activity.^{1–3} These elements substantiate the use of combined procedures for facial rejuvenation.

Neurotoxins (as botulinum toxin A) are the gold standard treatment for dynamic wrinkles of the upper face. Nevertheless, despite procedure safety and satisfactory results, the comparative performance of different brands provides

heterogeneous results, which can be due to different dosages, points of injection, dilution, the severity of the wrinkles, protocols of outcomes, and follow-up.⁴⁻⁶

Randomized controlled trials for the assessment of the efficacy of botulinum toxin utilize standardized dosages and points of injection to increase the internal validity of the study. Nevertheless, the variation of muscles used in the contraction of the face, as well as their intensity, can demand an individualized regimen of treatment to maximize the results.⁷⁻⁹

Despite the expert recommendations, in clinical practice, the protocols of aesthetic use of botulinum toxin (eg, dosages and points of injection) vary highly between countries, the assessment of injectors, and the patient's demands.¹⁰⁻¹² Actually, pragmatic and real-life studies offer the best evidence of the effectiveness of a treatment, measuring the extent to which an intervention does what is intended in routine circumstances.¹³

To date, there have been no real-life therapeutic series with botulinum toxin for aesthetic treatment of the face in which the subjects were systematically followed to assess its long-term benefit. This study aimed to assess the performance and length of the benefit treatment of glabellar and forehead lines with INCO in a real-life setting.

Patients and Methods

This prospective, evaluator-blinded, longitudinal real-life study enrolled 20 adult participants, who were indicated for treatment for upper facial dynamic lines (glabella and forehead) with botulinum toxin, in ten medical offices in Brazil from January 2021 to March 2022.

Inclusion criteria were age ranged between 30 and 50 years old, in accordance to follow-up by 180 days without other facial procedures, female-to-male inclusion rate of 3:1, without systemic comorbidities, breastfeeding, or pregnancy. Exclusion criteria were rosacea, facial acne, facial scars, facial dyschromias, dark circles, suntanning, severe skin laxity, obese, and autoimmune diseases.

The assessment and protocols of injection points and dosages were personalized by the injectors, according to their routine use and individual perception of adjustment needed at two weeks. Botulinum toxin was the only facial procedure the patients were subjected during the follow-up period.

The vials of 100U of incobotulinumtoxinA (INCO, Xeomin[®]/Bocouture[®], Merz Pharmaceuticals GmbH) were reconstituted in 1 or 2 mL of sterile saline solution shortly before the treatments.

The participants were photographed under maximum facial contraction before the application (D0) and after 15, 90, 120, and 180 days (D15, D90, D120, D180). The photos were randomly assessed by two blinded experienced raters to consensually grade the severity of dynamic lines according to the Merz Aesthetics Scales (MAS), a 5-point scale in which the scores range from 0 (no lines) to 4 (very severe lines).¹⁴

The main outcomes were any reduction of the MAS score and its reduction by 2 points from baseline (D0), or MAS = 0 if MAS = 1 at D0, assessed at D15, D90, and D120. Secondary outcomes were MAS scores at D180 lower than D0 and adverse events, as well as the association with MAS reduction at D180 according to age, INCO dosage, and MAS at D0.

The 95% confidence intervals (95% CI) were calculated for the percentiles of MAS reductions through bootstrapping (5000 resamples).¹⁵ The change in the MAS assessment before and after treatment was analyzed by Generalized Estimated Equations, and the multiple comparisons were corrected by the Šidák procedure.^{16,17} Correlations between the dose used to treat each region and MAS severity were explored by Spearman's (rho) rank correlation.¹⁸ The reduction of MAS at D180 in comparison with D0 was explored by a multivariate binary logistic regression model according to age, phototype, and MAS score at D0, and the effect size was represented by the odds ratio (OR).¹⁹

A *posteriori* sensitivity analysis was performed through the assessment of the female subjects of the sample.²⁰

Data were analyzed using IBM SPSS v25 software. Significance was set as a p-value ≤ 0.05 .²¹

The sample size was preliminarily estimated to detect up to 70% of participants with a reduction of MAS score by 2 points from baseline (D0), or MAS = 0 if MAS = 1 at D0, at D90. Considering a standard error of 20%, alpha = 0.05 and power = 0.80, resulting in 20 treatments.²²

The study complies with the Declaration of Helsinki. It was approved by the Research Ethics Committee of the Faculdade de Medicina de Botucatu, UNESP (Botucatu, São Paulo, Brazil), with approval no. 4.767.103, and all the participants gave written consent for data and image publication.

