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Dermal Thickness Increase and Aesthetic Improvement with Hybrid Product Combining Hyaluronic Acid and Calcium Hydroxyapatite: A Clinical and Sonographic Analysis

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Background: Hyaluronic acid filler injections are regarded as the gold standard procedure for facial rejuvenation. Standing as the second most injected cosmetic filler, calcium hydroxyapatite-based fillers are also widely used worldwide. However, to our knowledge, prospective studies assessing patient satisfaction and sonographic changes in dermal thickness after a single session of a hybrid filler combining hyaluronic acid and calcium hydroxyapatite have not been previously published.

Methods: This was a single-center, prospective, quasi-experimental study comprising 15 participants between 32 and 63 years of age. Each participant received a single-session treatment based on facial subcutaneous injections of HArmonyCa, a hybrid combination filler comprising hyaluronic acid and calcium hydroxyapatite. This study involved an intrapatient control design and a 120-day follow-up with clinical and sonographic assessment. For this purpose, standardized photographic images, high-frequency ultrasound evaluations, and physician- and patient-oriented overall aesthetic improvement scores were recorded at 0, 30, 90, and 120 following the procedure.

Results: According to our findings, 20% of the subjects had an exceptional improvement; 20%, "very improved"; and 60%, "improved." Intrapatient sonographic comparison showed a significant increase in dermal thickness, at 90 and 120 days, only on the side treated ($P < 0.001$).

Conclusion: In our clinical study, a single-session treatment with a hybrid product combining hyaluronic acid and calcium hydroxyapatite resulted in positive cosmetic satisfaction and increased dermal thickness. *(Plast Reconstr Surg Glob Open 2023; 11:e5055; doi: 10.1097/GOX.0000000000005055; Published online 15 June 2023.)*

INTRODUCTION

Currently, facial aging is thoroughly understood as a complex three-dimensional process affecting different anatomical layers. It typically encompasses a multitude of features, including muscle, bone, and fat remodeling but also cutaneous changes such as loss of elasticity and thinning of the skin.[1,2] Specifically an age-related decrease in dermal thickness, reduced production, and organization of collagen bundles are considered its main causes. Because collagen contributes to the major volume of the skin, stimulating neocollagenesis is paramount to increasing dermal thickness as well as enhancing the texture and laxity of the skin.[3]

Targeted neocollagenesis is an expected outcome from some semipermanent injectable fillers such as calcium hydroxyapatite (CaHA).[4]This cosmetic filler has been used in the last few decades exhibiting a good safety profile. CaHA is a natural substance regularly found in human teeth and bones; therefore, its synthetic microspheres are biocompatible and biodegradable.[5–7] Histologically, these particles induce histiocytic and fibroblastic tissue response

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in the dermis, thus increasing collagen formation at the injection sites.[8,9]

CaHA fillers are widely used worldwide, currently standing as the second most injected cosmetic filler. However, hyaluronic acid (HA)-based injectable fillers are the gold standard for volumization treatment in facial rejuvenation.[10,11] HA is a glycosaminoglycan composed of glucuronic acid and N-acetylglucosamine disaccharides. HA-based fillers are mainly composed of HA cross-linked, which could potentially stimulate collagen synthesis in some clinical contexts, besides providing soft tissue augmentation.[12,13]

Concerning aesthetic purposes, HA and CaHA fillers can be applied in the same treatment area, potentially adding beneficial outcomes. A premixed combination of them has also been widely used and, although the rheological properties are changed with this combination, great results with a satisfactory safety profile have been reported with this technique.[11,14,15]

The market of minimally invasive cosmetic procedures faced constant growth in the last few years. Hence, currently, both HA- and CaHA-based fillers top the ranking list of nonsurgical aesthetic procedures.[10,15] In this context, a cosmetic filler comprising both nonanimals, highly cross-linked hyaluronic acid and CaHA microspheres has been recently manufactured. This product is a hybrid product named HArmonyCa and is already approved in certain countries for cosmetic purposes.[14,16]

Prior clinical experience exhibited favorable outcomes regarding the use of this novel injectable filler for mid and lower-face rejuvenation.[17,18] Although theoretically neocollagenesis is expected at treated areas following its injection, to our knowledge, patient satisfaction and changes in dermal thickness after a single session of HArmonyCa have not been previously published. Therefore, these are the major goals of this study.

Takeaways

Question: To evaluate patient satisfaction and changes in dermal thickness after a single session of a hybrid combination filler comprising hyaluronic acid and calcium hydroxyapatite (HArmonyCa®).

Findings: Single-center prospective study comprising 15 participants. HArmonyCa® was injected, standardized images and ultrasound analysis at baseline and at 0, 30, 90, and 120 days following the intervention. According to our findings, 20% of the subjects had an exceptional improvement; 20%, "very improved"; and 60%, "improved". Ultrasonographic comparison showed a significant increase in dermal thickness, at 90 and at 120 days ($p < 0.001$).

Meaning: A single-session treatment with HArmonyCa® resulted in satisfaction and increased dermal thickness.

METHODS

This was a single-center, prospective, quasi-experimental controlled study conducted at Bravo Clinic (Rio de Janeiro, Brazil) between May 2022 and November 2022. We aimed to assess changes in dermal thickness following a hybrid soft-tissue filler single-session injection through a noninvasive evaluation with ultrasound.

The study sample consisted of one male and 14 female participants between 32 and 63 years of age. Patients' skin phototypes ranged from categories II to IV using the Fitzpatrick Skin Phototype Classification. A validated five-point grading scale to assess sagging skin of the jawline was used to categorize patients before intervention.[19] "Mild sagging" and "moderate sagging" patients were included in the study.

