RESEARCH

Patient perceived improvement and safety of new hyaluronic acid–based filler: A descriptive retrospective study

Ilaria Zollino[1], Antonella Franceschelli[2], Maria Luisa Garo[3]

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ABSTRACT

Background: Age-related loss of volume and imperfections in the face or neck can lead to psychological discomfort due to the stigmatization of these physical changes, potentially catalyzing uncomfortable interpersonal relationships. The aim of this retrospective study was to evaluate the patients' perceived improvement and safety regarding a new 2.5% (mg/ml) hyaluronic acid–based filler developed with a conservative cross-linking technology and purified by a dialysis procedure through water for injection (BDDE<0.1 ppm).

Methods: Patient's perceived improvement and treatment safety recorded from the first treatment to the last follow-up were retrospectively analyzed.

Results: Four hundred and seven patients (mean age: 51.4 ± 11.2) were included, totaling 1,070 treatments (2.63 ± 2.25 vials per subject). More than 98% of patients expressed high perceived improvement level. Mild and transient side effects (mainly hematoma and pain) occurred in 55% of patients; in many cases, these side effects disappeared without pharmacological treatment.

Conclusion: This new hyaluronic acid filler has proven capable of achieving aesthetic natural-looking results, maintaining high effectiveness in the long-term, and demonstrating an excellent safety and tolerability profile, even when repeated treatments are required.

Key Words: *Hyaluronic acid; Facial wrinkles; Facial folds; Stylema®; Long-term safety*

INTRODUCTION

Age-related loss of volume in the face or neck is considered a common aesthetic problem due to the increased ageing of the population. In the last decade, there has been an increasing demand for non-surgical aesthetic interventions, owing to the severe physical discomfort and associated stigmatization, unpleasant interpersonal relationships, anxiety, and depression related to aesthetic deficiencies or signs of ageing [1-3].

The ageing process starts at the age of 20 and accelerates after 40, influenced by various factors such as genetics, smoking, and/or sun exposure [4]. Typical signs of skin ageing are wrinkles, loss of elasticity, and a rough appearance [5.] For many years, various injectable fillers based on hyaluronic acid (HA) (~77%) have been used in aesthetic medicine as the gold standard in reducing and softening wrinkles along with increasing facial volume [4,6-10]. This is due to the optimal medium-term effect of HA, which can promote minimally invasive facial rejuvenation and volume correction, with low complication rates, low toxicity, and an immunogenicity profile, low cost, as well as HA versatility in various aesthetic applications [11-15].

HA is a linear, natural, biodegradable, and safely occurring polysaccharide that belongs to the class of biochemical compounds known as glycosaminoglycans [11]. It is one of the major constituents of the extracellular matrix found primarily in synovial fluids and the epithelial and connective tissues of vertebrates [16,17]. Its primary structure consists of a repeating carbohydrate sequence: [D-glucuronic acid β (1→3) N-acetyl-D-glucosamine β (1→4)]n [18]. Due to its high molecular weight, it has many unique physicochemical and mechanical properties such as regulation of inflammation, hygroscopicity, and lubrication [11,19]. The results from HA treatments are temporary and may last from six to 12 months or longer (up to 24 months), depending primarily on the concentration of HA, the cross-linking, the treated site, and the patient characteristics [20,11]. After 12–24 months, the body absorbs the HA particles naturally and gradually [8,21]. For the same, HA fillers with a concentration greater than 20 mg/ml are considered ideal; currently, FDA approved fillers have a HA concentration ranging from 15 mg/ml to 24 mg/ml [22,23].

To synthesize HA fillers and ensure long-lasting efficacy without compromising filler absorption, cross-linkers—small molecules consisting of a spacer and at least two functional groups—are designed to bridge HA chains to link them together to achieve the required elastic properties, projectivity, and the property of longevity by

[1]*Independent Researcher, Ferrara, Italy;* [2]*Independent Researcher, Rome, Italy;* [3]*Independent Researcher, Rome, Italy*

Correspondence: Maria Luisa Garo, Independent Researcher, 00199, Rome, Italy; marilu.garo@gmail.com

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slowing down enzymatic degradation kinetics [18]. Nowadays, BDDE (1,4-butanediol diglyceryl ether) is the standard cross-linker of HA in fillers for aesthetic medicine, thanks to its characteristic of easily reacting with HA to form a gel matrix by creating inactive and non-toxic covalent ether bonds with HA [24]. This rapid process allows the etherification of the HA chains by an inert diol molecule, no longer BDDE, as unreacted BDDE is immediately hydrolyzed during the cross-linking step into an inactive diol form; this is eliminated by further purification steps so that residual traces of BDDE are either undetectable in the final gel formulation or at least below the stated safety limit (18). To be safe, the FDA recommends a residual unreacted BDDE level to be less than 2 part per million (ppm), corresponding to <0.002 mg of BDDE in 1 mL of HA gel [25]. Cross-linked HA filler stimulates fibroblasts to produce collagen fibers via the TGF-β/Smad pathway, although the exact pathway is controversial. In the dermal microenvironment, collagen stimulation is associated with increased expression of the type II TGF-β receptor, the key regulator of type I procollagen synthesis in human skin. HA facilitates the interaction between CD44 and EGFR, promoting MAPK/ERK phosphorylation and inducing TGF-β1-dependent fibroblast proliferation [26]. HA can also promote the growth of elastic fibers. Different molecular weights of HA have varying effects on skin feel and viscosity. High molecular weight HA (molecular weight 1×10^6 Da) has anti-wrinkle, skin rejuvenation, free radical scavenging, and skin protection effects as it forms a better film and retains moisture. Alternatively, low molecular weight (1×10^4 Da – 1×10^6 Da) can penetrate the skin layer, repair damage to the muscle floor, and play a transdermal moisturizing role [27].

