Retrospective observational multicenter study on patients treated with a non-animal origin cross-linked hyaluronic acid with different molecular weights for nasolabial folds

Summary

Retrospective observational multicenter study on patients treated with a non-animal origin cross-linked hyaluronic acid with different molecular weights for nasolabial folds

Background: According to the American Academy of Aesthetic Plastic Surgeons, more than 11 million cosmetic surgical and nonsurgical procedures were performed by board-certified plastic surgeons, dermatologists and otolaryngologists in the United States, totaling more than 12 billion dollars. Of that total, more than 7 billion was spent on surgical procedures and more than 5 billion was spent on nonsurgical procedures. More than 1,872,172 people received Hyaluronic Acid (HA) injections in 2013. Moreover, filler treatments are the most popular procedures performed by dermatologists.

Objective: Evaluate, with a new imaging system, durability, efficacy and safety of a nasolabial fold treatment with a cross-linked HA of non-animal origin with different molecular weights. Material and methods: A cross-linked HA (25 mg/ml, 1000-2000 kDa, 23 ± 3 Newton [N]) extrusion force, 1,4-Butanediol Diglycidyl Ether (BDDE) content < 0.1 ppm) was used in order to perform the treatment. The product is commercially available with the trademark Aliaxin® GP (Global Performance) and distributed by BSA Farmaceutici Italia Srl. 25 female subjects aged 40 and 60 years with a photaging level III according Rubin or type III according Glogau were recruited for the treatment and 0.5 ml of the product were injected for single nasolabial fold. The aesthetic result and the duration of the aesthetic correction were evaluated by the analysis of the skin microreliefs through confocal microscopy and by Glogau’s Scale. Additionally, pictures of each patient were collected by Canon PowerShot G10 Digital Camera (14.7 megapixels) before and after treatment. Below the description of the experimental schedule: T0: Baseline, evaluation and treatment; T1: second visit, 4 months after the treatment; T2: third visit, 6 months after the treatment.

Results: From 25 patients, 150 silicone casts were obtained: 75 casts of the right nasolabial fold and 75 casts of the left nasolabial fold. Roughness arithmetical average (Ra) was assessed by profilometry. This parameter represents the arithmetic mean deviation of the profile points compared to the average value. The Ra of the right fold at T2 decreased by 50% versus T0 and by 40% compared to T1; Ra of the left fold at T2 decreased by the 45% versus T0 and by 35% compared to T1. No side effects were reported during the observation period beyond mild symptoms (pain, sensation of heat, reddening in the injection site) also described by the product technical sheet.

Conclusion: The results proved the efficacy and safety of the nasolabial folds treatment with the tested product and the durability of the aesthetic correction.

Key words: Hyaluronic acid fillers, Profilometry, Confocal microscopy, Aliaxin® GP.

Introduction

The dermal extracellular matrix is composed of a dense network of interlinked collagen and elastic fibers, containing water-retaining proteoglycans and glycosaminoglycans accounting for their biomechanical and viscoelastic properties.
Physiological ageing process and ultraviolet light exposure lead to a loss of dermal matrix volume and biomechanical properties due to a decrease in collagen and proteoglycan content and an increase in denatured and degraded collagen resulting in wrinkles, loss of elasticity and dryness. Consequently, several fillers for soft tissue augmentation procedures including treatment of nasolabial 2, perioral 3 and periorbital wrinkles 4 have been developed. HA dermal fillers have been extensively used for skin rejuvenation procedures in aesthetic medicine 5. HA is a glycosaminoglycan consisting of alternate repeating D-glucuronic acid and N-acetylglucosamine units and is distributed throughout the extracellular matrix, connective tissues and organs of all higher animals 6. HA enhances hydration in the extracellular space due to its ability to capture water molecules and to induce optimal physiological conditions in the extracellular matrix for proliferation and organization of dermal cells 7. HA, used as a filler, can have an avian or a bacterial origin with the latter being largely preferred for his high purity, increased viscosity and decreased potential to induce allergic reactions 8. Analysis of elastic fibers and collagen in the skin is a reliable tool to assess the efficacy of new HA-based compounds used for rejuvenation procedures. In fact, injection of Cross-Linked HA (CLHA) into dermal equivalent cultures results in elongation of fibroblasts together with type I collagen synthesis due to up regulation of transforming growth factor beta (TGF-β) pathway 9. Furthermore, HA injection significantly increases the synthesis of neo-collagen I and pro-collagen I and III mRNA compared to baseline, as observed 30 days post administration 10. CLHA injection into the buttocks skin of subjects aged over 70 years stimulated fibroblasts to ultimately produce type I collagen 9. HA fillers can be distinguished on the base of the particle size, cross-linking agent used, cross-linking degree, percentage of cross-linked HA and amount of free (unmodified) HA. In the present study, efficacy and safety of the nasolabial folds treatment with the tested product were evaluated together with the durability of the aesthetic correction.

**Materials and methods**

**Tested product**
CL HA (25 mg/ml, 1000-2000 kDa, 23 ± 3 Newton [N] extrusion force, 1,4-Butanediol Diglycidyl Ether (BDDE) content < 0.1 ppm) was used in order to perform the treatment. The product is commercially available with the trademark Aluixin® GP (Global Performance) and distributed by IBSA Farmaceutici Italia S.r.l.

