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**Carbon dioxide therapy and hyaluronic acid for cosmetic correction of the  
nasolabial folds**

Giuseppe Nisi M.D, Roberto Cuomo M.D., Cesare Brandi M.D., Luca Grimaldi M.D., Andrea Sisti  
M.D., Carlo D'Aniello M.D.,  
University of Siena, General and Specialistic Surgery Department, Plastic Surgery Division, "S.  
Maria alle Scotte" Polyclinic – Viale M. Bracci 2, 53100 Siena – Italy.

**Corrisponding author:**

Roberto Cuomo, MD

c/o Plastic Surgery Unit, "S. Maria alle Scotte" Polyclinic – Viale Bracci, 53100 – Siena, Italy

email: robertocuomo@outlook.com

Phone: 0039 333 2301668

## **Abstract**

**Background:** The main application of hyaluronic acid filling, in aesthetic medicine, is the augmentation of soft tissues. The carbon dioxide therapy, instead, improves quality and elasticity of the dermis and increases the oxygen release to the tissue through an enhancing of the Bohr's effect. The aim of the study is to compare the efficacy, tolerability and effect duration of hyaluronic acid fillers and the use of carbon dioxide therapy plus hyaluronic acid in the cosmetic correction of nasolabial folds.

**Materials and Methods:** Forty healthy female patients received a blinded and randomized treatment on nasolabial folds (hyaluronic acid in group A and hyaluronic acid plus subcutaneous injections of carbon dioxide in group B) for cosmetic correction of the nasolabial folds. The results were evaluated by two blinded plastic surgeons after the implant (1 week, 4 and 6 months) using a 1 to 5 graduated scale (GAIS) and, at the same times, to each patient was asked to express her opinion about the cosmetic result.

**Results:** Any long-term adverse reaction was reported. The blinded evaluation at 4 and 6 months from the implant shows in all patients a maintenance of a good cosmetic result higher for the side treated with carbon dioxide therapy plus hyaluronic acid.

**Conclusions:** At the control visit six months after the treatment, the patients treated with hyaluronic acid plus carbon dioxide therapy maintains a satisfactory aesthetic result while the nasolabial fold treated only with hyaluronic acid shows, in almost all patients, a come back to pre-treatment appearance.

**Keywords:** fillers, hyaluronic acid, carbon dioxide therapy, nasolabial folds.

**Level of evidence: II**

## **Introduction**

Hyaluronic acid (HA) is a polysaccharide, identical across all species and tissues, normally present in the body(1). The most striking features of hyaluronic acid are its remarkable physico-chemical characteristics, especially viscoelasticity, ability to bind and retain water and biological functions. For these reasons it plays an important role in tissue hydration, lubrication and cellular function and it is used in many medical fields(2). The main application of cross-linked hyaluronic acid-derived gels in aesthetic medicine is the augmentation of soft tissues especially for the cosmetic treatment of the aging face (nasolabial folds; glabellar and frontal wrinkles) and to increase and reshape lips. Its success is due to a very low incidence of adverse reactions compared with other soft tissue augmentation products but its point of weakness is represented by its relatively rapid enzymatic degradation with a subsequent need of periodic reimplants.

In order to increase the resistance of the natural hyaluronic acid to the enzymatic degradation and consequently to maintain the aesthetic result achieved with the intradermal implant for a longer time, the hyaluronic acid is submitted to a chemical process known as cross-linking, creating a viscoelastic polymer with an increase of its resistance to hyaluronidases.

Paliwal S. et al.(3) recently demonstrated, in a rodent model, that cross-linked HA-based filler, in addition to its well-understood space-filling function, stimulates the production of several extracellular matrix components, including dermal collagen and elastin, as a secondary effect.

Subcutaneous injections of carbon dioxide (CD) can promote the tissue oxygenation and vascularization through the Bohr effect and they can increase the dermis elasticity. Experiments on

rat skin showed that subcutaneous injection of CO<sub>2</sub> decreased the amount of Substance P and pro-Calcitonin Gene-Related Peptide (15 kDa) neuropeptides in rat skin, with no effects on the healing of acute skin wounds(4,5).

The aim of the study is to compare the, tolerability, cosmetic effect and correlations of hyaluronic filler and carbon dioxide injections in the cosmetic correction of nasolabial folds.

## **Materials and Methods**

Forty healthy female patients, mean age 45 years (range 38-52 years), were enrolled for this study. Inclusion criteria were represented by patients with moderate to severe nasolabial folds (grade 3 and 4) assessed using a 1 to 5 graduated scale (WSRS - Wrinkle Severity Rating Scale - table 1)(6).

