Minimally invasive Procedures for Facial Rejuvenation

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Botulinum Toxin Type a Treatment in Facial Rejuvenation

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Abstract

In this chapter we analyze the ever expanding potential uses of botulinum toxin in medical practice focusing on its cosmetic used in facial rejuvenation. We see how, from a single region and procedure for the treatment of the glabellar lines, the use of botulinum toxin has evolved into multiple areas, techniques, dosages and now new toxins in this ever expanding field. We focus on the use and dilutions of the two best-known botulinum toxin type A commercial products, making a distinction between their "label" and "off-label" uses. We consider that a thorough knowledge of the process of the facial aging and pertinent anatomy of the facial musculature is paramount to treat the patients successfully. We provide tips and hints on patient's management, from the selection through the assessment to the post-treatment care. We describe the clinical applications and preferred techniques used when injecting the mimetic facial and neck muscle with a detailed description for each clinical indication that maximize the treatment of the targeted muscles while minimizing potential complications. Finally we point out how the new concepts of aging and rejuvenation are opening new paths on the use of botulinum toxin together with other medical and surgical procedures in combined treatments.

Introduction

Botulinum Toxin type A (BTX-A) has dramatically transformed the aesthetic care of the aging face since its initial FDA approval in 2002 for the temporary treatment of glabellar lines. BTX-A injection continues to be the single most common aesthetic procedure in the USA and Europe, although its use is accompanied by growth in the use of other modalities, including dermal fillers [1]. The clinical uses of BTX-A are extensive and in continuous evolution. The "ideal" candidates are men and women between 40 and 60 years of age whose skin wrinkles have been formed by habitual muscle contraction [2]. Cosmetic indications have extended to include the treatment of hyperfunctional facial lines in the forehead, periorbital, perioral and neck regions, as well as facial asymmetry and muscle spasm. All of these aforementioned uses are non-FDA approved ("off-label"), though legal indications of BTX-A when managed

by licensed physicians. Botulinum toxin has also been employed to treat strabismus [3], cervical dystonia [4], hyperfuntional larynx [5], pain and headache [6], temporomandibular disorders [7], bruxism [8] and several other conditions [9]. The treatment with BTX-A belongs to the broad field of nonsurgical rejuvenation procedures who are directed at the texture and quality of the skin and volume deficiencies in the dermis and underlying soft tissues. The most important goal of BTX-A treatment in cosmetic medicine is to achieve a balance between dynamic rhytids caused by hyperactive muscles while maintaining natural facial animation. Different kinds of nonsurgical treatment of the facial aging are available and depend on the nature of the deformity (wrinkles, folds, furrows), on the skin layer in which the defect occurs or on the technique used to treat it (exfoliation, dermal and subcutaneous fillers, resurfacing, and chemodenervation). Optimal treatment often requires a multifaceted approach due to the many causes of wrinkles, various anatomic locations, individual patient preferences and the effects of aging; therefore the treatment should be individualized according to the causes and the patient's anatomy, goals and tolerance. Nonsurgical methods are often performed together and also in conjunction with surgical procedures to complement, enhance, or address a condition that cannot be corrected through surgery alone [10,11].

Structure and Mechanism of Action

Botulinum Toxin (BTX) specifically and physiologically denervates the muscle targeting the release of acetylcholine. BTX is produced by the anaerobic bacterium Clostridium Botulinum. It is a gram-positive, spore forming obligate anaerobe that is found naturally in the soil. It is produced by fermentation of strain C. Botulinum Type A grown in a culture medium. In order to be used as a drug the toxin has to be isolated, purified and stabilized. There are seven serotypes of botulinum neurotoxins (A, B, C1, D, E, F, and G) produced by different strains of C. botulinum with serotype C2 being cytotoxic and not neurotoxic. The human nervous system is susceptible to five toxin serotypes (A, B, E, F, G) and unaffected by the rest. Although all toxins have different molecular targets, their action leads to the blockade of the cholinergic nerves. However, only the A and B toxins are available as drugs. All of the botulinum neurotoxins are synthesized as single-chain proteins of approximately 150-kDa that must be nicked or cleaved by proteases into di-chain molecules of approximately 100-kDa and 50-kDa subunits in order to be active [12]. Cleavage results in a di-chain molecule consisting of an approximately 100-kDa heavy chain and an approximately 50-kDa light chain, linked by a disulfide bond (Figure 1) [13]. Botulinum producing organisms may be classified as proteolytic or non-proteolytic, denoting the presence or absence of endogenous enzymes that cleave the 150-kDa single chain neurotoxin into the active di-chain neurotoxin [14]. Type A-producing strains are proteolytic and nearly all of the toxin recovered from these organisms (>95 per cent) exists in the di-chain form and this makes Botulinum Toxin type A the most powerful and long lasting of all the subtypes [15]. Type B-producing strains may be either proteolytic or non-proteolytic. Proteolytic type B strains have been found to cleave approximately 30 per cent of the single-chain proteins, although the percentage nicked in the commercial product based on the B serotype may be significantly higher [16]. Clostridial strains that synthesize toxin serotypes E and F are nonproteolytic and the toxin they produce must be exposed to exogenous proteases in order to exert its activity [17,18]. Botulinum neurotoxins are produced as part of a multimeric protein complex consisting of the neurotoxin and associated hemagglutinin and non-hemagglutinin proteins [14]. The number and identity of the associated proteins vary by serotype and organism. The associated proteins serve to stabilize and protect the neurotoxin molecule from degradation [19,20]. Both A and B neurotoxins can be found in a 500-kD

form but A can also be found in a 900-kD form, and this has been the size reported for the crystallized type A toxin used clinically (Figure 2) [21,22]. The toxin produces chemodenervation by preventing release of acetylcholine at the neuromuscular junction of the peripheral nervous system and at ganglionic nerve terminals of the autonomic nervous system within 6 to 36 hours of exposure to muscle with maximum effect up to 7 to 14 days [11]. Acetylcholine is a common neural transmitter and stimulates striated as well as smooth muscles and the secretion of glands such as sweat glands. In both A and B neurotoxins the heavy chain acts like a "Key in the Lock" and is responsible for selective binding of the toxin molecule to high affinity external receptors situated on the membrane of presynaptic cholinergic nerve terminals. The light chain acts inside the cell to prevent acetylcholine release. Within the cell, the light chain of type A cleaves SNAP 25, a 25-kD synaptic cell-associated protein, while the light chain of type B cleaves Vesicle-Associated Membrane Protein (VAMP). This chain of events brings to the flaccid paralysis of the targeted muscle or the functional block of the glandular activity [23].

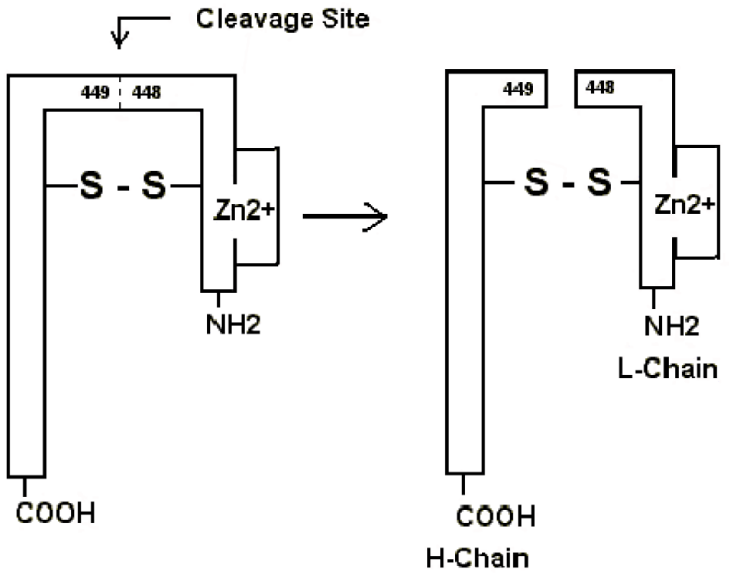

Figure 1: Schematic structure of the single-chain neurotoxin and cleavage site and its transformation into the active di-chain neurotoxin.