Results

The main demographic and clinical data of the participants are presented in [Table 1](#). Ages ranged from 31 to 50 years old, there was a predominance of women (80%), and most baseline MAS scores for dynamic lines were severe and very severe. The range of units of INCO used to treat the forehead was 7 to 44, and glabella was 17 to 35 ([Supplementary Table](#)); however, these doses were not correlated with the previous MAS severity ($\rho < 0.4$; $p > 0.085$).

The follow-ups of the participants are disclosed in the [Figures 1–3](#). According to the consensual blind assessment ([Figures 4 and 5](#)), the MAS scores from the forehead and glabella dynamic lines at D15, D90, and D120 were lower than their values at D0 ($p < 0.01$). The reduction of at least 1-point MAS scores from forehead dynamic lines was achieved in 90%, 85%, 70% of participants at D15, D90, and D120, respectively. For the glabellar lines, the MAS reduction was 85%, 95%, 80%, at D15, D90, and D120, respectively ([Figures 6 and 7](#)).

At D15, 18 (90%; 95% CI: 80–100%) participants have reached zero or a 2-point reduction on MAS score from the forehead and 16 (80%; 95% CI: 65–90%) for the glabella. These values from D90 were 14 (70%; 95% CI: 55–85%) for both sites. At D120, these values were 11 (55%; 95% CI: 35–75%) and 8 (40%; 95% CI: 25–55%) for the forehead and glabella, respectively.

At D180, 10 (50%; 95% CI: 30–70%) participants presented a MAS score for forehead or glabella dynamic lines lower than the assessed at D0. The long-term reduction of MAS scores for the glabella was not associated with age ($p > 0.07$) or dose of INCO ($p > 0.43$); however, the more intense MAS score at D0, the greater the chance of long-term improvement at the forehead (OR = 3.8; $p < 0.01$) and glabella (OR = 3.9; $p = 0.02$). There were no adverse effects from the treatments assessed at D15.

In the sensitivity analysis, only the 18 women were analyzed, and the results are quite comparable to the whole sample. The MAS reduction in forehead dynamic lines was achieved in 89%, 89%, 72%, and 50% of the participants at D15, D90, D120, and D180, respectively. The reduction for glabellar dynamic lines was achieved in 89%, 100%, 83%, and 50% of the participants at D15, D90, D120, and D180, respectively.

Table 1 Main Demographic and Clinical Data of the Participants (n = 20)

Variables	Values
Sex, n (%)	
Female	18 (90%)
Male	2 (10%)
Age, mean (SD)	38.4 (5.0)
Skin phototype, n (%)	
II	6 (30%)
III	9 (45%)
IV	5 (25%)
Baseline MAS – Forehead Lines Dynamic, n (%)	
I	3 (15%)
II	3 (15%)
III	3 (15%)
IV	11 (55%)
Units of IncobotulinumtoxinA at the forehead, mean (SD)	20.7 (7.3)
Points of injection at the forehead, mean (SD)	16.4 (4.0)
Baseline MAS – Glabella Lines Dynamic, n (%)	
I	2 (10%)
II	8 (40%)
III	6 (30%)
IV	4 (20%)
Units of IncobotulinumtoxinA at glabella, mean (SD)	23.2 (4.6)
Points of injection at glabella, mean (SD)	5.6 (1.2)



Figure 1 Sequential visits (previous to 180 days) from a participant who received 23 units of IncobotulinumtoxinA on the glabella. Photos under maximum contraction.

