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Effectiveness and Long-Acting of the Hyaluronic Acid Injected into the Nasolabial Fold Measured Through Magnetic Resonance Imaging

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Abstract
Background MRI analyzed quantitatively the HA injected into the NLF subcutaneous fat to correlate the gel diffusion and degradation with the morphological changes of the NLF appearance for twelve months. Measurements of the gel diffusion and degradation were taken by MRI as parameters to assess the clinical efficacy and long-acting of the HA in NLF rejuvenation.
Method HA was applied into the superficial compartment of the subcutaneous fat of twenty NLFs. Each NLF received three injection points, from the nasal ala toward the oral commissure, 1.0–1.5 cm distant from each other, according to the NLF length. A bolus injection technique without retrograde backflow applied per injection point 0.15–0.20 ml of HA for moderate. NLF and 0.20–0.25 ml for severe NLF. Patients were evaluated through MRI and clinically twenty-four hours, one month and twelve months after the HA application.
Results MRI, in T2-weighted, displayed the gel as a dense, spindle-shaped nodule as pattern of the gel diffusion, measuring its largest longitudinal and transverse axes. Twenty-four hours after HA application the longitudinal axis measured 1.79 cm, after one month 2.33 cm and at month twelve 0.91 cm. The transverse axis measured 0.92 cm at 24 hours, 1.13 cm after one month and 0.47 cm at month twelve.
Conclusion Despite reduction in size and denseness of the spindle-shaped nodule, the small amount of gel presenting into the subcutaneous fat after twelve months of the application evidenced the HA efficacy and long-acting in NLF rejuvenation.
Level of Evidence IV This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors -www.springer.com/00266.

Keywords Hyaluronic acid · Nasolabial fold · Nodule · Wrinkle · Denseness · Subcutaneous fat

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Introduction

The nasolabial fold (NLF) is a crease, running from the nasal ala to the oral commissure that may enlarge in depth and length with aging. The six-point grading scale is a reliable clinical tool in NLF assessment, ranging from 0-no wrinkles to 5-very deep wrinkle, redundant fold [1]. Identification of specific facial fat compartments is relevant to adequate placement of fillers in NLF rejuvenation. Subcutaneous fat at the NLF is distributed in superficial and deep compartments separated by the musculoaponeurotic system [2]. Hyaluronic acid (HA) is the most frequent soft-tissue filler used to adjust morphologic changes of the NLF. It is usually injected into the superficial compartment of the subcutaneous fat for improvement of the NLF [3]. There are several proceedings to evaluate efficacy and longevity of the HA into the subcutaneous fat. Clinical evaluation of HA into the subcutaneous fat has been widely reported, mostly for the NLF [4]. Adequate assessment of the HA into the subcutaneous fat must be quantitative, as histological analysis and imaging techniques. Ultrasound is used to evaluate the HA relationship with soft tissues and measure the gel diffusion [5]. Magnetic resonance imaging (MRI) provides more accurate definition and measurements of the gel diffusion. This happens due to the water high signal intensity of the HA on T2-weighted images [6]. The digital imaging and communications in medicine (DICOM), a format standard of the MRI devices, establish a common language between equipment of different brands [7]. The DICOM tool standardizes data provided by distinct MRI equipment, making them reliable. Measurements of the digital images provided by MRI are taken with the applicative RadiAnt DICOM Viewer [8].

MRI correlated the physiological diffusion and degradation of the HA with the morphological changes of the NLF appearance for twelve months. The intensity of the HA signal in T2-weighted sequence of MRI made easy to identify and measure the pattern of the gel diffusion into the subcutaneous fat to assess the HA efficacy and long-acting in NLF rejuvenation.

Fig. 1 Injection point made at the wrinkle edge with needle partially inserted under the NLF

Patients and Method

Ten female patients, ages ranging from 50 to 55 years old, mean age of 52.2 years old, with moderate and severe NLFs underwent HA filling for cosmetic purposes. Exclusion criteria were males, smokers, skin conditions, allergy, diabetes, medications and previous cosmetic procedures. As manufacturers standardize their products, to prevent unequal diffusion of the gel into the subcutaneous fat, which could interfere with the results, all patients used HA from a single manufacturer (S. THEPHARM, Co. Ltd., Seoul, Korea). Injections were done using a standard syringe size of 1.1ml containing the filler QTFILL PLUS - Sub Q®, a gel composed by 24 mg/ml of cohesive HA cross-linked with 1,4 butanediol diglycidyl ether (BDDE) 2.0 ppm and 0.3% lidocaine hydrochloride. Patients were followed for 12 months.