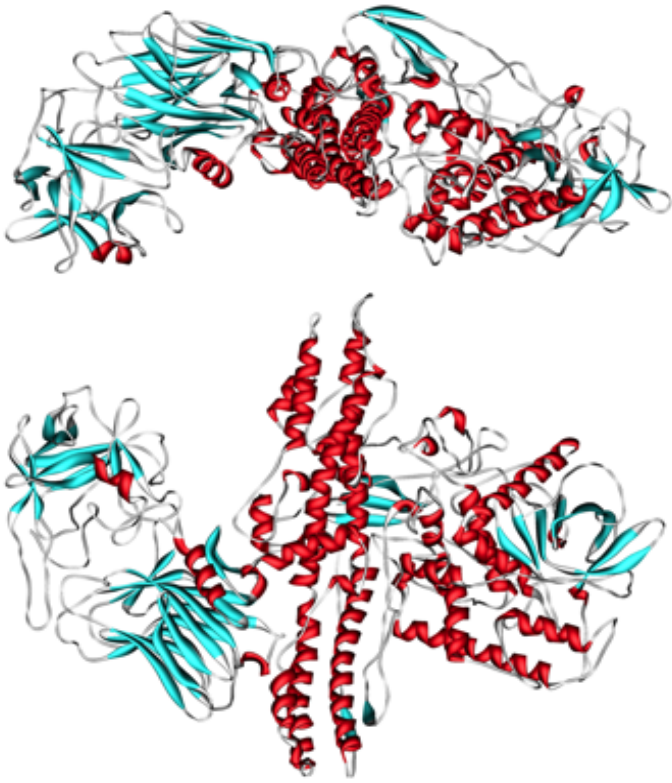

Figure 2: Molecular structure of botulinum neurotoxin 3D.

Botulinum Toxin blocks neuromuscular transmission through a three step process. First, the toxin binds to presynaptic cholinergic motor nerve terminals. Next, the toxin is internalized into the nerve terminal by endocytosis, where it eventually enters the cytoplasm. Finally, it inhibits acetylcholine release by cleaving a cytoplasmatic protein (Figure 3).

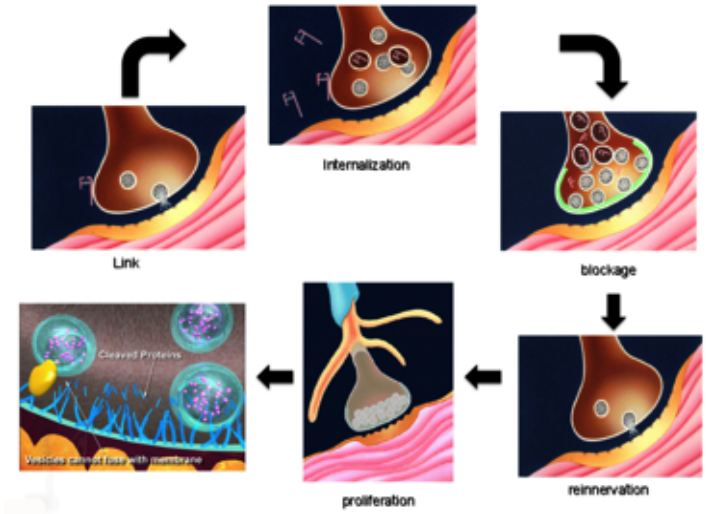

Figure 3: Mechanism of action.

At therapeutic doses the toxin induces paralysis limited to the injected muscle; however, the toxin has the potential to cause paralysis or weakness of adjacent muscles by diffusion and spread. Diffusion results from toxin moving down its concentration gradient, which is affected by local receptor concentration whereas spread depends on the solution volume and injection technique (physically pushing the toxin from the area of injection). Depending on the strength of the muscles treated and the dosages used, the duration of the effect varies from a couple of months to several months. The action of the drug slowly decreases over time as the affected axons sprout new nerve terminals which continually restore the impaired transmission. During this phase the damaged synapse itself will regenerate its function so that the sprouts regress as the clinical effects of the drug subside [24]. The muscular function gradually returns after 3 months and axonal sprouting and reorientation of muscle fibers prevent permanent paralysis of muscles that are treated repeatedly [11,25].

Toxicity

Botulinum toxin is one of the most potent toxins known. It is estimated that in a 70Kg adult, the lethal dose of crystalline botulinum toxin type A would be approximately 0.09 to 0.15 mg by the intravenous or intramuscular route, 0.7 to 0.9 mg by inhalation and 70 mg if ingested orally. The toxin does not penetrate intact skin, and person to person transmission does not occur [11]. The lethal dose of Botox/Vistabex (Onabotulinumtoxin A) is measured in units, with 1 unit being the lethal dose of toxin causing death in 50% of a group of 18 to 20gr female Swiss Webster mice within 3 days of intraperitoneal injection. The median lethal dose has been estimated to be 2700 (2500 to 3000) units in a 70 kg human, based on the median lethal dose of approximately 40 units/kg in primates [11]. Note that units of Dysport/Azzalure (Abobotulinumtoxin A) are expressed in Speywood Units (S.U.).

Different Products

The three most widely available BTX products approved for cosmetic indications are Onabotulinum Toxin A (OnT-A); Abobotulinum Toxin A (AbT-A) and Incobotulinum Toxin A (InT-A). Botox is the original OnT-A product, approved in 2002 by the FDA, it is produced by Allergan Inc. and it is marketed for cosmetic use in the US as BOTOX Cosmetic®, in Italy as Vistabex® and in the rest of Europe as Vistabel® [26]. Dysport® (Medicis Aesthetics Inc., Scottsdale, AZ, USA), approved in 2009 for cosmetic use [27] is currently marketed in Europe under the commercial name of Azzalure® (Galderma, Germany). More recently, another BTX-A formulation, under the non-proprietary name of incobotulinum toxin A (Xeomin® or Bocouture®; Merz Pharmaceuticals GmbH, Frankfurt, Germany) has received approvals for cosmetic use in many countries including Italy (Figure 4) [28]. In the Asian countries we can also find the recently released Chinese BTX-A (Prosigne®, Lanzhou Biological Products Institute, China) and Neuronox® (Medytox Inc., Ochang Science Complex, Chungcheongbukdo, Korea). Worth mentioning is the recently developed BTX-A toxin named PurTox® (Mentor Corporation, Santa Barbara, CA) which will be ready to hit the market real soon. Among these products, BOTOX is the most commonly used worldwide for all indications, followed by Dysport. NeuroBloc®, also marketed as Myobloc® (Solstice Neurosciences Inc.) is the only commercial available type B BTX. Although there is some data on its efficacy in aesthetic indications, it is not often used for these indications [29,30]. Cervical dystonia is currently its sole approved indication [31]. The authors' personal experience is limited to Vistabex® (or Botox Cosmetic®) and Azzalure® (Dysport®), which will then be the subject to this chapter. Both Azzalure®/Dysport® and Vistabex®/Botox® are supplied in a sterile vial, fitted with rubber stopper and sealed with an aluminum cap, containing botulinum toxin type A in the form of a sterile, lyophilized powder without preservatives in vacuum packed. Each vial of Dysport® contains 300 units of botulinum type A complex, 125 ug of human serum albumin, 2, 5mg of lactose, and trace amounts of cow's milk protein. A 500-unit vial of Dysport® is also available for cervical dystonia. Azzalure®, commercial trademark in Europe, is distributed in a 125-unit vial. Botox Cosmetic® (Vistabex®/Vistabel®) is supplied in a vial with 100 units of dried neurotoxin complex, 0.5mg of human albumin and 0.9mg of sodium chloride. A 50-unit vial of Botox Cosmetic® (Vistabex®/Vistabel®) is also available.

Figure 4: Most common used commercial products of Botulinum Toxin Type A.

It is important to note that 100 units of Botox cosmetic® is not equivalent to 100 units of Dysport®. Before use vials should be stored in a refrigerator at a temperature of 2-8°C and could be stored for up to 24 months. In order to be injected, the reconstitution is performed with sterile 0.9% unpreserved normal saline [32] although studies have demonstrated that preserved saline provides increased patient comfort without decreasing efficacy [33]. If the reconstituted product isn't injected immediately, it must be stored in the refrigerator for no longer than 24 hours, although some studies suggest that the activity can be maintained for up to 6 weeks after reconstitution [34]. Most practitioners discard unused reconstituted toxin after 1-7 days. Before injecting the rubber stopper can be removed from the vial before mixing to prevent foaming and the saline solution is introduced by means of a 1ml Luer-Lok syringe with a 22- or 25-gauge needle. The same needle is used to withdraw the reconstituted solution and is then replaced with a 30- or 32-gauge needle, which is used for injection.

Although there are no special handling precautions when using Botox, it is important to note that alcohol could neutralize the toxin. Therefore, if the practitioners use alcohol to prepare the skin, it should be allowed to dry before injection.