The exclusion criteria were the following: chronic or acute inflammation or infection near the injection site, known sensitivity to any injectable or topical component used in this study, pregnancy or breastfeeding, previous facial aesthetic surgical procedure, prior facial aesthetic procedure within six months before the study, any facial cosmetic procedure performed during the 120-day follow-up comprised in this study, preexisting autoimmune disease, history of severe allergy or anaphylactic reaction, and age below 18 years.

This study was thoroughly conducted in accordance with ethical standards stated in the Declaration of Helsinki, guaranteeing the safety, rights, and well-being of each participant.

All subjects were informed about the purpose of the study and its protocol and signed the informed consent form before enrolling in this study. The investigators also told them that they could leave the study at any time without any negative consequence.

TECHNICAL PROCEDURES

After an initial examination of the patient and thorough assessment and discussion of the risks for each patient, the following protocol was followed for each procedure.

Preprocedure skin antisepsis of the face was performed with 0.5% alcoholic chlorhexidine, followed by the application of a local anesthetic spray containing 10% lidocaine 30 minutes before the treatment, which was fully removed immediately before it started.

Each patient was then placed in a comfortable 45 degree supine position. HArmonyCa was injected subsequently in a perforation of the skin in the subzygomatic area, 5 cm below the lateral margin of the orbit, using a 21G needle, 0.80×50 mm, 1" (TSK Laboratory, Tochigi-Ken, Japan). Insertion of a 22G cannula, 0.70×50 mm 2" (TSK Laboratory, Tochigi-Ken, Japan).

Another puncture was made in the lower face, in the skin over the mandibular body at the anterior edge of the masseter muscle. A total of 2.5 mL of HArmonyCa was applied per patient, comprising two 1.25 mL syringes, one for each hemiface (Fig. 1).

The product was injected into the superficial subcutaneous layer of the posterior zygomatic region using the retrograde linear fanning technique, through the advancement of the cannula (total volume: 0.3 mL/side). Small bolus followed by retrograde injections in the

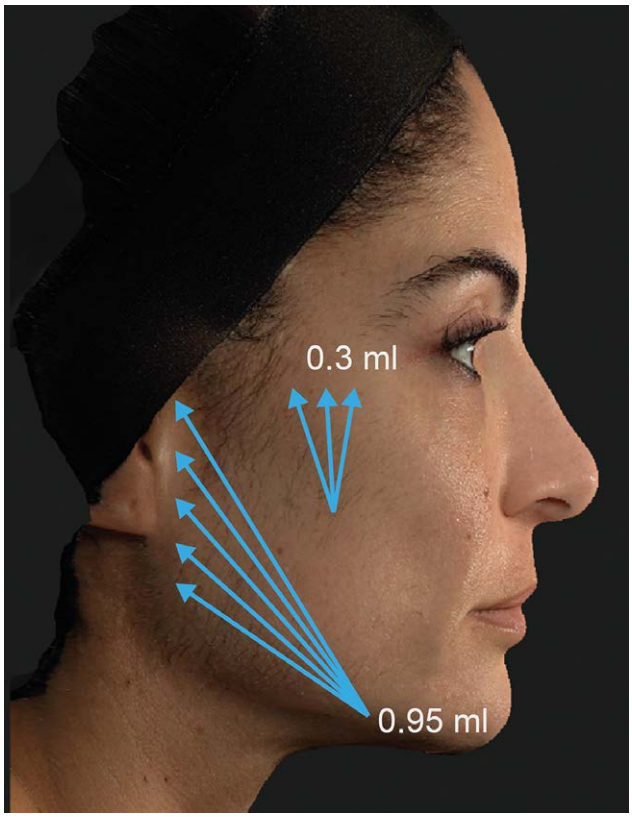

Fig. 1. Treatment plan.

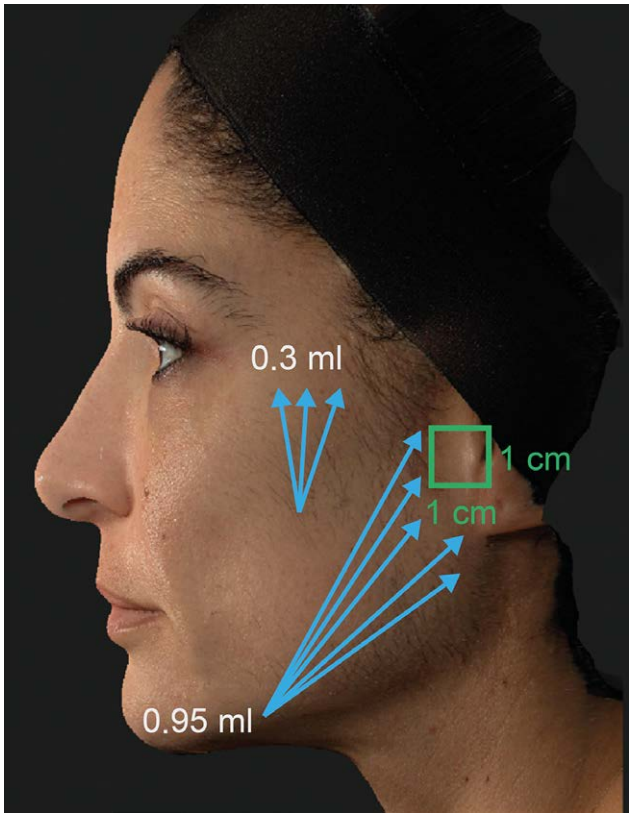

Fig. 2. Control area without treatment.

subcutaneous plane were also performed at the mandibular body and ramus regions as well as at the gonial angle, delivering 0.95 mL of HArmonyCa per side. Slight massaging and compression of the area where the skin performation occurred. A small area of approximately one square centimeter was spared from treatment in the left preauricular region of each patient, serving as a control in this study (Fig. 2).

