Dermatology Practical & Conceptual

Subjective and Objective Measurements of the Facial Effects of Microdoses of Botulinum Toxin

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Ethical Statement: This study was approved by the local Ethics Committee of Moinhos de Vento Hospital in Porto Alegre, Brazil and followed the Good Clinical Practices, the Declaration of Helsinki protocols and all national regulations.

ABSTRACT

Introduction: Studies have suggested that botulinum toxin A may improve skin quality, and application protocols using hyper-diluted doses of botulinum toxin (microdosing) have been studied as a way to achieve therapeutic goals without fully paralyzing the targeted muscles.

Objectives: To evaluate the effects of a combined protocol utilizing both the standard dosing and the microdosing of AbobotulinumtoxinA for the improvement of skin quality, measured by objective and subjective measurements.

Methods: Thirty patients were treated with botulinum toxin using both the standard technique and the microdosing technique. Objective (Sebumeter®, Mexameter® and digital dermoscopy pictures) and subjective (Global Aesthetic Improvement Scale and a clinical scale for evaluating the quality of facial skin) measurements of the effects in the treated areas were taken to assess the efficacy of the treatment.

Results: Digital dermoscopy showed a marked reduction of erythema and telangiectasias. Erythema and telangiectasias improved both on objective and subjective measurements. Skin oleosity, static rhytids, papules and pustules and enlarged pores improved on subjective measurements. Patient satisfaction was high (93%) despite the high rate of adverse events (56%).

Conclusions: The combined application of standard doses and microdoses of AbobotulinumtoxinA is effective in improving the overall quality of facial skin. The effects on erythema and telangiectasias suggest that it is an effective treatment option for patients with erythematotelangiectatic rosacea. When applying microdoses of botulinum toxin in the lower and mid-face, the doses and pattern of injection should be customized for each patient to reduce the occurrence of adverse events.

Introduction

Botulinum Toxin Type A (BTX-A) has been used for over 30 years to treat wrinkles and facial lines [1]. Injections of BTX-A are a simple, rapid, effective and safe method to improve the appearance of patients. Long term repeated treatments with BTX-A on the face treat dynamic rhytids and have additional positive effects, such as repositioning beauty defining facial structures (eg the eyebrows) and preventing static rhytids [2,3].

New off label indications for BTX-A are being explored, including the treatment of facial erythema, facial flushing, and acne [4-8]. One technique currently being studied is the microdosing of BTX-A, which is the injection of multiple microdroplets of hyper-diluted botulinum toxin into the dermis or in the interface between the dermis and the superficial subcutaneous. The lower concentrations and lower doses utilized in the microdosing technique are able to improve facial aesthetics while maintaining the muscular function in the treated areas [9].

Objectives

This study was designed to evaluate the effects of a combined protocol utilizing both the standard and the microdosing techniques of AbobotulinumtoxinA (Dysport ®) injections for the improvement of skin quality (wrinkles, erythema, telangiectasias, enlarged pores, and oiliness), using objective and subjective measurement methods, and to assess treatment safety and patient satisfaction.

Methods

Study Design

This was a prospective, single-center, open label trial. The participants were recruited at a research center in Porto Alegre, Brazil, and provided written informed consent. This study followed the Good Clinical Practices, Declaration of Helsinki protocols and all national regulations, and was approved by the local Ethics Committee of Associacao Hospitalar Moinhos de Vento.

The main inclusion criteria were patients aged between 18 and 70 years with at least one indication for cosmetic treatment with botulinum toxin in the upper face (glabellar, periorbital or forehead rhytids) and the presence of either rosacea (any kind), facial erythema, oily skin, enlarged pores or acne (any grade). Exclusion criteria included pregnancy or lactation, active infection on the treated areas, and having undergone previous invasive surgical procedures on the face, BTX-A treatment on the face less than 6 months before the study, or treatments to improve the overall quality of the skin less than 3 months before the study (eg chemical peelings, fillers, radiofrequency therapy, microfocused ultrasound).