The Stylema® HA (Uniderm Farmaceutici, Italy) product line was employed in the current study for personalized treatments, targeting wrinkle reduction, increased facial volume, and enhancement of the rejuvenation processes. These have been developed to provide a safe and minimally invasive method of restoring facial volume, reversible by hyaluronidase if treatment-emergent Adverse Events (AEs) occur. Manufactured in strict compliance with international regulations and ISO 13485: 2016 certified production protocol standards, the entire production process takes place in a clean room. Pure hyaluronic acid of the highest quality is used for all formulations, free of nucleic acids and protein residues, guaranteeing maximum biocompatibility. This process makes it possible to obtain an extremely homogeneous product that perfectly integrates into the skin, requiring lower amounts of BDDE chemical cross-linking agents.

Although the half-life of HA filler products depends on the presence and amount of cross-linking agents, some studies reported a correlation between filler half-life and the risk of early, late, and delayed adverse effects, along with volume loss in the 12-month period after treatment [8,20,21,28]. Adverse effects often occur weeks, months, or even years after injection, and are related to the site where the HA filler was injected; side effects are frequently reported after treatment in the nasolabial fold (35.6%), but side effects related to the lips, periorbital region, and perioral region have also been commonly reported [15]. To date, very few studies investigated the patients' satisfaction and safety profile of dermal fillers after a long-follow-up period in a real-world scenario on large samples. To this end, the aim of this study was to describe the patients' perceived improvement and safety of a new 2.5% (mg/ml) HA-based filler developed with a conservative cross-linking technology and purified by a dialysis procedure through water for injection (BDDE<0.1 ppm). Also, some specific treatment characteristics, such as the number of vials, the administration modality, and the treated area, were considered. To evaluate the safety of Stylema® fillers over a short and long period of time, in-depth monitoring of side effects was performed.

MATERIALS AND METHODS

Study design

A retrospective study was conducted on a sample of patients who required HA filler treatment because of the need of aesthetic improvement from January 2021 to September 2022. All four monophasic filler formulations were evaluated: Stylema® Light, Stylema® Medium, Stylema® Intense, and Stylema® Lips. The characteristics of the products are reported in (Table 1). While the manufacturing process is described in Supplementary materials (Supplementary material 1. The manufacturing process of the Stylema® filler.).

TABLE 1
Stylema® HA formulation

	Stylema® Light	**Stylema® Medium**	**Stylema® Intense**	**Stylema® Lips**
HA Concentration	2.5% (25 mg/ml)	2.5% (25 mg/ml)	2.5% (25 mg/ml)	2.5% (25 mg/ml)
Molecular Weight	2 M Da+1 M Da+0.5 M Da (+10% 0.5 M Da free from HA)	2 M Da+1 M Da	2M Da+1 M Da	2 M Da+1 M Da
Cross-Linking Grade	■■■□□	■■■■□	■■■■■	■■■■□
BDDE	Residual < 0.1 ppm	Residual < 0.1 ppm	Residual < 0.1 ppm	Residual < 0.1 ppm
Injection Level	Intradermal and hypodermal	Hypodermal	Supraperiosteal	Dermal, labial submucosal and hypodermic
Needle	27 G 19mm–30 G 13 mm	27 G 19 mm–27 G 13 mm	27 G 19 mm–27 G 13 mm	27 G 19 mm–27 G 13 mm
Viscoelastic moduli				
G'	30Pa	100Pa	200Pa	100Pa
G''	10Pa	20Pa	40Pa	20Pa

The manufacturing process of the Stylema® filler

Conservative cross-linking technique for Stylema® filler:

By acting on both the temperatures and the mixing time of the raw materials, the innovative process makes it possible to control the degree of cross-linking of the hyaluronic acid without causing depolymerisation and to use smaller amounts of the chemical cross-linking agent BDDE, making the product more malleable and safer.

By using lower amounts of the chemical cross-linking agent BDDE (< than 0.1 ppm on the remaining final product), the Conservative Cross-Linking Technology allows for a more homogeneous and very manageable final product that can be seamlessly integrated into the skin tissue.

Manufacturing process of the Stylema® filler:

The manufacturing process of the Stylema® filler consists of 8 different steps. Each of them is carried out in strict compliance with national and international regulations and using the highest safety standards.