Following treatment, each cannula entry site was covered with a small sterile adhesive dressing. The major postprocedure instructions were avoidance of massage or manipulation at the injection site as well as physical activity in the first 24 hours after the single-session treatment.

DATA COLLECTION AND ENDPOINTS

Investigators collected each participant's gender, age as well as skin phototype. Standardized photographic images were also captured at baseline and at 0, 30, 60, 90, and 120 days following the intervention, using the 3D Face Imaging System Vectra-H2 (Canfield Scientific, Parsippany, N.J.) and its vector analysis program, Markerless Tracking. It provides a dynamic assessment of skin surface changes. Skin surfaces are automatically aligned, tracked and mapped.

A board-certified dermatologist was engaged in this study as a blind investigator to evaluate changes on sagging skin of the jawline using an independently rated overall cosmetic improvement scale, the Physician Global Aesthetic Improvement Scale, at 120 days after the procedure. The Subject Global Aesthetic Improvement Scale was used to access patients' satisfaction as well.

Ultrasound analysis was performed immediately before the procedure and at 30, 90, and 120 days after this single-session treatment. Dermal thickness was assessed at preauricular regions through a linear 18 MHz transducer by one experienced radiologist using a high-frequency ultrasound LogicE device (GE Healthcare) with a high-frequency linear probe (L8-L18i-RS). This sonographic evaluation included both a treated area (right preauricular region) and a small area spared from treatment at the left preauricular region, considered the control in this study.

To sonographically assess the dermal thickness, the linear probe was statically placed at preauricular regions to measure the mean dermal thickness, by calculating the mean of five measures at this stable position of the transducer.

At each follow-up visit, all participants were asked about any adverse event using a structured questionnaire. Additionally, to thoroughly investigate potential adverse events, a study physician performed a meticulous visual inspection and ultrasound evaluation.

RESULTS

All 15 participants enrolled in this study completed the 120-day follow-up, and therefore were included in the statistical analysis.

The age of the participants ranged from 32 to 62 years, with a mean of 45.9±9.0 years. Patients' skin types, assessed through Fitzpatrick Skin Phototype Classification, were categorized as II (26.7%), III (60%), and IV (13.3%).

After aligning one standardized image from baseline and another at 120 days after the procedure (D120), using Vectra 3D Imaging System, the magnitude and direction of skin movement were precisely illustrated using vector arrows, demonstrating a prominent lifting effect of HArmonyCa, the red arrows are larger and predominant in the region of the lower third of the face, at the angle of the mandible as shown in Figures 3–12.

No additional applications or protocol deviations regarding volume or dilution were required. No infections, ischemia, or other relevant adverse effects were observed, but mild pain, ecchymosis, and local edema occurred after the injections. In one patient, there was an adverse event considered mild, in which a whitish papule appeared due to the accumulation of the product in the cannula inlet.

Regarding overall satisfaction following the procedure, at 120 days after the single-session treatment, all participants showed "very much improved," except for one patient who reported "much improvement." This subject was the same one who had an adverse event that required intervention. These subjective assessments were evaluated by using the Subject Global Aesthetic Improvement Scale (Table 1).

An independent board-certified dermatologist was responsible for qualitatively evaluating standardized three-dimensional images from each participant before and after the procedure to determine overall aesthetic improvement. The Physician Global Aesthetic Improvement Scale was the assessment tool used for this purpose. Results from this evaluation exhibited that all participants improved at 120 days after the procedure. Actually, 20% of the subjects had an exceptional improvement; 20%, "very improved"; and 60%, "improved" (Table 1).

Ultrasound evaluation was able to quantitatively determine dermal thickness changes after the procedure. An experienced radiologist assessed this parameter before

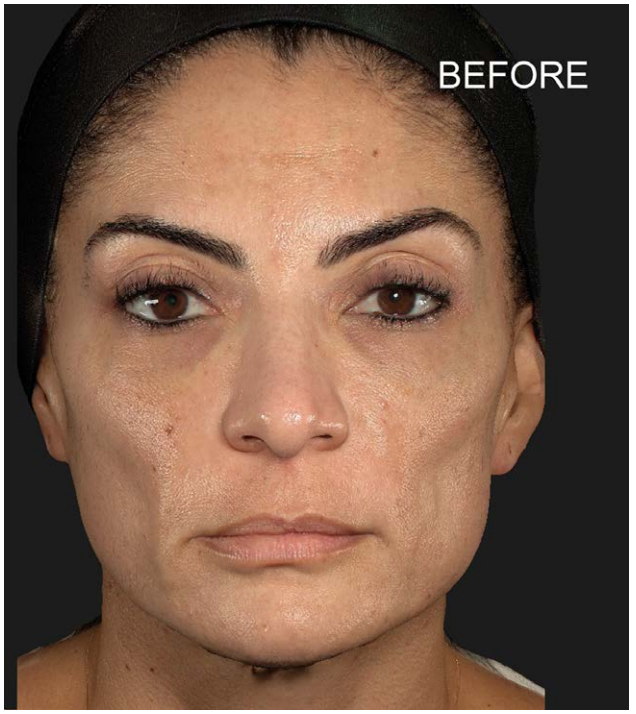

Fig. 3. Pretreatment using 3D face imaging system vectra-H2.

