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Contents

Editorial

Can the pandemic by COVID-19 (SARS-CoV-2 infection) increase the number of adverse effects after the use of dermal fillers?

On January 7th, 2020, the appearance of a new coronavirus (CoV) was officially reported in Wuhan (China). At the time, no one could have suspected the chain of events that, with unusual speed, would lead to the greatest pandemic affecting the world population today.

Medical literature on COVID-19 is currently overwhelming, with new findings being published every day. However, we are far from knowing all the intricate details about its mechanism of action, its physiopathology, the response it elicits on different subjects or even its symptoms. Questions are accumulating and we still have a lot of work ahead of us to learn about and fight this virus.

We do agree that it spreads easily, that it is not just a lung disease, and that it causes significant changes in the immune system.

We also know that there are many asymptomatic carriers and people who have suffered mild, and even moderate, forms of COVID-19, whose diagnosis could not be confirmed by the different tests available.

In Spain, as well as in other countries, the severity of the pandemic called for a mandatory confinement of the general population and the declaration of a state of emergency, which entailed the closure of Aesthetic Medicine clinics from mid-March until mid-May.

In general, those measures intended to ensure the safety of the medical body and patients have been recorded in a protocol.

However, we are solely responsible for the reevaluation of our actions with regard to potential risks that some of our therapies may entail.

In recent days, we have witnessed the increasingly number of warnings issued by several sources about possible complications that we will have to deal with in our daily practice. Despite that an analysis of said sources exceeds the aim of this brief text, a few are mentioned below:

- The *Joint Council of Cosmetic Practitioners* (JCCP) from the United Kingdom has proposed a recommendation guideline that states the following: "There is increasing evidence that dermal fillers given in the presence of any viral infection can increase the risk of delayed hypersensitivity reactions."
- The publication of a review in *J Cosmet Dermatol* (2020; 00:1-4): Aesthetic Dermatology Procedures in Coronavirus Days highlights the following: i) permanent filler materials and some resorbable ones may cause chronic inflammation; ii) in comparison, reactions are minor (in principle) when the material used is hyaluronic acid; iii) there are also late hypersensitivity reactions to hyaluronic acid; iv) viruses may activate cytokines and T cells, and promote a proinflammatory state, therefore they recommend: v) to return to antibiotic empirical treatment (macrolides or tetracyclines); vi) use needles with less caliber; and vii) avoid high-risk areas.

In order for this to be reflected in patients' medical history, their documentation must be duly adjusted and this new information must be included in the informed consent provided to the patient. The professional, for his/her part, must consider this possibility in terms of the care that patients may require after the performed procedure.

Reflections by Paloma Tejero, MD, PhD

In my personal experience, because of my doctoral thesis on adverse effects of filler materials in 2013 and because I was part of the SEME committee of adverse effects, my colleagues usually referred patients to me or consult with me on different issues regarding filling materials. From May to July 2020, I have received several reports of exacerbated inflammatory responses after implant placement. Some have called my attention, particularly: i) non-permanent fillers in the perioral area; and ii) two patients with granulomatous abscess of permanent fillers that had been inactive for 14 and 7 years, respectively. All patients had negative serology results, although they reported having been near patients with COVID. However, there are also studies that support the disappearance of antibodies after two or three months of exposure.

In the nearby future, we will be able to weigh on the usefulness of these events considering several factors, which will prevent biased observations: i) accept that AE reporting has been very low (or non-existent) during the months of confinement, and measure it; ii) assess and compare data against reported AEs between March and July of 2019; and iii) assess the frequency with which permanent fillers have AEs within five years of their implantation.

In a word: we currently have more questions than conclusions, but I believe it is important to be alert, try to minimize risks and conduct prospective studies that allow us to learn about the possible interaction between COVID-19 and our practice.

Reflections by Hernán Pinto, MD, PhD

As it happens with any other topic that becomes fashionable, words fly. However, this is a fashion that has been imposed by the circumstances we are living in. And each one of us must do whatever we can to improve our own lives and safety, as well as those of our families, friends, professional colleagues and patients.

Every day we learn more about this virus and COVID-19. But, as usual, a serious search for knowledge gets us answers that, in turn, raise more questions. And, unfortunately, the relationship is not linear: for each answer we get, several new questions emerge. That is, each day we know more than the day before but, at the same time, what we have left to learn also increases. The more we study, the more we have left to study. It is normal.

The protocolization of Aesthetic Medicine practices surrounding all the implications that this virus may have is now a necessity. However, the evidence we have is dissimilar and contradictory, both in terms of quality and conclusions. In a word, we don't know what it is going on. The creation of evidence from and for our collective has become fundamental because dermal fillers represent a high percentage of aesthetic-medical practice. That is why the Spanish Society of Aesthetic Medicine (SEME) will create a commission to study the relationship between Aesthetic Medicine

and the coronavirus (COVID-19), which will allow us to combine our efforts and, among other things, sponsor scientific evidence-based studies nationwide in order to ensure patients' safety.

Hernán Pinto
Main Handling Editor

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Newspaper article - in print* *if the city name is not part of the newspaper name, it may be added to the official name for clarity * if an article jumps from one page to a later page write the page numbers like D1, D5	Wolf W. State's mail-order drug plan launched. *Minneapolis Star Tribune.* May 14, 2004:1B.
Newspaper article - online	Pollack A. FDA approves new cystic fibrosis drug. *New York Times.* January 31, 2012. http://www.nytimes.com/2012/02/01/business/fda-approves-cystic-fibrosis-drug.html?ref=health Accessed February 1, 2012.
Websites	Outbreak notice: Cholera in Haiti. Centers for Disease Control and Prevention Web site. https://www.cdc.gov Published October 22, 2010. Updated January 9, 2012. Accessed February 1, 2012.
Entire book - in print	Modlin J, Jenkins P. *Decision Analysis in Planning for a Polio Outbreak in the United States.* San Francisco, CA: Pediatric Academic Societies; 2004.
Book chapter - in print	Solensky R. Drug allergy: desensitization and treatment of reactions to antibiotics and aspirin. In: Lockey P, ed. *Allergens and Allergen Immunotherapy.* 3rd ed. New York, NY: Marcel Dekker; 2004:585-606.

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Example Article

1. Zoellner J, Krzeski E, Harden S, Cook E, Allen K, Estabrooks PA. Qualitative application of the theory of planned behavior to understand beverage consumption behaviors among adults. J Acad Nutr Diet. 2012;112(11):1774-1784. doi: 10.1016/j.jand.2012.06.368.

In-Text Citation Example

LARGE INCREASES IN AMERICANS' CONSUMPTION OF sugar-sweetened beverages (SSB) have been a topic of concern. Between 1977 and 2002, the intake of "caloric" beverages doubled in the United States, with most recent data showing that children and adults in the United States consume about 172 and 175 kcal daily, respectively, from SSB,[1] it is estimated that SSB account for about 10% of total energy intake in adults[2,3]. High intake of SSB has....

References Section Example

References

1. Duffey KJ. Popkin BM. Shifts in patterns and consumptions of beverages between 1965 and 2002. *Obesity.* 2007:15(11):2739-2747.
2. Nielsen SJ. Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med.* 2004;27(3):205-210.
3. Drewnowski A. Bellisle F. Liquid calories, sugar, and body weight. *Am J Clin Nutr.* 2007;85(3):651-661.

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aesthetic medicine

Original Article

A prospective pilot study to evaluate the use of hyaluronidase in patients with hymenoptera venom allergy

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Short Title: Hyaluronidase and wasp allergy

Abstract

Background: Hyaluronic acid-based filler treatments for aesthetic purposes are widespread and constantly increasing in Italy and worldwide. In some specific complications of this filler, its removal by an enzyme, hyaluronidase, which is also one of the components of hymenoptera venom, is indicated. Following the hymenoptera puncture, venom specific IgE antibodies develop, which are the indicators of sensitization, the cause of a possible subsequent fatal anaphylactic reaction, following a new puncture.
Aim: the aim of this study is to verify whether hyaluronidase extracted from bovine, used to dissolve hyaluronic acid in case of complications, can cause allergic cross reactions in patients with hymenoptera venom allergy.
Methods: Skin tests with hyaluronidase, bee, vespid and hornet poisons were performed, before starting treatment in twenty patients with hymenoptera venom allergy requiring desensitizing therapy (Group A), and in five healthy volunteers (Group B). In Group A patients specific IgE to extracts of the whole venom of Apis mellifera, Vespula spp., Polistes spp. and Vespa crabro were detected, as well as to the molecular components of the same venoms.
Results: in all patients of both groups, the skin tests for hymenoptera venom and hyaluronidase gave a negative result, while in Group A patients a positivity to hymenoptera venom was detected and also confirmed with an increase in the specific IgE.
Conclusions: the hyaluronidase extracted from bovine utilized for the study did not cause cross reactivity in patients with hymenoptera venom allergy and can be used in complications due to hyaluronic acid-based fillers.

Keywords

Hyaluronidase, dermal filler, dermal filler complication, hymenoptera venom, wasp allergy

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Introduction

Hyaluronic acid (HA) is one of the main components of connective tissue, whose concentration in the body tends to decrease with advancing age and its lack leads to a weakening of the skin, favoring the formation of wrinkles and imperfections[1,2].
HA is synthesized by cell membrane enzymes called hyaluronic acid synthase (HAS) present in vertebrates, in some bacteria and in viruses. In vertebrates, there are three types: HAS1 and HAS2 that polymerize longer chains than HA (≥ 300 kDa); HAS3 that synthesizes shorter chains (<300 kDa)[3]. High molecular weight HA acts at the surface, ensuring effective hydration and binds to the stratum corneum components to form a film with a tensor and protective effect; the low molecular weight HA acts deeper down to repair tissue[4].
Due to its viscosity and mechanical properties, hyaluronic acid is used in skin fillers for aesthetic purposes, filling skin depressions and wrinkles by expanding the extracellular matrix.
Commonly available HA fillers differ in stiffness or G' (the stiffer products are applied deeper down and have a volumizing action); in cohesiveness, in the ability to bind water; in cross linking, a property that causes the crosslinking of HA with different compounds and increases its persistence in the tissues by decreasing the response to hyaluronidase, the enzyme responsible for HA degradation[5].
Complications from HA fillers occur at a rate of 0.5% (1 patient in 200) and include vascular compromise which despite being low in incidence, is very seriously feared as it causes tissue necrosis, edemas, granulomas, infections and nodules[6-9]. For the resolution of most complications, the recommendation to use hyaluronidase, an enzyme also present in the components of hymenoptera venom, carries the theoretical risk of triggering an allergic reaction in the patient when administered by injection[10,11].
The allergy to hymenoptera venom is responsible for approximately 20% of total cases of fatal anaphylaxis in different countries, with an estimate varying between 56-94% of the European adult population stung by a hymenoptera at least once during their lifetime, in one third of the cases by bee.
Following exposure to hymenoptera venom, the development of specific IgE towards one or more allergenic fractions of the venom, favored by atopic diathesis and genetic factors, may occur and correlates with a high level of total and specific IgE[12].
There is no reliable data in literature about treatment with dermal filler in patients with a history of hymenoptera venom allergy[13].
The aim of this prospective pilot study is to evaluate the indication for the use of bovine hyaluronidase in patients with complications from hyaluronic acid dermal fillers and with a confirmed allergy to hymenoptera venom, tested by measuring venom specific IgE.

Material and methods

The study was carried out at the Dermopathic Institute of the Immaculate (IDI) in Rome, during the period spanning June to December 2019; it was authorized by the Ethics Committee of the Hospital with number Aedes.IDI.2017, registration number n°.494/1.
Twenty patients with a history of hymenoptera venom allergy, requiring desensitizing therapy (Group A) according to a known protocol[14], were enrolled; 5 subjects not allergic to hymenoptera venom were included in the study as a control group (Group B).
No patients included in the study did not present comorbidities and did not take drugs.
All recruited patients received and signed an informed consent form.
All patients (Groups A and B) underwent prick and intradermal tests with the purified allergenic extracts of Apis mellifera, Vespula , Polistes and Vespa cabro (Anallergo, via N. Jotti 7, Scarperia and San Piero, FI, Italy; Allergy Therapeutics, via IV settembre 26, Settimo Milanese, Italy) and with hyaluronidase.
For the test, hyaluronidase produced by Bioindustria L.I.M. spa, Novi Ligure, Alessandria, Italy) was used. It consisted of 300 IU of lyophilized animal-derived hyaluronidase (bovine testes), with excipients 10 mg of lactose, reconstituted with 3 ml of 0.9% sodium chloride solution.
This solution was diluted in a ratio 1:2 with physiological solution to obtain 150 IU of hyaluronidase per dose. This final solution was used to perform the prick test on the flexor surface of the forearm by applying a drop of the solution on the skin, disinfected with benzalkonium chloride and ethanol.
A rapid reading intradermal test (ID) was also performed on both groups, with hyaluronidase solution used for the prick test, diluted 1:10 with physiological solution, to obtain a concentration of 0.1 mg/ml of substance, injecting 0.02 -0.05 ml of the solution until obtaining a 3 mm diameter. The test result is positive if after about 20 minutes a weal> 3 mm + erythema is elicited[17].
For each patient (Group A) after the skin tests, the serum immunoglobulin E (IgE), specific for the whole venom extracts of Apis mellifera, Vespula spp., Polistes spp. and Vespa cabro and for the molecular components of the same poisons of Apis m. (Api m1, Api m2, Api m3, Api m10), Vespula spp. (Ves v1 and Ves v5) and Polistes spp (Pol d5) were tested. In both cases, the ImmunoCap Thermofisher® method and allergens (whole extract and molecules) were used. Serum Tryptase was tested in each patient to rule out hidden systemic mastocytosis[15,16].
In Group A patients, tests were performed before starting desensitizing therapy.

Results

Group A patients were 14 males and 6 females with an average age of 54 years; control patients (Group B) were 3 men and 2 women with an average age of 55 years.
In Group A, 15 patients were found to be positive to Apis m poison., 3 to Vespula, 1 to Polistes and 1 to Vespa Cabro. Out of 15 patients allergic to Apis m. poison, 10 were found positive to Api m2 molecule (bee hyaluronidase), while all patients (Groups A and B) were found negative to hyaluronidase and control substance prick test, and positive to histamine (*Figure 1*).
All patients (Groups A and B) were negative to the skin test (*Table 1*).
The low levels of serum Tryptase showed that patients had experienced a true anaphylactic reaction after the hymenoptera puncture, as no one had cutaneous mastocytosis.