**Clinical study**
Six different dermatologists in Naples, Bologna and Catania participated in this retrospective multicenter observational study.

**Patients**
25 patients (25 women), aged 47.6 ± 1.3 (range: 40-60 years) were involved. All patients signed an informed consent before the beginning of the procedure. The inclusion criteria for this study was clinical evidence of moderate to severe wrinkles affecting the nasolabial folds, with a degree of photoageing level III according Rubin’s scale or type III according Glogau’s scale. Exclusion criteria were history of severe allergic disorders, cutaneous disease affecting the face such as acne and herpes, known hypersensitivity to HA, history of autoimmune disease, abnormal liver function, pregnancy, lactation and history of skin cancer; use of anticoagulant therapy such as aspirin or warfarin, use of topical steroids, retinoid medications, or anti-acne agents in the 4 weeks before to the treatment; microdermabrasion, chemical peels or use of fillers 24 weeks before the treatment.

**Treatment**
Patients received an injection of 0.5 ml of the product for each nasolabial fold requiring the anti-ageing treatment. Injections were performed with a linear retrograde technique. Once the procedure was completed, the injection site was manually massaged to allow the implanted material to adapt to the adjacent tissues. Visits were planned at 4 months (T1) and 6 (T2) months after the treatment. Patients were photographed before and after the injections and at T1 and T2.

**Profilometry and confocal microscopy**
Casts of the nasolabial folds, obtained using a silicone material, were collected from each patient after the injection in the treated site at T1 and T2. 150 silicone casts were collected: 75 of the right
Figure 1.
Ra is the arithmetic average of the absolute values of the deviations profile within the length of the base (L).

nasolabial fold and 75 of the left nasolabial fold. A confocal microscope was used in order to determine the surface roughness and the surface morphology of the folds with the analysis of each silicone cast. For the measurements of surfaces, the operator selects the defined area of the sample to be analyzed. All the images were collected under the following conditions: - Optical Magnification: 5x; - Lens Numerical Aperture: 0.15; - Lens Field of View: 2550 x 1910 micron; - Optical Resolution (x, y): 0.94 microns. From each acquired image was extracted the roughness profile and subsequently the Roughness arithmetical average (Ra) was calculated. Ra is the arithmetic average of the absolute values of the deviations profile within the length of the base (L) (Figure 1). The collected data was used to evaluate the roughness associated to the folds immediately after the treatment and at T1 and T2 (Figures 2, 3).

Figure 2.
Patient X: 3D view of the fold surface acquired by confocal microscope.

Figure 3.
3D images of surface wrinkles acquired by confocal microscope.
Results

In this study, all patients demonstrated aesthetic improvements well documented by the photographs taken before and immediately after injection of the tested product. Moreover, the photographs collected during the follow-up visits showed a good durability of the aesthetic correction of the nasolabial folds even 6 months after the treatment (Figures 4, 5). Combining the profilometric techniques with the confocal microscopy, we obtained an objective way to measure the durability of the aesthetic correction: Ra of the right fold at T2 decreased by the 50% versus T0 and by 40% compared to T1; Ra at T2 of the left fold decreased by the 45% versus T0 and by 35% compared to T1. 80% of the patients showed “exceptional improvement” at T1, as the evaluation with the GALS scale showed and this result didn’t change at T2.

All the patients were satisfied immediately after the procedure and no side effects were reported during the observation period beyond mild symptoms (pain, sensation of heat, reddening in the injection site) also described by the product technical sheet.

Discussion and Conclusion

Silicone skin casts of the treated area were collected in this study immediately after the treatment and at T1 and T2 of nasolabial folds with a cross-linked HA of non-animal origin with a combination of different molecular weights. Generally, the acquisition of the skin cast images is performed by a stereomicroscope connected to an analog camera, and the study of the
morphometric skin surface is used to evaluate the regularity of the skin texture surface and the changes of this aspect after filler treatments. The images of the replicas are then subject to the study of the Fourier spectrum, able to identify the mathematical descriptor parameters of the skin regularity degree in an objective and reproducible way. Moreover, the Fourier transformation of the skin texture surface image is evaluated by the mean gray values obtained along the x-axis and the y-axis; these averages are calculated from the parameters descriptors. A number of studies have been performed to evaluate the durability and safety of fillers using this methodology. The results of this study proved the efficacy and safety of the nasolabial folds treatment with the test product and the duration of the achieved aesthetic correction over a 6 month period. The durability of the aesthetic improvement was shown by this study combining, for the first time, profilometric techniques on skin casts with confocal microscopy. Confocal profilometry is a method that ensures reproducibility and comparability of the data, with standardized methods of recovery. This new non-invasive approach could be used as a guide for aesthetic physicians in the choice of different products, for different patients, in order optimize the choice and to better respond to the patient’s expectations. In fact, in aesthetic medicine it would be very important to define the parameters in order to assess the efficacy and durability of the most frequently used products on the market. Aesthetic physician communities should demand investigations of this type in order to set appropriate guidelines, enabling them the possibility to guarantee patient safety and satisfaction while avoiding, as much as possible, side effects.

References