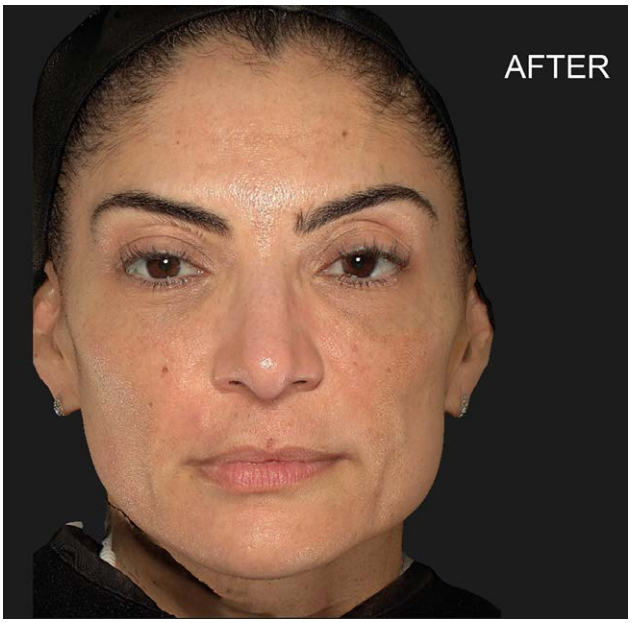

Fig. 4. Posttreatment using 3D face imaging system vectra-H2.

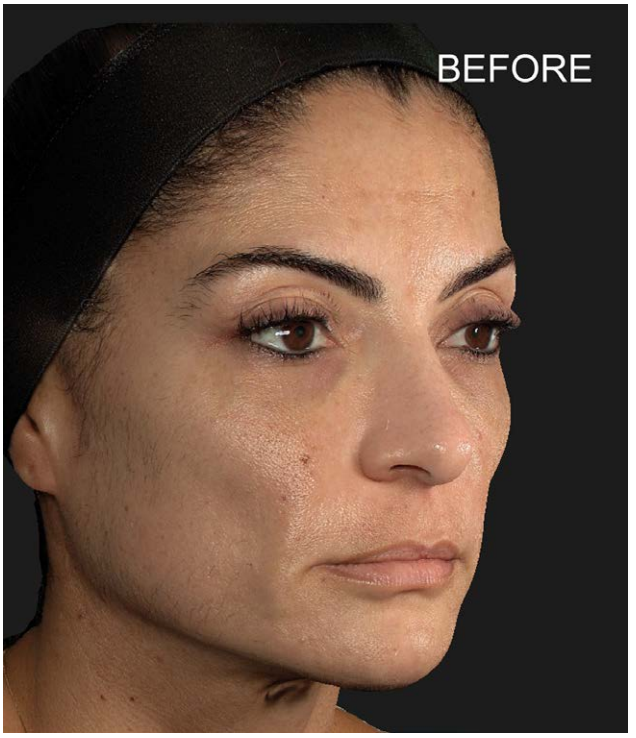

Fig. 5. Pretreatment using 3D face imaging system vectra-H2.

Fig. 6. Posttreatment using 3D face imaging system vectra-H2.

the procedure and at 30, 90, and 120 days after this intervention. The sonographic morphology of injected HArmonyCa exhibited a band-like hyperechoic deposit and some internal hypoechogenic areas with a hole-like appearance (Figs. 13–16).

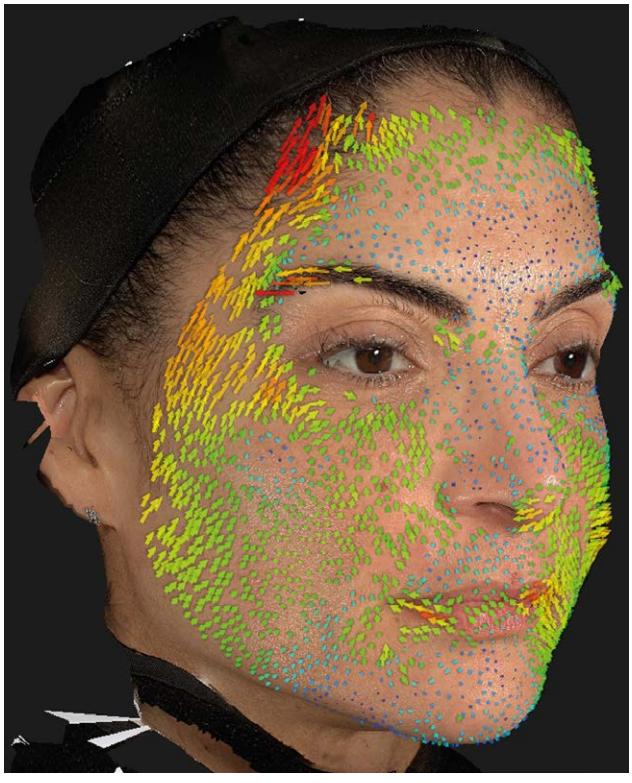

Fig. 7. Posttreatment using 3D face imaging system vectra-H2.

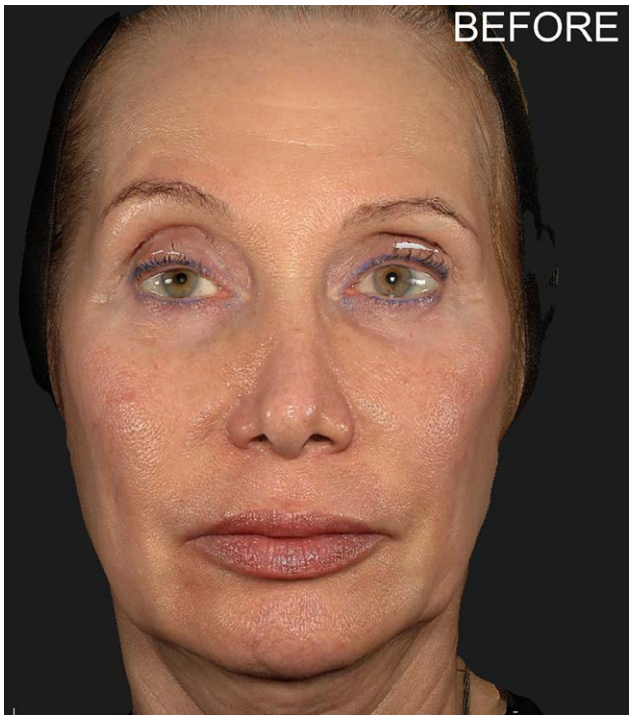

Fig. 8. Pretreatment using 3D face imaging system vectra-H2.