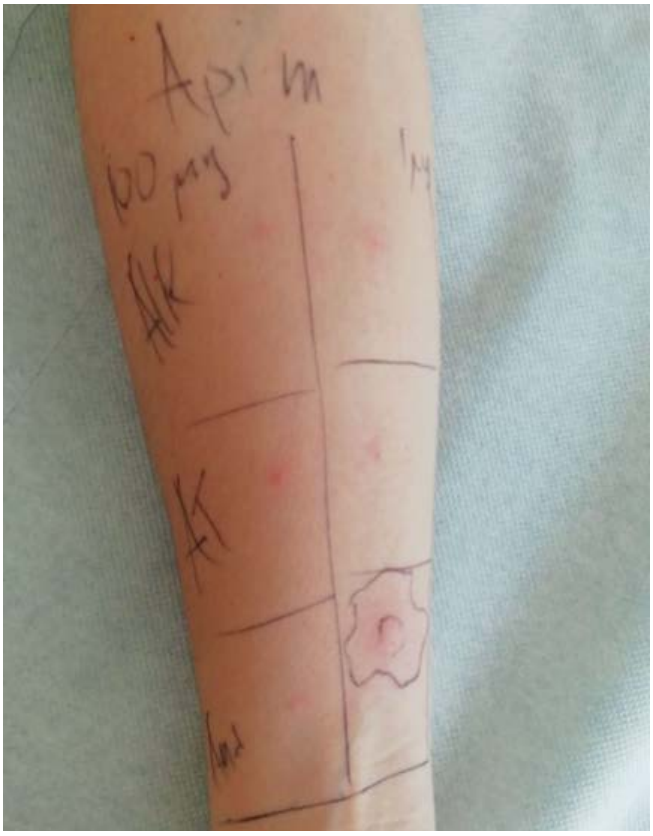

Figure 1 *- Skin tests: prick test (left) and intradermal test (right) in a patient positive to hymenoptera venom Api m2 (red circle) and negative to hyaluronidase (white circle).*

Discussion and conclusions

Hyal-2 and Hyal-1 are the major hyaluronidases in mammalian somatic tissues and they act together to degrade high molecular weight hyaluronan[18].
Meyer previously discovered the activity of these proteins and classified hyaluronidases into 3 groups[19]:
1) endo-β-Nacetylhexosaminidase, present in mammals, degrades the β-1,4 glycosidic bonds of HA (*Table 2*), producing tetrasaccharides. These enzymes act on HA,

Sex	Sensitizer	Specific IgE (kU/L)					Triptase	ST Hyaluronidase
		Apis	Vespula	Polistes	Vespa Cr.	Api m 2		
M	Apis	4,26	0	0	0	1,03	4,5	N
M	Apis	12,2	0,16	0,04	0,01	0,20	6,4	N
F	Apis	>100	0,35	0	0,09	0,10	5,6	N
M	Apis	0,44	0,05	0,09	0	0,08	4,4	N
M	Pol	0	0	0,6	0	0	4,8	N
F	Apis	9,57	0	0,01	0	0,07	6,3	N
F	Vesp	0,26	1,66	0,36	0	0	4,6	N
M	Apis	1,74	0,09	0	0	0,21	5,8	N
M	Apis	8,41	1,87	1,72	0,5	4,62	7,2	N
F	Vesp	3,28	19,7	5,7	0	0	7,3	N
M	Apis	8,16	2,02	1,93	0,19	0	6,5	N
M	Apis	2,2	0	0	0	0	4,4	N
M	Apis	8,17	0	0	0	0	7	N
F	Vesp	0,01	35,1	6,98	2,07	0	4,1	N
M	Apis	0	4,06	3,51	0	0,10	5,7	N
M	V .Cabro	0	1,35	1,36	46.3	0	3,9	N
M	Apis	40	4,76	2,06	1,14	4,72	5,9	N
M	Apis	2,54	0,03	0,04	0,22	2,35	4,6	N
M	Apis	0,67	5,8	13,9	0,97	0	4	N
F	Apis	15,9	0,23	0,29	0,26	0	7,2	N

Table 1 *- The table illustrates the sex of Group A patients, the venom they are sensitive to, serum specific IgE levels to whole venom extracts and to Api m2 (bee hyaluronidase), Triptase serum levels and the results of skin test to hyaluronidase.*

Allergen	Name/Function	MW [kDa]
Honeybee (Apis mellifera)		
Api m 1	Phospholipase A2	17
Api m 2	Hyaluronidase	45
Apim 3	Acid phosphatase	49
Apim 4	Melittin	3
Apim 5	Dipeptidyl peptidase IV	100
Apim 6	Protease inhibitor	8
Apim 7	Protease	39
Apim 8	Carboxylesterase	70
Apim 9	Carboxypeptidase	60
Api m 10	Icarapin 55 <1S a,c,Md,e	
Api m 11.0101	Major Royal Jelly Protein	8 55
Api m 11.0201	Major Royal Jelly Protein	8 60
Api m 12	Vitellogenin	200
Yellow jacket (Vespula vulgaris)		
Ves v 1	Phospholipase A1	35
Ves v 2.0101	Hyaluronidase	45
Ves v 2.0201	Hyaluronidase (inactive)	45
Ves v 3	Dipeptidyl peptidase IV	100
Ves v 5	Antigen 5	25
Ves v 6	Vitellogenin	200
European paper wasp (Polistes dominula)		
Pold 1	Phospholipase	34
Pold 2	Hyaluronidase	45
Pold 3	Dipeptidyl peptidase IV	100
Pold 4	Protease	33
Pold 5	Antigen 5	23

***Table 2** - List of the molecular allergens contained in venom extracts from Apis m, Vespula v. and Polistes d.). Api m2, Ves v2 and Pol d2 represent the hyaluronidases of these hymenoptera. Currently available tests detect only specific IgE to bee hyaluronidase (Api m2).*

chondroitin, chondroitin- 4,6-sulfate and dermatan sulfate and can be found in mammalian sperm and lysosomes, as well as in the venom of snakes, reptiles and hymenoptera. 2) endo-p-Dglucuronidase, present in the salivary glands of leeches and hookworms, that degrades the β-1,3 glycosidic bond, producing tetra- and hexasaccharides. 3) microbial hyaluronidase, or hyaluronate lyase. These enzymes have been isolated from several microorganisms (Clostridium, Micrococcus, Streptococcus and Streptomyces).

Hyaluronidases can also be divided into two additional groups based on their pH dependent activity:

· Acid active hyaluronidases. This group of enzymes express their activity between pH 3 and 4.

· Neutral active hyaluronidases, active between pH 5 and 8. Snake venom and bee venom hyaluronidases belong to this group[20].

Hyaluronidases for medical use were initially derived either from raw extracts of ovine or bovine testicular tissue (bovine testicular hyaluronidase), or from Streptococcus agalactiae hyaluronate lyase[21]. A recombinant human hyaluronidase, considered to be less immunogenic has recently been introduced on the market[10,11].

Depending on living conditions and life style, it is estimated that 56-94% of the adult population has been stung by a hymenoptera at least once in their lifetime in Europe, by bees in a third of cases. Allergy to hymenoptera venom is responsible for approximately 20% of total fatal anaphylaxis cases in different countries[12].

The components of hymenoptera venom capable of inducing an allergic reaction are generally glycoproteins, with a molecular weight between 10 and 50 kDa3. Many of these allergens are well known and have been sequenced, and some are already available in a recombinant form.

The main allergens of bee venom are phospholipase A2 (Api m 1), isolated and also identified in the bumblebee (Bom p 1, Bom t 1), hyaluronidase and acid phosphatase. The major allergens of Vespids are phospholipase A1;

hyaluronidase, which has about 50% sequence identity with its homologous of Apidi 1; and antigen 5, which is present in the venom of all Vespids[22].
Bee venom hyaluronidase (Hya) specifically degrades HA in the extracellular matrix of the skin, thereby facilitating the penetration of the venom constituents into the body. Native Hya isolated from bee venom is a single polypeptide consisting of 350 residues[23].
Bee venom hyaluronidase shares a sequence identity of more than 50% with hyaluronidases from other hymenoptera[24] such as wasps[25]. Bee venom hyaluronidase is also homologous to several mammalian enzymes such as those found in humans (almost 30% identity), including glycosylphosphatidylinositol (GPI) membrane-bound PH-20 protein and human lysosomal enzymes Hyal-1 and Hyal-2, which are involved in the turnover of hyaluronic acid[26]. Based on the similarity of the sequences and the mechanistic pathway involved in their activity, insect and mammals hyaluronidases have been classified as belonging to the same family of glycosides hydrolases.
Bee and wasp poisons contain proteins that can induce life-threatening allergic reactions in humans, with extremely variable symptoms, from a mild local reaction to a systemic reaction, up to anaphylactic shock[27].
In recent years, thanks to the technology of recombinant allergens derived from molecular biology studies applied to allergic pathology, the analysis of IgE reactivity to the individual molecular components of an allergenic extract is now possible. This allows us to carry out a Component Resolved Diagnosis (CRD), in other words to identify the reactivity profile of a subject sensitized to the individual allergenic components, increasing the specificity[28]. Apis mellifera venom is certainly the most extensively characterized hymenoptera venom. So far, 12 HBV allergens are included in the official list of allergen nomenclature of the WHO/International Union of Immunology Societies[29,30] (*Table 2*).
Contrary to Api m 2 hyaluronidase, which is an important allergen, the homologous Ves v 2 seems to have limited relevance and sensitization is reported in 5% -25% of patients allergic to Vespula poison[31].
The clinical history of the patient requiring dermal filler treatment must verify the presence of factors that contraindicate treatment such as chronic or neoplastic diseases, severe or multiple allergies and ongoing skin infections, infections of the head and neck district (such as sinusitis, periodontal disease and dental infections, infections of the oropharyngeal cavity), connective tissue diseases. Chronic pathological conditions such as ulcerative rectocolitis, Crohn's disease, repeated urinary tract infections, liver, kidney and thyroid alterations must be investigated and evaluated by the medical doctor6. The indication for the use of hyaluronidase in the treatment of filler complications constitutes off-label use and requires specific authorization from the patient.
Finally, in non-emergency situations and in the presence of a positive history of hymenoptera bite allergy, skin tests can be undertaken by an allergist. When an urgent administration of hyaluronidase is required, as in the case of vascular ischemia after hyaluronic acid injection, the risks and benefits of lacking a skin test must be assessed.
Our results show that patients with hymenoptera venom allergy did not present cross allergy reactions with the bovine-derived hyaluronidase used in this study. Although the sample is small, the pilot study can be considered significant because the prevalence of systemic reactions from hymenoptera bites among adults varies from 0.5% to 3.3% in the United States, while European epidemiological studies report the occurrence of systemic reactions between 0.3% and 7.5%. Of all these reactions, about 1% are anaphylactic[32].
In conclusion, the results of our study show that, from an allergological perspective, the use of bovine-derived hyaluronidase in patients allergic to hymenoptera venom is safe, also in emergency situations.

Acknowledgments

The Authors declare that they have no conflict of interest.

Disclosure

The Authors did not receive any funds and certify that there is no actual or potential conflict of interest in relation to this article.

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aesthetic medicine

Original Article

Improving on laser: biorevitalization of stretch marks, the polynucleotides infiltrations combined with CO_2 laser option

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Abstract

Introduction: Ablative CO_2 laser is extensively used in the esthetic management of striae albae. The goal of this exploratory, intra-subject-controlled study was to investigate whether combining the dermal remodeling efficacy of polynucleotide infiltrations with the resurfacing efficacy of the CO_2 laser might offer further benefits compared to laser resurfacing.

Methods: Eighteen mature striae albae from three women were randomized to one of three treatment options: polynucleotides dermal infiltrations, polynucleotides infiltrations combined with three CO_2 laser sessions; untreated controls. Endpoint: comparison of striae albae width and wrinkling (Antera® 3D CS skin imaging technology) before the first treatment session and after 3 weeks of follow-up.

Results: Almost a 30% mean overall reduction in stretch mark depth was achieved with polynucleotides dermal infiltrations. The mean depth of medium-wrinkled and thin striae further improved with the polynucleotide infiltrations / laser combination (-44.3% and -42.3%, respectively).

Conclusions: The esthetic efficacy of polynucleotides dermal infiltrations on mature striae albae confirmed the results of previous studies. Combining the resurfacing efficacy of CO_2 laser treatment with the trophic power of polynucleotides may improve esthetic outcomes, although validation in controlled studies is required.

Keywords

Stretch marks, striae albae, striae gravidarum, polynucleotides, CO_2 laser

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Introduction

The unsightly dermal scarring caused by stretch marks, first investigated in depth in 1936, develops in up to 90% of primigravidae and is the commonest and most irksome aesthetic sequel of late pregnancy, usually occurring after the sixth month.
These therapeutically challenging lesions are also known as striae distensae (striae gravidarum); the thighs, buttocks, breasts and the abdomen are the most frequent sites of development[1-5].
Stretch marks develop to look like mature atrophic scars that have permanently lost all viscoelastic properties. Early stages of development (striae rubrae) are associated with the disruption of the normal elastic fiber network, loss of both the vertical fibrillin fibers subjacent to the dermal-epidermal junction, and inflammatory changes, such as perivascular lymphocytes and dilated dermal venules[6-10].
Epidermal and dermal atrophy, loss of rete ridges and vascularity, and densely packed, thin and horizontal collagen bundles are the histologic markers of the final, atrophic stages of stretch mark scarring (striae albae)[8,9]. Based on these morphologic findings, the primary goals of stretch marks management should be to reduce inflammatory redness, swelling and irritation in striae rubrae, and to increase collagen and elastin fiber production in striae albae[2,9,10].
Twenty years ago, the dermal infiltration of highly purified fractions of DNA polynucleotides extracted from trout gonads (PN HPT, "Polynucleotides Highly Purified Technology") was first reported to enhance the proliferation and trophism of human skin fibroblasts and the remodeling of fibrillary and amorphous matrix[11,12]. PN HPT have since been extensively used in Aesthetic Medicine for skin rejuvenation[13], and the regeneration of several skin fractions, including collagen, elastin fibrils and glycosaminoglycans, has been documented after PN HPT infiltration[11,12]. Several reports of stretch mark revitalization with PN HPT have also been published over the last years[14-19].
The non-ablative fractional laser (NAFL) technique, most commonly performed with a 2940-nm erbium-doped yttrium aluminum garnet (Er:YAG) laser, is known to induce the re-pigmentation of striae albae and like PN HPT, to stimulate the production of new collagen and elastin[20].
An interesting hypothesis deserving verification is whether a course of PN HPT dermal infiltrations to stimulate new collagen and elastin deposition in areas of striae albae may synergize with the acknowledged resurfacing and collagen-tightening efficacy of ablative CO_2 laser therapy with surgical handpiece.
Combining the dermal remodeling efficacy of PN HPT infiltrations with the resurfacing performances of CO_2 laser treatment might improve the remodeling outcomes of the NAFL technique. This combination might hopefully also overcome the frustrating lack of consistently predictable benefits in the treatment of atrophic striae albae.
Based on this rationale, the herein described pilot study aimed to explore if combining a three-session CO_2 laser cycle of treatment with an eight-session cycle of PN HPT infiltrations could lead to increased aesthetic benefits in women with mature striae albae, compared to PN HPT infiltrations alone. The intended purpose of the study was purely of an exploratory nature and if positive, preliminary to well-designed, wider studies will be required.

Methods

General design
Intra-subject-controlled exploratory comparison of aesthetic efficacy; 3 treatment groups:
- Combined treatment with PN HPT intradermal infiltrations associated with ablative CO_2 laser resurfacing (laser device with surgical maniple).
- Monotherapy with PN HPT intradermal infiltrations.
- Untreated controls.

Study subjects
Three non-pregnant non-lactating women, aged from 40 to 55 years, with multiple abdominal striae albae from previous pregnancies.