Step 1 - Raw materials:

High purity hyaluronic acid, which at this stage is still in powder form, is placed in special containers, weighed, sealed, and sent to the sterile liquid filler department where the more viscous hyaluronic acid solution is produced.

Step 2 - Cross-linking:

In this first step, highly purified hyaluronic acid, free of nucleic acids and protein residues, is mixed with the cross-linking agent BDDE at low temperatures and over a long period of time (about 12 hours). This special manufacturing process, called cross-linking, enables the preservation of the specific viscoelastic properties of hyaluronic acid and, more importantly, the use of lower amounts of the chemical cross-linking agent BDDE, resulting in a product of the highest quality and a very high degree of biocompatibility.

Step 3 – Purification:

The mixture is purified by a dialysis process using WFI (Water for Injection). This process eliminates any remaining BDDE molecules and ensures a product with a high degree of purity and low toxicity.

Step 4 – Mixing:

The product is mixed with a buffered solution to obtain a neutral pH.

Step 5 – Filling:

The resulting mixture is placed in the tank of a filling machine in the absence of oxygen, where it rests for several hours waiting for laboratory results certifying that the quality and purity requirements have been met. Once the controls have been passed, the product is ready to be bottled with an automatic filling machine.

Step 6 – Sterilization:

The pre-filled syringes are then sterilized in an autoclave. This is done by applying moist heat at 121^0 for 15 minutes. Once the sterilisation process is complete, the syringes are held ready until the results of the quality control tests are available.

Step 7 - Quality control and product release:

In this step, final checks are carried out to confirm that the quality and safety requirements for the release of the finished product have been met:

1. Extrudability and pH control
2. External tests in accredited laboratories
3. Sterility and LAL tests
4. Visual atomisation

Step 8 – Packaging:

In this step, the syringe is placed in a thermoformed blister pack, sealed with medical grade paper and placed in the packaging. Finally, the product is packaged and taken to the warehouse where it is stored at the unexpected storage temperatures of the shipment.

Participants

Included patients were women aged ≥ 18 years who completed at least 12 months of follow-up. Data were retrospectively extracted from patients' medical charts. Exclusion criteria were as follows:

1. Hypersensitivity to the product, lidocaine, or HA, as determined by a skin test or a history of anaphylaxis or severe allergic reaction.
2. Bleeding disorders such as hepatic dysfunction, coagulopathy, or the need to take antithrombotic or aspirin during the study.
3. Immunosuppressed patients and patients with autoimmune diseases.
4. Previous rejuvenation treatments such as HA injections, botulinum toxin injections, chemical peeling, or other plastic surgery.
5. Pregnant or lactating women.
6. Hypertrophic scars, keloids, or scars in the nasolabial fold area.
7. Patients with a medical or drug history that may affect the results.
8. Patients who refused to sign the informed consent form.

The study was conducted in a private setting in accordance with good clinical practice guidelines and the recommendations of the ethical principles of the World Medical Association Declaration of Helsinki for Medical Research Involving Human Subjects, as revised by Fortaleza (2013). Written informed consent was obtained from all patients. In Italy, no ethics committee is required for studies conducted in a private setting.

Treatment

Two experienced investigators (medical doctors with extensive experience in esthetic medicine: A.F. and I.Z.) performed the treatments, monitored the patients during the follow-up period, and assessed and evaluated possible side effects. Subjects were treated with one or more HA fillers based on the patient's aesthetic needs in predefined areas, and according to clinical judgement (e.g., safety: full face, cheekbones, nasolabial fold, marionette, lips, barcode, chin, jaw, nose, or other facial regions.) Specifically, before treatment, patients underwent a brief general examination and were informed about possible complications and side effects. The investigators selected the appropriate treatment according to the patients' treatment goals, the investigators' experience, and the manufacturer's instructions for use. The volume injected, needle/cannula type, injection technique, and number of treatments and post-injection massages to achieve optimal correction and reduce post-treatment discomfort were at the discretion of the investigator.

Anesthesia was used according to the area to be treated and the pain threshold level of the patient. Notably, pain perception is also

location-dependent (e.g. the lip area is very sensitive). On this basis, each investigator decided the best technique, the number of required vials, the region to be treated, the application technique (cannula, needle, or both), and the use of anesthetic according to the procedure, the rheologic properties of the fillers, and patient's aesthetic expectations.

The goal of each treatment was to achieve a clinically significant improvement in facial appearance with the least amount of product, striking a balance between aesthetic improvement and patient's satisfaction. The faces of all the patients were digitally photographed at rest during each visit. Possible abnormal reactions during treatment were closely monitored and documented. Side effects and adverse events were managed according to the current guidelines [29-31].

Patient's perceived improvement

A five-point Likert scale (1=not at all satisfied to 5=completely satisfied) assessed the degree of patient perceived improvement in aesthetic appearance and feelings of self-confidence, attractiveness, and well-being at the end of the follow-up period.