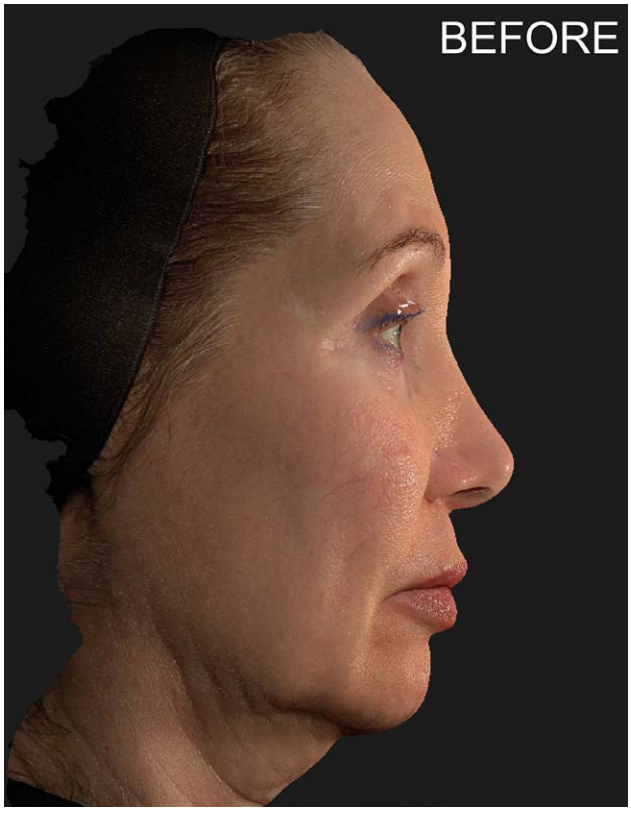

Fig. 9. Pretreatment using 3D face imaging system vectra-H2.

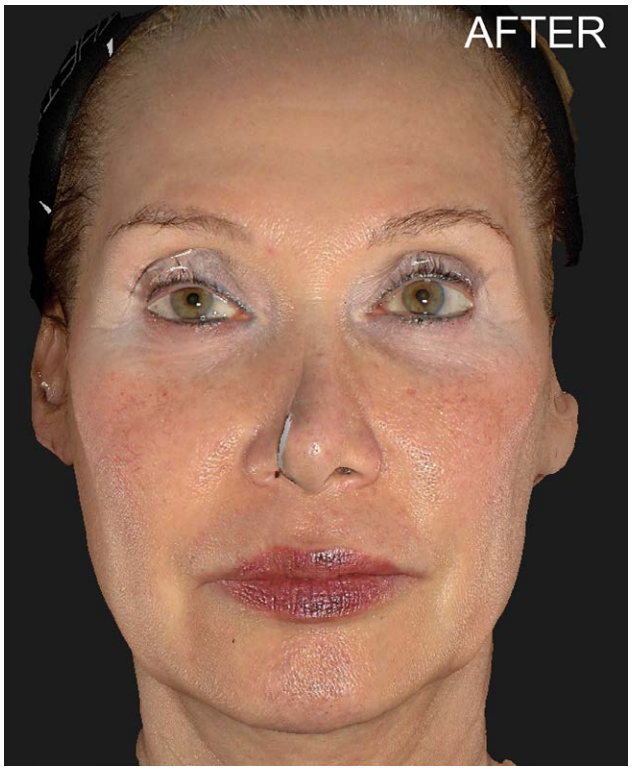

Fig. 10. Posttreatment using 3D face imaging system vectra-H2.

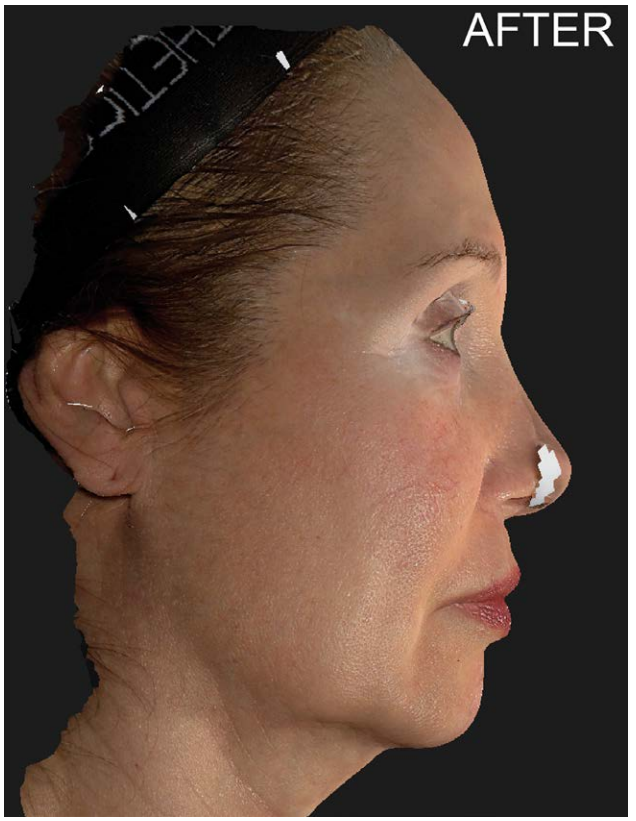

Fig. 11. Posttreatment using 3D face imaging system vectra-H2.