Assessments

The primary outcomes of this study were the clinical improvement in skin quality, the percentage of patients that improved in the Global Aesthetic Improvement Scale (GAIS), and the frequency of adverse events. Secondary outcomes were patient satisfaction and changes in facial erythema, in skin oiliness, and in clinical characteristics of the skin assessed by digital dermoscopy.

Patients were evaluated at baseline/procedure (day 1), at touch up (day 21) and at two follow up visits (days 56 and 84). Skin quality was evaluated using a non-validated clinical scale developed by the researchers for this study, which rated the participants' erythema, superficial static rhytids, enlarged pores, papules or pustules, and skin oiliness as imperceptible (0), mild (0.5) or marked (1). The sum of all criteria classified the quality of the skin as great (0 to 1), good (1.5 to 2.5), average (3 to 4) or bad (4.5 to 5).

Photographs with a high-definition camera were taken at all visits, with patients at rest and while performing dynamic movements of the face. The pictures were taken at the same setting, with the same lighting, and by the same trained evaluator. They were used by three investigators (two dermatologists and one general practitioner) to clinically assess the patients and to fill the GAIS. The GAIS was also self-reported by each patient at every visit following the procedure.

Spectrometer measurements (Mexameter® MX 18) were used to objectively evaluate the patients erythema. At every visit, four measurements were taken at the left side of the forehead and other four measurements were taken at the left malar area of the patients. The mean value of the measurements of each area was recorded for statistical analysis. Skin oiliness was objectively quantified with Sebumeter® on the

forehead, chin, and on the tip of the nose, always on the midline of the face.

Digital dermoscopy was performed with Medicam® (FotoFinder Body Studio ATBM Master®, FotoFinder Systems GmbH). Pictures were taken with polarized and non-polarized lights 1cm lateral to the left alar-facial sulcus at 20x magnification, using an open front cap to avoid compression of the superficial vessels of the skin. The pictures were analyzed by the evaluators to assess the presence of erythema, oily skin, enlarged pores and telangiectasias, using their overall clinical impression to grade them as "improved" or "not improved".

Objective measurements were taken after the patients rested for at least 10 minutes in a climatized room at controlled temperature (22±2°C).

Adverse events were evaluated at every visit. Patients filled a satisfaction questionnaire at every visit after the procedure.

Procedure

All patients were simultaneously treated with the standard technique for at least one indication in the upper face (glabellar, forehead or periorbital rhytids) and with the microdosing technique.

For the standard technique, 300 units of AbobotulinumtoxinA (Dysport®) were reconstituted in 1.2mL of 0.9% saline solution for a concentration of 250U/mL (2.5U/0.01ml) and applied in intramuscular injections using an ultra-fine 0.3cc needle (30G). The number of units of botulinum toxin per injection point and the location where the points were applied varied according to the investigator's clinical assessment of the patients. The total dose applied in each area was different for each patient, but always followed the international consensus recommendations [7,8]. Doses of 40U to 50U were used to treat the glabella, of 45U to 65U to treat the periorbital area, of 5U to 15U to treat the forehead, and of 10U to 15U to treat the chin. The total dose utilized ranged from 120U to 210U for each patient.

For the microdosing technique, an additional 0.75mL of 0.9% saline solution was added to each 0.25mL of the previous reconstituted toxin (2.5U/0.01mL solution), resulting in a final concentration of 0.625U/0.01mL. The microdoses were applied intradermally following a standardized protocol: an addition to the standard treatment, each patient received an additional dose of 62.5U of Dysport® distributed in about 100 points of injection (Figure 1) - approximately 20U to 25U in each third of the face. Each injection consisted of a volume of 0.01ml (0.625U) per injection site.

