Clinical and instrumental evaluation of a cross-linked hyaluronic acid filler dermal injection: effects on nasolabial folds skin biophysical parameters and augmentation from a single-dose, monocentric, open-label trial

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ABSTRACT
BACKGROUND: When a hyaluronic acid dermal device to fill soft tissues is chosen, efficacy, safety and durability are key concerns. This is an open-label prospective study to instrumentally evaluate the effects of HA filler dermal injection on nasolabial folds skin biophysical parameters and augmentation.

METHODS: A single Italian site treated female subjects aged 40-55, for nasolabial folds, with a single standardized injection. The outcome was evaluated with objective quantitative measurements after 90 (T1) and 180 days (T2) from the injection comparing to baseline (T0) by means of Corneometer (skin hydration measurement), Cutometer (skin elasticity measurement), and Visioface devices for digital and UV computerized image analysis. Secondary endpoints were safety assessment, subject investigator satisfaction with the intervention. Assessment of aesthetic results included photographic documentation.

RESULTS: The computerized image analysis confirmed the clinical assessment showing statistically significant reduction in nasolabial folds both at T1 and T2. Visioface® indexes showed a marked and statistical significant response. An excellent profile of satisfaction of the product at T2 from investigators and patients was recorded. Skin hydration and elasticity did not show significant changes.

CONCLUSIONS: In our study, a standardized HA filler dermal injection on nasolabial folds did not influence skin biophysical parameters such as skin hydration and elasticity. Nasolabial folds showed a persistent and significative response at T2 confirmed by instrumental evaluation. The tolerability and safety profile of the product was excellent.

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Skin ageing, a process that has been better understood in recent years, has led to the widespread use of a variety of biocompatible soft tissue fillers, used to cosmetically improve defects and deficiencies. The use of these preparations has become common, especially for the correction of wrinkles and folds. Currently available dermal fillers include bovine and human collagens, Hyaluronic Acid (HA) preparations of animal or biosynthetic origin, poly-L-lactic acid products, polymethylmethacrylate, and calcium hydroxyapatite.1-3 Skin ageing processes result in reduced dermal vascularization and in decreased fibroblast biosynthesis of important extracellular matrix components; this process results in loss of skin turgidity and elasticity.4 In particular, the amount of HA, a glycosaminoglycan with...
marked ability to maintain tissue hydration, is deeply impaired in mature skin.5

HA is a polysaccharide, which is naturally present in the human body. Its main function consists in maintaining the correct hydration of the tissues, thanks to its intrinsic capacity to bind a large amount of water and to induce optimal conditions for proliferation of dermal cells.6

ALIAXIN®GP Global Performance is a medical device for intradermal treatment, available in prefilled syringes for local injection. This product contains a highly purified cross-linked HA sodium salt, with a mix of different molecular weights ranging from 1000 to 2000 kDa. Other components of the product are: sodium phosphate and water for injectable preparations.

The medical device also has a high safety profile and documented quality. The non-clinical efficacy and safety of this medical device has been documented in both in vitro tests and in vivo studies, as well as in some clinical experiences.7,9

Overall the product, correcting lines and wrinkles, is expected to show the clinical performance of a filler able to rejuvenate skin.

The aim of the study was to confirm clinical efficacy using a prospective design and an objective quantitative primary outcome measure. Instrumental measurements were performed by means of noninvasive skin diagnosis techniques, useful tools for monitoring, evaluation and therapeutical follow-up in esthetic medical procedures.10, 11

Materials and methods

This study was a single-dose, monocentric, open-label not-controlled study, to assess the degree of improvement in nasolabial-folds three and six months after injection of ALIAXIN®GP, distributed by IBSA Farmaceutici Italia Srl, via dei Martiri di Cefalonia 2 – Lodi (LO). The composition of each syringe, according to the International Nomenclature for Cosmetic Ingredients, is Cross-Linked Hyaluronic Acid 25 mg/mL and 1 g of phosphate buffer and water for injectable solution.

Thirty female subjects, aged 40-55, seeking correction of nasolabial folds were enrolled. Subjects had to be available and able to return to the study site for post-procedural follow-up examinations and give written consent.

Exclusion criteria were (as per protocol): previous injection of permanent filler in the area; contraindication or known allergy to the device’s components or to the treatment; known severe allergies manifested by a history of anaphylaxis, or history or presence of severe multiple allergies; any serious skin disease, e.g., eczema and psoriasis of the face, severe rosacea, scleroderma, local infections and severe acne; cancerous or precancerous lesions in either the right or left NLF or the lips; diabetes, coagulation disorders, connective tissue disorders, lipodystrophy or other serious systemic disease; infection with HIV or receiving immunosuppressive therapy; pregnancy or breast-feeding; known alcohol or drug abuse; participation in the study of an investigational drug or device.