Operative Technique

The filler QTFILL PLUS—Sub Q® was injected on twenty NLFs of ten patients. Three injection points were distributed on each NLF, starting 0.5 cm from the nasal ala and directed to the oral commissure, distant 1.0 cm from each other for moderate NLF and 1.5 cm for severe NLF. A bolus injection technique without retrograde backflow applied, per injection point, 0.15 ml to 0.20 ml of HA for moderate NLF and 0.20 ml to 0.25 ml of HA for severe NLF. Injection points were made at the wrinkle edge with the needle partially inserted into the NLF subcutaneous fat (Fig. 1). Superficial application of the HA achieved an accurate NLF leveling with the surrounding tissues (Fig. 2). Table 1 summarizes patients distribution and volume of HA injected.

MRI Evaluation

Quantitative evaluation of the gel was performed through MRI with DICOM format viewer, in T2-weighted (Fig. 3).

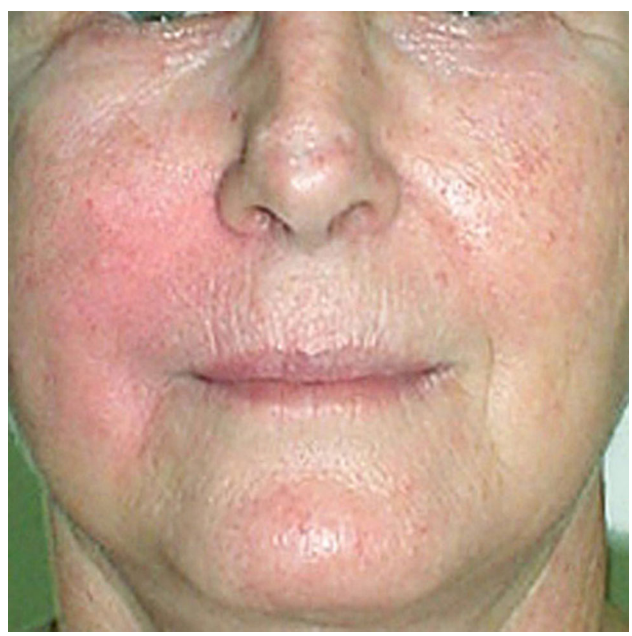

Fig. 2 Accurate leveling of the right NLF with surrounding tissues following HA application

Table 1 Nasolabial fold: patients distribution and outcomes

	Age	Nasolabial fold grading		Volume injected (ml)	
		Right	Left	Right	Left
1	54	Moderate	Severe	0.50	0.65
2	55	Severe	Severe	0.65	0.65
3	50	Moderate	Moderate	0.40	0.40
4	52	Moderate	Severe	0.50	0.60
5	50	Moderate	Moderate	0.40	0.45
6	52	Moderate	Moderate	0.40	0.40
7	51	Moderate	Moderate	0.40	0.45
8	54	Severe	Moderate	0.60	0.45
9	51	Severe	Severe	0.60	0.60
10	53	Moderate	Moderate	0.45	0.50

Diffusion and subsequent degradation of the gel were evaluated on the axial plane with the axial sections supported on the nasal spine to standardize the images for all patients. The applicative RadiAnt DICOM Viewer evaluated quantitatively the images provided by MRI, measuring the linear dimensions of the gel twenty-four hours, one month and twelve months after the HA application. Measurements were settled by the largest longitudinal and transverse axis of the gel diffusion without the need for the axes to be perpendicular to each other (Fig. 4).

Statistical Analysis

Pearson product-moment correlation coefficient measured the linear correlation of the gel diffusion into the right and left NLFs subcutaneous fat one month and twelve months after HA application. The "r" of Pearson ranged from -1 to $+1$, where 0 is no linear correlation, $+1$ is total positive linear correlation and -1 is total negative linear correlation [9]. A bar graph displayed the largest longitudinal and transverse axis of the gel diffusion into the right and left NLFs subcutaneous fat twenty-four hours, one month and twelve months after HA application. The graph compared multiple data, displaying them side by side through vertical rectangular bars with lengths proportional to the values that they represent [10].

Results

Twenty NLFs received injections of HA into the superficial compartment of the subcutaneous fat, creating a same pattern of gel diffusion in all of them. It acted as a pillar, holding up the NLF at the level of the surrounding tissues, improving its appearance in all patients (Fig. 5). According to the six-point grading scale, thirteen NLFs were moderate or moderately deep wrinkle and seven NLFs were severe or deep wrinkle with well-define edges. The volume of HA injected per NLF was distributed between the injection points, changing from one point to another as the wrinkle