Statistical Methods

Thirty patients were enrolled for this study through convenience sampling. Statistical analysis to determine sample size was not performed prior to the study. Data was described as mean ± standard deviation (SD) or median and interquartile range (IQR), depending on its distribution (evaluated through histogram). The significance of the variation of erythema levels measured by Mexameter® MX 18 and of sebum levels measured by Sebumeter® were tested using the paired samples Wilcoxon test, as the variable's distribution were not symmetric. Multiple testing corrections were not performed, and so the p-values of the secondary outcomes must be regarded as exploratory. The results were considered statistically significant when the P value was lower than 0.05.

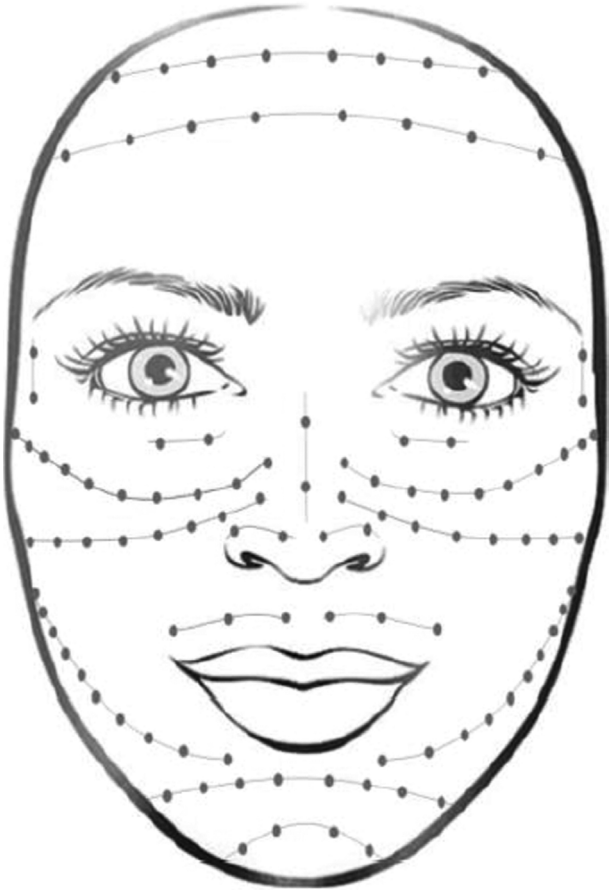

Figure 1. Injection points for the microdoses of Botulinum Toxin. The 100 points of injection were distanced 1cm from each other, were applied as symmetrically as possible and were evenly distributed between the left and right sides and the 3 thirds of the face.

Results

Twenty seven of thirty participants completed all visits; none of the dropouts were motivated by adverse events. Demographic data of the participants is shown in Table 1.

Figure 2 shows the changes in facial skin quality pre and post treatment as assessed by the clinical scale. In the final assessment, 56% of the patients improved their facial erythema (initially present in 23 patients), 56% improved their superficial static rhytids (initially present in 21 patients),

Table 1. Demographic data (N = 30).

Characteristics		
Age, years mean (±SD)		42.1 (±13.2)
Gender, N (%)	Female	28 (93.3%)
	Male	2 (6.7%)
Race, N (%)	Caucasian	29 (96.7%)
	Multiracial	1 (3.3%)
Phototype, N (%)	II	10 (33.3%)
	III	19 (63.3%)
	IV	1 (3.3%)
Smoking, N (%)	Non-smokers	29 (96.7%)
	Smokers	1 (3.3%)

SD = standard deviation.

41% improved their papules or pustules (initially present in 19 patients), and 30% improved their enlarged pores (Initially present in 19 patients). Skin oiliness (initially present in 26 patients) was reduced in 70% of patients. Overall, 85% of the subjects improved their final score on the scale.

On the GAIS, 89% (24) of the patients improved when assessed by the investigators, and 93% (25) self-reported an improvement of their appearance. Only one patient reported "worsening of their appearance" by the end of the treatment, which was due to an adverse event (reduction of smile amplitude), but none had a worsening of their appearance according to the investigators.