Dilution Considerations

The recommended dilution for Botox Cosmetic®/Vistabex® ranges from 1 to 4 ml per 100 unit vials (0.5-2 ml per 50 unit vial). The manufacturer's recommendations and initial FDA approval for the treatment of glabellar lines were based on 2.5 ml (0.1 ml = 4 units) of unpreserved normal saline solution per 100 units [35]. The maximum total recommended dose is 300 to 400 units at any one session and not more than 400 units over a 3-month period [11]. The recommended dilution for the 300 unit vial of Dysport® is 2.5 ml of 0.9% sodium chloride to yield a solution equivalent to 10 units per 0.08 ml. It can also be reconstituted with 1.5 ml of sodium chloride for a solution of 10 units per 0.05 ml. Each 500-unit vial is reconstituted with 5 ml of saline to yield a solution of 10 units per 0.1 ml. The recommended dilution for the 125-unit vial of Azzalure® is 0.63 ml of normal saline to prepare a solution of 10 units per 0.05 ml [26]. The maximum total recommended dose is 1000 units at any one session. As aforementioned we have to be aware of the differences in dosing between Botox Cosmetic®/Vistabex® and Dysport®/Azzalure®, since the units are not interchangeable. Azzalure®/Dysport® is less active on a unit-per-unit comparison with Botox Cosmetic®/Vistabex®, which means that more units of Azzalure®/Dysport® are required to achieve similar clinical effects. The FDA mandated in 2009 that all BTX-A product labels clarify that the potency units for each product are specific to each preparation, however, in common practice many providers have used a dose (unit) equivalent ratio of Botox® and Dysport® of 1:2.5 or 1:3 suggested in the literature. Note that the use of this conversion ratio is most appropriate when considering the safety profile of the product, not its efficacy [36-39]. For what concern Incobotulinum toxin A (Xeomin®/Bocouture®) the recommended dilution with unpreserved saline is 1.25 ml per 50 units, giving a final concentration of toxin of 40 units/ml [40]. In terms of potency, Xeomin®/Bocouture® appear to exhibit a 1:1 dose ratio when comparing with Botox [41]. The reconstitution, dilution, and storage are matters of physician preference and the providers will develop a familiarity with the efficacy of each formulation of BTX-A through experience (Table 1).

Type and Concentration of Botulinum Toxin	
Type of Botulinum Toxin	**Concentration (units/ml)**

Dysport® 300 - unit vial	
1 ml	30/0.1
1.5 ml	20/0.1
2.5 ml	12/0.1
3 ml	10/0.1
Azzalure® 125 - unit vial	
0.63 ml	10/00.5
Botox Cosmetic® Vistabex® , Vistabel® 100 -unit vial	
2 ml	5/0.1
2.5 ml	4/0.1
4 ml	2.5/0.1
Botox Cosmetic® Vistabex® , Vistabel® 50 - unit vial	
1 ml	5/0.1
2 ml	2.5/0.1

Table 1: Recommended dilutions for Botox Cosmetic®/Vistabex® and Azzalure®/Dysport® [11].

After intramuscular injection, botulinum may spread or diffuse. "Spread" occurs immediately after the injection itself, which is related to technique, volume of injection, and needle size. "Diffusion" occurs over several days as the toxin passively moves away from the injection site [42]. Generally Azzalure®/Dysport® has a larger area of action, and there has been ongoing debate regarding the difference in onset between Botox Cosmetic®/Vistabex® and Azzalure®/Dysport® [43]. Based on the concept of diffusion and spread it has to be kept in mind that the mimic muscles of the face are in a very intimate relationship in between themselves, therefore, to achieve the most accurate effect on the targeted muscle we should use highly concentrated doses of the drug, injected by means of very small volumes of solution [44]. In general large dilutions with lower concentrations of toxin require larger volumes of injections to achieve the desired result, which can increase the potential for spread of the toxin to unwanted surrounding areas. The general trends currently agree on the use of low volumes on high concentrations of toxin [45].

Infiltration Technique

A 30-gauge 1-inch needle is used to perform the injections. Topical anesthesia with ice or other agents may be beneficial to decrease pain associated with injections but is not necessary. In order to estimate the expected benefit from botulinum toxin A injection, a glabellar "spread test" may be performed prior to injecting by spreading the glabellar wrinkles apart with the thumb and index fingers. Patients with sebaceous skin and deep dermal scarring that are not improved with manual spreading usually respond poorly to BTX-A injection. With the exception of the perioral and periocular areas, injections should be made into the muscle belly perpendicular to the skin. The needle is advanced to the periosteum and then withdrawn slightly to place the needle in the muscle. It is useful to use facial animation to mark the areas that require treatment. Injections should be made with the patient reclined to 45°C even though some authors prefer the patient to sit in the upright position or lay down completely. When injecting, the skin should be slightly stretched to assist in identifying superficial vessels that can be avoided to decrease the risk for ecchymosis. If a bruise starts to develop, the

practitioner should hold 5-10 minutes of pressure to avoid a hematoma, which could lead to migration of the toxin. The injection should be performed slowly in order to minimize discomfort. Patient should be told not to massage the area as it may cause diffusion of the drug and result in weakness of unintended muscles. Patients should also be advised to contract the treated areas as it may increase local uptake of the toxin [11]. It is preferable to start treatment with the lowest dose so to test patient responsiveness and, if required, to increase botulinum units in succeeding sessions.

Microinjection Technique

The microinjection technique is used to administer low doses of botulinum toxin very superficially. BTX-A applied by microinjection technique in the crow's feet area will decrease the risk of an involuntary co-treatment of the m. zygomaticus major. The microinjection technique follows an intradermal approach; small amounts of toxin (less than 0.025 ml) are injected approximately 1 cm apart, very superficially, in a technique comparable to the intradermal skin test.

Patient Selection

Experience has changed the way BTX-A is now used. Patient's feedback after the injections and the analyses of results has led to the understanding that muscular inhibition does not necessarily promote a cosmetic upgrade. The feared "frozen look" belongs to the past and both patients and injectors understand that a natural look is desired. Treatment with BTX-A injection should be individualized. The duration of effect changes from patient to patient because every individual is different and so is muscular behavior. Some of the patients are symmetrical while others are quite asymmetrical. Other patients have single muscle insertions while others may have multiple muscle insertions, which may also vary the choice of treatment. Certain patient characteristics and anatomic features help to define good, or conversely, less acceptable candidates for botulinum toxin injection. De Maio et al., classify the patients into three groups before treatment, based on their muscle tonus; kinetic, hyperkinetic and hypertonic. The Kinetic patients are the easiest to treat, especially for the beginner, and the duration effect is the longest among the groups. The hyperkinetic patients are victims of excessive muscular contraction and they usually return for treatment twice or three times a year depending on when the effect starts to fade, being the most common group for BTX treatment. The last ones, the hypertonic patients represent the negative result of lack of control of the hyperkinetic ones. They are the group that particularly needs treatment and usually get frustrated with. The result in these patients is the shortest of all groups and they should be told immediately about the limitations of treatment with BTX-A alone and should be treated with fillers or other surgical methods [46,47].

Patient Pre-Treatment Assessment

The practitioner has to take record of the medical history of the patient including prior treatments with botulinum toxin, topical agents or injectable fillers. The facial features have to be evaluated both at rest and in maximal facial animation to best understand the anatomic extent and relative strength of the target muscle. This information will dictate modifications in the dose required for the desired effect [9]. The patient is instructed to accentuate the specific facial lines to be treated by frowning, squinting and elevating the brow. Clinician

should set realistic expectations and discuss if off-label use is planned. The patient should be fully aware of the cause of his or her condition and the potential role of botulinum toxin in improving it; the practitioner should explain that the toxin's mechanism of action is to address hyperkinetic muscles of facial expression, causing them to relax and therefore releasing the overlying wrinkle rather than filling it in or sanding it down and that the effect of the toxin may not manifest for 24-72 hours after injection, with optimal result seen at 2 weeks. The patient should be informed that the results typically last 2-3 months, even though some authors have reported results lasting 6 months or more [34,48-52] and also that an assessment and possible retreatment should be done 14 days post-injection when the maximum effect of the toxin has been reached. Planning for concurrent dermal filler or subsequent surgical treatment can be discussed. Most important is to evaluate the patient for pre-existing ptosis in order to discuss with him/her the actual risk/benefit of the treatment. Patients are instructed to avoid using aspirin products, vitamin E, nonsteroidal anti-inflammatory agents and dietary supplements that predispose them to bleeding or coagulation problems, for 10 to 14 days before treatment to minimize ecchymosis [53,54]. Pretreatment photography is highly recommended to document any pre-existing asymmetry, to assess efficacy and to guide pre and post-injection treatment plans. As with any aesthetic procedure photodocumentation is fundamental to help resolve any issues raised by the patient following treatment. An informed consent is required, with detailed mention of possible complications. In general the main side effects that a patient may encounter include: pain at the injection site, bruising and unexpected weakness of muscle groups (for example eyelid ptosis) [55,56].

Contraindications to BTX treatment

To avoid adverse events certain contraindications have to be ruled out prior to the treatment with botulinum toxin. Patients with hypersensitivity to the ingredients (Albumin, sodium chloride, botulinum toxin); neuromuscular disease (myasthenia gravis, Easton-Lambert syndrome, motor neuron disease); patients treated with BTX concurrent with other drugs that interfere with neuromuscular transmission such as antibiotics (aminoglycosides, lincosamides, polymixins), penicillamine, quinine, calcium channel blockers, neuromuscular blocking agents (atracurium, succinylcholine), anticholinesterases, magnesium sulfate and quinidine, all of which may increase the paralytic effect of the toxin; pregnant or lactating women; patients receiving anticoagulation therapy/aspirin; patient with inappropriate anatomy (skin laxity, photodamage) and finally patient with poor psychological adjustment or unrealistic expectations (dysmorphism) [11,57-59].