Fig. 3 MRI, in T2-weighted, displayed a hyperintense signal of the HA

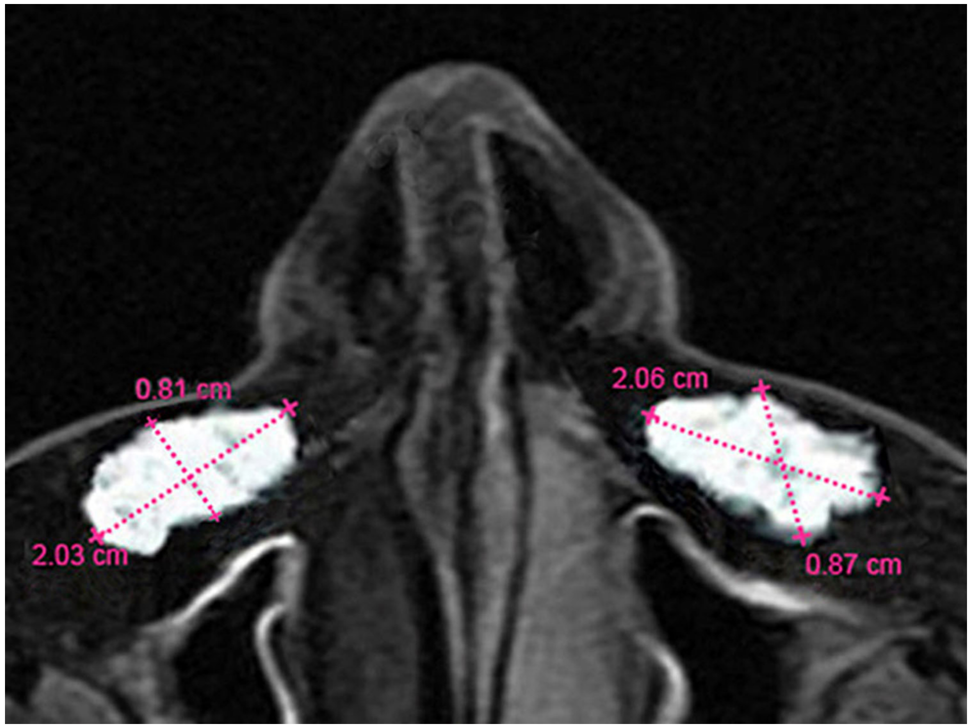

Fig. 4 Measurements of the largest longitudinal and transverse axis of the spindle-shaped nodule

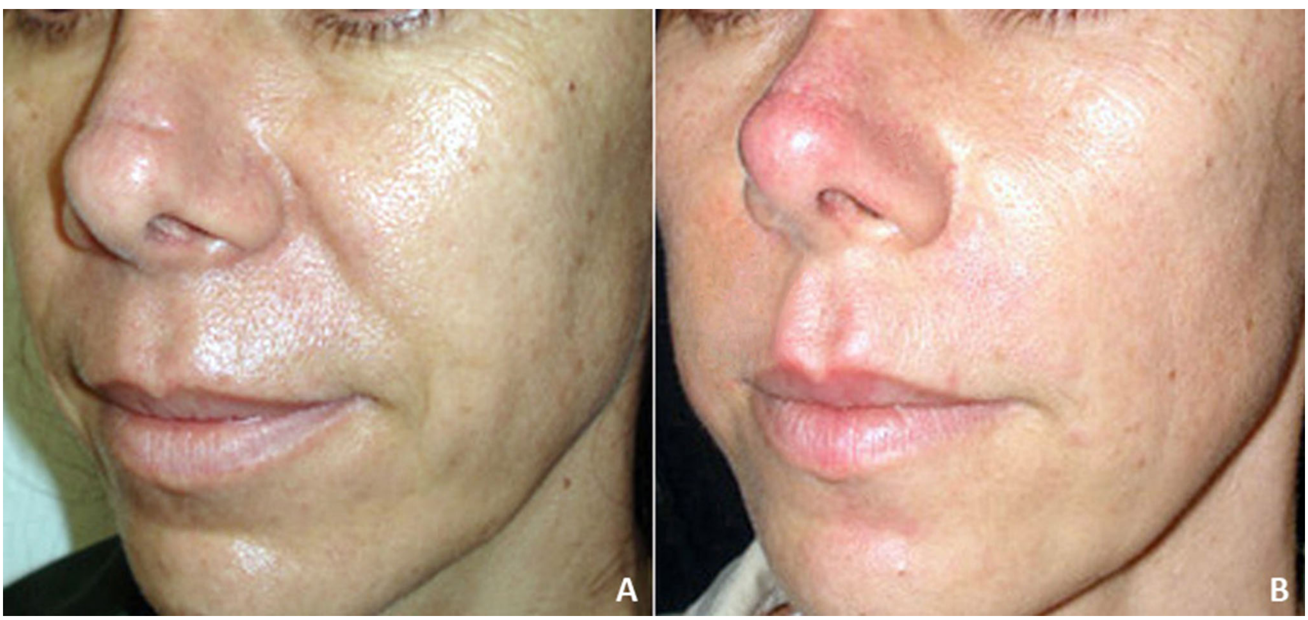

Fig. 5 **a** A 50-year-old female with moderate NLFs. **b** No visible NLFs one month after HA application

depth and edges length. Six moderate NLFs received 0.40 ml, four 0.45 ml and three 0.50 ml, average of 0.44 ml of HA per NLF. Four severe NLFs received 0.60 ml and three 0.65 ml, average of 0.62 ml of HA per NLF.