Out of the 28 patients, 16 (57%) had adverse events related to the treatment. Eleven participants had an isolated

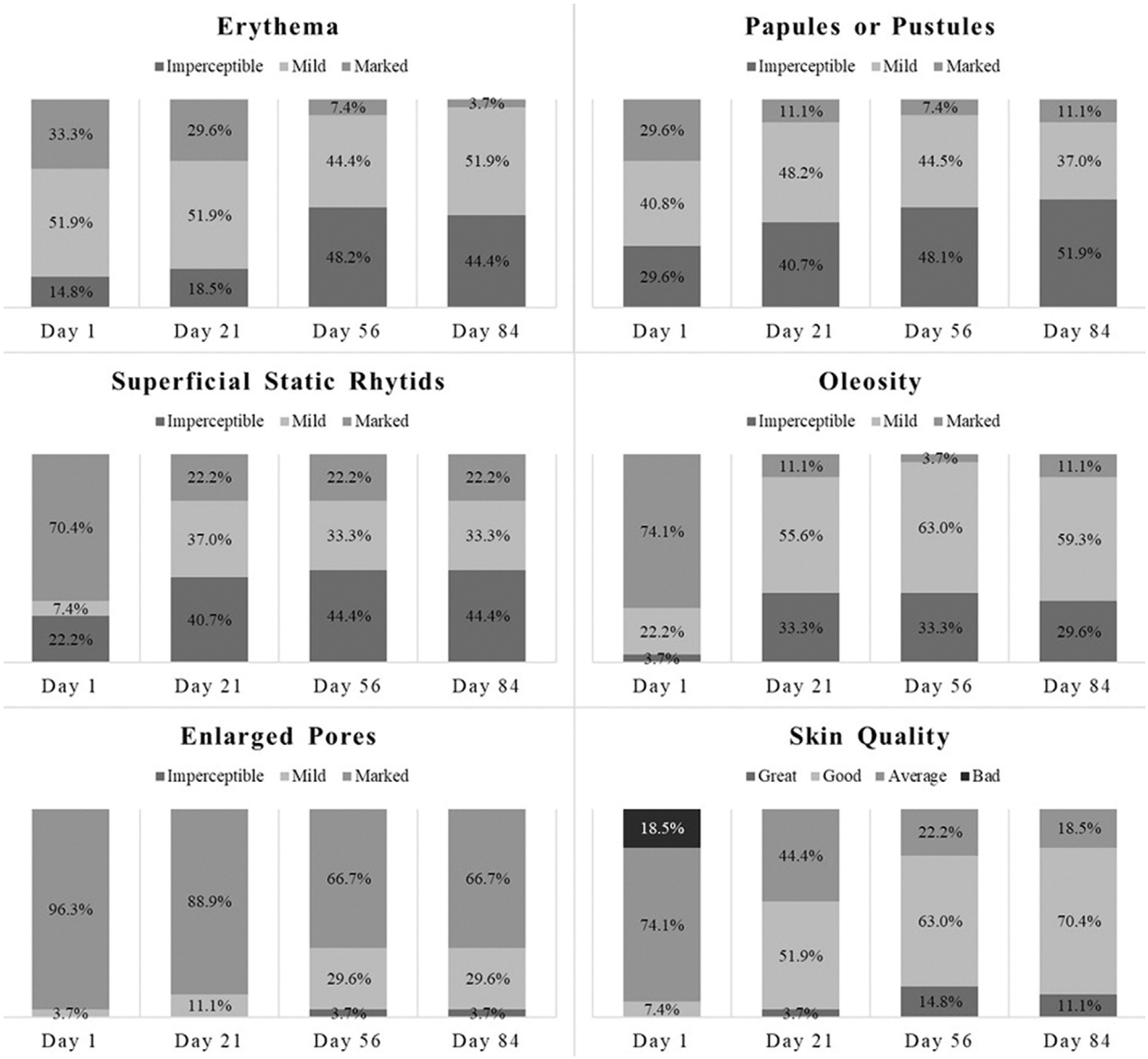

Figure 2. Changes in skin quality according to the clinical scale.