Markings

Markings are usually not necessary because the patient is asked to repeatedly contract the muscle to identify sites for injection. If the patient is having BTX-A injections intraoperatively, markings are made over the body of the muscle, not over the deepest dermal rhytid.

Anatomy of the Facial Musculature

The use of botulinum toxin for rhytids or to reshape or reposition structures requires an in-depth understanding of the function and the location of the muscle of the face, their interactions (agonist versus antagonist) and the unique anatomic features of each region of the face (Figure 5).

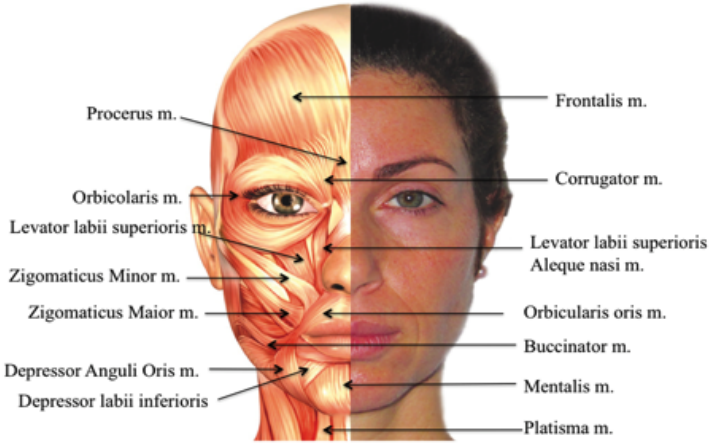

Figure 5: Pertinent anatomy of the facial musculature.

Anatomy of the forehead

The venter frontalis of the m.occipitofrontalis is part of the m. epicranius. It derives from the skin of the eyebrows and glabella and intervenes with the fibers of the m. orbicularis oculi. It follows upwards where it inserts into the galea aponeurotica, the extended tendon of the m. epicranius. This muscle leads, when contracted, to the horizontal lines of the forehead. It raises the eyebrow and the upper lid and by this makes the eye look open and much bigger [60,61].

Anatomy of the eye region

The *m. orbicularis oculi* is innervated by the temporal and zygomatic branches of the facial nerve. The *m. orbicularis* arises from the nasal portion of the frontal bone, the frontal process of the maxilla and the medial palpebral ligament. It is composed of three portions: orbital, palpebral and lacrimal. The orbital portion forms the majority of the muscle bulk. Fibers are arranged in an elliptical pattern and present no interruption laterally. The superior orbital portion of the orbicularis oculi runs more superficially than the m. corrugators and blends medially into the frontalis. Laterally, the muscle extends over the temporal fascia. Inferiorly, it continues and covers the upper portion of the m. masseter. More medially, at the inferior orbital margin, its extension covers the elevators of the upper lip. The palpebral portion originates from the medial palpebral ligament and adjacent bone. It is subdivided into preseptal and pretarsal portions. The pretarsal fibers spread across the eyelids, the preseptal fibers course in front of the orbital septum and both fiber interdigitate laterally with the lateral palpebral raphe. The ciliary bundle is a small group of fine fibers lying at the palpebral margin. The lacrimal portion has both superficial and deep heads that arise from the medial palpebral ligament and the posterior lacrimal crest. The fibers extend laterally to attach to the tarsi and to the lateral palpebral raphe [60,61].

Anatomy of the glabellar region

Glabellar lines are created by three muscles: the *m. depressor supercilii*, *m. corrugators* and the *m. procerus*. The *m. depressor supercilii* is the medial part of the orbicularis oculi pars orbitalis. It derives from the ligament palpebrale mediale and inserts in a fan shape cranially in the dermis of the medial part of the eyebrow. Contracting the *m. depressor supercilii* will draw the eyebrows down and will give this person a menacing expression. The *m. corrugator's supercilii*, also seen as an independent deeper part of the orbicularis oculi pars orbitalis derives from the medial orbital ring and gradually proceeds laterally to where the muscle inserts above the middle of the eyebrow in the dermis. Contracting the m. corrugators supercilii leads to vertical lines between the eyebrows. The *m. procerus* originates from the bridge of the nose and inserts into the skin of the glabella. Its fibers are interwoven with the frontalis ventral fibers of the m. occipitofrontalis. Contracting the *m. procerus* will induce a horizontal line between the eyebrows [60-63].

Anatomy of the upper third of the face

The facial upper third extends from the hairline to the top of the eyebrows. In men with receding hairlines, the upper part of the forehead equals the superior aspect of the frontalis muscle. Its normal resting tension is responsible for the normal position of the eyebrows. The galea aponeurotica covers the skull, just beneath the fat. The *m. frontalis* is the anterior part of the occipitofrontalis muscle. In front of the coronal suture, the aponeurosis gives origin to and is partly hidden by the frontalis bellies, which descend without any bone attachment to blend with the m. orbicularis oculi. The medial fibers of the *m. frontalis* blend with the m. procerus fibers and become contiguous at the nasal level. The *m. frontalis* has two halves and in the superior aspect of the midline forehead there is no muscle, but a fascial band. The usual action of the *m. frontalis* is to raise the eyebrows in the expression of surprise and even higher with freight, and to furrow the forehead with transverse line with thought. The eyebrows have one elevator and three opponents as depressors: the m. corrugators, the m. procerus and the m. orbicularis oculi [60,61].

Anatomy of the Nose – "Bunny lines"

The skin is thinner and more mobile in the upper two thirds of the nose, and it is thicker and more adherent in the lower third. The nose contains three main muscles: the m. procerus, the *m. nasalis* and the *m. depressor septi nasi.* The *m. procerus* draws the medial part of the eyebrow down. The m. depressor speti nasi drops the tip of the nose when contracted and the *m. nasalis* is the most important one for promoting the bunny lines. Although the *m. levator labii superioris alaeque nasi* is not an intrinsic nasal muscle, it may contribute to the bunny lines due to its medial fibers. The *m. nasalis* originates in the transition from the nasal bone with the maxilla and inserts into the aponeurosis of the nasal dorsum. It looks like an upside-down horseshoe, with the curved part formed by transverse fibers on the nasal dorsum. Its action is to narrow the nostrils. The transverse fibers of the *m. nasalis* lead, when contracted, to the lateral nasal lines (bunny lines) and to additional lines in the internal infra-ocular region. The two lower parts of the *m. nasalis* run vertically down the sides of the nose and their action is to open the nostrils. The *m. depressor septi nasi* is the most important muscle that acts on the position of the nose tip. Its origin is at the base of the nasal septum and it blends with the fibers of the orbicularis oris. Its fibers are longitudinal and, with contraction, it shortens the upper lip and can decrease tip projection on animation [60,61].

Anatomy of the nasiolabial fold

The nasolabial fold extends from the upper lateral part of the nasal flare down to the oral commissure. It can vary from individual to individual: be complete absent or flat or even very deep with skin excess and premaxillary deficiency. It can stop laterally to the oral commissure o go downward to the chin area. Nasolabial fold can result from more than one cause. It can result from the loss of skin thickness over the sulcus; from the presence of redundant skin drooping over the sulcus; from excessive fat deposits laterally to the sulcus; from ptosis and laxity over the malar fat pad and from muscular hyperactivity. The muscles at the nasolabial level, from medially to laterally, are the *m. levator labii superioris alaeque nasi*, m. levator labii superioris, *m. zygomaticus* minor, *m. zygomaticus* major and at a deeper level, the *m. anguli oris*. It is important to underline that the zygomaticus major has little or no effect on the nasolabial fold. The *m. levator labii superioris* is the main elevator of the upper lip and functions to create and move the middle portion of the nasolabial fold. It originates from the lower margin of the orbit, above the infraorbital foramen and below the orbicularis oculi. It continues downward between the levator *labii superioris alaeque nasi* and *zygomaticus* minor and inserts into the central and lateral aspects of the upper lip. It elevates and everts the upper lip. The *m. levator labii superioris alaeque nasi* originates from the frontal process of the maxilla and descends and divides itself into two muscle bundles: the most medial smaller fibers insert into the nasal cartilage and the skin of the nose and a larger and more lateral bundle continues downward and inserts into the upper lip, merging its fibers with the *m. levator labii superioris* and with the *m. orbicularis oris*. The *m. levator labii superioris alaeque nasi* creates the medial most upper portion of the nasolabial fold. Its medial nasal muscle bundle dilates the nostril and displaces the sulcus laterally, elevating the nasolabial fold. The labial muscle bundles evert and elevate the upper lip [60,61].