Clinical Findings

The depth of the NLF before application was used as parameter to evaluate the clinical acting of the HA (Fig. 6a). One month after HA application the NLF was leveled with the surrounding tissues, recovering the mid-face symmetry and balance (Fig. 6b). At month twelve, the level of the NLF with the surrounding tissues was about

Fig. 6 **a** A 54-year-old female with severe NLFs. **b** No visible NLFs one month after HA application. **c** At month twelve, NLFs two-thirds less than one month after application

two-third lesser than the month one after application, indicating yet a small amount of gel into the subcutaneous fat (Fig. 6c). Efficacy and long-acting of the HA was evident comparing the NLF before application with those seen at month one and month twelve after application.

Digital Images Findings

MRI, in T2-weighted, displayed the pattern of the HA gel diffusion into the NLF subcutaneous fat as a dense spindle-shaped nodule. Size, brightness and denseness of the spindle-shaped nodule one month after application were greater than twenty-four hours after application (Fig. 7a, c). The morphologic changes of the spindle-shaped nodule occurred by the high water content absorbed by the HA in the first month of the application. Size, brightness and denseness of the spindle-shaped nodule at month twelve were significantly smaller than one month after application (Fig. 7e). Despite decrease in size and denseness of the spindle-shaped nodule, its presence into the subcutaneous fat after twelve months evidenced the long-acting of the HA. The physiological changes of the spindle-shaped nodule displayed in MRI twenty-four hours, one month and twelve months after HA application guided the changes of the NLF appearance in the same period (Fig. 7).

Measurements of the Spindle-Shaped Nodule

Table 2 summarizes measurements of the longitudinal and transverse axes of the spindle-shaped nodule of HA twenty-four hours, one month and twelve months after application.

Longitudinal Measurements—The "*r*" of Pearson of 0.9998 and 0.9917 for right and left NLFs indicated a strong positive correlation of the largest longitudinal axis of the spindle-shaped nodule at twenty-four hours and one month after HA application. At twenty-four hours, the longitudinal axis ranged from 1.63 cm to 1.95 cm, average of 1.79 cm, and after one month ranged from 2.01 cm to 2.74 cm, average of 2.33 cm (Fig. 8a, b). The high capacity of the HA to absorb water in the first month after application increased the longitudinal axis in 0.54 cm. At month twelve, the longitudinal axis ranged from 0.77 cm to 1.08 cm, average of 0.91 cm, indicating yet a small amount of the gel into the NLF subcutaneous fat (Fig. 8c). A vertical bar graph displayed, per patient, the measurements of the longitudinal axis on each one of the NLFs, arranging them side by side twenty-four hours, one month and twelve months after HA application (Fig. 9).

Transverse Measurements—The "*r*" of Pearson of 1.0000 for right and left NLFs indicated a strong positive correlation of the largest transverse axis of the spindle-shaped nodule at twenty-four hours and one month after HA application. At twenty-four hours the transverse axis ranged from 0.73 to 1.23 cm, average of 0.92 cm, and after one month ranged from 0.87 to 1.47 cm, average of 1.13 cm (Fig. 8a, b). The high capacity of the HA to absorb water in the first month after application increased the transverse axis in 0.21cm. At month twelve the transverse axis ranged from 0.37 cm to 0.54 cm, average of 0.47 cm, indicating yet a small amount of the gel into the NLF subcutaneous fat (Fig. 8c). A vertical bar graph displayed, per patient, the measurements of the transverse axis on each one of the NLFs, arranging them side by side twenty-four hours, one month and twelve months after HA application (Fig. 10).