Exclusion criteria were represented by patients with a history of sensitivity to hyaluronic acid, inflammation, infection, acute or chronic skin disease in the area of the injection. Were also excluded patients with absent, mild or extreme nasolabial folds (grade 1, 2 and 5 of the WSRS), and patients submitted to other cosmetic (further augmentation injections, laser resurfacing, chemical peelings, botulinum toxin type A injections) and surgical (face-lift procedures, lipofilling, zygomatic implants) procedures during 6 months before the implant. To be enrolled in the study was asked to all patients to sign an informed consent about the modalities of the study and to abstain from other cosmetic and surgical procedures for the 6 months following the implant.

For this study we used an hydrogel filler composed of cross-linked hyaluronic acid. This filler is presented as a sterile 1.0 ml volume in a single-use 1.0 ml glass syringe. Cross-linking should ensure a higher resistance to enzymatic degradation with a longer lasting maintenance of the cosmetic correction.

The patients received a randomized assignment in two group: Group A and Group B (20 patients each group). All patients received an injection of cross-linked hyaluronic gel for cosmetic correction of the nasolabial folds but the patients of Group B received in addition ten subcutaneous injections of carbon dioxide, every three day for one month before the filler and after seven days the filler was injected. All the implants were performed by the same surgeon with the same intra-dermal linear threading technique.

Neither the surgeon nor the team of the measurements were aware of which patient received the injections of CD.

Before the treatment, a bilateral infraorbital regional nerve block was performed in all patients, using 1 ml of 2% Lidocaine by intraoral injection to minimize the injection discomfort. In all cases, the amount of material necessary to achieve a complete correction of the wrinkle without any overcorrection was injected. The results were evaluated by two blinded plastic surgeons one week after the implant and at the follow-up visit at 4 and 6 months after the injections using a 1 to 5 graduated scale (GAIS - Global Aesthetic Improvement Scale - table 2)(7). At the same times to each patient was asked to express her opinion on satisfaction about the cosmetic result using a 1 to 5 graduated scale named (PAIS - Patient's Aesthetic Improvement Scale - table 3), specifically designed for the study. High resolution digital photos were taken before the treatment, immediately after and at each follow-up visit.

## Results

Any long-term adverse reaction, such as granulomas, inflammations, allergic and infectious reactions was reported in all 40 patients. Transient minimal injection related reactions as edema or erythema were reported in all cases but they were limited to the site of injection. The blinded physicians' evaluation reported, at one week after the implant, a GAIS score of 5 in all patients for both areas (table 4); at four months the average value was 3,9 for the area in the Group B (18 patients with score of 4 and 2 patients with a score of 3), while for Group A 2,85 (17 patients with a score of 3 and 3 patients with a score of 2); at six months the average value was 3,2 for the area pretreated with carbon dioxide (16 patients with a score of 3 and 4 patients with a score of 4), while for the area treated without CO<sub>2</sub> was 2,1 (18 patients with a score of 2 and 2 patients with a score of 3) (Fig. 1). An indirect confirmation of what reported from the physician evaluation arrived from the results of PAIS (table 5); in particular one week after the implant the score was 5 in all patients in both groups.

At four months the average score was 3,8 for the group pretreated with carbon dioxide (16 patients with a score of 4 and 4 patients with a score of 3), while for the Group A was 2,6 (12 patients with a score of 3 and 8 patients with a score of 2). At six months the score decreased to an average value of 3,05 for the Group B (19 patients with a score of 3 and 1 patients with a score of 4) and 2,05 (19 patients with a score of 2 and 1 patients with a score of 3) for the Group A.

## **Discussion**

HA-based dermal fillers have gained rapid acceptance for treating facial wrinkles and deep tissue folds. Filling with HA showed positive effects in decreasing the clinical signs of skin aging and in improving the face volumes(8).

A recent meta-analysis from Huang X. et al.(9), proved both safety and efficacy for HA fillers for the correction of nasolabial folds.

Monophasic fillers demonstrated a significantly better efficacy than biphasic fillers over the 6 month follow-up period, while biphasic fillers showed higher tolerance than monophasic fillers.

HA-based soft tissue fillers are biocompatible, nontoxic and non-allergenic, the risk of complications is mainly related to the immunological status of the patient(10,11).

Subcutaneous carbon dioxide injections are often used in aesthetic medicine. CO2 therapy improves local parameters of circulation, reduces localized adiposities(12,13) and can be used for the treatment of chronic wounds(14).

Paolo F. et al.(15), in 2012, reported a reduction of facial fine lines and wrinkles as well as a decrease in periorbital hyperpigmentation, using CD therapy. A few side effects were observed but they were all transient and did not require discontinuation of treatment.