Outcomes

The primary outcome was the patients' perceived improvement, while the secondary outcomes were the effectiveness of the treatment and the safety profile.

The effectiveness of the treatment was evaluated for each participant during the follow-up by the two investigators. It was assessed using the validated Wrinkle Severity Rating Scale (WSRS) (1=fold absent, 2=superficial, 3=moderate, 4=serious, 5=very serious) and Global Aesthetic Improvement Scale (GAIS) (0=worse to 4=much improved) [32,33]. These assessments were made one month after treatment and at the last follow-up.

The side effects were monitored throughout the duration of the study, with particular attention to early and delayed side effects. The assessment of the safety and tolerability of the products used relied on the subjects' spontaneous reports of adverse effects and the assessment of their general health status at each visit. Adverse Events (AEs) and Serious Adverse Events (SAEs) that were judged to be more severe or longer lasting than routinely observed were recorded.

Statistical analysis

The sample size was not predetermined; all patients were treated from January 2021–September 2022. Data are presented as relative frequencies, percentages, or mean ± standard deviation. Safety and efficacy were analyzed descriptively, and statistical analyses were performed using STATA18 (Stata Corp., University Station, TX, USA).

RESULTS

Participant's characteristics

Four hundred and seven consecutive female patients (mean age: 51.4 ± 11.2 years) treated from January 2021 to September 2022 were included in the study. The complete characteristics of the patients are reported in (Table 2).

TABLE 2
Patient's characteristics

No. of patients	**407**
Age (years)	
Mean (SD)	51.4 (11.2)
Range (min–max)	(19–81)
Follow-up (months)	
Mean (SD)	12 (0.9)
Range (min–max)	(12–22)

Treatment

About 274 (67.3%) patients received treatment in only one area, 71 (17.4%) patients received treatment in two areas, 38 (9.3%) patients received treatment in three areas, and the remaining 24 (5.9%) patients received treatment in four or five areas (Table 3). Nearly half of the patients were treated with only one vial, 85 (20.9%) patients received treatment with two to three vials, and the remaining patients were treated with more than three vials. The light filler concentration was used in 128 (31.4%) of patients, most of whom were treated with only one vial (78.9%, n=101). One hundred and forty-four (35.4%) patients were treated with the medium formulation, while 125 (30.7%) patients were treated with the lip formulation, and 183 (45.2%) patients were treated with the intense formulation. A same participant could have received more than one treatment. The investigators used a needle in more than half of the patients (51.6%, n=210), both a needle and a cannula in 26.5% (n=108) of the subjects, while only the cannula was used in 21.9% (n=89) of the patients. Anesthesia was required in 54.5% (n=222) of women.

TABLE 3
Treatment's description and administration modality n (%)

	Frequency	**Percentage**
Total treated regions for patient		
One region	274	(67.3)
Two regions	71	(17.4)
Three regions	38	(9.3)
Four regions	21	(5.2)
Five regions	3	(0.7)
Number vials for patient		
1 vial	203	(49.9)
2–3 vials	85	(20.9)
4–5 vials	63	(15.5)
≥ 6 vials	56	(13.8)
Stylema® Light	128	(31.4)
Number Stylema® Light vials for patient		
1 vial	101	(78.9)
2 vials	20	(15.6)
3 vials	7	(5.5)
Stylema® Medium	144	(35.4)
Number Stylema® Medium vials for patient		

1 vial	117	(81.3)
2 vials	22	(15.3)
3 vials	4	(2.8)
4 vials	1	(0.6)
Stylema® Lips	125	(30.7)
Number Stylema® Lips vials for patient		
1 vial	123	(98.4)
2 vials	2	(1.6)
Stylema® Intense	183	(45.2)
Number Stylema® Intense vials for patient		
1 vial	114	(62.0)
2 vials	34	(18.5)
3 vials	16	(8.7)
4 vials	11	(6.0)
5 vials	2	(1.1)
6 vials	6	(3.2)
7 vials	1	(0.5)
Administration Modality		
Cannula Only	89	(21.9)
Needle Only	210	(51.6)
Needle+Cannula	108	(26.5)
Anesthesia	**222**	**(54.5)**
Topical anesthesia*	159	(39.1)
Injective anesthesia*	100	(24.6)
Topical and Injective anesthesia*	37	(16.7)

The most frequently treated sites were the lips and the nasolabial fold (Table 4). Specifically, 44% (n=179) of patients were treated in the lip region, and 39.1% (n=159) of patients were treated in the nasolabial fold region. Treatments of the marionette region and barcode were performed in 27.8% (n=113) and 21.9% (n=89) of patients, respectively.