Anatomy of the oral region

The orbicularis oris is a sphincter around the mouth. It is a bilateral circumferential muscle that closes and puckers the mouth and forms a purse string. It anchors to the nasal septum and the maxilla above and to the medial part of the mandible below. The deeper layer of the orbicularis oris are the fibers of the buccinators and are reinforced by the incisive bundles. From the skin, short oblique fibers traverse the thickness of the lip in the direction of the mucosa. The more superficial layer is formed by the insertion of seven small muscles: five elevators and two depressors. At the corner of the mouth there is an area denominated modiolus, it is where the muscles that elevate and depress the lip interdigitate. The elevators consist of the *m. zygomaticus* major and minor, *m. levator labii inferioris*, *m. levator labii superioris alaeque nasi* and *m. levator anguli oris*. The zygomaticus major muscle originates from the zygoma (anterior to the zygomaticotemporal suture) and runs inferiorly and medially to the angle of the mouth and contributes to the modiolus. The zygomatic minor muscle arises from the malar bone (behind the maxillary suture) and passes downward and inward and in continuity with the *m. orbicularis oris* at the outer margin of the *m. levator labii superioris*. The action of the *m. zygomaticus* major is to elevate the corner of the mouth and it has little or no effect on the nasolabial fold. It is both the *m. levator labii superioris* and the *m. levator labii superioris alaeque nasi* that create and move the middle and medial most portions on the nasolabial fold, respectively. The main elevator of the lip is the *m. levator labii superioris* and it arises from the lower margin of the orbit just above the infraorbital foramen and its fibers insert into the midportion of the nasolabial fold. The *m. levator labii superioris alaeque nasi* arises

from the frontal process of the maxilla and inserts on the alar cartilage and medial upper lip. It dilates the nares and everts and elevates the medial upper lip. It deepens the medial upper nasolabial fold. The depressors consist of the *m. depressor anguli oris* and the *m. mentalis*. The *m. depressor anguli oris* belongs to the most superficial part of the perioral muscles of the lower lip and chin. It is a triangular muscle that derives from the base of the mandible and continues laterally and cranially. It inserts in the fibers of the corner of the mouth where it interweaves with the elevators of the mouth, the *m. levator anguli oris* and the m. zygomaticus major. The *m. depressor anguli oris*, together with the fibers from the platysma, drags the corner of the mouth down. This movement will induce a visible crease ("Marionette Lines") that descends from the corner of the mouth and gives the total face a dissatisfied and sullen expression. The *m. mentalis* belongs to the muscles of the perpendicular system of the perioral area and is the most medial and deepest muscle of this area. It derives from the lower incisors and inserts transversally in the dermis of the chin. The muscles from both sides crisscross each other. While contracted, the chin may show a "cobblestone" patter. Moreover, the mentolabial crease might be increased while showing the lower lip forward [60,61].

Anatomy of the lip

The lips comprise the red part of the mouth as well as the skin adjacent to it. Both parts must be considered as an anatomic unit that reaches from the nose to the chin. Perfect lip structure in the mucosa and skin consist of a "V-shaped" Cupid's bow, a pronounced vermillion and medial tubercle as well as ascendant lines in the oral commissures. The ratio between the upper and lower lips, at golden proportions, is 1:1.618. A very important topographic landmark is the philtrum. The midpoint of the upper cutaneous lip is highlighted by the two vertically oriented ridges of the philtrum. The Cupid's bow is the concavity at the base of the philtrum. The skin of the upper lip is very thin and lacks subcutaneous fat. The lack of additional support of this area together with extensive muscular movement of the main muscles may lead to pronounced wrinkles. The *m. orbicularis oris* is the major muscle of the lips. It has circumferential fibers that are responsible for the sphincter function of the mouth [60,61].

Anatomy of the neck region

The platysma is the largest mimic muscle. It originates at the border of the lower jaw, covering the chin up to the angulus mandibulae. The lateral fibers of this muscle extend over the angulus mandibulae in the area of the lower cheek and also radiate towards the corner of the mouth, where the interwine with the other muscles of the modiolus. The caudal part of the platysma runs as a broad thin sheet of muscles towards the clavicle and inserts approximately around the second rib at the fascia pectoralis. The platysma does not usually cover the medial area where the cartilage of the larynx can be found. The platysma covers the superficial fascia of the neck and is closely connected to the skin. It draws the lower jaw and the corners of the mouth down, expands the skin of the neck and extends the skin in vertical lines. In the area of the upper thorax, contraction of the platysma might cause diagonal wrinkles. When treating the platysma, the practitioner has to pay attention to the close relationship to the group of supra and infrahyoid muscles and to the outer larynx muscles. Apart from the diagonal *m. sternocleidomastoideus*, only the fascia of the neck will separate the platysma from the muscles of the larynx [60,61].

Treatment of Anatomic Areas

The first cosmetic indication for botulinum toxin A was the treatment of glabellar lines for which Botox® and Dysport® received the U.S. FDA approval in 2002 and 2009 respectively. Both have been approved for "the temporary improvement in the appearance of moderate to severe glabellar lines associated with corrugators and/or procerus muscle activity in adults younger than 65 years [27,64]. Xeomin®/Bocouture® recently received cosmetic approval for the treatment of glabellar lines in Germany, UK, France, Italy and Spain [28]. "Off-label" clinical indications have expanded to include hyperfunctional facial lines on the bridge of the nose, forehead, crow's feet, around the mouth, over the chin and bands in the neck. Additionally, non-cosmetic indications have developed for weakening mimetic facial muscles for disorders such as hemifacial spasm and facial dyskinesis. Further indications have been developed in the field of chronic pain management, which includes Temporomandibular Disorder (TMD) and headache [65,66]. Regarding the doses of toxin used for cosmetic purposes, it was concluded by a consensus committee that the dose of BTX-A should be adjusted in different anatomic areas depending mostly on muscle mass (contracted vs resting) and the desired degree and duration of effect [67]. Facial proportions may be an important consideration, for example, when injecting the frontalis to treat forehead lines. Other factors exist and may be interrelated; for example, wrinkle severity increases with age and men usually have larger muscles than women. Observing muscle action is the most important method for locating injection points in almost every anatomic area, followed by anatomic landmarks.

FDA Approved Uses

Glabellar complex/Vertical frown lines

Glabellar lines form as the result of frowning and may also persist in repose as approximately vertical, static lines between the eyebrows. This area may be treated by the "novice" injector, although a basic degree of training is needed before injecting in any area [67]. Medial brow depressors that form horizontal (procerus and depressor supercilii muscle) and vertical (corrugator's supercilii muscle) lines in the glabellar area are targeted. The aim of the treatment is to reduce the vertical as well as the horizontal lines of the glabella. Injections points for the glabella are best determined by observing muscle contraction, although palpation of the muscles and making references to superficial landmarks sometimes can be useful, as can bony landmarks and anatomic diagrams. The most commonly used dose according to the literature is 20U of onabotuilinumtoxinA (Botox®/Vistabel®/Vistabex®) [68,69] and 50U of abobotulinumtoxinA (Dysport®/Azzalure®) [70-74] divided between five injection sites. This dose does not have to be divided equally among injection sites. These dosages are mainly referred to women patients but it has to be kept in mind that, on average, men need higher dose of botulinum toxin A than women to receive equivalent efficacy due to a larger mass being treated [9,75]. Injections should be made intramuscularly and perpendicular to the skin surface. Care should be used to avoid injecting superior to the target muscles, which can cause brow ptosis by weakening the frontalis [67]. In most patients, the first injection point is used to treat the *m. procerus* in the middle of an imaginary cross between the contralateral eyebrows and the medial corner of the eyelid. This area is massaged horizontally with the thumb, which safely distributes some toxin into the depressor supracilii [76]. The two most important points for treating the glabella are the injection points in the corrugator's muscles which are located 0.5-1cm above the medial orbital rim in extension of the exit of the *n. supraorbitalis* (the

medial canthus can be used as a marker). After injecting in this location the needle is partially withdrawn but the tip kept beneath the skin, then repositioned until it angles superiorly and the tip advanced until it is approximately 1cm above the previous injection site and continue with the injection (Figure 6). A useful tip is to place the non-dominant index finger on the undersurface of the medial brow while injecting as an aid to avoid excessively deep insertion of the needle through the orbital septum and subsequent weakening of the levator palpebri muscle [9]. This "two hands" technique is also useful in identifying the bulk of the targeted muscle group that requires treatment. Injections should be no placed any further lateral than the midpupillary line to avoid loss of facial expressivity from weakening the lateral frontalis muscle and thus changing brow contour.