Procedures
Six well-individualized mature striae albae were selected and numbered in each woman (*Figures 1 and 2*) to allow easy identification after treatment; each selected stretch mark was to be more than 5 cm long and no more than 1 cm wide at the widest point. All histories of PN HPT intolerance, psoriasis, keloids, vasculitis, locally active infections, or contraindications to laser treatments led to the exclusion of some candidate women. The 18 selected striae albae were randomized (WinPepi software) to one of the three intra-subject treatment options being evaluated. Two striae albae were kept as untreated controls (Group "Controls"), while two stretch marks from each woman were treated with infiltrative PN HPT monotherapy (Group "PN HPT"). Two more stretch marks from the same woman were treated with the PN HPT infiltrations and CO_2 laser combination (Group "Combined").

PN HPT protocol
Infiltrative sessions (Plinest Body®, Mastelli S.r.l., Sanremo, Italy; intradermal PN HPTs dose: about 1 mL/cm^2 equivalent to about 2 mg PN HPT per cm^2; vial concentration, 8 mg in 4 ml) were performed every week in the first month of the study, and every two weeks for two more months, for a total of eight PN HPT infiltrations in 11 weeks.

CO_2 laser with surgical handpiece protocol
Monthly sessions over 3 months, with the power of the CO_2 laser device set to 1.0 Watt. Fluence was variable within the fairly high range used for skin resurfacing (7 to 17 Joules/cm^2).

Clinical assessment, timing and parameters
Basal photographs of all randomized striae albae and quantitative evaluations of their width and wrinkling were collected before the first treatment session. The research-grade, camera-equipped Antera® 3D CS optical imaging device used (Miravex Limited, Dublin, Ireland)

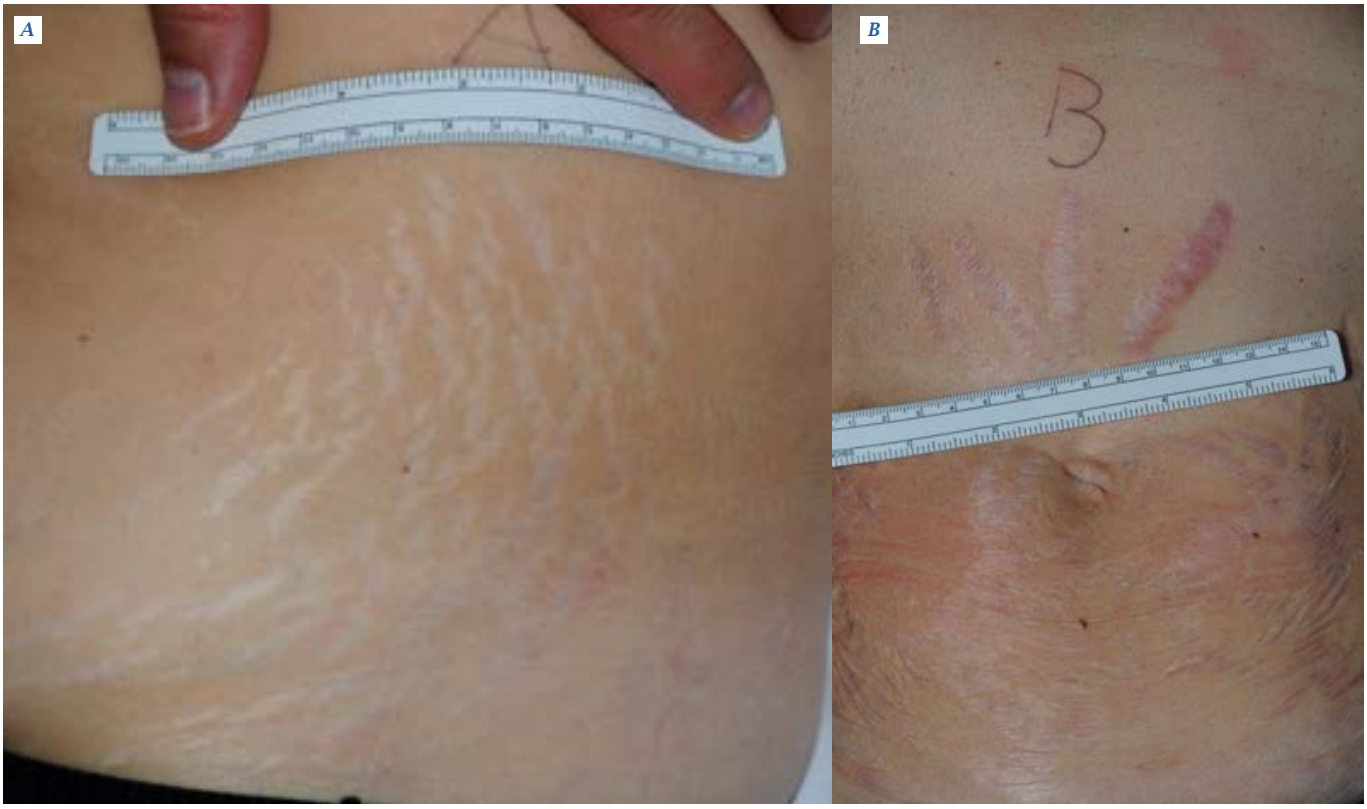


Figure 1 *- Selection of candidate striae albae (no more than 5 cm long and 1 cm wide at the widest point) in subjects A (left) and B (right).*

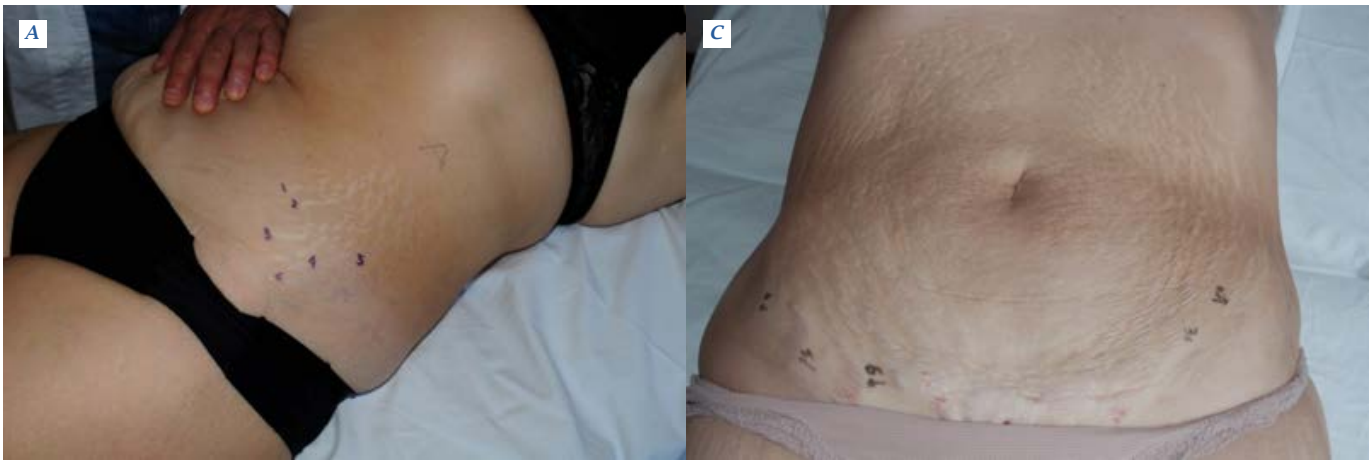


Figure 2 *- Numbering of selected striae albae before randomization in subjects A (left) and C (right).*

enables tridimensional measurements and basal vs. end-of-treatment numerical and graphical comparisons of skin roughness and parameters of pores, wrinkles and stretch marks. Operated as a colorimeter, the Antera® 3D CS device also measures the average concentration and uniformity of melanin and hemoglobin, as well as hyper- and hypo-concentration thereof[21,22]. Photographs and quantitative evaluations were repeated at the end of the study, namely three weeks after the end of the planned 11-week treatment sessions. The three treated women were also asked to fill out a subjective impression of efficacy and tolerability questionnaire, both before and after treatment, while the CO_2 laser operator was asked to fill out a clinical evaluation questionnaire. The steering committee of the Aesthetic Medicine and Surgery Clinic, Udine (Italy) peer-reviewed and approved all study materials for any ethical problems, including the informed consent form, study protocol and case report forms; the study was office-based and carried out in accordance with the principles of the Declaration of Helsinki. *Table 1* summarizes the timing of study evaluations at baseline, before the 11-week period of randomized treatments, and at the end of the 3-week follow-up.

Week	ANTERA® photograph	Photograph	Medical assessment	Woman's self- assessment
0	Yes	Yes	Yes	Yes
1 to 10				
11				
12				
13				
14	Yes	Yes	Yes	Yes

Statistics

Due to the small number of assessments, the non-parametric Mann-Whitney test was applied to quantitative study parameters (end-of-study vs. basal mean depths of striae albae and mean improvements of stretch marks depths).

Results

All the enrolled women completed the study; all were of Caucasian ethnicity and 39, 53, and 54 years old. No more than some minimal change, with depth increasing by 5% to 9% in some striae albae (mean depth increase, 5.6%), was observed at the end of the follow-up period in untreated controls. The PN HPT infiltration option (Group "PN HPT") confirmed the PN HPT remodeling efficacy, with a mean overall reduction in stretch mark depth of almost 30%.

The mean depth of both medium-wrinkled and thin striae improved the most with PN HPT dermal infiltrations combined with ablative CO_2 laser (Group "Combined") (*Figure 3*). *Figure 4* shows two examples of morphologic and quantitative changes of representative thin and medium-wrinkled mature striae albae before and after combined treatment (PN HPT dermal infiltrations and ablative CO_2 laser) as evaluated with the camera-equipped Antera® 3D CS optical imaging device. In comparison, *Figure 5* shows good results achieved with monotherapy with bio-trophic and bio-reactivating PN HPT without laser resurfacing, while *Figure 6* illustrates the lack of skin texture improvements in one of the stretch mark which was untreated for control purposes.

In questionnaires, all women reported their subjective impression of some improvement in both look and touch with PN HPT monotherapy, but all women agreed on much more appreciable improvements with the PN HPT infiltrations and CO_2 laser combination. The overall women's judgment was one of "poor" improvement, heavily influenced by the unchanged or worsened control striae. Conversely, the investigator's overall judgment was not influenced by the untreated control stretch marks and indicated a "strong" improvement for all three study subjects. Neither the women nor the investigator reported irritation, discomfort or any other side effect during and after treatments.

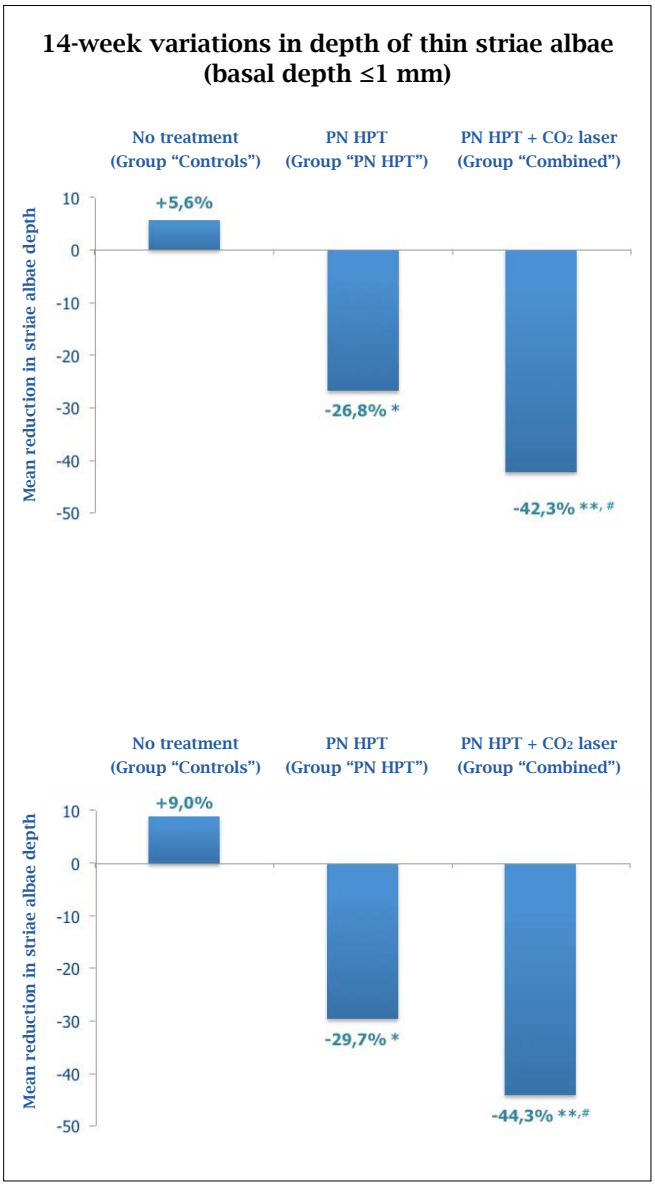
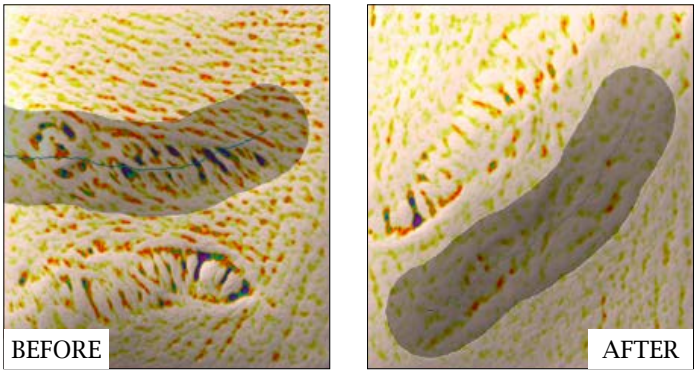


Figure 3 *- Mean percent changes in the depth of thin and medium-wrinkled mature striae albae (depth before treatment, respectively, ≤ 1 and ≤ 2 mm) at the end of the follow-up period: assessment with the Antera® 3D CS camera-equipped optical imaging device after 11 weeks of treament with dermal PN HPT infiltrations (Group "PN HPT") or the PN HPT and CO_2 laser combination (Group "Combined") vs. untreated controls (group "Controls"). * p <0.05 and ** p <0.01 vs. basal evaluation; # p <0.05 vs. monotherapy with PN HPT infiltrations.*

Mature thin stretch mark

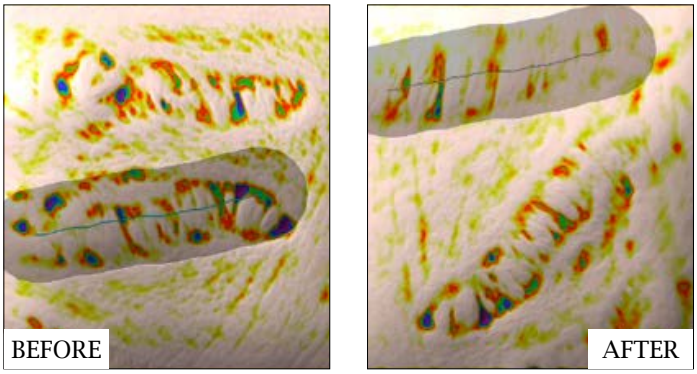


Antera® 3D total score:
Basal 71.5, final 30.7 (-57.1%)