TABLE 4
Treatment location n (%)

	Frequency	Percentage
Full face	27.0	(6.6)
Cheekbone	71.0	(17.5)
Nasolabial folds	159.0	(39.1)
Marionette	113.0	(27.8)
Lips	179.0	(44.0)
Barcode	89.0	(21.9)
Chin	36.0	(8.8)
Jaw	20.0	(4.9)
Nose	17.0	(4.2)
Other	48.0	(11.8)

Patient's satisfaction

After 12 months, more than 99% of patients were satisfied with the received treatment: patients treated with light and medium formulations reported the highest satisfaction rate (100%). When the lip area was treated, more than 99% of patients reported a high satisfaction rate (satisfied or completely satisfied). The same level of satisfaction was also observed for intense treatment (97.3%). Notably, the number of vials received did not affect the patients' satisfaction; it was found that a higher number of vials did not result in a loss of satisfaction rate (4–5 vials: 98.4, ≥ 6 vials: 100%). Patients treated with cannula reported only a slight decrease in satisfaction rate (96.6%) compared to those who preferred needle usage in their procedure (98.6%). The type of anesthesia did not affect the patients' satisfaction rate, which remained high (topical anesthesia: 98.7%, injective anesthesia: 99%, both anesthesia types: 97.3%). Satisfaction rates were not influenced by treatment site. A slight decrease in satisfaction rates (95.8%) was observed in patients who were treated at the cheekbone compared to other anatomical sites, where the satisfaction rate was above 97%. Specifically, the rate of very satisfied patients decreased slightly when the treatment was administered on the cheekbone (66.2%), marionette (69%), and chin (63.9%). The full results on participant satisfaction rates are reported in (Table 5).

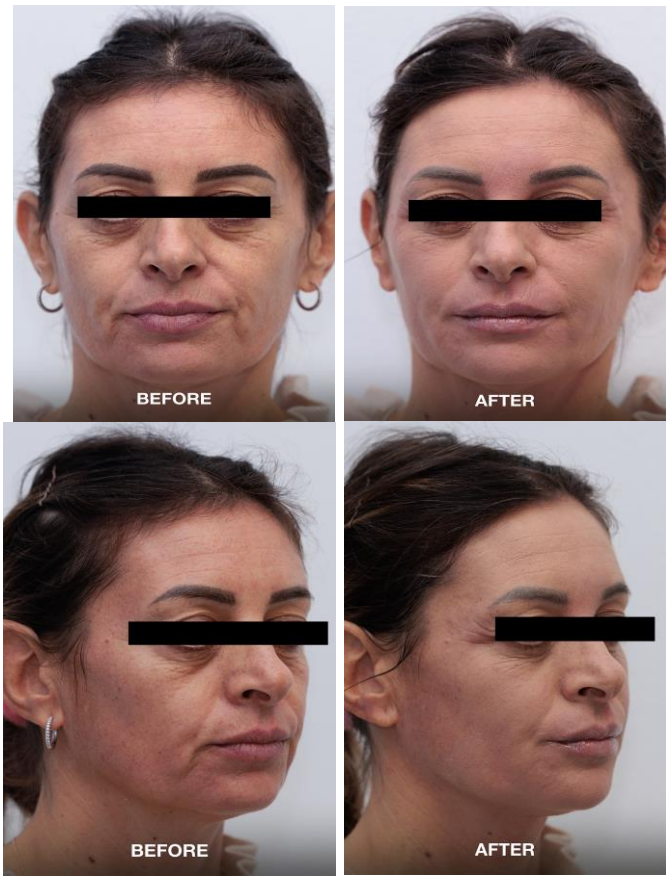
TABLE 5
Patient's satisfaction rates by treatment factors, n (%)

	Not satisfied	Poorly satisfied	Satisfied	Completely satisfied
Total Sample	2 (0.5)	4 (1.0)	83 (20.4)	318 (78.1)
Treatments				
Light	0 (0.0)	0 (0.0)	34 (26.6)	94 (73.4)
Medium	0 (0.0)	0 (0.0)	28 (19.4)	116 (80.6)
Intense	1 (0.5)	4 (2.2)	42 (23.0)	136 (74.3)
Lips	1 (0.8)	0 (0.0)	14 (11.2)	110 (88.0)
No. of Vials				
1 vial	1 (0.5)	2 (1.0)	30 (14.8)	170 (83.7)
2–3 vials	1 (1.2)	1 (1.2)	24 (28.2)	59 (69.4)
4–5 vials	0 (0.0)	1 (1.6)	21 (33.3)	41 (65.1)
≥ 6 vials	0 (0.0)	0 (0.0)	8 (14.3)	48 (85.7)
Technique				
Cannula	1 (1.1)	2 (2.2)	22 (24.7)	64 (71.9)
Needle	1 (0.5)	2 (1.0)	37 (17.6)	170 (81.0)
Cannula+Needle	0 (0.0)	0 (0.0)	24 (22.2)	84 (77.8)
Anesthesia	1 (0.5)	1 (0.5)	39 (17.6)	181 (81.5)
Topical **Anesthesia**	1 (0.6)	1 (0.6)	25 (15.7)	132 (83.0)
Injective **Anesthesia**	1 (1.0)	0 (0.0)	21 (21.0)	78 (78.0)
Both Anesthesia	1 (2.7)	0 (0.0)	7 (18.9)	29 (78.4)
Treatment location				
Full Face	0 (0.0)	0 (0.0)	1 (3.7)	26 (96.3)
Cheekbone	1 (1.4)	2 (2.8)	21 (29.6)	47 (66.2)
Nasogeniene	0 (0.0)	3 (1.9)	41 (25.8)	115 (72.3)
Marionette	0 (0.0)	3 (2.7)	32 (28.3)	78 (69.0)
Lips	1 (0.6)	0 (0.0)	25 (14.0)	153 (85.5)