The clinical effect of treatment was evaluated by investigators and patients assessment calculated by a four point scale: excellent, good, sufficient and insufficient.

At each visit, digital photographs were taken using a Visioface® Quick device (Courage - Khazaka electronic GmbH, Köln, Germany), which enable an accurate computerized image analysis of the skin. Visioface is a standard technique already used in several studies related to cosmetic procedures and devices;10, 12, 13 analyses of different skin surface parameters can be performed on the image (wrinkles, skin color, pores, spots). Visioface takes high resolution full face photographs under standardized conditions, being useful for the documentation and analysis of treatments. Stable, long lasting and homogenous illumination of the face is obtained by 210 white light LEDs, without developing heat, and a high resolution reflex camera is integrated (18 mega pixel). Removable head and chin rest for the exact position for frontal sidewise images is part of the device. Special software-tools allow to calculate the surface area (pixel²) and volume (pixel³) of selected wrinkles (Figure 1).

The study was instrumentally documented with corneometry (Corneometer® CM 825 - Courage - Khazaka Electronic, Köln, Germany) to record skin capacitance variations; Cutometer (Cutometer® MPA 580- Courage - Khazaka Electronic) to measure skin elasticity.

Assessments

Subjects received the single treatment injection two weeks after the screening visit and returned for evaluations three and six months later.

The complete flow-chart of the study is displayed in Table I.
signed product was injected into both nasolabial folds of each subject: 1 mL syringe of the product was administered for both the right and left nasolabial folds (0.5 mL each) with a linear threading technique in order to standardize the dosing.

Outcome measures

**Efficacy**

The primary outcome measure was the nasolabial folds reduction at T1 and at T2, assessed with instrumental devices.

Two other parameters were also employed: skin hydration and skin elasticity (measured with Visioscan R2 parameter as a percentage of skin return after mechanical deformation (full return R2=1)). Secondary efficacy endpoints were two qualitative scales: patient and physician satisfaction regarding the use of the product.

**Safety**

The assessment of safety was based on the frequency of adverse events (AEs), based on the evaluation of local tolerability and on the vital signs measurements. Ad-
verse events were defined according to Good Clinical Practice and to EN-ISO 14155 and were recorded and reported either if spontaneously revealed by the subject or observed by the Investigator.

**Statistical analysis**

Populations to be analysed were the Intention to Treatment (ITT) and Per Protocol. The latter was defined as the subset of the ITT populations without major protocol violations. The Last Observation Carried Forward principle was to be applied for missing observations.

All patients to whom the product was administered were to be included in the safety population.

For the ITT population and for any endpoints, a descriptive analysis was to be produced.

Adverse events data had to be presented in frequency tables (overall and by intensity).

**Results**

A total of thirty Caucasian female volunteers (age 49.0±3.4 years) were enrolled, and after agreeing to participate in the study and signing the informed consent, followed the procedures and after two weeks, received the expected injection of HA.

All subjects were fully compliant with the procedures and no major protocol violations were recorded. There were no missing data derived from the Visioface® assessments nor from skin hydration and elasticity measurements.

No adverse events were recorded, apart from mild transient redness in 30% of patient (resolving in few hours after injection) and bruising in the 10%.

The four continuous variables coming from VisioFace® assessments were summarized through mean, standard deviation, median and range (Tables II, III).

Figure 2.—Counts of dry and normal skin at different visits.

![Figure 2](image)

Figure 3.—Mean percentages of skin elasticity (R2) at different visits.

![Figure 3](image)

![Table II](image)

**Table II.**—Mean and SD of Visioface assessment at different visits.

<table>
<thead>
<tr>
<th>Parameters (mean and SD)</th>
<th>T0 (treatment administration)</th>
<th>T1 (90 days)</th>
<th>T2 (180 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume right nasolabial fold (pixel³)</td>
<td>114.96 (63.75)</td>
<td>84.58 (58.38)</td>
<td>92.93 (59.43)</td>
</tr>
<tr>
<td>Volume left nasolabial fold (pixel³)</td>
<td>126.6 (67.13)</td>
<td>79.82 (52.13)</td>
<td>95.92 (60.04)</td>
</tr>
<tr>
<td>Surface right nasolabial fold (pixel²)</td>
<td>9.7 (4.54)</td>
<td>7.1 (4.34)</td>
<td>7.73 (4.51)</td>
</tr>
<tr>
<td>Surface left nasolabial fold (pixel²)</td>
<td>10.36 (4.62)</td>
<td>6.84 (4.05)</td>
<td>7.86 (4.41)</td>
</tr>
</tbody>
</table>