adverse event, four had 2 adverse events and one had 3 adverse events. Five different kinds of adverse events were observed: minor bruises (7 occurrences), eyebrow asymmetries (6 occurrences), reduction of smile amplitude (5 occurrences), smile asymmetries (3 occurrences), and one case of smile asymmetry associated with a difficulty of moving the lower lip. Minor bruises had a median duration of 4 days, lasting from 2 to 7 days; eyebrow asymmetries and smile asymmetries had a median duration of 20 days, lasting from 14 to 43 days (not counting one outlier smile asymmetry which lasted for 4 months); reductions of smile amplitude had a median duration of 60 days, lasting from 30 to 78 days.

Objective measurements of erythema on the malar region (assessed in the 27 patients who completed the study) showed a significant reduction (P = 0.041) of the median, from 440 (IQR 382.5 to 479.25) pre-treatment to 409 (IQR 364.5 to 458) on day 84. Statistical analysis of the measurements of erythema on the forehead and of sebum on all regions assessed were not significant.

On the satisfaction questionnaire, 93% of the patients reported an improvement of their appearance. 93% declared being satisfied with the treatment and 89% said they would like to receive the same treatment again in the future.

Table 2 shows the changes in skin quality as evaluated by digital dermoscopy. Improvement on erythema and telangiectasias was imperceptible on day 21, evident on day 56, and persisted until the end of the study. A subset of patients worsened on day 21 and later improved when compared to the baseline. The improvement in the caliber of the telangiectasias was greater on small caliber vessels and lasted until the end of the study.

Conclusions

Several studies suggested that injections of microdoses of BTX-A may be an effective procedure for the management of excess facial sebum, facial pores, papules and pustules, facial erythema, and rosacea [6,10,11]. The generalizability of the results of those studies, however, is limited due to the heterogeneity in the population being studied, the distinct treatment protocols being evaluated, the assessment methods utilized to measure the outcomes and the different brands of the BTX-A studied [6].

Our results suggest that microdoses of AbobotulinumtoxinA can improve skin quality, especially malar erythema, starting 3 weeks from the treatment and maintaining the results for at least 12 weeks, as skin quality was improved when assessed by the clinical scale and by digital dermoscopy. This finding is in accordance with previous literature [5,6].

It is noteworthy that digital dermoscopy revealed not only a reduction of the erythema, but also an improvement of the caliber of facial telangiectasias (Figures 3-5). This improvement was evident on day 56 and persisted until the end of the study. Telangiectasias have traditionally been treated with light-based therapies [12,13], but it is postulated that BTX-A can improve them by modulating blood vessel dilation, as it inhibits the release of acetylcholine from peripheral autonomic nerves of the cutaneous vasodilatory system, by blockading substance P and calcitonin gene-related peptide (which acts as inflammatory mediators) [14], and by inhibiting mast cell degranulation (which releases proinflammatory cytokines) [15]. The digital dermoscopy evaluation performed in our study revealed an evident reduction in the caliber of the telangiectasias, an effect which persistent until the end of the study. This is a new finding which has not been reported in previous studies [6,11].

Enlarged pores were evaluated with non-polarized light and without a liquid interface, as the lighting of the non-polarized light allowed better visualization of the pores. Our results suggest a reduction in the size of the pores in a subset of patients, but it is important to note that the absence of a liquid interface makes it harder to reproduce the pictures, thus limiting this evaluation.

Table 2. Skin quality evaluated by digital dermoscopy.