Depth of stria alba:
Basal 0.121 mm, final 0.039 mm

Width of stria alba:
Basal 7.26 mm, final 8.18 mm

Mature medium-wrinkled stretch mark

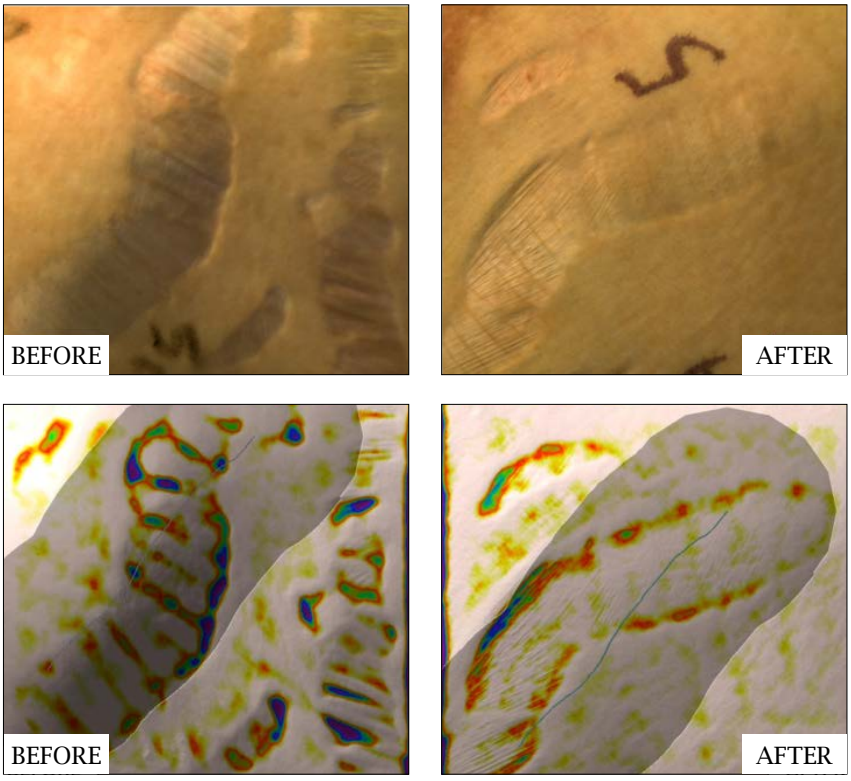


Antera® 3D total score:
Basal 164.0, final 95.4 (-41.8%)

Depth of stria alba:
Basal 0.199 mm, final 0.122 mm

Width of stria alba:
Basal 7.81 mm, final 7.51 mm

Figure 4 *- Antera® 3D CS total score, depth, and width of B subject thin stria alba 1 (upper image) and medium-wrinled stria alba 2 (lower image) before and after combined treatment with PN HPT infiltrations and ablative CO_2 laser; quantitative basal vs. end-of-study assessments.*



Antera® 3D total score:
Basal 314.6, final 188.3 (–58.6%)

Depth of stria alba:
Basal 0.414 mm, final 0.192 mm

Width of stria alba:
Basal13.5 mm, final 14.3 mm

Figure 5 *- Photographic documentation (upper image) and Antera® 3D CS total score, depth, and width of C subject medium-wrinkled stria alba 5 (lower image) before and after monotherapy with PN HPT infiltrations without ablative CO_2 laser; quantitative basal vs. end-of-study assessments.*

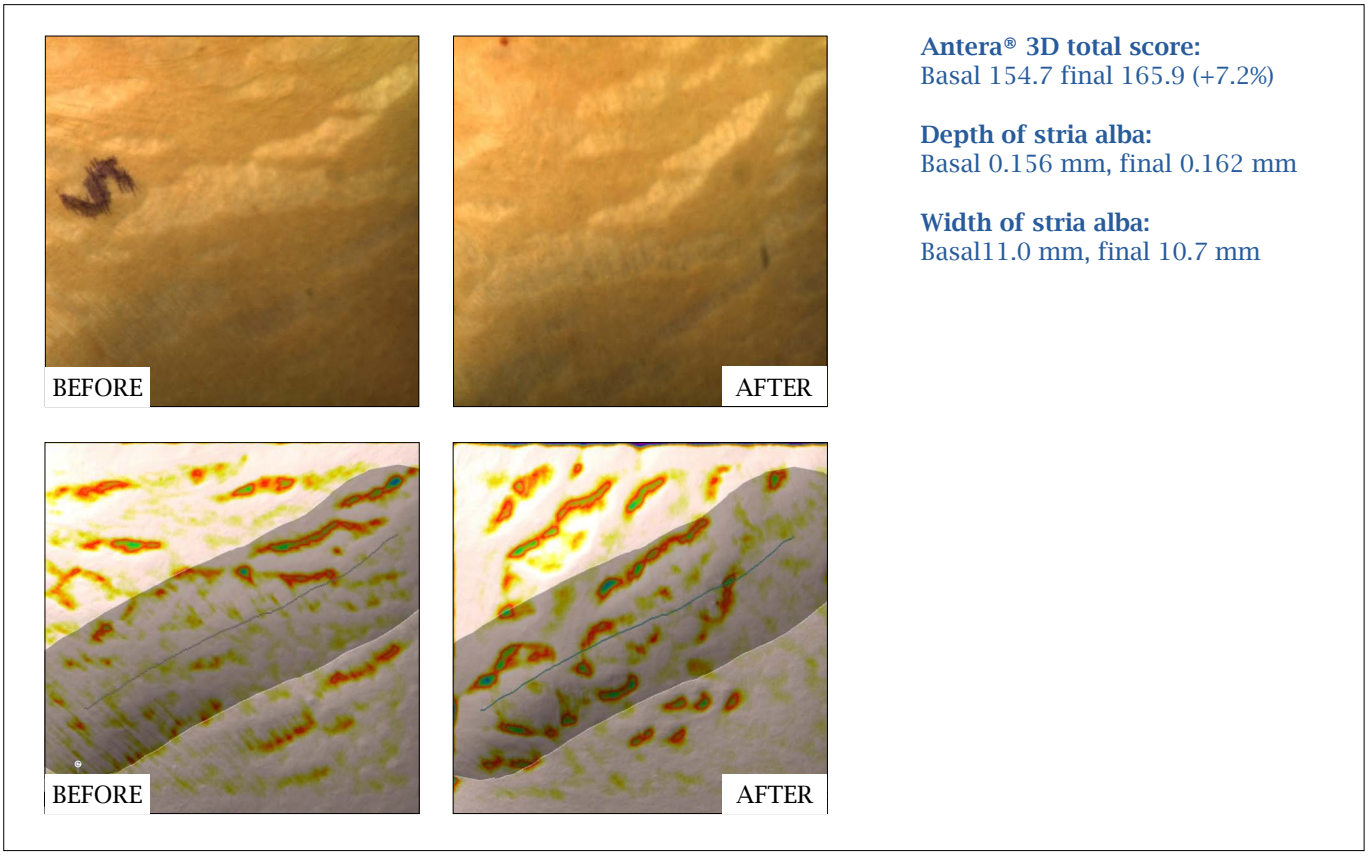


Figure 6 *- Example of untreated control: photographic documentation (upper image) and Antera® 3D CS total score, depth, and width of A subject medium-wrinkled stria alba 5 (lower image); quantitative basal vs. end-of-study assessments.*

Discussion

Stretch marks may be a minor clinical problem, but they are a major problem in terms of self-esteem and self-confidence in affected individuals. This may be especially true for women with striae gravidarum, but adolescents of both genders are no exceptions. The thighs and lumbosacral region are the most common sites where stretch marks develop in adolescent males[1,2]. Metabolic disorders such as obesity and Cushing's disease, chronic liver disease, anorexia nervosa, the use and abuse of topical or systemic corticosteroids, and even some aesthetic surgery procedures such as breast and gluteal augmentation are commonly associated with stretch marks. However, striae gravidarum are the most common type of stretch mark[1,2].

Early-phase striae rubrae, usually pink-red to purple, often slightly raised and itchy, respond better to treatment than more seasoned, whitish and atrophic striae albae; many studies with topical trenitoin confirm this rule[6]. Laser devices, although a breakthrough in the treatment of stretch marks, have been no exception: the increased vascularity of striae rubrae has meant higher levels of target oxy-hemoglobin chromophore, thus resulting in better early aesthetic outcomes[6,8]. The rule of unsatisfactory results in mature striae albae has also proved true for the most recent devices even if their attraction to vascular targets is strong, for instance the 1064-nm long-pulsed neodymium-doped yttrium aluminum garnet (Nd:YAG) laser[6,8].

Ablative lasers have long been the gold standard for the treatment of striae albae[6,9]. A landmark study in 2012 with an ablative fractional 10,600-nm CO_2 laser in subjects with striae albae and skin type III and IV showed better clinical improvements than topical treatment with either 0.05% tretinoin cream or 10% glycolic acid peels[23]. Unfortunately, improvements with CO_2 and other ablative lasers are often limited and once again, overall aesthetic benefits are quite poor[6,9].

The newer NAFL resurfacing paradigm of low spot density combined with high fluences is FDA-approved for the treatment of acne and surgical scars, among other indications[6]. Given the similarities between atrophic scars and striae albae, collagen and elastin deposition in the dermis was unsurprisingly shown in several studies on NAFL treatment of striae albae[24-28].

Combining the acknowledged resurfacing and collagen-tightening efficacy of the CO_2 laser with the powerful fibroblast reactivating power of polynucleotides was the leitmotiv behind this exploratory study. In other words, could the combination improve on the acknowledged dermal remodeling efficacy of the ablative CO_2 laser treatment? The persistent bio-revitalizing and dermal trophic effects of highly purified PN HPT have long been used in Aesthetic Medicine, including for the correction of depressed scars and striae distensae[11-19]. PN HPT, acting as sources of nucleotides and nucleosides, promote cell growth and improve skin trophism[11-13]. In this study, as in previous ones, the intradermal infiltration of PN HPT was associated with an aesthetically significant degree

of stretch mark flattening (about 30%)[13-15]. Combining PN HPT infiltrations with their strong dermal bio-trophic and reactivating potential to a standard course of ablative CO_2 laser resurfacing (laser device with surgical handpiece) more than doubled the reduction of mean depth of mature medium-wrinkled striae albae (from -20.7% to -44.3%). The final aesthetic outcome was slightly lower on average for thin striae albae (from -26.8% to -42.3%). The Antera® 3D CS optical imaging device enabled highly accurate quantitative assessments and eliminated any subjective bias introduced by the investigator. The study, carried out in three subjects with the randomization of six stretch marks per subject (two stretch marks per treatment), was of an exploratory nature. It was planned to be no more than a pilot study to tentatively probe the rationale of the new combined treatment option (laser ablation plus dermally infiltrated PN HPT) and was conceived with a very discriminating intra-subject experimental design. The goal was to minimize the variance associated with unknown confounding factors and to give credibility to outcomes, however preliminary. The number of treated striae distensae, six per subject, was also fair and suitable for exploratory probing purposes. The encouraging suggestions of the study thus appear quite solid, despite requiring confirmation in further long-term and more extensive randomized studies.
In short, there are benefits to be gained from combining the strong bio-reactivating potential of PN HPT dermal infiltrations with the acknowledged resurfacing efficacy of the ablative CO_2 laser device with surgical handpiece. Any future confirmative study will have to include fully validated evaluations of women's satisfaction, gratification, and self-esteem. Investigating whether the combined PN HPT dermal infiltrations / CO_2 laser treatment option might offer some benefits over CO_2 laser ablation, non-ablative fractional (NAFL) and non-fractional laser treatments are other issues that are worth developing on in the future.

Conclusions

Could the fibroblast trophic power of repeated PN HPT infiltrations in the superficial derma of mature striae albae areas usefully complement the resurfacing efficacy of ablative CO_2 laser treatment? The question that inspired the exploratory study herein described has received some preliminary support. Full validation will require further well-designed, controlled studies.

List of Abbreviations

CO_2 carbon dioxide

DNA Deoxyribonucleic Acid

Er:YAG Erbium-doped Yttrium Aluminium Garnet (laser)

FDA U.S. Food and Drug Administration

NAFL Non-Ablative Fractional Laser

Nd:YAG pulsed neodymium-doped yttrium aluminium garnet (laser)

PN HPT Polynucleotides Highly Purified Technology

Acknowledgements

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Contributions of authors

Gianfranco Matera, the main author, directly contributed to the conception of the protocol and was responsible for explaining the aims of this exploratory investigation to the three female participants. He was also personally responsible for obtaining the informed consent of all enrolled subjects, for carrying out all procedures and investigations, and for interpreting outcomes.
Nicholas Dodici, the second author, an Internal Medicine resident physician at the University of Udine Medical School, Udine (Italy), assisted the main author in all procedures, particularly in registering and filing outcomes of procedures and helping with the Antera® 3D CS skin imaging technology.
Mauro Raichi, corresponding author and manuscript submitter, an independent bioinformatics and statistics consultant with full medical qualifications, made crucial contributions to the fine details of the study design. He also had full responsibility for identifying the conservative statistical strategy best suited to a study with exploratory ambitions only, and for data analysis. His responsibilities also included drafting the manuscript, obtaining getting the approval and imprimatur of other authors.
All authors approved the submitted version of the manuscript and are personally accountable for their own contributions as well as for the accuracy and integrity of all the clinical work leading to the manuscript's submission.

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aesthetic medicine

Original Article

Non-ablative capacitive resistive 448 khz radiofrequency for wrinkle reduction pilot study

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Abstract
Background: anti-aging non-invasive cosmetic procedures are a common way of addressing cosmetic concerns raised by patients. Radiofrequency treatment is a well-established form of non-surgical cosmetic improvement, which is used in particular for wrinkle reduction.
Objective: the aim of this study was to determine the effectiveness of repeated treatments of 448 khz monopolar capacitive / resistive radiofrequency in facial wrinkle reduction.
Methods: we recruited 32 healthy volunteers to undergo six radiofrequency treatments over a four-week treatment period. No other treatment for facial wrinkles was performed during the study period. Treatment was performed with temperature control of the skin, to prevent skin burns (40-42oC). The results were evaluated two months and three months after the completion of treatment, using the Fitzpatrick facial wrinkle scale on standardized photographs. A skin digital analyser was used to objectively assess the evolution of wrinkles. Subjective questionnaires were also usedfor patients and professionals to rate improvement, effectiveness and friend recommendation. Statistical significance was determined by the Paired student's t-test ($p<0.05$).
Results: there was a significant reduction of wrinkle size and depth, with an improvement of at least one point on the Fitzpatrick wrinkle scale in 80% of participants. No undesirable side effects were reported.
Conclusions: the use of a 448 kHz monopolar capacitive / resistive radiofrequency with Temperature Monitoring Control has proven to be safe and effective in the improvement of wrinkle appearance up to three months after treatment.