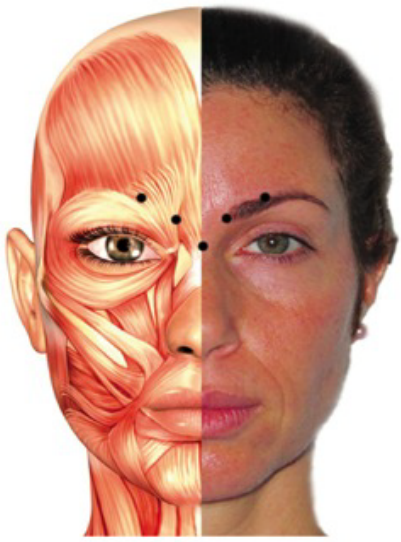

Figure 6: Injection points for glabellar treatment.

Complications: Injecting within 1cm of the bony margin of the orbit or near the supraorbital notch can cause eyelid ptosis by weakening the *m. levator palpebrae superioris* and this is certainly the effect least wanted [45]. Some authors have suggested that low-volume; high-concentration injection is one technique that can be used to minimize diffusion of the toxin to undesired muscles [57]. Ptosis can occur as early as 48 hours or as late as 7 to 10 days post-treatment. The degree of ptosis worsens throughout the day as the muscle fatigues. Proper injection technique is the best means to avoiding blepharoptosis. Pharmacological improvement can be accomplished with topical alpha-adrenergic agonists. Muller's muscle is stimulated with apraclonidine or phenylephrine eye drops administered several times per day [77-80]. Keep in mind that eyelid ptosis is temporary and usually subsides after a few weeks.

"Off-Label" Uses

Frontalis/Horizontal forehead lines

Forehead rhytids develop from hyperfunctional habitual activity of the frontalis muscle. One of the most undesired effects of over treating this muscle is the brow ptosis which is one

of the major signs of aging. The medial fibers of the frontalis muscle are generally stronger than the attenuated lateral fibers and form deeper rhytids. Injecting only the medial frontalis muscle can yield a canted brow contour with a scowled expression. The horizontal rhytids are marked when the frontalis muscle is in maximal contraction. The number of injections varies from four to ten, depending on the severity of the rhytids with a dose range of 10 to 15U of onabotulinumtoxinA and 20 to 30U of abobotulinumtoxinA divided among all sites [9]. Injections are done perpendicular into the muscle 2cm above the brow and should be distributed in the middle of the forehead area. The more lateral points of injection will determine the degree of movement of the eyebrow; if the lateral injection point is placed in the midpupillary line, the lateral parts of the frontalis muscle will lift the lateral parts of the eyebrows upward which is preferable in female patients, whereas in male patients the lateral injection point should be placed in a line with the lateral corner of the eye (Figure 7) [81]. Complete immobilization of the forehead is often not desired, even if some lines remain, because it can prevent normal facial expression. The goal should be to weaken the muscle only [67]. Often the forehead is treated together with the glabella. When treated at the same time, the total dose might be reduced to avoid a frozen expression.

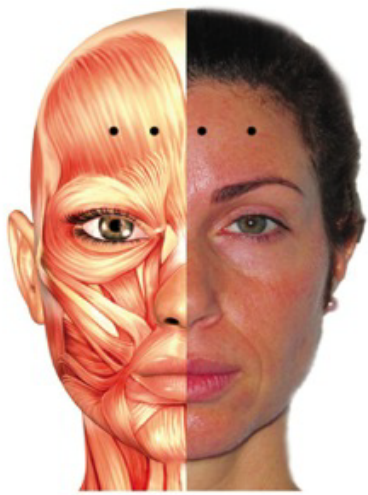

Figure 7: Injections points for forehead treatment.

Complications: Brow ptosis is the most common and unwanted adverse event. It may result from injecting the frontalis muscle within 1cm of the supraorbital rim, not simultaneously weakening the brow depressors, and from excessive delivery of toxin to the frontalis muscle, particularly in the elderly patient with undiagnosed senile brow ptosis [9]. This “caveman droop” is difficult to fully correct because the frontalis is the only muscle that can elevate the brows. For this reason it should be avoided to treat the lower part of the forehead [67]. If the frontalis muscle is not weakened at the same time as the glabella, excessive brow elevation can occur; especially in hypertonic patients the lateral movement of the frontalis muscle will produce more visible wrinkles or make the existing wrinkles more visible. This effect, variously called “Spock”, “Joker” or “Mephisto” eyebrow, can be corrected with an injection of approximately 2U of onabotulinumtoxinA or 5U of abobotulinumtoxinA into the frontalis muscle above the point of maximum contraction [78].

Brow lift

The aging process causes gradual descent of the forehead and brow, especially its lateral third. This makes the individual look tired and aged leading to a negative appearance. Eyebrow mal-positioning may cause upper eyelid fullness that may be targeted insufficiently by blepharoplasty alone. Eyebrow asymmetry is very common in middle-aged women and this makes eyebrow elevation a very important part of the world of cosmetic procedures. Eyebrow position differs between men and women. In women, the eyebrow should be positioned above the supraorbital rim, while in men, it lies at the rim. The medial and lateral ends of the eyebrow should lie at the same horizontal level. In general, the ideal shape of the brow has been described with the crest of the arch over the lateral canthus, with some aesthetic refinement based on each patient's facial shape [82]. The position and shape of the brow is a dynamic balance between elevator and depressor forces. The frontalis muscle is the only brow elevator and is opposed by the *orbicularis oculi*, *depressor supercilii*, *procerus* and *corrugators supercilii* muscles. As time passes, the shape and vertical position of the brow changes as muscles weaken and the forces of gravity take hold. The aim of the treatment is to lift the lateral eyebrow. The medial aspect could also be lifted in selected cases. Eyebrows can be elevated by injection of the depressors and allowing elevators of the brow to act unopposed. We describe here three techniques that could help the practitioner in achieving this result.

The first technique is suitable for mild lateral eyebrow lifting when the opponent elevating lateral fibers of the frontalis muscle are strong enough to produce the lifting effect with the antagonist blocking. One injection is placed into the upper lateral fibers of the orbicularis oculi pars orbitalis muscle approximately 0.5cm above the orbital rim. The recommended dose range is 3-4U of onabotulinumtoxinA and 10-12U of abobotulinumtoxinA per point of injection (Figure 8) [83-88].

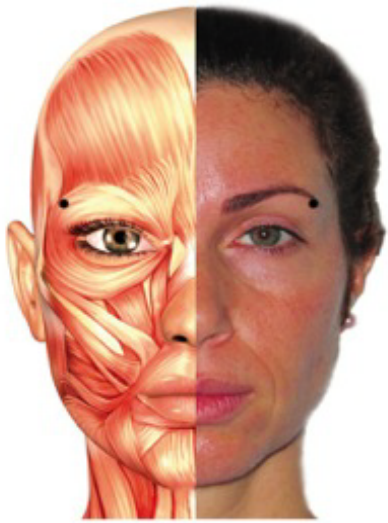

Figure 8: Injection points for brow lift (Technique 1).

The second technique is generally used when only the lateral aspect of the eyebrow needs elevation and there are not many horizontal fibers in the forehead, only in the midline. It consists of the full blocking of the medial fibers of the depressors and partial blocking of the medial fibers of the frontalis muscle. Seven injections point are made (FIG X) treating the corrugatores muscles with a dose range of 3-5U of onabotulinumtoxinA (10-15U of abobotulinumtoxinA) per point, the procerus muscle with 3-5U (10-15U of abobotulinumtoxinA) and the medial frontalis muscle fibers with 2-6U (6-15U) in two points (Figure 9) [83].

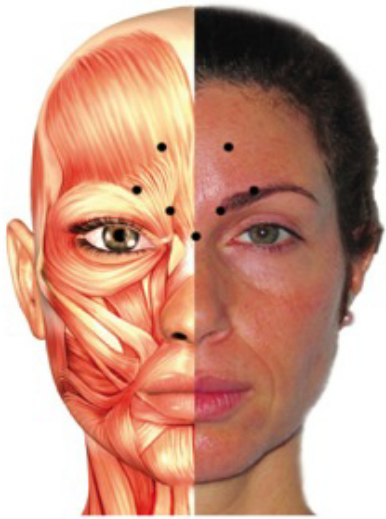

Figure 9: Injection points for brow lift (Technique 2).

The third technique is the most appropriate for medial, intermediate and lateral eyebrow lifting and it consists of the use of three to five injection points within the hair of the eyebrow. The injections should be superficial, with the needle pointing upwards and located approximately 0.5cm above the bony orbital rim to avoid intraorbital diffusion of botulinum toxin A. For lateral lifting only, three points are injected laterally to the supra-orbital foramen at the hemipupillary line. The dose range is of 10-15U of onabotulinumtoxinA and 30-40U of abobotulinumtoxinA divided between the three sites over the supero-lateral portion of the orbicularis oculi muscle [83-88]. If medial and lateral eyebrow lifting is desired, the toxin should be distributed in five injections sites within the whole eyebrow (Figure 10).