Evaluated Characteristic	Percentage of patients with that finding on baseline evaluation	Out of the patients which presented with that finding, the percentage which showed an improvement when compared to the baseline		
		Day 21	Day 56	Day 84
Erythema	93.3%	10.7%	77.8%	81.5%
Telangiectasias	93.3%	10.7%	74.1%	74.1%
Enlarged pores	66.7%	3.5%	18.5%	25.9%
Comedones	37.0%	3.5%	22.2%	25.9%

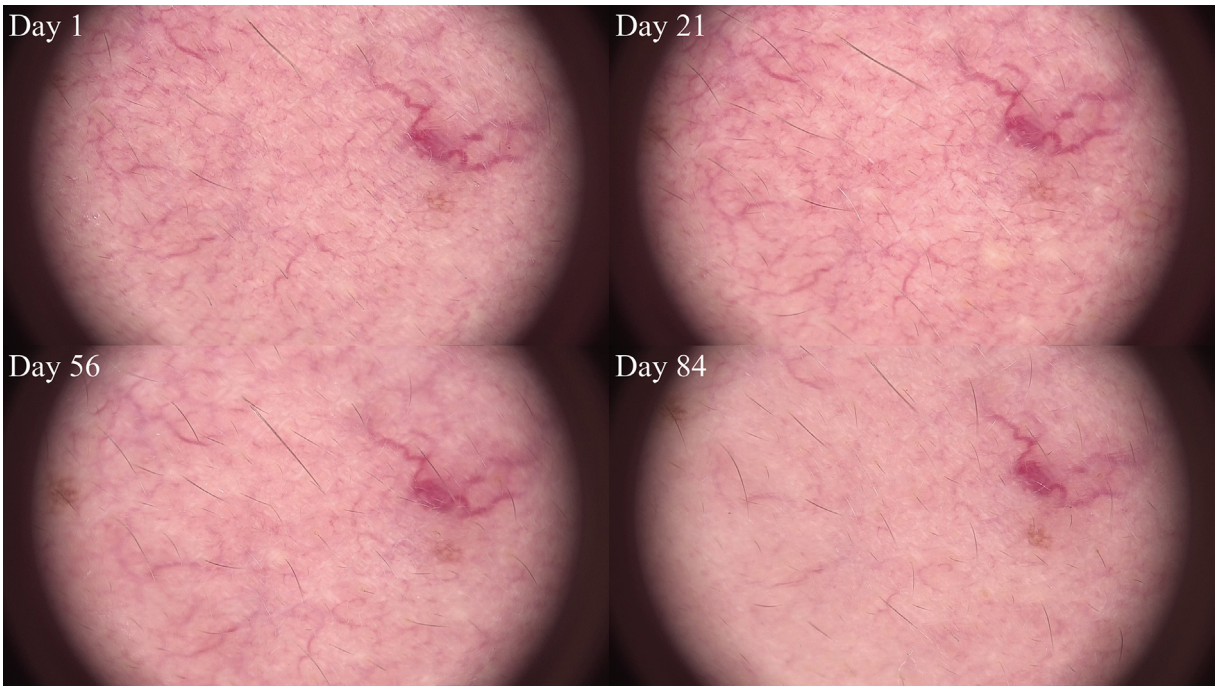

Figure 3. Polarized pictures taken with digital dermoscopy (20x magnification). Using the large telangiectasia as a reference point, it is possible to perceive a marked reduction in erythema and in the caliber of small vessels of the skin. The improvement was present on day 56 and lasted until day 84.