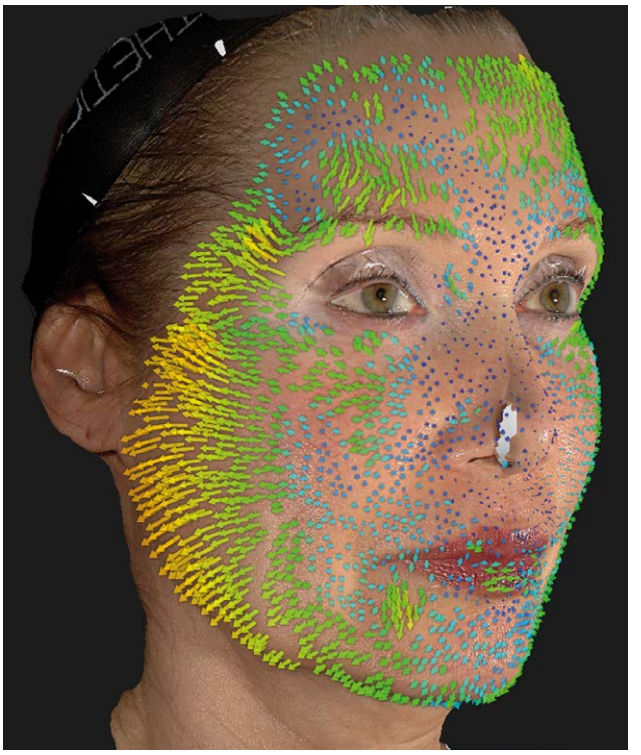

Fig. 12. Posttreatment using 3D face imaging system vectra-H2.

Table 1. Subject Global Aesthetic Improvement Scale Scores

Score	Rating	30 d (N = 15)	60 d (N = 15)	90 d (N = 15)	120 d (N = 15)
5	Worse	0	0	0	0
4	Mildly improved	0	0	0	0
3	Improved	0	0	0	0
2	Much improved	0	0	1	1
1	Very much improved	15	15	14	14

The evaluation of the dermal thickness performed through ultrasonography demonstrated that there was no difference between the treated and untreated sides at baseline (D0) ($P = 0.221$). Means ranged from 1.47 ± 0.08 at baseline (pretreatment) to 1.48 ± 0.09 if we consider 30 days after treatment. The variation was not significant ($P = 0.334$). At 90 days, the mean was 1.56 ± 0.08 and at 120 days it was 1.68 ± 0.08. The intrapatient comparison showed a significant increase in dermal thickness only on the side treated; note that all variations analyzed two by two were significant ($P < 0.001$), except between pretreatment and at 30 days (Table 2, Fig. 17).

Localized and self-limited side effects such as mild edema, pain, and ecchymosis were seldom reported. No vascular occlusive events or infections following the procedure were noted. Also, no clinical or sonographic signs of granuloma formation were detected in this assessment. Only one patient had an adverse event, developing a small papule in the lower face. Percutaneous needle puncture

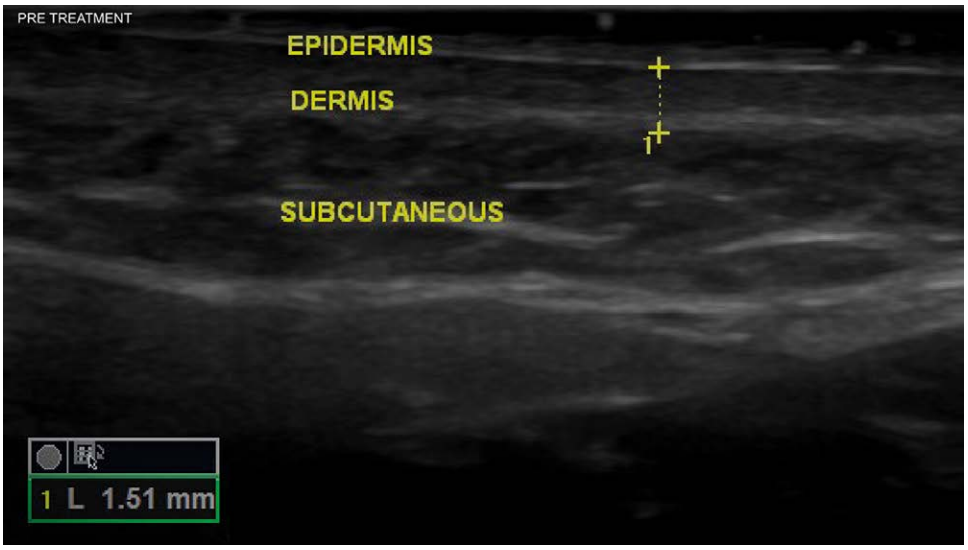

Fig. 13. Pretreatment with ultrasonographic analysis.