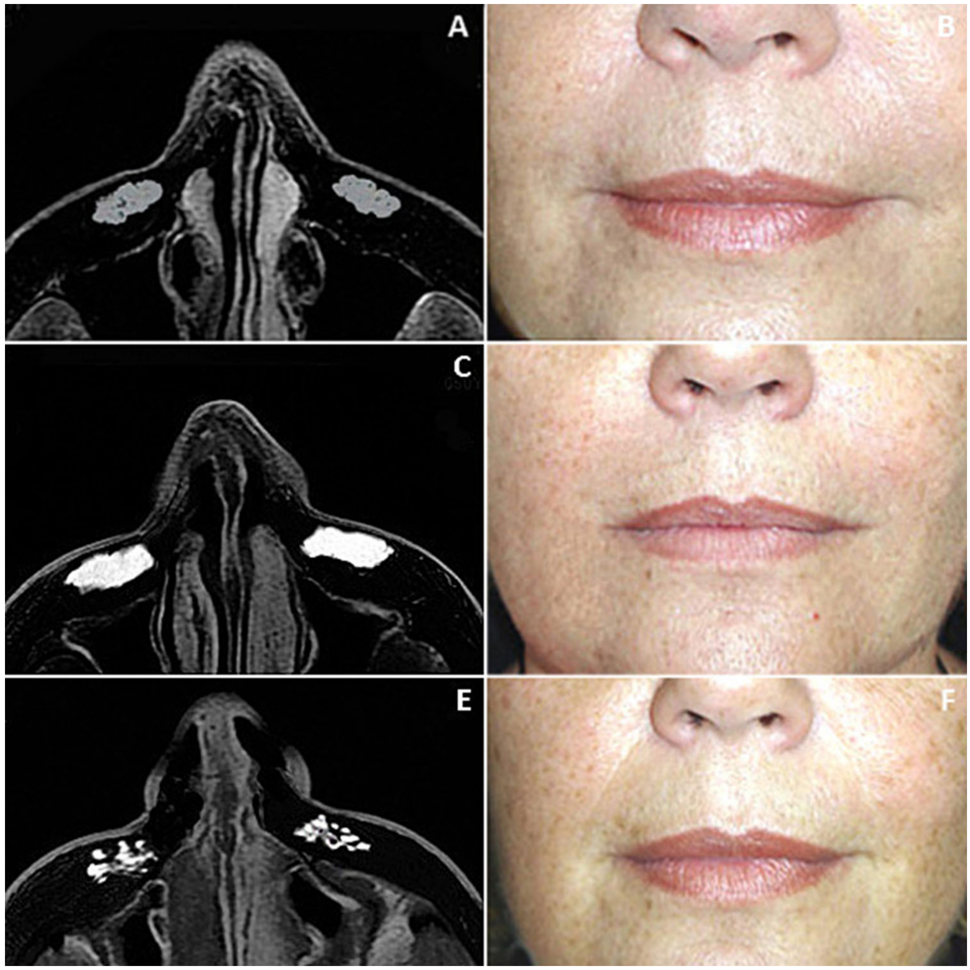

Fig. 7 Brightness, denseness and size of the spindle-shaped nodule correlated with NLF appearance, **a**, **b** at twenty-four hours, **c**, **d** one month and **e**, **f** twelve months after HA application.

Table 2 MRI measurements of HA into the subcutaneous fat of the nasolabial fold

	Age	Right nasolabial fold						Left nasolabial fold					
		Longitudinal Measure (cm)			Transverse Measure (cm)			Longitudinal Measure (cm)			Transverse Measure (cm)		
		24 hs	1 month	12 months	24 hs	1 month	12 months	24 hs	1 month	12 months	24 hs	1 month	12 months
1	54	1,96	2,39	0,93	1,07	1,31	0,46	1,91	2,36	1,05	0,89	1,10	0,48
2	55	2,08	2,57	0,99	1,09	1,35	0,52	2,08	2,51	0,99	0,99	1,16	0,53
3	50	1,93	2,30	0,88	1,23	1,47	0,49	1,84	2,30	0,89	1,02	1,28	0,51
4	52	1,81	2,23	0,83	0,80	0,99	0,41	1,85	2,28	0,96	0,88	1,09	0,42
5	50	1,86	2,23	0,86	0,75	0,89	0,37	1,90	2,27	0,88	0,73	0,87	0,38
6	52	1,84	2,19	0,82	0,89	1,06	0,43	1,73	2,08	0,81	0,84	1,01	0,46
7	51	1,83	2,26	0,88	0,90	1,11	0,49	1,82	2,28	0,88	0,78	0,98	0,51
8	54	2,07	2,62	0,99	1,03	1,31	0,51	1,78	2,19	0,85	0,81	0,98	0,51
9	51	2,14	2,74	1,08	1,05	1,34	0,53	2,10	2,66	1,00	0,99	1,25	0,54
10	53	1,95	2,15	0,77	0,87	1,07	0,41	1,63	2,01	0,83	0,78	0,96	0,40