Keywords
Monopolar radiofrequency, capacitive, resistive, skin tightening, anti-aging, wrinkles

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Introduction

Antiaging treatments and skin care in general are becoming very popular. The concern for safety and a desire to avoid down time and the risk associated with surgery has led to the increased presence of non-invasive procedures on the market, such as non- ablative radiofrequency (RF) and different types of laser and light, used to promote facial rejuvenation[1-4].
Despite the popularity of laser and pulsed lights, their use have many limitations, such as skin colour, depth of action as well as many side effects and post treatment down time in ablative lasers[2,4-7]; other techniques, like radiofrequency, are not affected by such limitations[8].
The aim of RF is to increase tissue temperature (hyperthermia). The effects of hyperthermia are directly related to the type of tissue, the temperature reached as well as exposure time[9-11]. The induction of fibroblast proliferation[12-14] and activation of neocollagenesis[12,13] are just some of numerous effects this induces.
The use of radiofrequency (RF) in anti-aging treatments is well established, due to its action on collagen remodelling, skin tightening and wrinkle attenuation[15,16]. In order to optimise working parameters of a 448 kHz monopolar capacitive (CAP) / resistive (RES) radiofrequency (448 kHz CRET) device in Facial Treatment Methodology for Wrinkles, a pilot multicentre evaluation with a thermal control application protocol has been undertaken.
The aim of the study was to determine the effect of RF treatment on the skin following multiple treatments.

Materials and methods

Study Design

This was a Spanish and United Kingdom multicentre prospective study. The study population included 32 healthy volunteers (30 women and two men), suffering from wrinkles aged from 31 to 83 years old and with skin photo types from II to IV (Fitzpatrick scale)[17], population inclusion criteria can be seen at *Table 1*.
Volunteers' wrinkles were classified according to the Fitzpatrick Wrinkle Classification and Degree of Elastosis criteria[18] (*Table 2*). Volunteers received 6 treatment sessions (30 minutes per session) over a 4-week treatment period free of charge. Sessions were completed under temperature controlled conditions to ensure that the local temperature achieved and maintained was ≥ 40oC (40- 42oC) in each area.
Results were evaluated after the treatment, two and three months after the completion of the treatment.

RF Device and accessories

An INDIBA® device (INDIBA S.A., Barcelona, Spain) was used as an RF source. An IR Thermometer (Fluke 62 MAX+) was used to ensure the desired temperature (40° C) was achieved. Finally, a skin analyser was used (Antera 3D®) to measure wrinkles (total size, depth, width and maximum depth).

Effectiveness evaluation

Effectiveness was assessed by standardized facial photographs analysed blindly by independent examiners. Images were taken prior to treatment, after treatment and two and three months later, as follow up pictures after the last session. Treatment results were evaluated with subjective questionnaires for both patients and professionals, to rate: improvement, effectiveness and friend recommendation. A skin analyser was used to digitally measure wrinkles, to objectively assess general global facial condition and obtain data on total size, depth, width, and maximum depth. Statistical significance was determined by the Paired student's t-test.

Include	Exclude / Avoid	Contraindicated for IDC Treatment
Male or female > 18 years old with a Fitzpatrick Skin Type I-IV Presenting wrinkle score (Fitzpatrick Wrinkle Classification System or similar) of 4-9	Blepharoplasty, surgical face lift (12 monts) or chemical peel treatment within the last 6 months Hyaluronic facial filler or boyulinum toxin injection within the last 6 months (Or Collagen - Spain) Current wrinkle reduction treatment Future facial treatment with any of the above until 3 months post final IDC TCM session Know hypersensibility to · Radiofrequency · TheraCream™ (including active ingredients) · Nickel, chromium	Pacemaker or any electronic implant Pregnancy Areas of broken skin on facial region (recent burns, abscesses, open wounds) Thrombophlebitis Removable denture (In resistive mode)

Table 1 *- Inclusion criteria for the enrolment of volunteers in the study.*

Class	Wrinkling	Score	Degree of Elastosis
I	Fine wrinkles	1-3	Mild (fine textural changes with subtly accentuated skin lines)
II	Fine to moderate depth wrinkles Moderate number of lines	4-6	Moderate (distinct popular elastosis-distinct papules with yellow translucency under direct lightening - and dyschromia)
III	Fine to deep wrinkles Numerous lines With or without redundant skin folds	7-9	Severe (multipapular and confluent elastosis - thickened yellow and pallid - approaching or consistent with cutis rhomboidalis)

Table 2 *- Fitzpatrick wrinkle scale and elastosis degree[18].*

Safety evaluation
Safety was assessed by subjective questionnaires (pleasantness, tolerance and erythema) filled out by the professionals and the volunteers, as well as a record of undesirable side effects. Output power was to be reduced when erythema was ≥ 4 (scale from 0 to 5 as maximum erythema), or when pain/tolerability was ≥ 9 (scale from 0 to 10 as maximum pain). Therapists were asked to maintain a subjective dialogue with the volunteers throughout the session to confirm their comfort.

Treatment protocol
All volunteers underwent full facial treatment, and temperature was monitored to ensure 40° C was achieved during the treatment. Volunteers lied in a horizontal position, a return plate was placed under the back in a dorsal location. RF was applied by means of CAP and RES electrodes. The face was divided into 5 different zones, in the beginning every zone was treated with the CAP electrode for two minutes with the aim of reaching a temperature of at least 40° C (measured with an infrared thermometer), after each zone was treated, for four minutes, with the RES electrode. Initial output power per zone was protocolled to ensure desired temperature. Rotating non-stop manoeuvres were used to move the electrode, with 50-70 changes of direction per minute. Precise parameters, such as treatment time, initial power guide and final power guide was provided for each area in the face.

Results

Study Population
All 32 subjects enrolled into the study completed all 6 RF sessions, although three cases were excluded for not fitting the inclusion criteria. The age of patients actually included in the study ranged from 37 to 83 years old with an average age of 56 ±11 y.o. Skin Fitzpatrick photo types distribution was: 2.4% Type I, 61.0% Type II, 24.4% Type III and 12.2% Type IV. The Fitzpatrick wrinkle score distribution of patients before the treatment is shown in *Table 3*.

Fitzpatrick Wrinkle Evaluation
Overall, the mean basal wrinkle degree was Fitzpatrick 6.5 (±1.5), at the end of the treatment, the mean degree decreased to 5.8 (±1.6) and at three months follow up, after the end of treatment, it had decreased to 5.3 (±1.4), percentage distribution can be seen at *Table 3*.

Class	Score	Basal	3 months after treatment
I	1		
	2		2 (7,1 %)
	3	1 (3,6 %)	5 (17,9 %)
II	4	2 (7,1 %)	4 (14,3 %)
	5	5 (17,9 %)	8 (28,6 %)
	6	6 (21,4 %)	4 (14,3 %)
III	7	8 (28,6 %)	2 (7,1 %)
	8	5 (17,9 %)	3 (10,7 %)
	9	1 (3,6 %)	

Table 3 *- Fitzpatrick Wrinkle Classification assessment at baseline and 3 months after the last treatment.*

Paired student's t-test showed statistical significance in Fitzpatrick Wrinkle degree reduction at the end of the treatment (p=0.002), at two months follow up (p=0.000) and at three months follow up (p=0.000). Images showing the basal state (before treatment) and the outcome at three months after completion of treatment can be seen from *Figure 1* to *Figure 4*.

Effectiveness
Not all patients who underwent 448 kHz CRET treatment were tested with the skin analyser, the data of eleven patients was collected after the end of treatment. The average age of tested patients was 57.9± 11.7 years old. Data was collected from different parts of the face, as each part was not analysed separately. Paired student's t-test showed a significant total size reduction (p=0.000) of wrinkles as depth reduction (p=0.001); maximum depth reduction was not significant, nor was the slight increase in width after the conclusion of treatment (*Table 4*).

	Total size	Depth mm	Width mm	Max. depth
Mean loss	4,93	0,02	- 0,01	0,03
% loss	16,10	15,48	- 0,57	18,23

Table 4 *- Wrinkle mean loss and percentage of loss one month after starting the treatment. After a Paired student's t-test, total size reduction was statically significant (p=0.000) as depth reduction (p=0.001) (N=11).*

Examples of the Skin analyser images are shown in *Figures 5* to *8*.
According to the subjective questionnaires, Professionals declared no change in 11% of the patients, 58% improved, 21% showed much improvement and 11% improved very much (*Table 5*). According to patients, 26% didn't see any improvement at all, 42% improved somewhat, 11% moderately and 21% strongly. The treatment was felt as moderately or strongly pleasant by 95% of the patients (*Table 6*). A mean value of 3 was obtained for the Erythema questionnaire, where 0 was no erythema and 5 intense erythema.

Safety
The treatment proved to be safe and no undesirable side effects were reported. Regarding tolerability, pain intensity was ranked as 2, with 0 indicating no pain and 10 worst possible pain. In general the treatment was well tolerated by most of the patients (*Table 6*).
There were no withdrawals.

THERAPIST EVAL.	Worst	No change	Improved	Much improved	Very much improved
Improvement	0	2 (8,7 %)	11 (47,8 %)	8 (34,8 %)	2 (8,7 %)

Table 5 *- Results of the subjective therapist questioner to evaluate the efficiency of INDIBA® treatment on wrinkles per treated patient (N=23).*

SELF EVALUATION	No improvement	Some	Moderate	Much
Improvement	5 (17,9 %)	11 (39,3 %)	4 (14,3 %)	8 (28,6 %)
Atractive	4 (14,3 %)	12 (42,9 %)	5 (17,9 %)	7 (25,0 %)
Pleasant	1 (3,6 %)	1 (3,6 %)	8 (28,6 %)	18 (64,3 %)
Recommend to friends	1 (3,6 %)	3 (10,7 %)	8 (28,6 %)	16 (57,1 %)