Barcode	0 (0.0)	0 (0.0)	21 (23.6)	68 (76.4)
Chin	0 (0.0)	1 (2.8)	12 (33.3)	23 (63.9)
Jaw	0 (0.0)	0 (0.0)	6 (30.0)	14 (70.0)
Nose	0 (0.0)	0 (0.0)	2 (11.8)	15 (88.2)
Other	0 (0.0)	1 (2.1)	13 (27.7)	33 (70.2)

Effectiveness

Using GAIS, the investigators found that after 12 months, only one participant's condition had worsened from the baseline after treatment. No changes were noted in three patients. In the remaining 403 patients, the investigators noted improvement from baseline (26.3% improved and 72.7% very improved).



(A)

(B)

Figure 1) *Presentation of two cases - A) Treatment of the middle and III lower face with a needle and cannula: bilateral zygomatic region, nasolabial folds, lips, and jaw; B) Treatment of lower third with needle and cannula: Nasolabial folds, labial folds, and lips.*

WSRS scores after 12 months (1.2 ± 0.8) were nearly identical to those registered one month after the treatment (1.1 ± 0.9) (p=0.094). No statistically significant differences emerged both in WSRS and GAIS considering different formulations, administration modalities, or treatment locations (data not reported). Some cases are shown in (Figure 1).

Safety

Overall, about 70% of patients reported mild and transient side effects immediately after treatment, mainly resolved without pharmacological treatment. Specifically, 45.5% of the total sample reported only one side effect, while 5.4% and 4.7% of the total sample reported two and three side effects, respectively. No delayed side effects and AEs were reported.

Hematoma

Mild and transient hematomas were reported by 31.7% of the total sample (Table 6). When adverse events were analyzed by specific formulation, hematomas did not occur in more than 70% of patients treated with the medium, intense, or light formulations. As the number of vials increased, the percentage of patients with hematomas remained below 40%. More than 80% of patients treated with the cannula reported no hematomas. The percentage was similar for patients treated with the cannula alone (only 40% of patients reported hematomas) and for those treated using a combination of cannula and needle (less than 30% reported hematomas). For anesthetic use, more than 70% of patients reported no hematomas. When examining the specific type of anesthesia, less than 30% of patients treated with topical or injectable anesthesia reported hematomas.

TABLE 6
Side effects: Hematoma

	Frequencies	%

Total (n=407)	129	(31.7%)
Treatment		
Light (n=128)	53	(41.4%)
Medium (n=144)	43	(29.9%)
Intense (n=183)	47	(25.7%)
Lips (n=125)	36	(28.8%)
Number of vials		
1 vial (n=203)	80	(39.4%)
2–3 vials (n=85)	21	(24.7%)
4–5 vials (n=63)	12	(19.0%)
≥ 6 vials (n=63)	16	(25.4%)
Administration		
Cannula (n=89)	15	(16.9%)
Needle (n=210)	84	(40.0%)
Cannula+Needle (n=108)	30	(27.8%)
Anesthesia (n=222)	65	(29.3%)
Topical Anesthesia (n=179)	50	(27.9%)
Injective Anesthesia (n=100)	28	(28.0%)
Both Anesthesia (n=37)	13	(35.1%)

Pain

More than 20% of patients reported pain as the first effect in the post-treatment phase (Table 7). The duration of the symptoms did not exceed 2 days. Specifically, 32% and 36% of women reported pain after treatment with the light and lips formulations, respectively. However, in both cases, a significant proportion of patients (more than 64%) reported no pain. As the number of vials increased, the frequency of pain increased accordingly: more than 40% of patients treated with four to five vials, and 54% of patients treated with more than five vials reported pain. The use of a needle or cannula alone did not increase the frequency of pain. On the contrary, 43.5% of patients treated with a needle and cannula reported pain. More than 68% of patients treated with anesthesia reported no pain in the post-treatment period; only those who received topical and injectable anesthesia reported pain (59.5%).