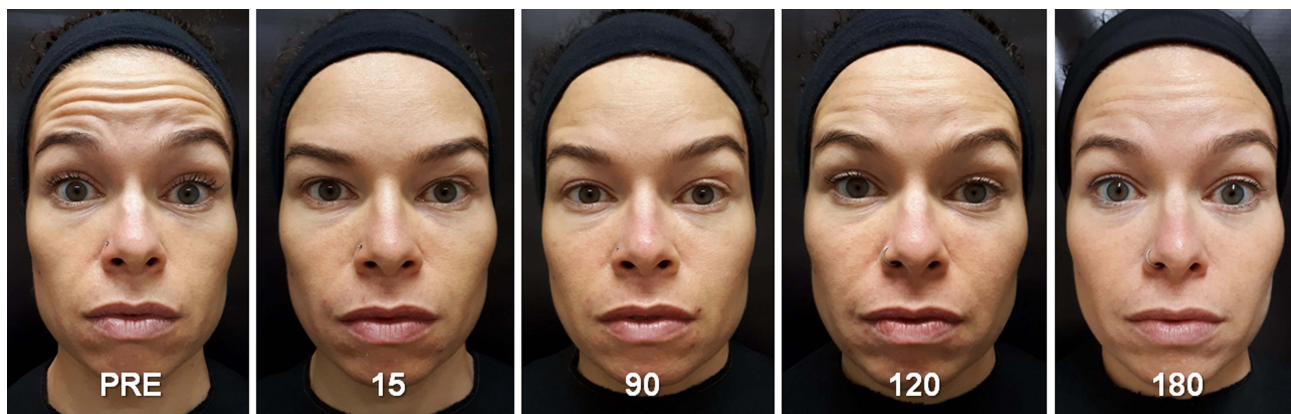


Figure 2 Sequential visits (previous to 180 days) from a participant who received 22 units of IncobotulinumtoxinA on the forehead. Photos under maximum contraction.



Figure 3 Sequential visits (previous to 180 days) from a participant who received 44 units of IncobotulinumtoxinA on the forehead. Photos under maximum contraction.

Discussion

This case series brings real-life data regarding the treatment of forehead and dynamic glabella lines with INCO. By tailoring the treatment of these regions according to the individualized needs, we demonstrated sustained benefits for up to 70% of the participants after 120 days, despite a wide variation in dosage and points of application.

Real-life data from aesthetic treatments incorporates pragmatic results that can differ from the outcomes achieved in standardized procedures from randomized controlled trials because the results are influenced by the assistant assessment,