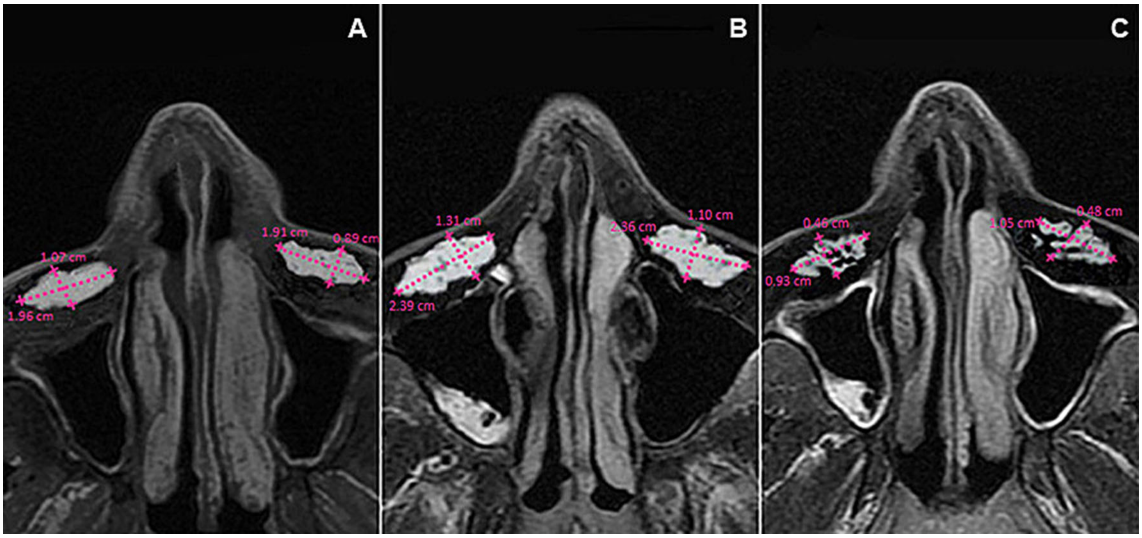

Fig. 8 Measures of the largest longitudinal and transverse axis of the spindle-shaped nodule, **a** at twenty-four hours, **b** one month and **c** twelve months after HA application