TABLE 7
Side effect: Pain

	Frequency	%
Total (n=407)	82	(20.1%)
Treatment		
Light (n=128)	41	(32.0%)
Medium (n=144)	36	(25.0%)
Intense (n=183)	49	(26.8%)
Lips (n=125)	45	(36.0%)
Number of vials		
1 vial (n=203)	4	(2.0%)
2–3 vials (n=85)	18	(21.2%)
4–5 vials (n=63)	26	(41.3%)
≥ 6 vials (n=63)	34	(54.0%)
Administration		
Cannula (n=89)	18	(20.2%)
Needle (n=210)	17	(8.1%)
Cannula+Needle (n=108)	47	(43.5%)
Anesthesia (n=222)	**69**	**(31.1%)**
Topical Anesthesia (n=179)	58	(32.4%)
Injective Anesthesia (n=100)	33	(33.0%)
Both Anesthesia (n=37)	22	(59.5%)

Redness

About 11% of the patients reported redness after the treatments, which subsided within a few days (Table 8). More than 80% of patients treated with the light formulation showed no signs of redness after treatment; similar positive percentages were also observed with the medium (84%), intense (84.7%), or lip (86.4%) formulations. As the number of vials increased, the percentage of patients showing redness increased accordingly (2–3 vials: 15.3%, 4–5 vials: 23.8%, ≥ 6 vials: 25.4%). A quarter of patients treated with a cannula and needle showed redness in the post-treatment phase. In addition, simultaneous use of topical and injectable anesthesia was also found to be a potential risk factor for redness. In fact, almost 30% of patients reported redness in the post-treatment phase.

TABLE 8
Side effect: Redness

	Redness	%
Total (n=407)	47	(11.5%)
Treatment		
Light (n=128)	25	(19.5%)
Medium (n=144)	23	(16.0%)
Intense (n=183)	28	(15.3%)
Lips (n=125)	17	(13.6%)
Number of vials		
1 vial (n=203)	3	(1.5%)
2–3 vials (n=85)	13	(15.3%)
4–5 vials (n=63)	15	(23.8%)
≥ 6 vials (n=63)	16	(25.4%)
Administration		
Cannula (n=89)	12	(13.5%)
Needle (n=210)	8	(3.8%)
Cannula+Needle (n=108)	27	(25.0%)
Anesthesia (n=222)	36	(16.2%)
Topical Anesthesia (n=179)	28	(15.6%)
Injective Anesthesia (n=100)	19	(19.0%)
Both Anesthesia (n=37)	11	(29.7%)

Swelling and nodules

Only 4.2% of patients reported swelling, and less than 3% had nodules. The occurrence of swelling and nodules was not dependent

on the type of treatment, number of vials, route of administration, or anesthesia (Table 9).

TABLE 9
Side effects: Swelling and nodules

	Swelling		Nodules	
Total (n=407)	17	(4.2%)	11	(2.7%)
Treatment				
Light (n=128)	4	(3.1%)	10	(7.8%)
Medium (n=144)	5	(3.5%)	4	(2.8%)
Intense (n=183)	9	(4.9%)	9	(4.9%)
Lips (n=125)	7	(5.6%)	5	(4.0%)
Number of vials				
1 vial (n=203)	9	(4.4%)	0	(0.0%)
2–3 vials (n=85)	2	(2.4%)	2	(2.4%)
4–5 vials (n=63)	3	(4.8%)	3	(4.8%)
≥ 6 vials (n=63)	3	(4.8%)	6	(9.5%)
Administration				
Cannula (n=89)	1	(1.1%)	0	(0.0%)
Needle (n=210)	11	(5.2%)	3	(1.4%)
Cannula+Needle (n=108)	5	(4.6%)	8	(7.4%)
Anesthesia (n=222)	14	(6.3%)	11	(5.0%)
Topical Anesthesia (n=179)	14	(7.8%)	11	(6.1%)
Injective Anesthesia (n=100)	3	(3.0%)	8	(8.0%)
Both Anesthesia (n=37)	3	(8.1%)	8	(21.6%)

DISCUSSION

Aesthetic treatments with HA to enhance facial volume and to compensate for signs of ageing have become the standard in aesthetic treatments [34]. Treatments of the entire face, specific areas of the face, or other areas of the body, such as the hands, are minimally invasive procedures, allowing for rapid restoration of volume with minimal recovery time and a favorable safety profile. In 2020, according to the American Society of Plastic Surgeon, more than 3.4 million non-invasive dermal fillers were performed. Presently, HA fillers are considered the preferred choice, thanks to their natural-looking results, good tolerability, and relatively long-lasting effect without requiring skin-allergy tests.

An ideal HA filler should have the following characteristics: easy to inject, non-allergenic, non-carcinogenic, have long-term efficacy, integrate well into tissues, be cohesive, and exhibit excellent bio-compatibility. It should also have no early or delayed side effects, no migration, and minimal inflammatory response. Additionally, it should also allow a very short recovery time and degrade slowly [18, 35]. In real world scenarios, studies on HA dermal fillers' effectiveness have short follow-up periods and are conducted on small sample sizes. Over the long term, relevant volume losses have been reported along with delayed side effects [21,15].

To this end, the aim of this study was to evaluate the patient's perceived satisfaction, effectiveness, and safety profile of a new 2.5% (mg/ml) HA–based filler, developed with a conservative cross-linking technology and purified by a dialysis procedure through water for injection (BDDE<0.1 ppm). Overall, the tested HA filler is well-tolerated and effective for up to 12 months.