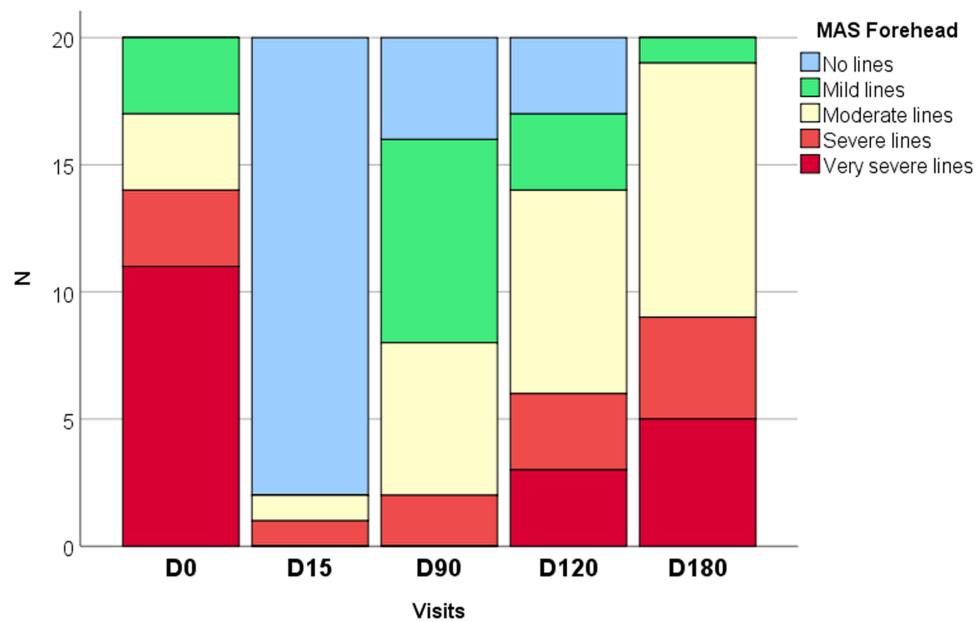


Figure 4 MAS scores of dynamic lines of the forehead at D0, D15, D90, D120, and D180 (n = 20).

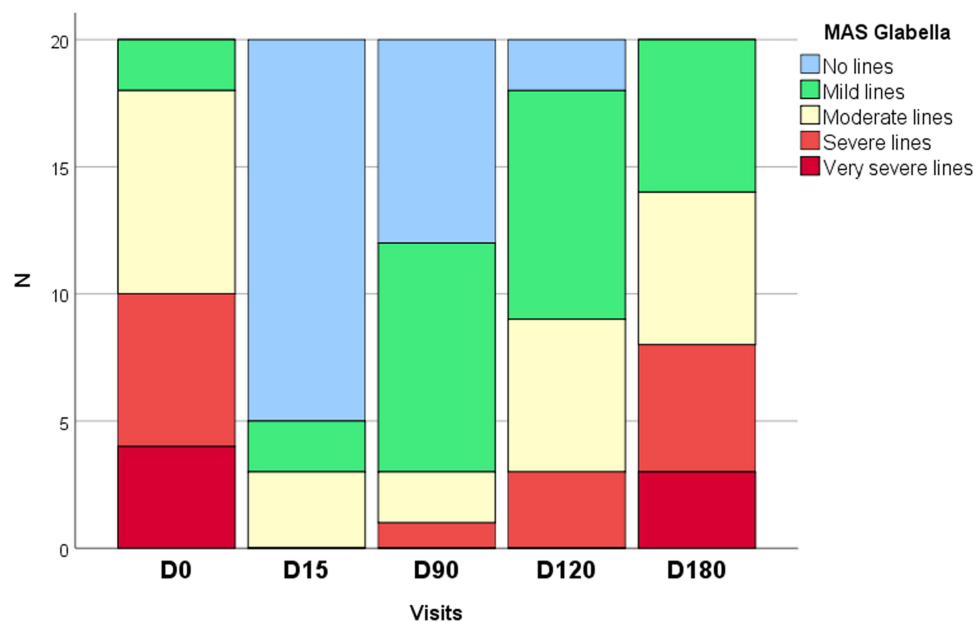


Figure 5 MAS scores of dynamic lines of the glabella at D0, D15, D90, D120, and D180 (n = 20).

the treatment regimen, and patient demands. Nevertheless, these results are more reliable regarding the expected effectiveness in clinical practice, when the aesthetic procedures are personalized. Furthermore, the individualization of the assessment for the treatment of upper face dynamic wrinkles with INCO can result in better outcomes than the homogeneous approach for all patients and can potentially decrease the rate of adverse effects.^{23,24}

There has been a lack of blinded comparative (head-to-head) studies, with homogeneous outcomes, on assessing long-term effects of botulinum toxin A among brands and different techniques.⁵

In a standardized injection protocol of abobotulinumtoxin A in 22 Brazilian patients, the evaluator-blinded 120 days of any improvement for forehead and glabellar lines, at maximum contraction, was evidenced in 70.4% and 83.9% of the subjects, respectively. For the 180-days follow-up, the reduction of contraction scores for forehead and glabellar lines