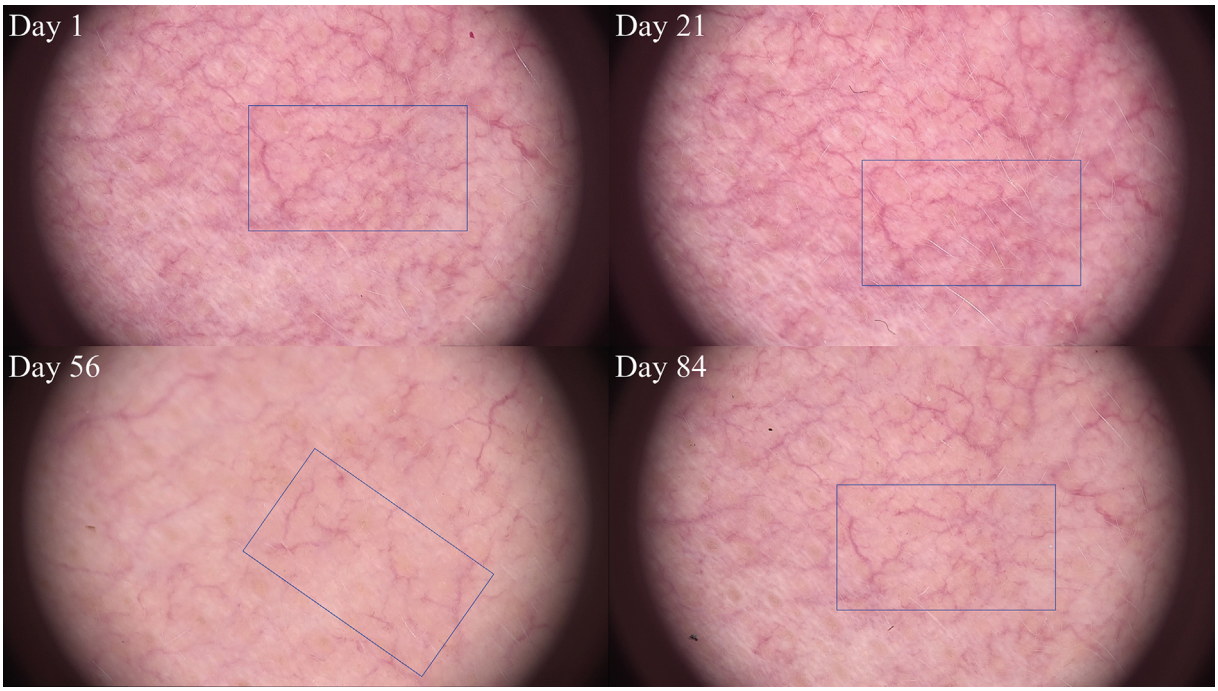

Figure 4. Polarized pictures taken with digital dermoscopy (20x magnification). The rectangle highlights an improvement in telangiectasias, starting on day 56 and persisting until day 84. A global reduction in erythema also occurred at the same time period.

Our findings are in accordance with other studies which used different treatment protocols of AbobotulinumtoxinA injections for the treatment of malar erythema, including the injection of 15 to 45 units in the affected areas (dilution of 100U/ml) and of 20 to 50 units per cheek (dilution of 100U/ml) [6]. Our protocol combines both the standard technique and the microdosing technique, being able to address both the facial wrinkles and facial erythema/telangiectasias in the same treatment session. Our study also documented the evolution of these effects in facial skin, as

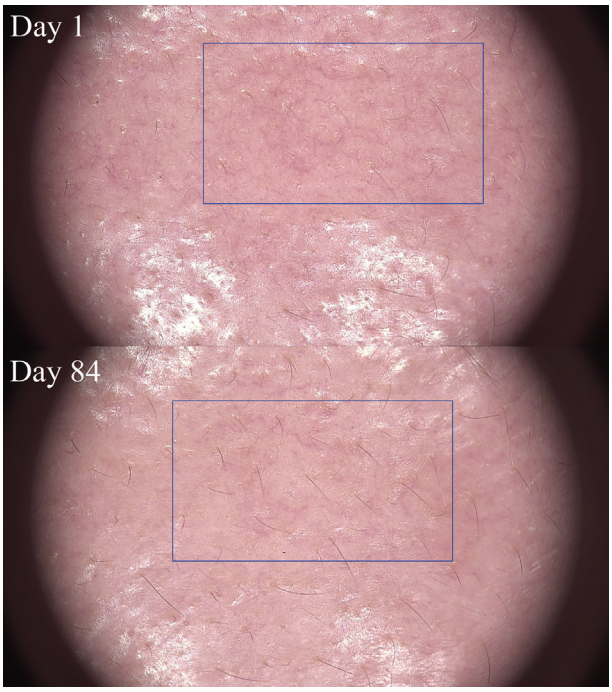

Figure 5. Non-polarized pictures taken with digital dermoscopy (20x magnification). A reduction in the size of the pores is seen in the area with light reflections. The rectangle highlights a reduction in the telangiectasias.

the objective and subjective measurements were taken from baseline to the final visit of the participants.