Table 6 *- Results of the patients' subjective perception questioner(N=28).*

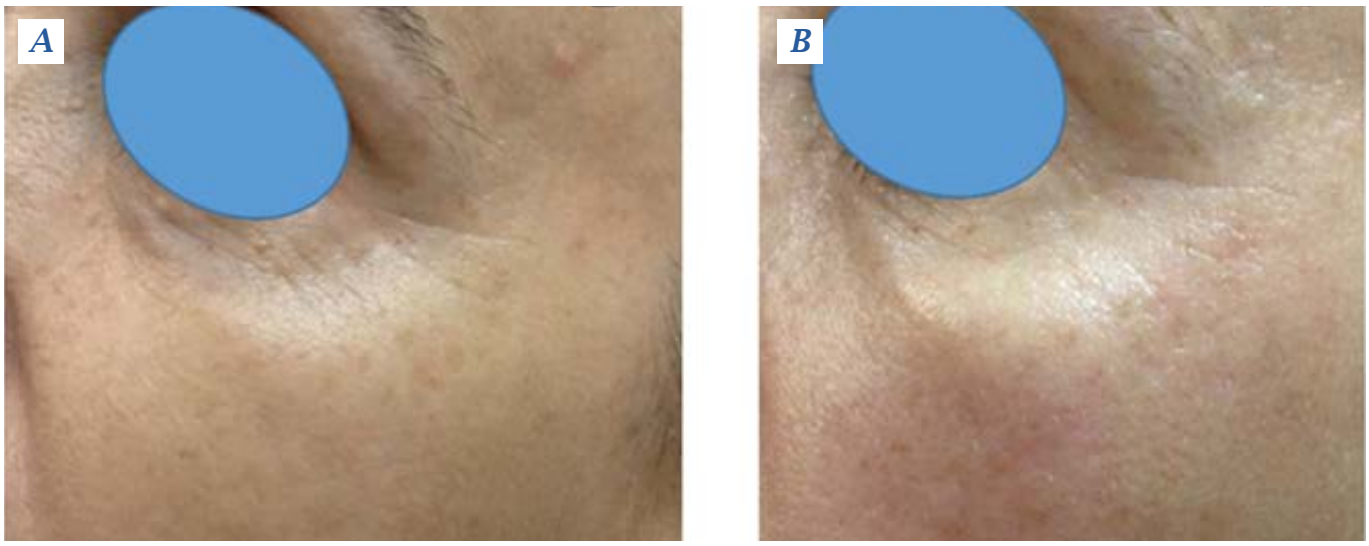

Figure 1 *- 63-year old patient before (B) and 3 months after (A) the last tx.*

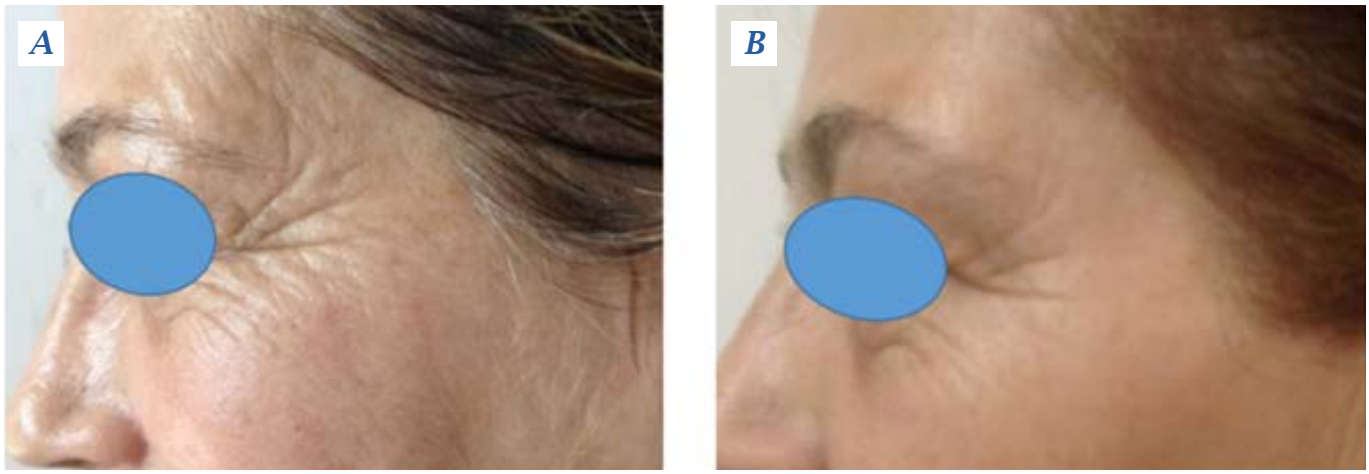

Figure 2 *- 55-year old patient before (B) and 3 months after (A) the last tx.*

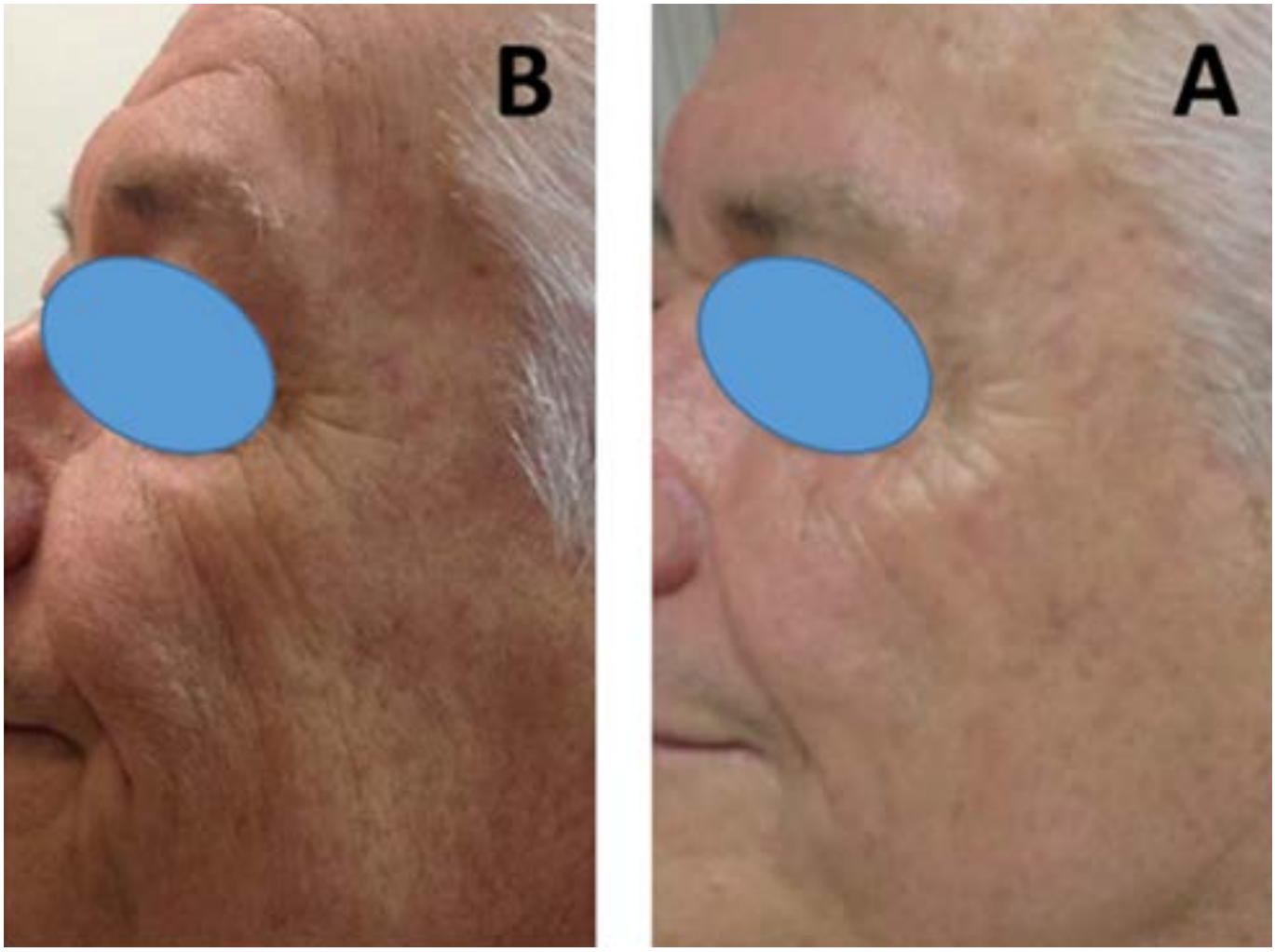

Figure 3 *- 74-year old patient before (B) and 3 months after (A) the last tx.*

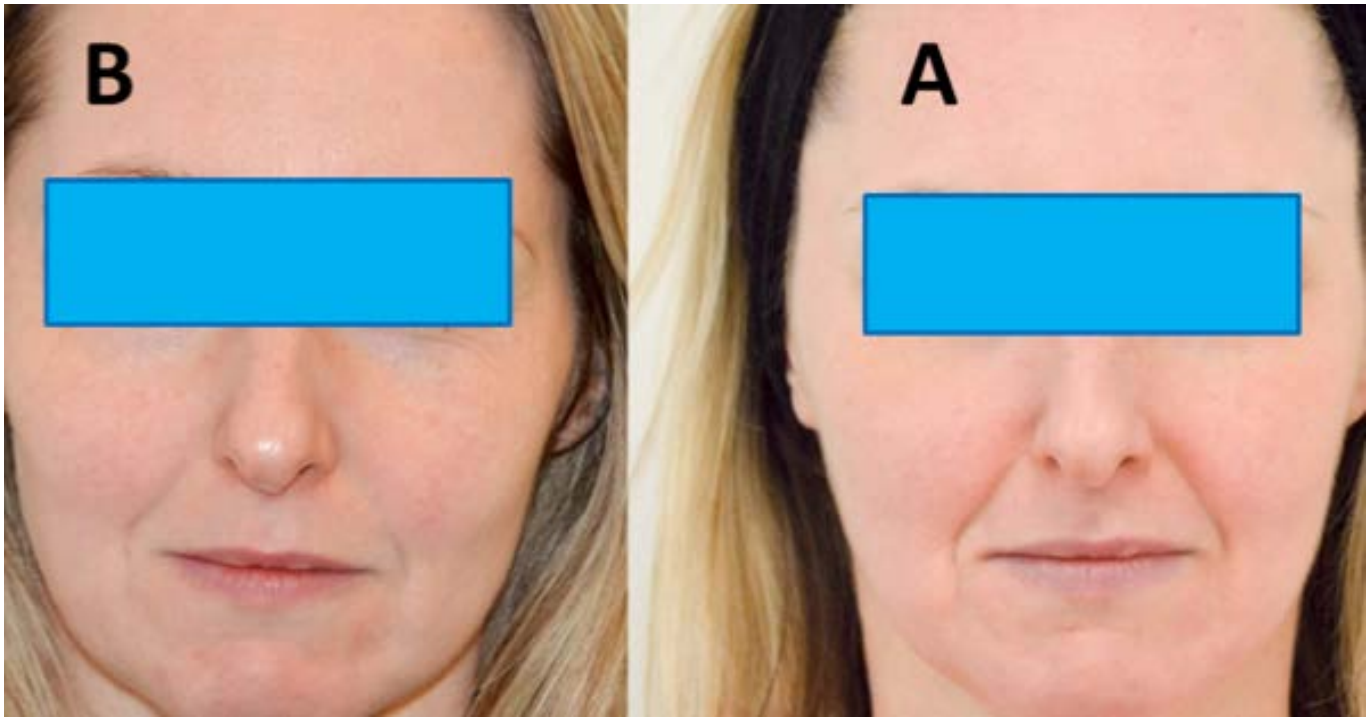

Figure 4 *- 74-year old patient before (B) and 3 months after (A) the last tx.*

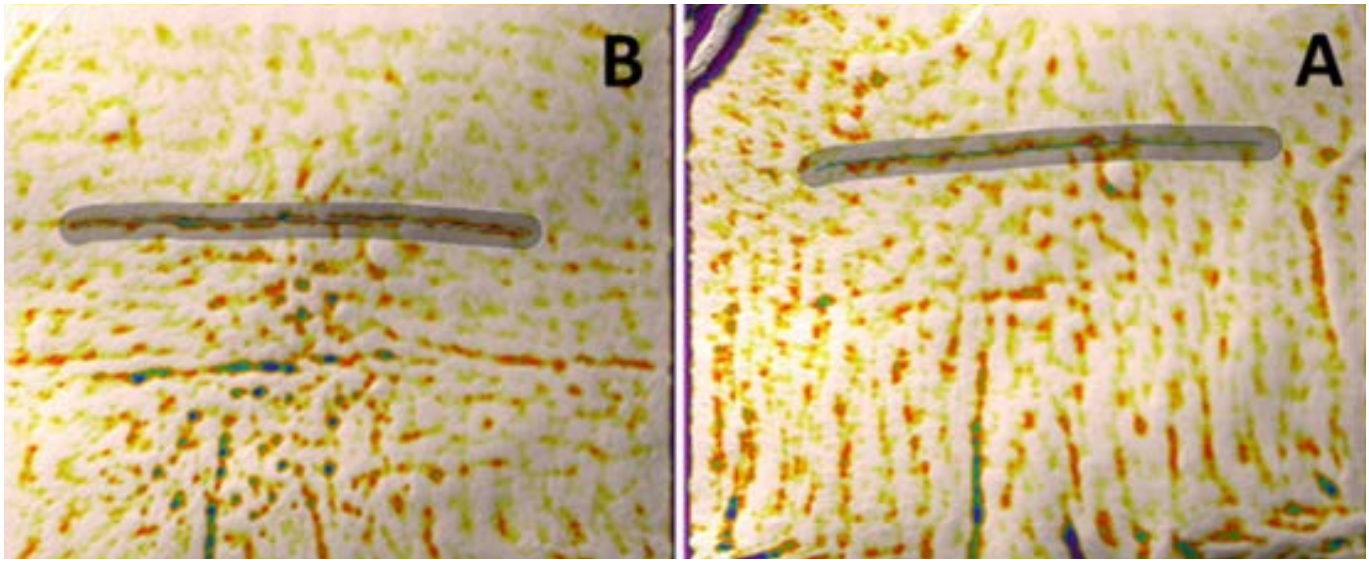

Figure 5 *- 45 y.o. Wrinkle measures before the treatment (B): total size 15.4 / depth 0.0517 mm / width 1.65 mm / maximum depth 0.080. Measures after the treatment (A): total size 12.6 / depth 0.0356 mm / width 1.83 mm / maximum depth 0.059.*

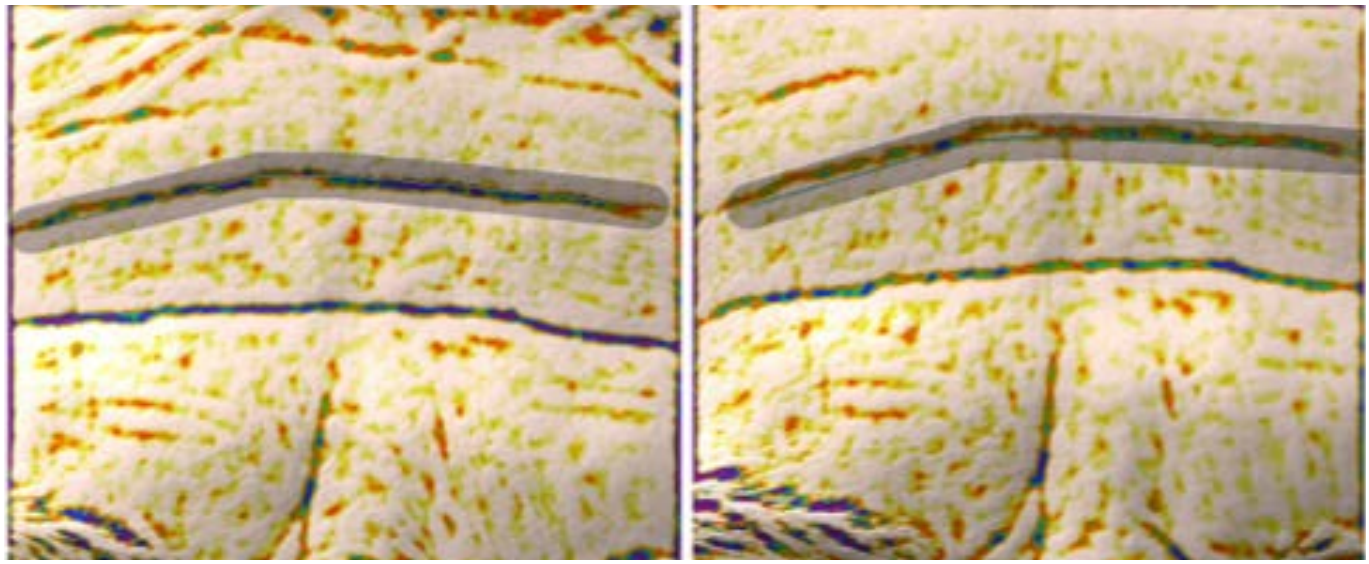

Figure 6 *- 54 y.o. Wrinkle measures before the treatment (B): total size 26.1 / depth 0.0859 mm / width 1.9 mm / Maximum depth 0.202. Measures after the treatment (A): total size 22.3 / depth 0.0651 mm / width 2.13 mm / maximum depth 0.089.*

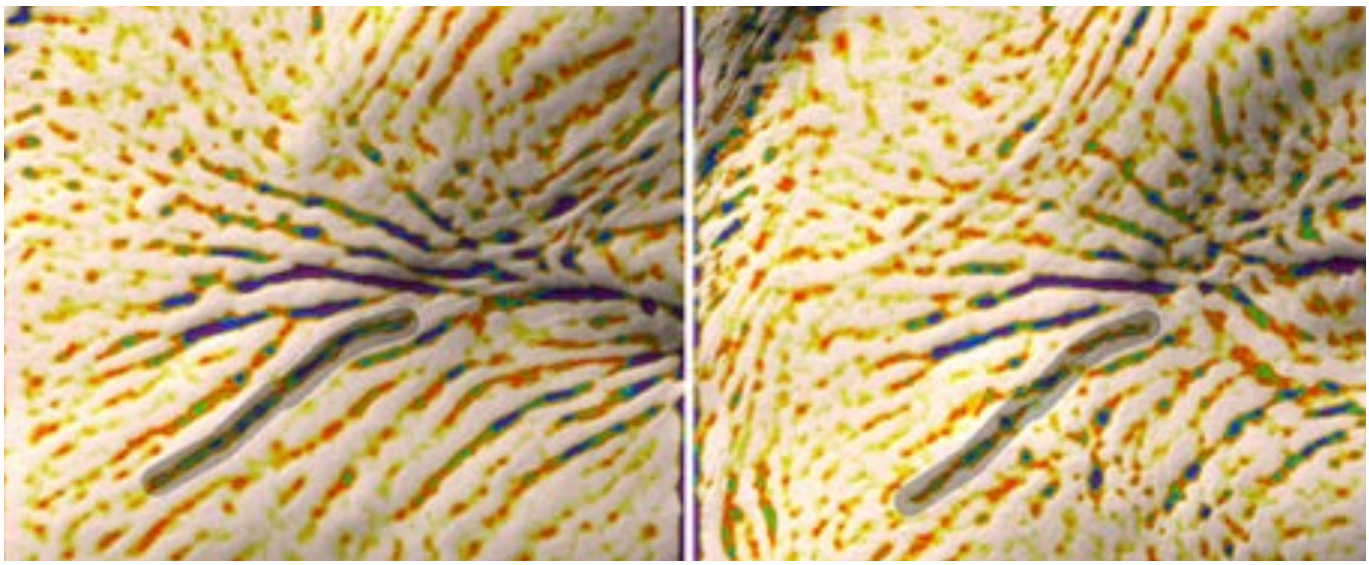

Figure 7 *- 69 y.o. Wrinkle measures before the treatment (B): total size 25.8 / depth 0.0803 mm / width 1.5 mm / maximum depth 0.112. Measures after the treatment (A): total size 18.7 / depth 0.0674 mm / width 1.45 mm / maximum depth 0.116.*