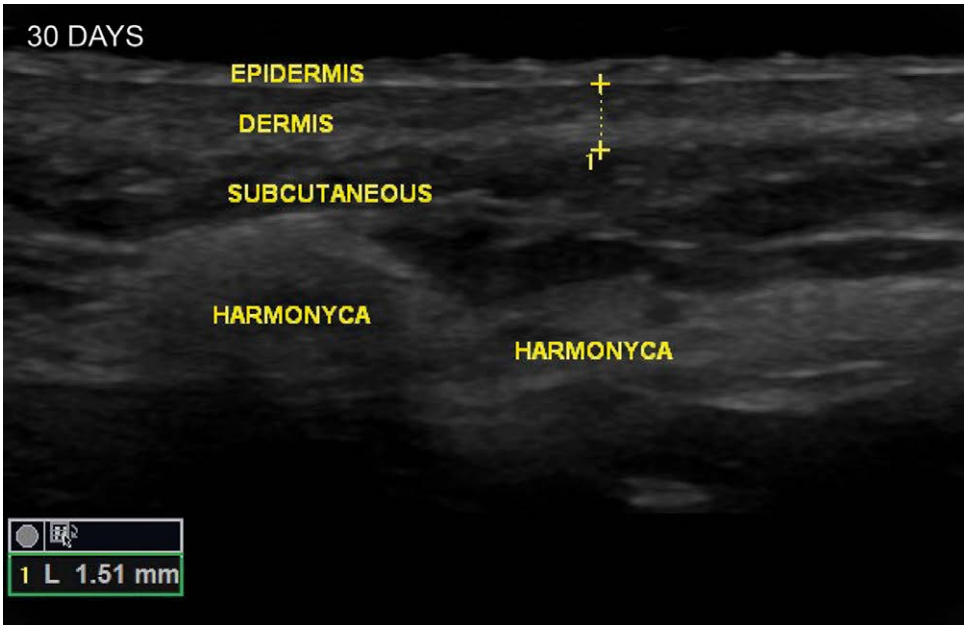

Fig. 14. Post 30 days treatment with ultrasonographic analysis.

at the papule site followed by gentle extraction was performed with no recurrences.

DISCUSSION

The overall quality of the dermis as well as its thickness experience a gradual age-related decrease, especially from the age of 50. The diameter of the collagen bundles tends to decrease, whereas the space between fibers increases.[20] In this sense, CaHA has a well-documented ability to induce neocollagenesis within the dermis, which persists even when CaHA molecules are hyperdiluted.[9,21,22]

Comparative assessments among fillers demonstrate that rheology alone is not sufficient to thoroughly understand cosmetic filler performance.[23] However, it was previously demonstrated in a rodent model that a combination of CaHA microspheres with cross-linked HA in a single product has a significantly higher lift capacity than CaHA-based fillers without HA molecules. It was also demonstrated that HArmonyCa shows a greater tissue integration in the first week after its injection.[24]

The soft tissue–lifting effect of hyaluronic acid-based fillers has been previously reported in a cadaveric experimental model. This prior study also analyzed standardized images obtained using the three-dimensional surface imaging system Vectra H1 system (Canfield Scientific, Fairfield, N.J.), similar to our study. However, only immediate postprocedure response was analyzed.[25]

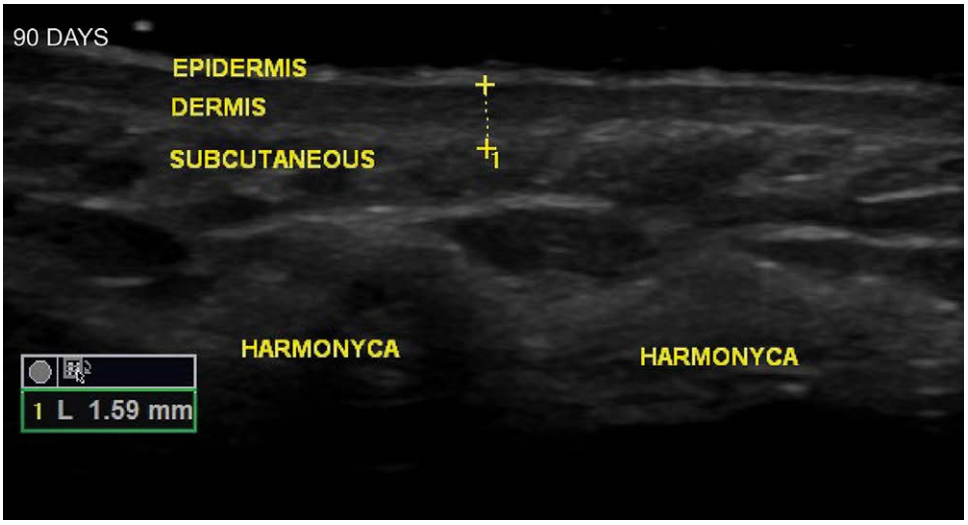

Fig. 15. Post 90 days treatment with ultrasonographic analysis.

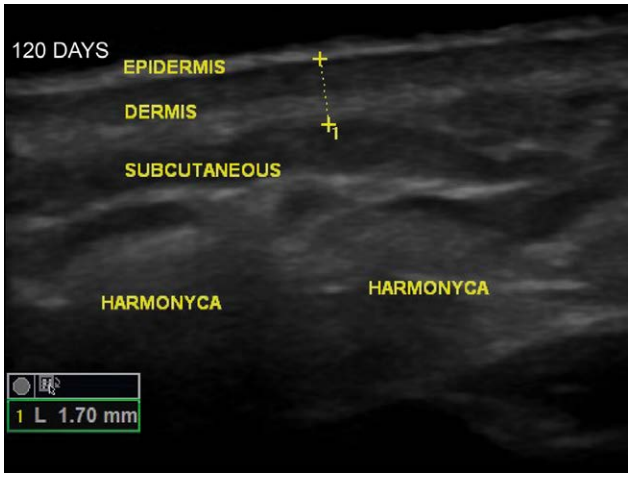

Fig. 16. Post 120 days treatment with ultrasonographic analysis.

Markerless tracking provides dynamic assessment of skin surface changes. After automatically aligning a pair of 3D images of pre- and postprocedure photos, the skin surfaces are tracked and mapped. Colored vector arrows provide a precise indication of the direction and magnitude of skin movement, which is interesting for evaluating treatment results. The red arrows show greater lifting, and the blue arrows, the regions with less lifting. In the case of the patients evaluated in this study, this technology can confirm the patient's improvements with detailed evidence through the review of the results and refinement of the technique.

Our study demonstrated a medium-term tissue-lifting effect using three-dimensional standardized images; the red arrows are larger and predominant in the region of the lower third of the face, at the angle of the mandible. To our knowledge, the lift capacity of HArmonyCa in human subjects has not been previously investigated.