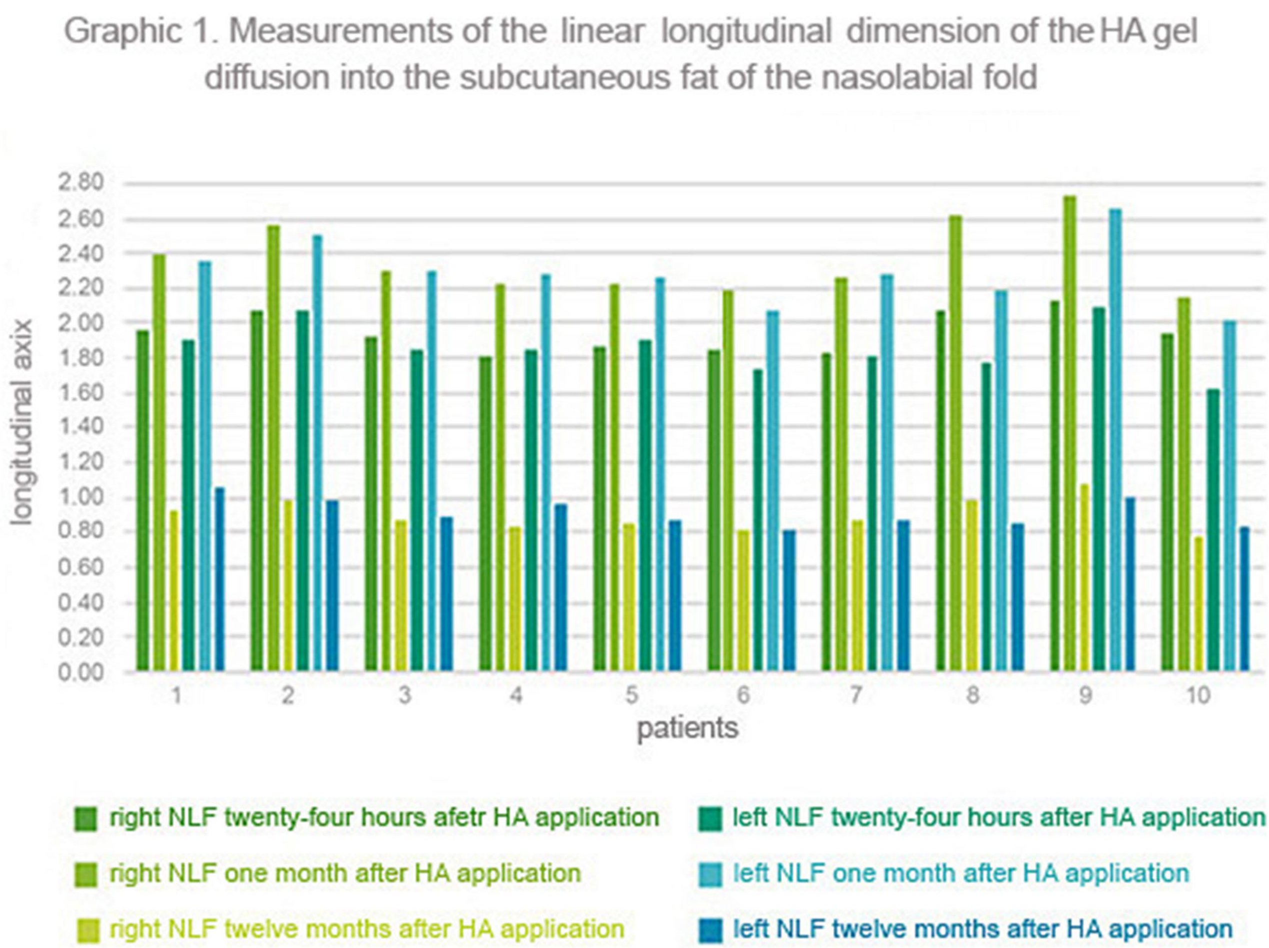

Fig. 9 Vertical bar graph distributed the values of the longitudinal axis side by side, providing an overview of the HA degradation on both NLFs of each patient

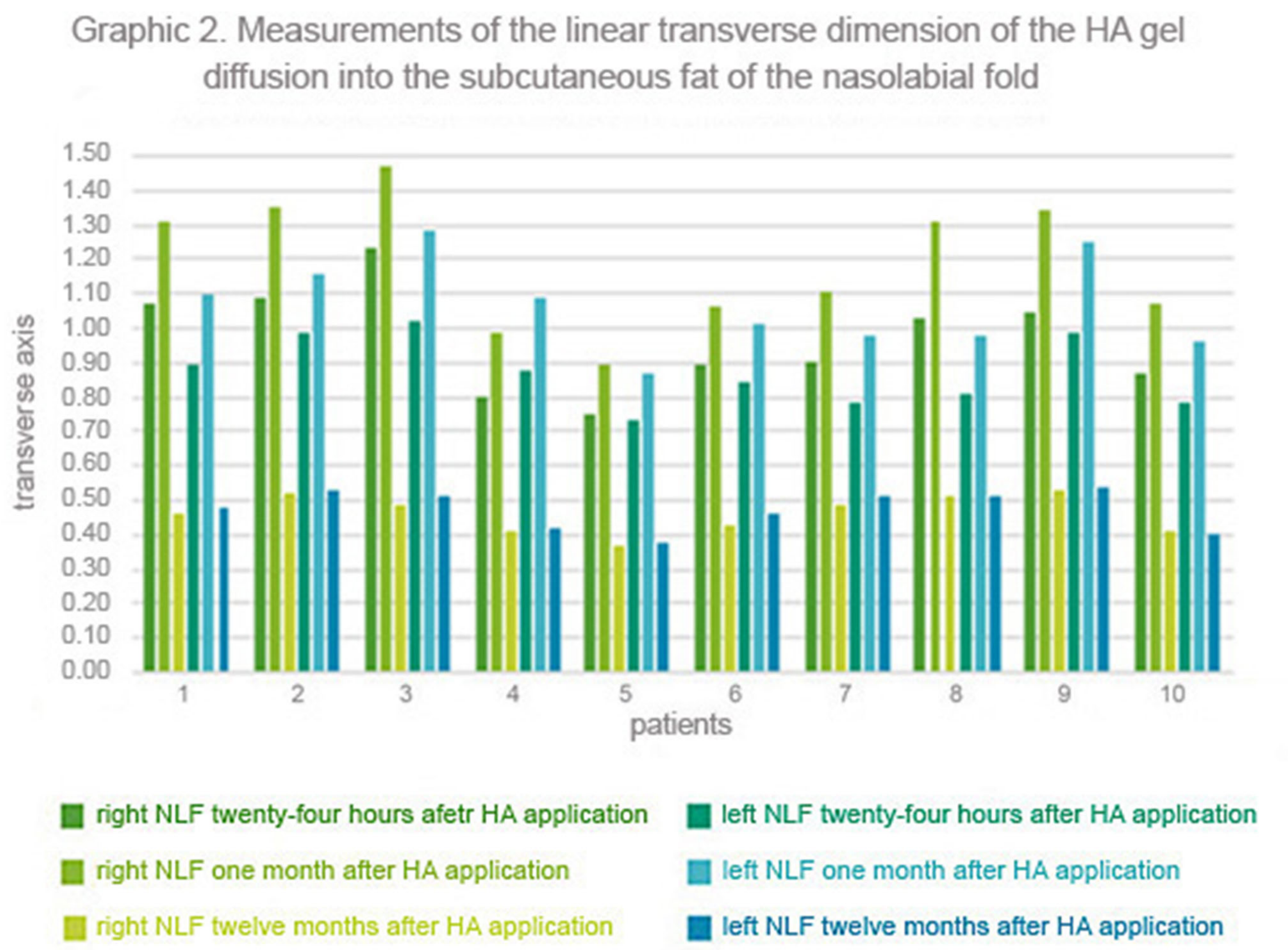

Fig. 10 Vertical bar graph distributed the values of the transverse axis side by side, providing an overview of the HA degradation on both NLFs of each patient