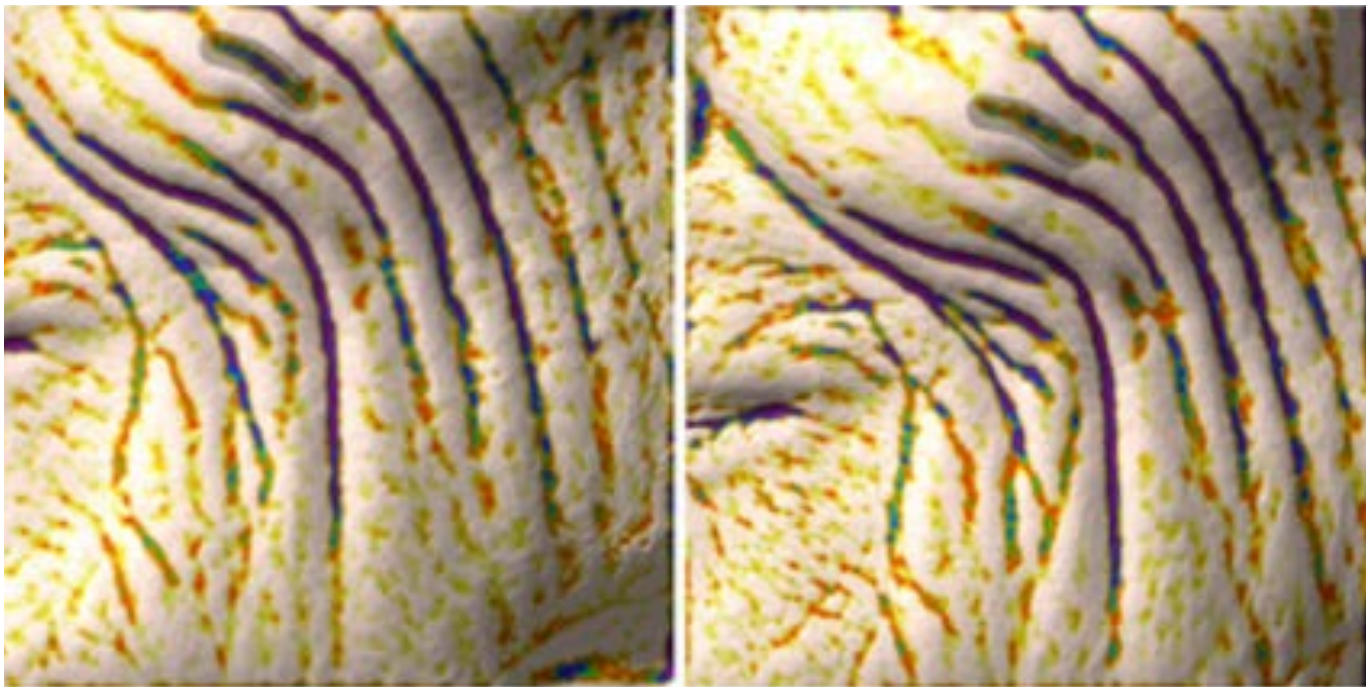

Figure 8 *- 83 y.o. Wrinkle measures before the treatment (B): total size 48.9 / depth 0.144 mm / width 1.8 mm / maximum depth 0.191. Measures after the treatment (A): total size 39.7 / depth 0.11 mm / width 1.81 mm / maximum depth 0.143.*

Discussion

Treatment with a 448 kHz CRET for wrinkles has proven to be a safe technology, according to the both professional and patient assessments obtained using questionnaires.
Although pictures in many patients did not clearly convey the relevant improvement, all evaluations showed an improvement in the appearance of wrinkles.
Skin analysis showed a 16% reduction in total wrinkle size, a 15% decrease of depth and an 18% reduction in maximum depth; width was the only parameter which increased by 0.6%. The subjective therapist assessment claims reduced appearance of wrinkles in 89% of patients; in self-evaluation, 74% of patients stated they experienced some degree of improvement, 42.9% of patients considered the result of the treatment to be a moderate or extensive improvement.
What appears contradictory at first glance is the increase of mean wrinkle width (0.57 %) in contrast with all other measurements, which decreased (total size, depth and maximum depth). This could be a result of the relaxation of depth, tracking the tissue and bringing both sides of the wrinkle closer; resurfacing would relax the sides and make them spread away from the centre of the wrinkle, giving a false appearance of widened wrinkles.
The present results are to be attributed to the effect of hyperthermia on tissues, as it has been reported that collagen denaturation starts at 40° C; collagen coagulation leads to skin shrinkage and in this process there is a microinflammatory stimulation of fibroblasts which stimulates neocollagenesis and neoelastinogenesis, resulting in skin tightening[19].

Conclusions

The use of 448 kHz CRET with Temperature Monitoring Control has been proven to be safe and effective in reducing the appearance of wrinkles for up to three months after treatment.
Further studies would help to evaluate the risks and benefits of different temperature ranges as well as the regime protocol for sessions.

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aesthetic medicine

Case Report

Gummy smile correction with Botulinum Toxin-A: a case report

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Abstract
Smiles are significant in interpersonal relationships and can impact first impressions. Excessive gingival exposure while smiling, known as "gummy smile", often causes psychological complexes and affects self-esteem. The interdisciplinary treatment of gummy smile can be challenging, especially in patients with high aesthetic expectations. A case of a 24-year-old female patient presenting a gummy smile corrected with the use of botulinum toxin type A is described. A single injection of botulinum toxin type A reduced the gummy smile by 5 mm. The applied technique proved to be a useful method in the reduction of gummy smile, an effective alternative to surgical procedures in selected clinical cases.

Keywords
Botulinum toxin, aesthetics, gingiva, smiling, lip

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Introduction

An attractive smile is one of the main features which affects confidence, first impressions and social relationships. It is a part of communication expressing positive emotions, such as joy or kindness. An aesthetically pleasing smile is determined by the balanced relationship of lips, teeth and a healthy, harmonious gingiva. An imbalance between these structures may be manifested as excessive gingival exposure while smiling, known as gummy smile (GS) (*Figure 1*).

This disability is usually associated with complexes and aesthetic problems. A perfect smile demonstrates full crowns of the upper teeth and 1-2 mm of the gingiva below the upper lip. Aesthetically acceptable gingiva exposure does not exceed 3 mm[1], and a smile with greater gum presentation is known as GS[2].

Despite the fact that GS does not affect the stomatognathic system, it can have a great impact on patient well-being and interpersonal relationships.

The determination of the smile line and gum line are important in the diagnosis of GS. The classification of a smile is determined by the position of the upper lip and the smile line:

- low smile line - less than 75% of the crown of upper anterior teeth are visible;
- average smile line - optimal aesthetics - 75-100% of the upper teeth crown and interdental gingiva are visible during a natural smile;
- high smile line - full crowns of the anterior upper teeth and a large area of the gums are visible during a moderately wide smile[3-5].

The aetiology of GS includes both extra- and intraoral factors. Extraoral determinants include excessive vertical growth of the maxilla (EVM), short upper lip and the excessive contraction of muscles, such as the levator labii superioris or the levator labii superioris alaeque nasi. Intraoral factors include altered passive eruption (APE), compensatory over- eruption of teeth, a combination of both types of eruption[2] and orthodontic defects[6]. According to Ezquerr et al., the causes of GS are categorized into: (1) gingival - related to APE; (2) bony - comprising EVM; and (3) muscular - generated by the excessive contraction of the levator labii superioris[3].

According to the classification of Mazzuco and Hexsel, introduced in 2010, GS can be classified as anterior, posterior, mixed or asymmetric (*Table 1*)[7].

Due to its multifactorial aetiology, GS treatment usually requires a multidisciplinary approach. It should be preceded by careful and detailed diagnostics. Gummy smile caused by APE requires the removal of excessive gingiva with or without osteoctomy, whereas gingival smile associated with EVM entails both orthodontic and surgical treatment[2]. In the case of GS associated with excessive lip muscle contraction, the partial resectioning of the levator labii superioris can be a therapeutic solution[8]. An alternative to surgery can be considered, involving the application of intramuscular botulinum toxin type A (BTA) injection producing temporary effects for 3 to 6 months[9].

TYPE OF GUMMY SMILE	CLINICAL APPEARANCE
ANTERIOR	Major gum exposure (>3 mm) in area between canine teeth
POSTERIOR	Major gum exposure (>3 mm) posterior to canines, with normal exposure (<3 mm) in anterior region
MIXED	Excessive gum exposure in both areas (anterior and posterior)
ASYMMETRIC	Excessive or more apparent gum exposure on one side only

Table 1 *- Gummy smile classification according to Mazzuco and Hexsel[7].*

Case report

A twenty-four-year-old female patient presented a problem with excessive exposure of the gingiva while smiling. The patient reported orthodontic treatment, completed in July 2017, and composite veneers on upper canines and incisors. Upon clinical examination, mixed GS (excessive gum exposure both anterior and posterior to canine segments) was found. The vertical dimension of the maxilla did not deviate from the normal range, the heights of upper, middle and inferior horizontal facial thirds were 60 mm, 62 mm and 61 mm, respectively. Upper lip height was 21 mm (subnasale - stomion distance). Gingival exposure while smiling was measured as 6 mm over the tooth 11, and 7 mm over tooth 21. The patient did not agree to a gingivectomy, gingivoplasty or replacement of composite veneers of upper incisors. Gummy smile correction with BTA was recommended to reduce excessive muscle contraction, thus minimising gingival exposure. The patient signed a consent form before the procedure. Photographs were taken before the procedure. *Figure 2* shows the position of the lips at rest and at maximum smile. Two Yonsei points were located and marked 1 cm laterally from the nose wing and 3 cm above the angle of the mouth, BTA injected in these points affects three muscles - levator labii superioris alaeque nasi, zygomaticus minor and levator labii superioris[10]. A topical skin anaesthesia (Emla cream 5%, lidocaine and prilocaine, Aspen Pharma) was applied on the planned injection areas, 20 minutes before injection. The reconstitution of BTA (VISTABEL, Allergan) was carried out in accordance with the principles of good practice and with particular regard for aseptic principles. Botulinum toxin type A was reconstituted in 1,25 ml 0.9% saline injection without preservatives. To prevent BTA denaturation, the solution was prepared by slow injection of 0.9% saline into the vial, which was spun gently to prevent bubble formation.

After reconstitution, a visual inspection of the solution was performed.

A clear, colourless solution without particles was obtained,

which was then used for the procedure.
The injection was administered at the centre of the highest activity located in the lower part of the levator labii superioris (*Figure 3*). Two dose units of BTA were used for one point.
At the follow-up examination visit two weeks after the procedure, the patient did not report any postoperative disturbances. No side effects or complications were observed in clinical examination. Gingiva exposure was 1 mm above the incisors when smiling, a 5 mm (the tooth 11) and 6 mm (the tooth 21) reduction were obtained (*Figure 4*). The patient was satisfied with the correction of GS and no additional BTA injections were necessary.