It is worth mentioning that beauty provides remarkable social and professional advantages.[26] In this regard, minimally invasive cosmetic procedures using hyaluronic acid resulted in positive patient satisfaction, which was also correlated to brain mapping.[27] Hence, the significantly positive overall satisfaction after the HArmonyCa injection observed in our study is following what was previously demonstrated with HA-based dermal fillers.

Noninvasive evaluation of skin thickness using ultrasound devices has been studied for the past few decades.[28–30] Currently, high-frequency ultrasound is considered a reliable method to assess dermal thickness in healthy adults in different clinical contexts.[31,32] Concerning facial aesthetic rejuvenation, a significant increase in dermal thickness has been previously demonstrated with a combination technique comprising both HA- and CaHA-based fillers.[33] However, considering that rheological properties are altered when CaHA microspheres and cross-linked HA coexist in the same syringe, changes in dermal thickness following treatment with this premixed combination have not yet been investigated, to our knowledge. For that matter, our study demonstrated that a single-session treatment using HArmonyCa, a novel HA and CaHA combination filler, resulted in a significant increase in dermal thickness.

One participant in the present study exhibited an adverse event that required intervention. A painless normochromic papule developed at a cannula entry site had sonographic features related to injected HArmonyCa. According to our evaluation, this event probably resulted from a minor superficialization of the product at a puncture site. No other procedure-related granulomas or any other adverse events were detected in our study. Moreover, a retrospective study enrolling 87 subjects who received an injectable treatment of HA and CaHA combination filler did not observe granuloma, allergic reaction or infection as adverse events.[34]

This study does have some limitations concerning the extension of its findings to the general population. First,

Table 2. Pre-auricular Dermal Thickness Measurement Assessed by High-frequency Ultrasound throughout the Follow-up

	Subjects Side	Pre-treatment	30 d
1	Right	1.51 mm	1.51 mm
	Left	1.51 mm	1.51 mm
2	Right	1.48 mm	1.48 mm
	Left	1.48 mm	1.48 mm
3	Right	1.52 mm	1.52 mm
	Left	1.52 mm	1.52 mm
4	Right	1.56 mm	1.56 mm
	Left	1.55 mm	1.55 mm
5	Right	1.47 mm	1.47 mm
	Left	1.47 mm	1.47 mm
6	Right	1.55 mm	1.55 mm
	Left	1.55 mm	1.55 mm
7	Right	1.43 mm	1.43 mm
	Left	1.43 mm	1.43 mm
8	Right	1.43 mm	1.43 mm
	Left	1.43 mm	1.43 mm
9	Right	1.33 mm	1.33 mm
	Left	1.35 mm	1.35 mm
10	Right	1.39 mm	1.39 mm
	Left	1.39 mm	1.39 mm
11	Right	1.39 mm	1.39 mm
	Left	1.39 mm	1.39 mm
12	Right	1.59 mm	1.59 mm
	Left	1.59 mm	1.59 mm
13	Right	1.57 mm	1.59 mm
	Left	1.59 mm	1.59 mm
14	Right	1.55 mm	1.55 mm
	Left	1.55 mm	1.55 mm
15	Right	1.35 mm	1.35 mm
	Left	1.36 mm	1.36 mm

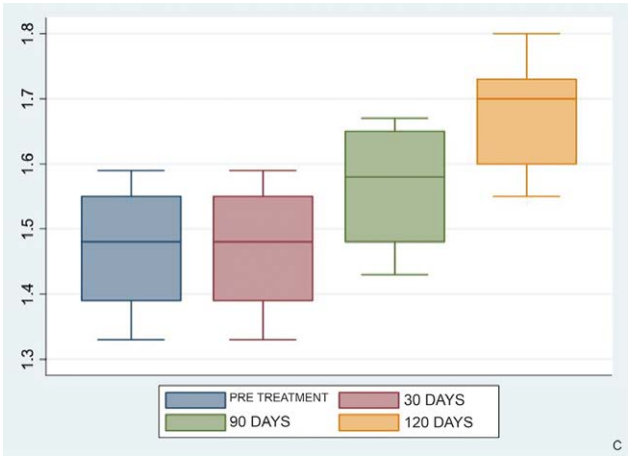

Fig. 17. Box plot synthesizing central and dispersion measures in four evaluation moments.

it is a single-center-based study, and secondly, regarding the 15 participants enrolled in this study, 14 were women and only one subject was a man. Thus, further studies are necessary to investigate whether the clinical and sonographic outcomes from this study are valid in long-term scenarios.

CONCLUSIONS

In our clinical study, a single-session treatment with this hybrid filler combining hyaluronic acid and calcium hydroxyapatite resulted in positive cosmetic satisfaction among the participants and increased dermal thickness objectively assessed through ultrasound. Moreover, a medium-term tissue-lifting effect was also achieved. Therefore, this quasi-experimental controlled study showed, in human subjects, aesthetic benefits of a product that aims not only to provide volume augmentation but also dermal thickness increase.

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DISCLOSURES

Dr. Bruna S.F. Bravo is a medical consultant of Merz, Allergan, and Lóreal. All the other authors have no financial interests to declare in relation to the content of this article.

PATIENT CONSENT

The patient provided written consent for the use of her image.

ACKNOWLEDGMENTS

We acknowledge all patients who participated in this article and declare that all of them have given written informed consent to the publication of their case detail. The authors confirm that the ethical policies of the Journal, as noted on the Journal's author guidelines page, have been adhered to. All participants have signed the informed consent form, and they participated in our study voluntarily. No ethical approval was required, reinforcing that it followed all the ethical principals of studies. The authors ensure that participants' rights are protected.

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