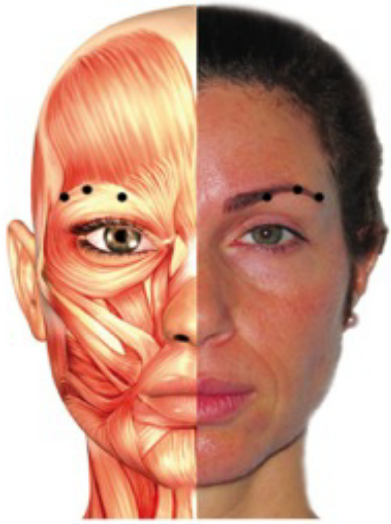

Figure 10: Injection points for brow lift (Technique 3).

Complications: The main concern when injecting the orbicularis oculi muscle is the risk of diffusion of botulinum toxin to surrounding muscles. Diplopia can occur when the lateral rectus muscle is reached by diffusion, eyelid ptosis when the levator palpebri muscle is reached or excessive brow elevation when the frontalis muscle is caught by diffusion [89]. The third technique aforementioned may lead to upper eyelid ptosis if the toxin is injected too deep and the needle directed downwards. Sculpting of the lateral brow with BTX-A is unpredictable in the amount of elevation achieved in each individual with a given dose. Using a conservative dose with reinjection in 2 weeks is a safe and controlled manner of addressing this problem.

Orbicularis oculi/Crow's Feet

The aging process in the eye area may lead to skin excess, eye bags, static and dynamic wrinkles and pigmentation disturbances. The wrinkling is usually noticed when smiling and localized at the lateral part of the lower eyelid. Static wrinkles are caused by skin photo-damage and could be present in young people. Eyelid wrinkling may also result from the desiccating effect of wind and from smoking. Patients with light-colored eyes are more sensitive to daylight and as a consequence, squinting in bright sunlight may mechanically contribute to the lateral periorbital wrinkles. Patients with thick skin present deeper wrinkles and the more atrophic the skin is, the greater the quantity of fine wrinkles that may be found. Moreover, eyebrow ptosis may contribute to upper lid excess and skin wrinkling. The orbicularis oculi muscle functions for voluntary and involuntary closing of the eyelids. Periorbital rhytids, or crow's feet, result from the hyperkinetic sphincter function of this muscle. Its lateral portion has direct dermal insertion, which creates fine senile wrinkles in a radial pattern. The aim of the treatment with BTX-A is to weaken the sphincter contraction of the orbicularis oculi so to soften the skin lines and yield a refreshing appearance to the eyes. The precise location and depth of injection is very important to avoid possible complications such as lid or lip ptosis. As we know from

the anatomy, the *zygomaticus* major and *levator labii superioris* muscles blend with the deep surface of the orbicularis oculi muscle, and together serve to elevate the upper lip and oral commissure. Chemodenervation of these adjacent muscles could lead to lip ptosis [89,90]. To avoid this complication, a careful selection of injection sites is mandatory. Good lighting and stretching the skin may help the practitioner in avoiding perforating blood vessels that could lead to bruising and ecchymosis. Injection points are usually three to five and are located based on observed muscle action and superficial landmarks. Care should be used to inject along an arc 1cm from the lateral and inferior bony rim in order to mitigate the risk of intraorbital diffusion and inadvertent lid ptosis [55,91]. As the periorbital skin is thin, the needle must be inserted almost parallel to the skin and the botulinum toxin will diffuse to the underlying muscle; with deeper injection, it is more likely to produce skin bruising. The total starting dose is typically 12U of onabotulinumtoxinA [92] or 30U of abobotulinumtoxinA [93], divided equally per injection site (Figure 11). Injecting into the periorbital area is also useful for lifting the lateral aspect of the eyebrow. For this reason, in common practice, a combined treatment of glabellar lines, periorbital rhytids and a subtle temporal brow-lift is given in a single session (Figure 12-17).

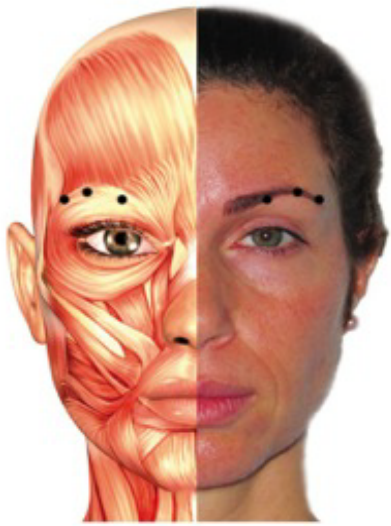

Figure 11: Injection points for crow's feet treatment.

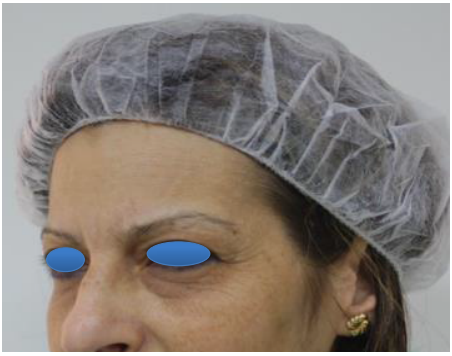

Figure 12: Pre-treatment.

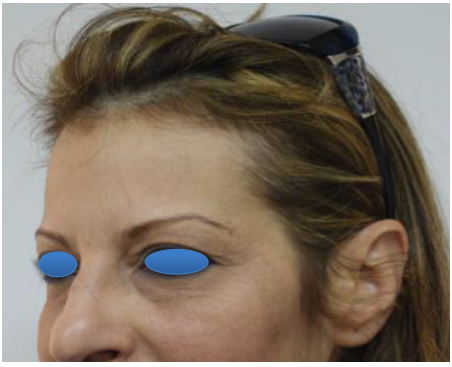

Figure 13: Post treatment (forhead, glabellar, and periocular – Vistabex 50 U).

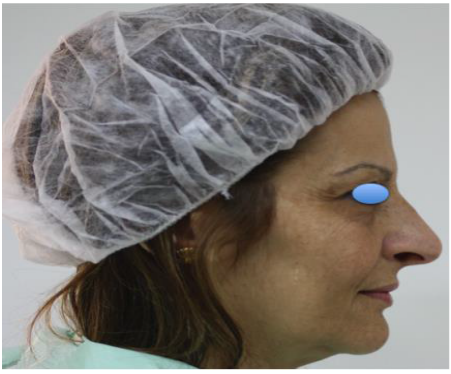

Figure14: Pretreatment lateral view.

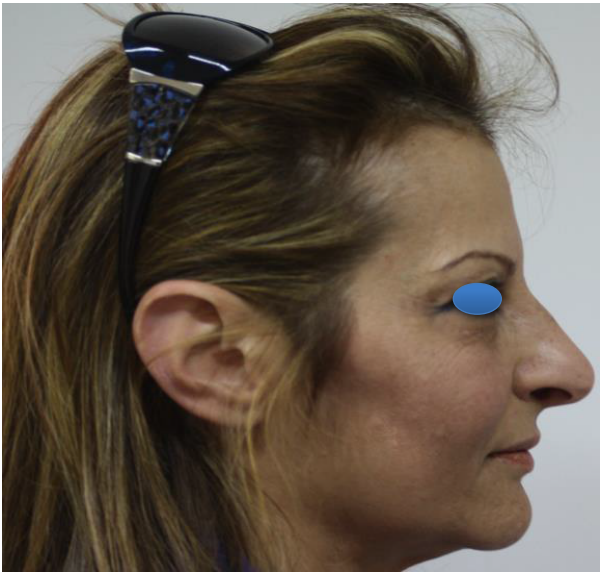

Figure 15: Post treatment lateral view (3 months).

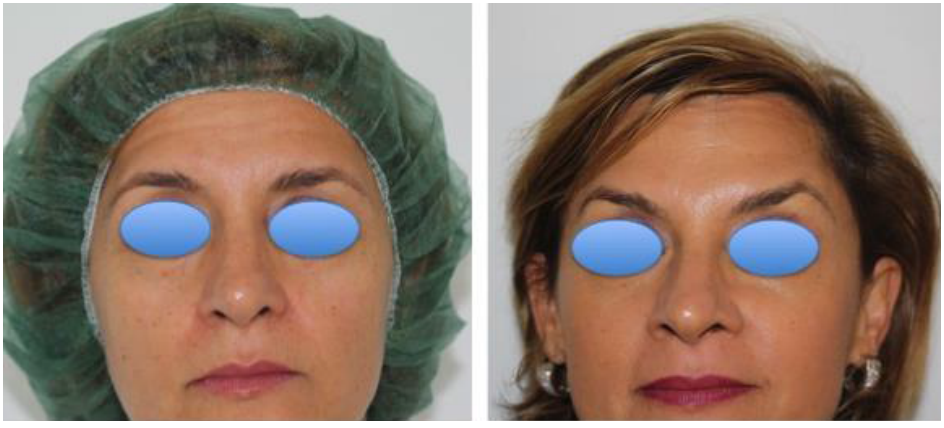

Figure 16: Pretreatment - On the left; Post-treatment (forehead, glabellar, and periocular - Vistabex 50 U) 3 months - On the right.