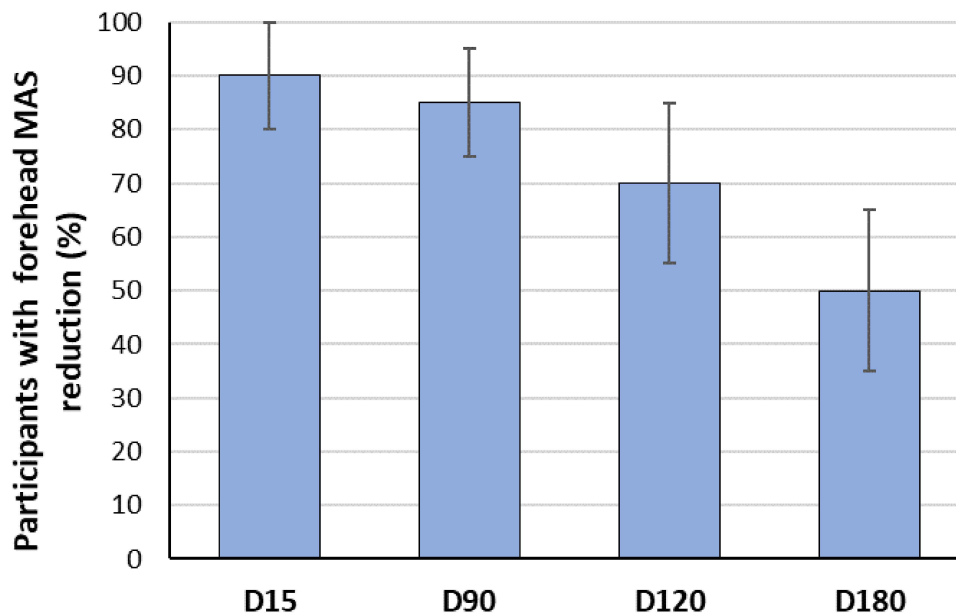


Figure 6 Percent (95% CI) of participants with any improvement in forehead MAS dynamic lines severity, according to the visits (n = 20).

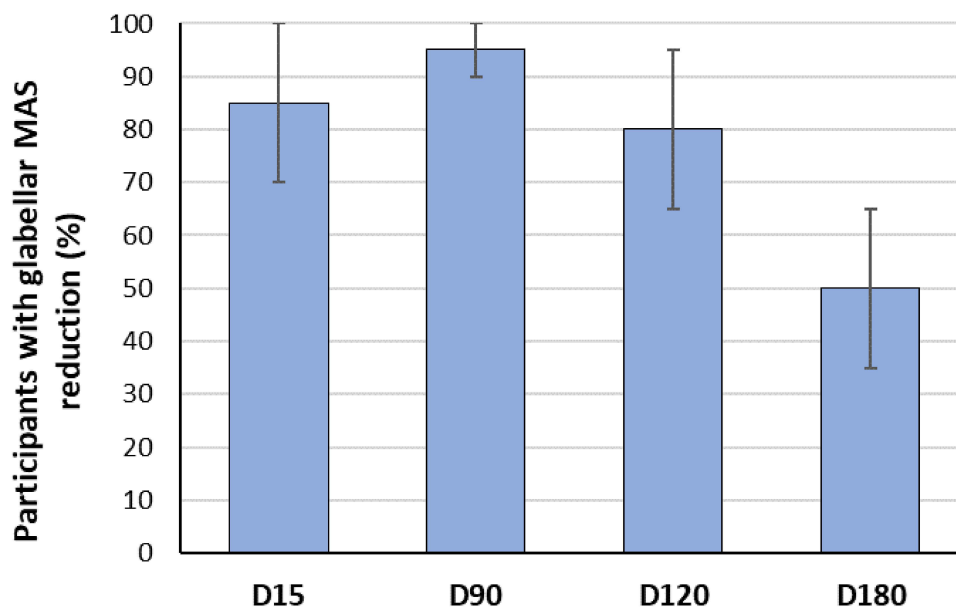


Figure 7 Percent (95% CI) of participants with any improvement in glabellar MAS dynamic lines severity, according to the visits (n = 20).

was sustained at 22.2% and 35.5% of the subjects, respectively.²⁵ These results are quite parallel with the demonstrated in this case series.