Discussion

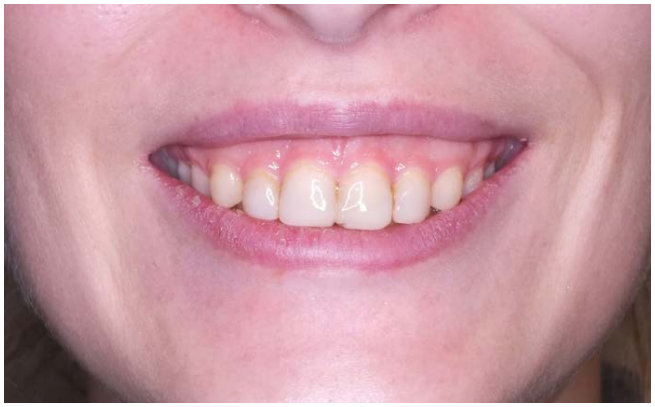

Figure 1 *- Gummy smile.*

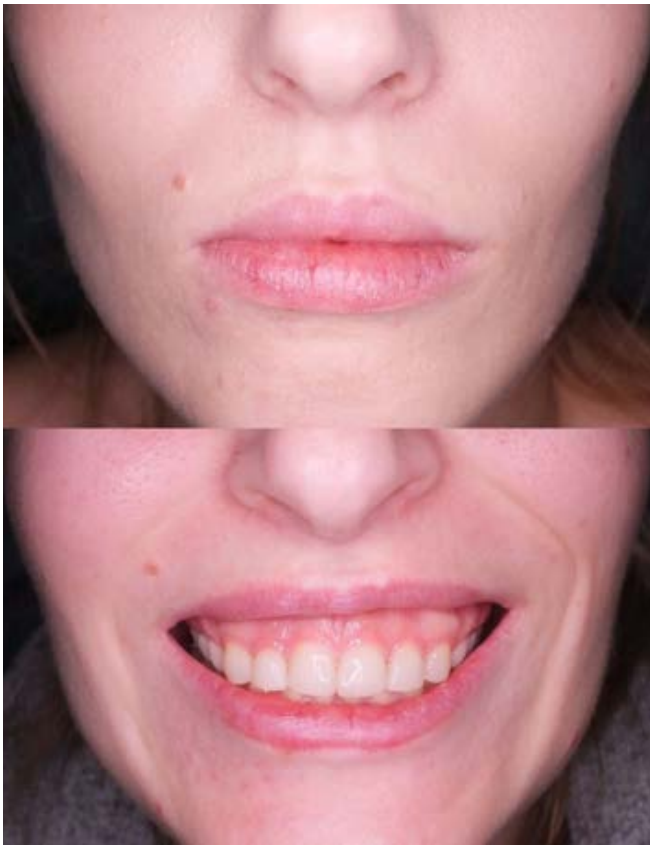

Figure 2 *- Position of the lips at rest and at the maximum smile.*

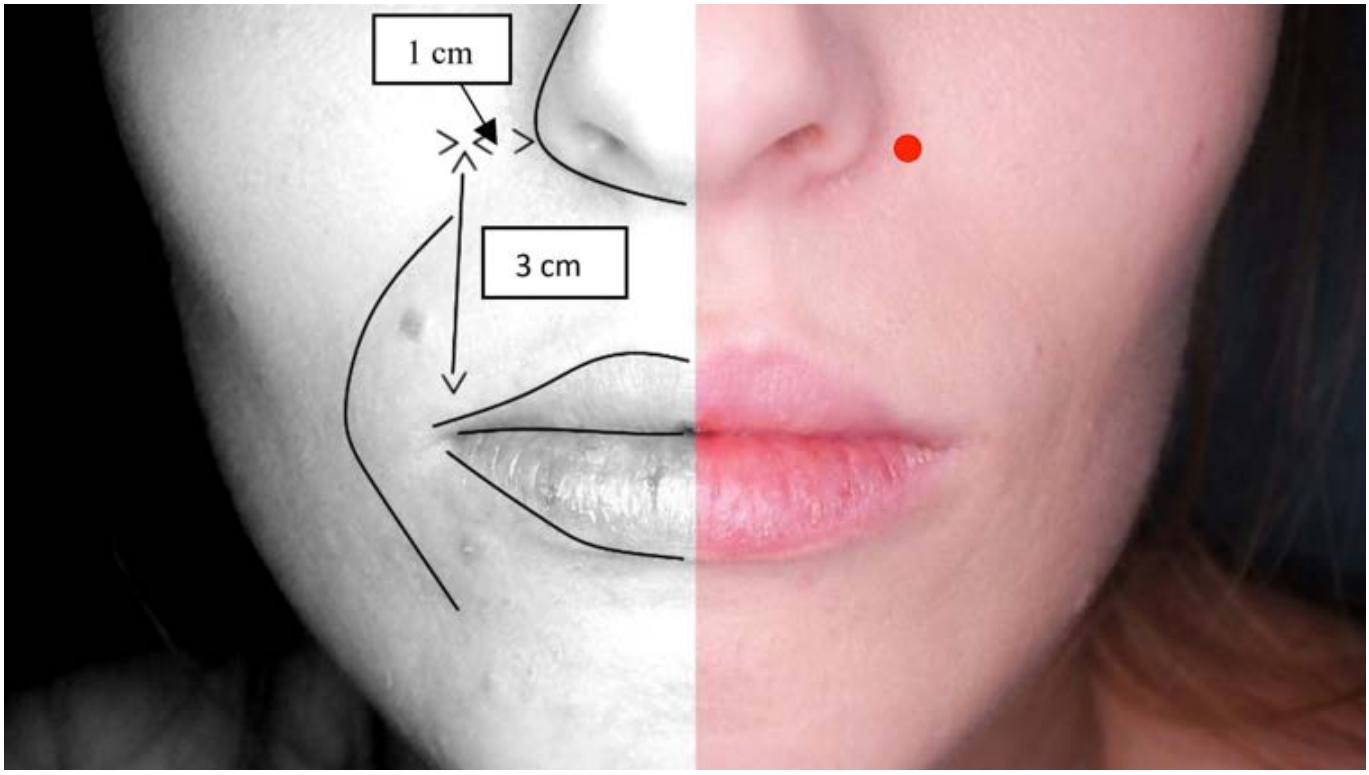

Figure 3 *- Place of injection of BTA.*

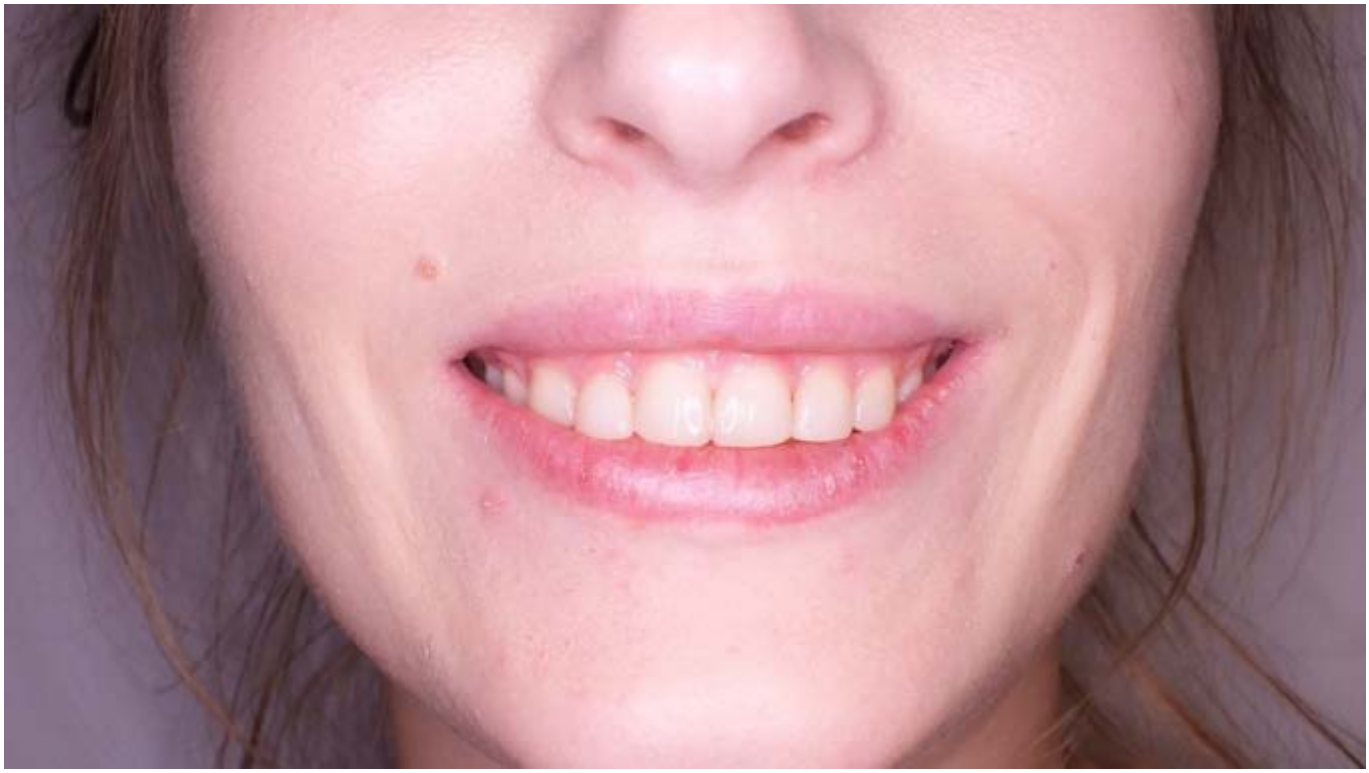

Figure 4 *- Correction of gummy smile – 2 weeks after injection of BTA.*

Gummy smile affects approximately 6% of the population[11]. Due to different aetiology, the treatment method should be chosen after a clinical examination and detailed diagnosis. In this study, the BTA injection technique was chosen because of lip muscle contraction, while other important parameters were normal.

The upper lip height was within the range of average values for Caucasian women (20 to 22 mm)[12], while the ratio of the upper, middle and inferior horizontal facial thirds were close to parameters defining a harmonious face[6]. There were no contraindications for BTA injection. Botulinum toxin affects the neuromuscular junction and presynaptic membrane of cholinergic neurons, where it inhibits the release of acetylcholine and causes temporary muscular paralysis or organ function loss. Generally BTA is only used for medical indications. The first effects are visible about 24 hours after injection, and the maximum therapeutic effect is observed after 2 weeks. Although muscle function returns to normal approximately three months after the injection, due to the functional recovery of neuromuscular junctions, clinical results can persist much longer, for as long as 6 months after the procedure, due to i.e. atrophy[13].

Diaspro et al. assessed a method of GS reduction with hyaluronic acid. Patients who displayed at least 3 mm gingiva in the maxilla were included in the study. The procedure was completed with the injection of hyaluronic acid into the paranasal region, 3 mm laterally from the nose wing. The authors stated that hyaluronic acid injections were dangerous in this area due to blood vessels presence and the risk of vascular complications[14]. The use of BTA does not induce such a risk. The most commonly used method of treatment of GS associated with EVM is LeFort I surgery[15,16] while for GS caused by APE is gingivectomy with or without osteotomy[17,18,19]. Excessive lip muscle contraction can be treated by removing part of the vestibular mucosa[20,21], partially resectioning the levator muscles or subperiosteal dissection of the levator labii superioris[22]. Moderate GS related to an inefficient, short lip can be successfully treated by surgical reduction of the muscle activity of the zygomaticus major, orbicularis oculi, levator anguli oris, levator labii superioris, and levator labii superioris alaeque nasi[23]. In the past, the myotomy of muscles involved in smiling was recommended as an independent procedure. Nowadays, lip repositioning is performed together with rhinoplasty as a part of plastic surgery procedures, and is rarely used as a method for GS correction[24]. In 1979, Litton and Fournier described the surgical correction of GS and short lip, which involved the correction of the levator lip muscles[25]. Miskinyar treated GS with myomectomy and the partial resectioning of one or both levator labii superioris[26]. Lip repositioning surgery for patients with insufficient attached gingiva width and with severe vertical maxilla growth is not recommended[8]. Laser therapy is another approach for the treatment of GS[27,28]. Narayanan et al. presented two subjects in whom diode laser (810 nm) in continuous mode with a power between 0.8 and 1.5 watts was applied for gingivoplasty[28]. Dental laser therapy involves minimally invasive procedures and is a well tolerated method[27,28]. Excessive correction should be avoided in this technique, as the upper lip lengthens with age[29]. Another method considered for GS correction is silicone implant placement between the muscles of the upper lip and the anterior nasal spine[30]. However, all of these GS treatment methods are invasive and irreversible, whereas the use of BTA presented in this study is minimally invasive, safe and

has a transient effect. Botulinum toxin type A can be an alternative treatment for patients with GS caused by excessive muscle contraction, who do not agree to invasive surgery. The use of BTA can support surgical treatment. The procedure is suitable for patients who expect a temporary correction, as the effects of BTA persists from 3 to 6 months[9,31].

Conclusion

Gummy smile is an aesthetic problem that affects many patients. Suitable treatment planning and the appropriate selection of treatment methods should be preceded by thorough clinical examination and profound diagnosis, with the identification of etiological factors. A wide range of GS treatment methods are available, though some are invasive. Injections of botulinum toxin type A constitute a valuable alternative to surgical treatment of GS with muscular aetiology.

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The authors declare that they have no conflict of interest.

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aesthetic medicine

Case Report

Body reshaping in a young woman on a very low calorie ketogenic diet with protein replacement: a case report

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Abstract

Objective: weight regain is the most common consequence of dieting; therefore weight loss should be strictly associated with Fat Mass (FM) loss and with the preservation of Fat Free Mass (FFM). The aim of the case report was to evaluate the effectiveness of a Very Low Calorie Ketogenic Diet (VLCKD) with protein replacement to preserve lean body mass.
Materials and methods: our patient was a 44-year old woman seeking body reshaping after pregnancy. We analyzed her blood tests, collected her anthropometric data and performed bioelectrical impedance. She followed a VLCKD with protein replacement for four weeks.
Results: after four weeks of treatment, the patient lost more than 5% of body weight, exclusively as FM and extracellular water. All body circumference and Body Mass Index values were improved.
Conclusion: the case report demonstrates the efficacy of the VLCKD in terms of FM loss and body reshaping. Protein replacement is useful for ensuring correct protein intake while preserving lean body mass.

Keywords

VLCKD, ketogenic diet, weight loss, body reshaping, fat mass, lean mass

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Introduction

Recently, Ketogenic Diets (KD) have emerged in literature suggesting their therapeutic potential in several diseases.
Ketosis is a physiological process which occurs when the supply of glucose is restricted.
Ketone bodies are produced by the liver to serve, together with free fatty acids, as a readily oxidizable fuel in various tissues[1]. KDs include dietary treatments characterized by a reduction of carbohydrates (usually less than 50 g/die) and a relative percentage increase in fat and protein[2]. It has been demonstrated that Very Low Calorie Ketogenic Diets (VLCKD) are effective in weight loss because of reduced energy intake and the use of energy derived from protein, an 'expensive' process for the body[3].
Moreover, VLCKD with amino acid supplementation is associated with weight loss at the expense of Fat Mass only, preserving Fat Free Mass, unlike a restricted-calorie but not ketogenic diet[4]. Avoiding weight regain is the biggest challenge when dieting and VLCKD has proved to be an effective treatment because only Fat Mass is reduced, with no changes to resting metabolic rate[5].

Meterials and Methods

The patient was a 44 year-old woman seeking weight loss after pregnancy. She was not breastfeeding.
She was diagnosed with hypothyroidism and she was taking levothyroxine (100 mg/die); her blood pressure was normal. Anthropometric measurements were taken.
Patient body weight was 72 kg, measured to the nearest 0.1 kg on electronic devices (SECA®) in underwear and without shoes. The height, calculated with a stadiometer (SECA®) to the nearest 0.1 cm was 165 cm, Body Mass Index (BMI) was calculated as weight (kg)/ height (m2) and was 26.4. According to BMI categorization, the patient was classified as overweight (BMI > 24.9)[6].
Body circumferences were evaluated with a measuring tape; waist circumference, a central obesity parameter, was 83 cm, abdomen circumference was 103 cm and hip circumference was 106 cm. Based on a < 80 cm waist circumference upper reference range for women, the patient was classified as having abdominal obesity[7].
We performed Bioelectrical Impedance Analysis (BIA) to evaluate body composition using Nutribox®.
Nutribox® is a single- frequency BIA (SF-BIA) at 50-kHz.
The 50-kHz serial model is the most common model used for in vivo analysis of body water compartments, based on resistance (R) and reactance (X) as measured at 50-kHz[8]. Whole-body BIA allows the determination of Fat Free Mass and total body water in subjects free from significant fluid and electrolyte abnormalities, when using appropriate population, age or pathology-specific BIA equations and established procedures[9].
In addition, BIA gives an indirect estimate of Fat Mass (calculated as the difference between body weight and Fat Free Mass)[10]. SF-BIA results were taken into account to prescribe a suitable VLCKD diet with amino acid replacement which the patient followed for four weeks, after which the same instruments were applied in the same conditions to test for any changes.
The energy intake was < 1000 kcal/day with 55% of energy from fat, < 10% of calories from saturated fat, 5% of energy from carbohydrates (< 20 g), and 40% of energy from protein, corresponding to 1.15 g/kg of body weight per day. A 60% daily protein intake was achieved using an amino acid supplement made of isolated whey protein (Macresces, Italfarmacia, Rome).
Diet adherence was tested through urinary ketone excretion as measured by keto-sticks.

Results

The blood test (*Table 1*) showed high total cholesterol and LDL cholesterol and a vitamin D deficiency, according to current guidelines[11].

BUN	28 mg/100 ml
Blood glucose	87 mg / 100 ml
Insulin	5.62 IU/ml
Creatinine	1.00 mg/ dl
AST	20 U/l
ALT	18 U/l
HbA1c	5.20 %
HDL Cholesterol	52 mg/ 100 ml
LDL Cholesterol	136 mg/ 100 ml
Total Cholesterol	212 mg/100 ml
Triglycerides	139 mg/100 ml
Uric acid	4.9 mg/100 ml
Vitamin D	13.3 ng/ml

Table 1 *- The table shows patient blood test results.*

At the end of the 4 week VLCKD treatment a weight loss of four kg was observed, with BMI = 25, waist circumference = 79 cm, abdomen circumference = 100 cm and hip circumference = 100 cm. SF-BIA outcomes were compared to the first evaluation (*Table 2*).

	T0	T1
	Baseline	After 4 weeks of VLCKD
Resistance (R)	471	484
Reactance (Xc)	44	48
Fat Free Mass	25.6 kg	25.8 kg
Fat Mass	19.4 kg	16.8 kg
Total body water	38.5 L	37.5 L
Extracellular water	27.1 kg	25.5 kg
Phase angle	5.3	5.7

Table 2 *- The table shows BIA outcomes at baseline and after four weeks of treatment.*

Discussion

After clinical assessment, a VLCKD was recommended.
A diet low in carbohydrates seems to improve lipid blood profiles. VLCKD can modulate cholesterol endogenous synthesis and increase High Density Lipoprotein[12,13].
VLCKD was an effective dietary treatment for Fat Mass loss. Interestingly, amino acid replacement was effective in preserving Fat Free Mass, confirming the hypothesis that Fat Mass is the only target of a VLCKD. Weight loss associated with the loss of Fat Mass but not of Fat Free Mass led to effective body reshaping.
Furthermore, anthropometric measurements showed a waist circumference of < 80 cm, thus decreasing the risk of central obesity. BMI was also improved.

Conclusions

The patient lost more than 5% of body weight; said weight loss was a consequence of Fat Mass and extracellular water loss alone.
VLCKD proved to be an effective therapy tool for Fat Mass loss and body reshaping as it also preserved Fat Free Mass.

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Obituary

In memory of Dr. Olga Sergeevna Panova

Dr. Olga Sergeevna Panova

It is with great regret we announce the loss of the colleague and friend Dr. Olga Panova, Dermatologist, President of the Russian Society of Aesthetic Medicine, and a great lady.
She had been also the President of the Union Internationale de Médecine Esthétique - UIME from 2013 to 2015, to which her Society belongs since 2001.

She was an excellent colleague and a wonderful person. We will always remember her smile and kindness.

UIME is closed to her husband Anatoly and her daughter Katya.

Courses and Congresses

Due to the Covid-19 related medical emergency, this page is suspended until further notice

aesthetic medicine