From a Pubmed-based literature search, only Chin S.H. et al.(16), in 2013, assessed selective dermal rejuvenation using sequential intradermal injections of CD and HA as a treatment of facial wrinkles. This study included 36 cases of facial wrinkles in 34 patients. The follow-up period was 3 to 11 months. Temporary adverse effects were injection-site pain, mild edema, and redness. Most cases showed obvious improvement in skin thickness, elasticity, and smoothening. Complications included irregularities and hyperpigmentation in 3 cases, and 91% were highly satisfied with the anti-wrinkle treatment.

In our prospective study, filling agents showed an immediate optimal cosmetic result that reduced in time. At 4 months, HA showed a higher cosmetic efficacy and patient satisfaction than patients in

group B. A good cosmetic result was reported with both treatments for the first 4 months; at the control visit (six months of follow-up) only the patients treated with CD therapy plus HA maintained a satisfactory aesthetic result while the nasolabial fold treated only with HA showed a come back to pre-treatment appearance.

## **Conclusion**

Carbon dioxide therapy plus hyaluronic acid filler is a minimal invasive modality for the rejuvenation of the nasolabial folds.

This method is a safe, economical, and clinically effective anti-wrinkle treatment. We would recommend this sequential therapy to get long-lasting results.

## **Acknowledgement**

All authors declare that no conflict of interest is present.

**Table 1** Wrinkle Severity Rating Scale (WSRS). Grade Description\*

Grade	Description
1	Absent: no visible fold; continuous skin line
2	Mild: shallow but visible fold with a slight indentation; minor facial feature. Implant is expected to produce a slight improvement in appearance.
3	Moderate: moderately deep folds; clear facial feature visible at normal appearance but not when stretched. excellent correction is expected from injectable implant.
4	Severe: very long and deep folds; prominent facial feature. Less than 2 mm visible fold when stretched; significant improvement is expected from injectable implant.
5	Extreme: extremely deep and long folds, detrimental to facial appearance; 2–4 mm visible V-shaped fold when stretched; unlikely to have satisfactory correction with injectable implant alone.

\*Adapted from Day JD et al.(6)

**Table 2** Global Aesthetic Improvement Scale (GAIS). Grade Description\*

Grade	Description
1	worse
2	No change
3	somewhat improved
4	moderately improved
5	very much improved

\*Adapted from DeLorenzi C et al.(7)

**Table 3** Patient's Aesthetic Improvement Scale (PAIS). Grade Description

Grade	Description
1	worse than before treatment
2	no change
3	minimal improvement
4	good improvement
5	optimal improvement

**Table 4** Percentage distribution of patients based on GAIS Score. HA= hyaluronic acid, CD= carbon dioxide.

	1 week		4 months		6 months	
	Percentage (No. of patients)		Percentage (No. of patients)		Percentage (No. of patients)	
	Group A (HA)	Group B (HA + CD)	Group A (HA)	Group B (HA + CD)	Group A (HA)	Group B (HA + CD)
Worse (Grade 1)						
No change (Grade 2)				15% (3/20)		90% (18/20)
Somewhat improved (Grade 3)			10% (2/20)	85% (17/20)	80% (16/20)	10% (2/20)
Moderately improved (Grade 4)			90% (18/20)		20% (4/20)	
Very much improved (Grade 5)	100% (20/20)	100% (20/20)				

**Table 5** Percentage distribution of patients based on PAIS Score. HA= hyaluronic acid, CD= carbon dioxide.

	1 week		4 months		6 months	
	Percentage (No. of patients)		Percentage (No. of patients)		Percentage (No. of patients)	
	Group A (HA)	Group B (HA + CD)	Group A (HA)	Group B (HA + CD)	Group A (HA)	Group B (HA + CD)
Worse than before treatment (Grade 1)						
No change (Grade 2)				40% (8/20)		95% (19/20)
Minimal improvement (Grade 3)			20% (4/20)	60% (12/20)	95% (19/20)	5% (1/20)
Good improvement (Grade 4)			80% (16/20)		5% (1/20)	
Optimal improvement (Grade 5)	100% (20/20)	100% (20/20)				

Figures

**Fig. 1.** LIPS: Patient not treated with CD. **a and b:** before the treatment with HA; **c and d:** same patient, 6 months after the treatment with HA;

HA= hyaluronic acid, CD= carbon dioxide

**Fig 2. LIPS:** Patient treated with HA and CD **a:** at 1 week after treatment **b** after 6 months

**Fig 3 and Fig 4** Nasolabial folds: Patient treated with HA and CD **a:** at 1 week after treatment **b** after 6 months

**Fig 5.** Nasolabial folds: Patient treated only with HA **a:** at 1 week after treatment **b** after 6 months

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