![Table III](image)

**Table III.**—Median and range of Visioface assessment at different visits.

<table>
<thead>
<tr>
<th>Parameters (median and range)</th>
<th>T0 (treatment administration)</th>
<th>T1 (90 days)</th>
<th>T2 (180 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume right nasolabial fold (pixel³)</td>
<td>107.34 (36.6-347)</td>
<td>72.1 (19.74-310.75)</td>
<td>80.9 (20.72-311.35)</td>
</tr>
<tr>
<td>Volume left nasolabial fold (pixel³)</td>
<td>109.9 (35.11-292)</td>
<td>59.2 (17.7-220.5)</td>
<td>77.8 (19.93-232.21)</td>
</tr>
<tr>
<td>Surface right nasolabial fold (pixel²)</td>
<td>9.63 (4.12-26)</td>
<td>6.12 (1.75-22.51)</td>
<td>6.75 (2.21-23.16)</td>
</tr>
<tr>
<td>Surface left nasolabial fold (pixel²)</td>
<td>9.25 (3.64-21.72)</td>
<td>5.3 (1.81-16.82)</td>
<td>6.65 (2.16-18.9)</td>
</tr>
</tbody>
</table>
Figure 4.—Visioface volume right nasolabial fold (Friedman Test P value: 4.92e-12).

Figure 5.—Visioface volume left nasolabial fold (Friedman Test P value: 2.763e-13).
Figure 6.—Visioface surface right nasolabial fold (Friedman Test P value: 2.763e-13).

Figure 7.—Visioface surface nasolabial fold (Friedman Test P value: 3.834e-11).
parallel plots and differential boxplots for all four variables were depicted, with the correspondent P-values. In Figure 8 a Visioface digital image example is provided.

An excellent good profile of satisfaction of the product both from investigators and patients at T2 was recorded (Figure 9).

Discussion

When a hyaluronic acid dermal device to fill soft tissues is chosen, efficacy, safety and durability are key concerns.

In cosmetic dermatology, studies supporting the safety and efficacy of fillers for skin ageing besides clinical evidence are limited. Most of these evaluated the effects of hyaluronic acid dermal fillers injections mainly by clinical assessment based on scoring scales (Average Wrinkle Severity Rating Scale -WSRS-, the Global Aesthetic Improvement Scale -GAISS-, and Facial Volume loss Scale -FVLS-).14-16.

Sparavigna et al. 17 performed both clinical and instrumental assessments after a cross-linked hyaluronic acid filler dermal injection, including skin hydration and image analysis (3D) of nasolabial folds confirming the clinical efficacy of the treatment. The values of skin hydration were significantly improved at one, six and nine months after the treatment. The skin profilometry of wrinkles showed significant reduction in the roughness parameter up to 6 months from the injection.

In our prospective design study we used an objective quantitative primary outcome measure to confirm clinical efficacy of the cross-linked hyaluronic acid filler examined. Instrumental measurements were performed
on skin hydration, elasticity and topography by means of noninvasive skin diagnosis techniques. In this open label study we evaluated a sample of 30 volunteers, with a narrow age range (40-55 years), up to 180 days after standardized HA injection. No missing data on primary outcome measures or major protocol violations were observed. Clinical and patient valuations of the outcome showed high satisfaction with study treatment (Figure 9).

The objective Visioface® indexes showed a marked and statistical significant response 90 days after the injection (T1). At 180 days (T2) the improvement from injection was still evident, even if as expected, less marked than after 90 days.

Skin hydration and elasticity did not show significant changes, except for a shifting of few patients classified from dry skin towards normal skin.

Strong points of the present study were the marked, persistent and statistical significant corrective response of nasolabial folds, coupled with an excellent tolerability and safety profile. The main limitation of the study was the non-controlled nature of the trial, but this fact should be adequately compensated by the objectivity of the primary efficacy outcome measures.

Conclusions

This study provided instrumental information about the excellent efficacy/safety ratio of the study product. A standardized HA filler dermal injection on nasolabial folds did not influence skin biophysical parameters such as skin hydration and elasticity.

References


Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.