The analysis of the primary outcomes showed that more than 98% of patients were satisfied with the treatment after 12 months from the last received treatment, even when the treatment was given with the light formulation. Patients' satisfaction has been considered a relevant parameter that is closely associated with feelings of attractiveness, comfort and self-confidence; thus, providing an overall increase in the quality of life and self-esteem, along with an improvement in psychological outcomes [36].

The efficacy of the product emerged from the assessment of the investigators, reporting a very high satisfaction rate. The combination of different formulations, along with the great malleability and the potential to adjust product amount according to the patients' needs, increased HA's effectiveness and allowed the operators to properly use all the rheological properties of the product to treat facial wrinkles.

The tolerability profile of the investigated filler also showed a very high level. Although nearly 70% of patients reported some kind of side effects immediately after treatment, including local reactions at the injection site, mainly hematoma, redness, and pain, the duration and severity of these effects remained limited and mild as reported in a previous study [16]. No one experienced any AEs. No delayed reactions occurred over the long period (>12 months). This is in line with the current literature which has proved that AEs in patients who were treated with HA fillers include immediate reactions such as oedema and erythema, paraesthesia, pain, bruising, and hematoma (29), primarily due to the mechanical trauma associated with the procedures [37]. Hematoma and pain were the most common side effects reported in this study, involving 37.1% and 20.1% of patients, respectively. The results are encouraging, because the incidence was lower than that of the 50%–100% of local reactions at the injection site commonly reported in other similar studies [38,39]. Combining these side-effect rates with the number of treatments, no relevant increase in side effects occurred.

Indeed, in the detailed analysis of side-effect occurrence, depending on the treatment type or vial count, we observed a non-relevant change in the hematoma rate with an increase in treatment intensity or vial count, whether using a needle or cannula. In contrast, we observed a higher prevalence of pain when the number of vials increased, and when a combination of cannula and needle (43.5%) was used during the same session. Pain is commonly reported by patients undergoing cosmetic procedures with dermal fillers, and it can increase using a needle [40,41]. With this in mind, we hypothesize that pain may have increased with repeated treatments and with combined administration of cannula and needle due to a developed hypersensitivity in patients [31]. Nevertheless, the pain disappeared after a few days and, in many cases, without the need for pharmacological treatment. Similarly, the combination of different anesthetics resulted in a higher incidence of pain; this could be the result of the patient's greater sensitivity, as proven by the need to use a higher dose of anesthesia in comparison to when the treatment started. Indeed, although the incidence of complications was

dependent on the injection site, as a "sensitive" area may have a higher risk of side effects, we did not observe a change in the rates of side effects depending on the injection site [37]. The observed pain was always the local response of the treated area to the treatment itself, and not the symptoms of more serious complications, such as vascular occlusion [31]. Given its temporary onset, it was no perceived as a negative element to reduce their satisfaction.

The study has some limitations: First, patients were selected across a wide age range and were exclusively female. Second, the study design may have introduced some bias, such as selection bias or instructional bias. Third, the retrospective approach prevented us to have objective measurements of improvements, requiring more stringent study designs like Randomized Controlled Trials (RCTs) for future research. Therefore, future studies could incorporate different and more robust study designs, such as RCTs or a prospective approach. They should also include male populations deepen the analysis with objective measurements.

The study presents several strengths. Firstly, it replicates a real-world clinical scenario, providing a valid source of data to assess patients' care and outcomes in routine clinical practice. Secondly, the follow-up extended beyond the 12-month mark (up to 22 months), an important element in the evaluation of the effectiveness of HA-based filler, considering its rapid absorption. Thirdly, the study was conducted on a very large sample size, unusual in dermal filler evaluations. Fourthly, reported side effects, despite being temporary and non-severe, did not impact patient satisfaction. Finally, the results of the study may assist aesthetic clinicians in planning long-term treatments to achieve optimal and predictable treatment outcomes.

CONCLUSIONS

In summary, this study demonstrates that patients perceive Stylema® filler as highly effective and safe for correcting various facial defects, even when applied to sensitive areas like the lips, and even when a substantial number of treatments or vials are required to meet the patients' aesthetic expectations. The product's remarkable malleability and the possibility to tailor the dosage individual patients needs enhance the efficacy of HA without raising the risk of side effects.

Supplementary materials

Supplementary Materials S1: The manufacturing process of the Stylema® filler.

Author contributions

Conceptualization, I.Z., A.F. and M.L.G.; methodology, I.Z., A.F. and M.L.G.; software, I.Z., A.F. and M.L.G.; validation, I.Z., A.F. and M.L.G.; formal analysis, M.L.G..; investigation, I.Z. and A.F.; resources, I.Z., A.F. and M.L.G.; data curation, I.Z., A.F. and M.L.G.; writing—original draft preparation, I.Z. and M.L.G.; writing—review and editing, A.F.; visualization, I.Z., A.F. and M.L.G.; supervision, M.L.G..; project administration, M.L.G..; funding acquisition, I.Z and A.F. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of interest

Authors are scientific consultants for Uniderm Farmaceutici srl and receives compensation.

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