Another Brazilian series with 110 subjects reported the results of the treatment of the upper face with a standardized dosage of Lanzhou botulinum toxin type A. The investigators perceived any improvement after 120 days in 37% of the subjects. Interestingly, the perception of long-term improvement after 180 days was higher by the patients (50%) than by the investigator (20%).²⁶

A multicenter trial that enrolled 300 subjects treated with a standardized dose of abobotulinumtoxinA for glabellar dynamic lines resulted in 60% of the subjects with any reduction on contraction scores at D120, and 31% of the subjects evidenced only mild lines at maximum contraction at D120.²⁷ A meta-analysis that polled the data from four trials, with 523

onabotulinumtoxinA-treated subjects with a standard dosage on glabella, resulted in 50% of the subjects with any reduction of contraction scales after 120 days of the treatments.²⁸ In a multicenter trial with the standardized injection protocol of onabotulinumtoxinA in 203 patients from United States, the evaluator-blinded 120 days' improvement (mild lines at maximum contraction) for glabellar lines were evidenced in 26.2% of the subjects.²⁹ Nevertheless, only head-to-head trials can compare and adequately explore the different brands in their long-term performance.

Our results showed that the patients with more severe dynamic lines have a greater long-term benefit, but not those who were injected with a greater dosage. As long as INCO promotes a definitive blockade in neuromuscular transmission, this result suggests that the number of points of application decided on the basis of the assessment of muscle contraction and the dosage of injection can result in better long-term results. It means that the right assessment and application technique are paramount for the success of the treatment and that the characteristics of INCO allow individualized approaches and future new technique development.³⁰

The aesthetic treatment of the upper face with botulinum toxin A promotes an overall improvement in skin and facial appearance. The long-term benefit can be due to the decrease of the muscle contraction, which leads to its mass reduction and a lower strength in its recovery. This phenomenon can be more pronounced in the more severe cases.³¹ A behavioral conditioning of lower muscle contraction to perform face expressions cannot be excluded, as the greater positive reinforcement following botulinum toxin A treatment should occur in the more severe cases.^{32,33}

This study has limitations for being neither randomized nor controlled, as well as it was underpowered for the analysis of subgroups regarding age, gender and skin phototype. Nevertheless, these limitations do not hinder the assessment of pragmatic results regarding the INCO performance for the treatment of glabellar and forehead dynamic lines in routine circumstances. Pragmatic double-blinded, randomized controlled trials are warranted on long-term outcomes for the comparison of techniques and brands of botulinum toxin A for the treatment of dynamic wrinkles of the upper face.

Conclusion

In this real-life series, as much as 70% of the patients sustained a reduction of contraction scores after 120 days of the treatment with INCO for dynamic glabellar and forehead lines. Half of the patients evidenced prolonged benefit at 180 days. The patients with more severe dynamic lines have a greater long-term benefit.

Acknowledgments

The patients in this study who given written informed consent to publication of their case details and images. The authors thank Prof. Hélio Miot for protocol development support, ethics approval, editorial and writing assistance.

Ethics Statement

The study was approved by the Research Ethics Committee of the Faculdade de Medicina de Botucatu, UNESP (Botucatu, São Paulo, Brazil) – approval no. 4.767.103.

Informed Consent Statement

Written informed consent was obtained from patients to publish their data in this paper.

Disclosure

Ana da Cunha is an employed of Merz. Rossana de Vasconcelos, David di Sessa, Gabriel Sampaio, Pitila Ramalhoto, Bruno Zampieri, Bárbara Deus, Suyan Vasconcellos, Talitha Bellote, Juliano de Carvalho, Giseli Petrone, Gustavo Moreira Vinícius Figueredo are medical speakers of Merz Aesthetic. The authors report no other conflicts of interest in this work.

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