Although the skin oiliness improved after the treatment when assessed by the investigators using the clinical scale, no significant difference was observed in the objective measurements with Sebumeter®. This finding should be interpreted with caution, as our study might not have had the statistical power to detect a significant difference and because sebum production is known to vary according to different factors which are hard to control in spite of using a climatized setting, such as sweating, changes in the sleep cycle, menstrual period and the time of the day in which the measurements were taken [16]. Also, the fact that the production of sebum is not the same in different areas of the face makes it possible that the variation in sebum production responsible for the perception of the reduced skin oiliness in the investigators assessment may have happened in areas which were not objectively quantified in this study.

A total of 22 adverse events occurred in 16 (57%) of the patients, a rate higher than what has been reported in studies using only the standard technique [11]. These events were probably related to the sum of the effects caused by both techniques: the standard aesthetic treatment, ranging from 120U to 210U for each patient, plus the 62.5U used for microinjections. Moreover, to standardize the technique, 1/3 of the total microdoses was used in each third of the face. Ideally, the doses in the lower face should be lower than in the upper face, as the muscles of the lower face are very sensitive to lower doses of the toxins and any additional dose to the standard treatment is able to promote side effects. Thirteen (43%) adverse events were short termed, as they were either bruises resulting from the injections or asymmetries which were corrected in the touch up. Nine adverse events, however, lasted longer. They were located on the lower face and were all attributed to the sum of the units used in the microdosing technique over the standard technique. The adverse event which lasted longer was a reduction in the amplitude of the smile which, despite lasting more than 100 days, progressively improved as the treatment effects wore off.

To reduce the rate of adverse events, customized techniques with lower doses and less injection points should be used when applying microdoses of botulinum toxins in the perioral region. The total dose of microinjections and the number of injection sites should ideally be customized, as are other cosmetic treatments with botulinum toxins. In our study, the same doses used for each third of the face and the symmetrical injections of microdoses were a problem, especially for patients with marked facial asymmetries, as they require a more personalized approach to the treatment.

Despite the high rate of adverse events, patient satisfaction was very high due to the efficacy of the treatment, both in treating facial rhytids and in improving the quality of the skin. Eighty nine percent of the patients reported that they would like to repeat the treatment if given the chance and only one patient reported being dissatisfied with the results. The dissatisfaction of this patient was attributed to an adverse event (long-term reduction in the smile amplitude) and outweighed the benefits of the improvement she had in the quality of her skin. This corroborates that perioral adverse events cause intense discomfort to patients and that the dose applied in the perioral area should be lower than the dose used in this trial (approximately 20U). Customization of the number and injection sites are always important in aesthetic treatments.

Our study, however, has limitations. The small sample size and the non-probabilistic sample limits the interpretation of the statistical analysis, while the combined protocol of using the standard technique combined with the microdosing technique confounds the ability to judge which technique led to a perceived improvement in the areas which were treated with both techniques (glabellar, periorbital, chin and forehead).

Our results suggest that the combined protocol of standard dosing and microdosing of AbobotulinumtoxinA is an effective treatment for facial wrinkles and for fine lines of the lower eyelid and perioral region, while also being able to improve the overall quality of the skin in the injected areas. The therapeutic effects on erythema and the improvement of facial telangiectasias suggest that it may be a treatment

option for patients with erythematotelangiectatic rosacea. Given the high rate of adverse events in the perioral area using this combined protocol, caution should be taken with doses and number of injection sites when injecting this area. Customized doses and sites of injections can give optimal results and reduce the occurrence of adverse events.

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