Discussion

Selection of patients involved only females to prevent unequal diffusion of the HA gel caused by female and male differences regarding thickness and elasticity of the skin and subcutaneous fat, wrinkle, hydration, pH, water loss and circulation [11]. The small difference between the age of the patients was important to prevent distortion of the HA gel diffusion. Knowledge of the subcutaneous fat compartments allowed to establish the concept of compartment-specific volume augmentation [12]. The specific volume average of 0.44 ml for moderate NLF and 0.62 ml for severe NLF, apart to level the NLF with the surrounding tissues, maintained the HA for twelve months into the superficial compartment of the subcutaneous fat. The volume injected in each NLF was distributed differently among the three injection points, according to the NLF wrinkle deepness and lengthiness. The HA cohesivity, defined as the capacity of a filler not to dissociate, because of the affinity of its molecules for each other, was relevant for the gel diffusion into the tissue [13]. Concentration of 24 mg/ml of the HA cross-linked 2.0 ppm provided a gel cohesivity enough to stabilize the spindle-shaped of the gel for twelve months into the subcutaneous fat. Gel cohesivity was featured by the denseness of the spindle-shaped nodule in MRI. Notwithstanding the long-term clinical improvement of the NLF, was necessary a proper understanding of the physiological diffusion and degradation of the HA into the subcutaneous fat. Despite the numerous proceedings for assessing fillers, imaging techniques offered particularities that made them more specific to evaluate the HA into the tissues, mainly MRI. It was initially used to measure the volume of the HA gel injected into the subcutaneous fat of rats and to observe its subsequent degradation [14]. Facial MRI can provide, clearly and consistently, images to identify, measure and analyze symmetry of the HA diffusion, mostly for the NLF [15]. Axial sections of MRI were supported on the nasal spine to ensure minimal variation in the measures of the longitudinal and transverse axes of the spindle-shaped nodule. MRI correlated the longitudinal and transverse measurements of the spindle-shaped nodule with the appearance of the NLF for twelve months, allowing to understand the NLF morphological changes after HA application, thereby, was able to evaluate quantitatively the HA long-acting in NLF rejuvenation. The main advantage of MRI was the T2-weighted sequence, which suppressed uniformly the fat, making it appear dark and HA with hyperintense signal [16]. The high-intensity of the signal was due to the HA to have in MRI an appearance similar to the water. In addition, the hygroscopic property of the HA fillers enabled them to increase correction up to 15% after injection [17]. The capacity of the HA to absorb water right after application was showed in MRI by the increase in the spindle-shaped nodule axes in the first month after application. The longitudinal and transverse axes at month one were 23.18% and 18.58% greater than at twenty-four hours after application. Denseness and brightness of the spindle-shaped nodule at month one greater than at twenty-four hours after application also displayed the volume gained by the HA in the first month after injection. The stability of the volume gained depended on the speed of the enzymatic degradation. Enzymatic degradation of the HA into the subcutaneous fat was made at the peripheral surface and inside of the pattern of gel diffusion [18]. The small surface and high denseness of the spindle-shaped nodule reduced the area of the enzymatic activity delaying the HA degradation, ensuring its long-term acting. The longitudinal and transverse axes at month twelve 61.11% and 58.41% smaller than one month after application indicated the slow degradation of the HA within twelve months.

Conclusion

Application into the superficial compartment of the subcutaneous fat, apart to level the NLF with the surrounding tissues, maintained the HA for twelve months into the tissue. MRI correlated the longitudinal and transverse measurements of the spindle-shaped nodule with the appearance of the NLF for twelve months, allowing to understand the NLF morphological changes in the period. The T2-weighted sequence of MRI suppressed uniformly the fat, emphasizing the white color of the HA, making easy its measurements. Despite the reduction in size and denseness of the spindle-shaped nodule, the small amount of gel presenting into the subcutaneous fat after twelve months evidenced the HA efficacy and long-acting in the NLF rejuvenation.

Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest or financial ties to disclose.

Human and Animal Rights Statement All procedures performed in this study involving humans participants were in accordance with the ethical standards of the ACAInstitute of Assistance in Plastic Surgery of São Paulo research committee and with the 1964 Helsinki Declaration and Medical Research Involving Human Subjects and its latter amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all patients.

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