Figure 17: Pretreatment - On the left; Post-treatment (forehead, glabellar, and periocular - Vistabex 50 U) 3 months - On the right.

Lower eyelid

When observing the lower eyelid care should be taken to check for the quality of skin, presence of eye bags and wrinkling. Eye bags may result from the laxity of the orbicularis oculi and are considered to be pseudo-herniation. It is not advisable to inject botulinum toxin in patients with prominent eye bags; surgery would be the best option in these cases. The skin wrinkling in the lower eyelid results from the hyperkinetic behavior of the palpebral portion of the orbicularis oculi muscle. The pretarsal portion of the muscle may produce orbicularis hypertrophy which reduces the palpebral aperture. Injecting BTX-A would soften the bulging at this site and promotes eye widening. A “snap test” to measure lower lid skin laxity should be performed because a poor response can be expected if the skin does not snap back into place after downward tugging [94]. The best injection site in the lower eyelid is at the pretarsal in the midpupillary line and as well as improving lower eyelid wrinkling, it produces a widening of the eye which leads to aesthetic enhancement. A microinjection technique for one to two injection points is recommended. The needle should be inserted parallel to the skin so that a very superficial papule is seen. The total dose should be 1-2U of onabotulinumtoxinA or 2-4U of abobotulinumtoxinA (Figure 18) [95,96]. Treating the lower eyelids is not for the novice injector, due to the delicacy of this area, but is effective with experience and reasonable skill [67].

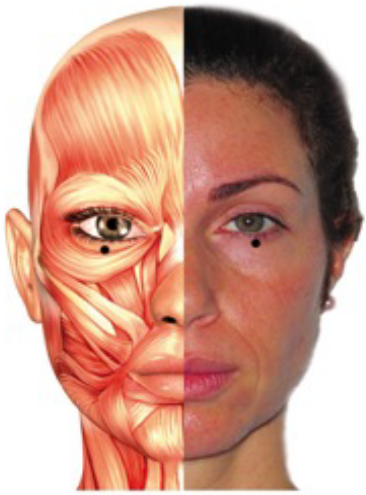

Figure 18: Injection points for lower lid treatment.

Complications: Care should be taken to avoid injecting patients with dry eyes, morning eyelid edema or poor skin elasticity. All patients should have a positive "snap test" [94]. Patients with with skin excess, eye bags, sclera show, static and dynamic wrinkles and pigmented spots will not be 100% satisfied with the use of botulinum toxin A and they will tend to see only the negative aspects of this treatment. Ecchymosis and bruising may result from injection into the lower eyelid or deeper injections at the crow's feet. Use of ice bags is recommended. Upper lip asymmetry or cheek ptosis may result from injecting into the lowest extensions of the crow's feet at the zygomaticus major muscle. Usually these complications result from injecting too deep [89,90]. Similar to the treatment of the frontalis muscle, the goal should be to weaken the muscle rather than cause complete immobility since excessive blocking of the palpebral portion may affect the lacrimal pump mechanism, forced eyelid closure and the blink reflex. This may lead to dry eyes and corneal exposure especially in older patients. When injecting the lower eyelid care should be taken to inject in the midpupillary line because lateral injections to this point would lead to eyelid ectropion and rounded lateral canthus, whereas medial injections may cause epiphora and dry eyes. Moreover, excessive blocking of the palpebral portion of the orbicularis oculi may lead to impairment of eye closure, for both voluntary and involuntary functions [96,97].

Perinasal lines/ Bunny lines

The perinasal or bunny lines are wrinkles that fan over the nasal dorsum and sidewalls from hyperkinetic function of the nasalis muscle, seen predominantly in patients with thin skin. These lines may be naturally present in some patients during animation like smiling, laughing, frowning and even speaking. They may appear or become more prominent after treatment with BTX-A, especially when the glabella and crow's feet are treated, leading to the so-called "Botulinum Toxin sign". This is due to the fact that in some patients when the frontalis, the corrugatores, the procerus and the orbicularis muscles are blocked with

botulinum toxin injections, the untreated nasalis muscles react with over-contraction being itself a synergistic muscle when the eye and nose complex is under animation, therefore leading to wrinkle formation. Observed muscle action is considered most important for locating injection points. During animation, patients should be asked to laugh, to sniff and to squint intensely. Injections are placed in an intramuscular plane centered over each nasal bone medial to the nasofacial junction, which corresponds to the bulk of the nasalis muscle. It has to be paid attention not to inject too close to the nasofacial junction so the botulinum toxin does not spread laterally to the *levator labii superioris alaeque nasi* muscle and cause lip ptosis (Figure 19). Care should be taken with blood vessels at this level; otherwise bruising may result, so stretching the skin to identify blood vessels prior to injection and slight pressure after injection is recommended. One injection site on each side is used to treat the "bunny lines". A total dose of 2-5U of onabotulinumtoxinA or 6-15U of abobotulinumtoxinA should be distributed on both lateral sides [67,98,99].

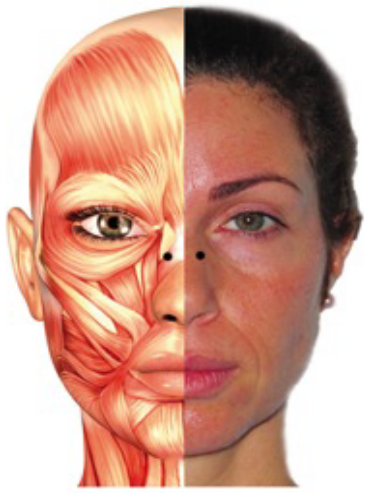

Figure 19: Injection points for Bunny Lines treatment.

Complications: As aforementioned it is important not to inject too laterally down the nasal sidewalls otherwise the levator labii superioris alaeque nasi may be blocked and upper lip ptosis and asymmetry could result. The most common complication is the presence of ecchymosis or hematoma due to injection into the angular vessels [9]. Diplopia may occur from inadvertent blocking of the rectus inferior or medialis.

Gummy Smile

Gummy smile is defined by having an excessive gum line exposure with a full smile where the gingiva above the canines can be seen. Patients with a short distance between the nasal base and Cupid's bow as well as those with a facial convex profile with a prominent nose and underdeveloped chin are prone to exhibit this kind of smile. Deep nasolabial folds are also found in these patients. The levator labii superioris muscle that elevates the upper lip and

the *levator labii superioris alaeque nasi* muscle that elevates the medial part of the upper lip and the nasal flare are responsible for the gummy smile [100]. As a matter of fact, in patients with gummy smile it is very common to see the inversion of the upper lip when smiling which makes these patients usually bad candidates for upper lip augmentation with fillers only. The ideal treatment would be a combination of filler and botulinum toxin. As the golden proportion establishes, that the upper lip should cover the upper third of the central incisors, the aim of treating gummy smile with botulinum toxin is to avoid gingiva showing at rest and to reduce excessive gum exposure during a smile. The patient should be asked to smile at maximum contraction, then two injection sites on each side of the face are given. Both are deep intramuscular injections but remain superficial to the periosteum. The first injection site is at the nasofacial junction for treatment of the alaeque nasi muscle. The second site is approximately 2cm inferior to the bony orbital rim just medial to the midpupillary line for treatment of the levator labii superioris muscle (Figure 20). Care must be taken to not weaken the zygomaticus major muscle by placing the injection too lateral over the malar eminence. A starting dose of 2U of onabotulinumtoxinA or 5U of abobotulinumtoxinA per side is recommended. After 15 days the patients should be evaluated and treated if necessary [9].

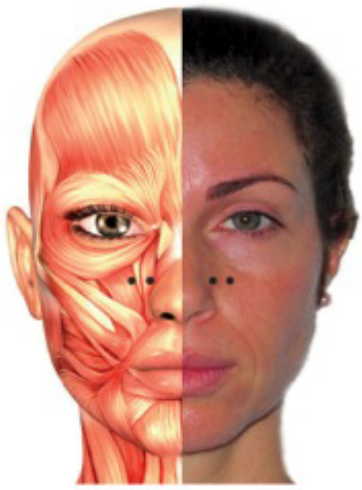

Figure 20: Injection points for Gummy Smile treatment.

Complications: Asymmetries and upper lip drooping are the most common complications. Asymmetries should be corrected with administration of 25% of the initial dose and the outcome evaluated after one week. Excessive drooping of the medial part of the upper lip may happen if excessive blocking is undertaken, resulting in the "joker" smile due to excessive lateral pulling of the zygomaticus major muscle [98]. In order to reduce the possibility of complications the ideal candidate would be a patient with the open lip posture and with a short upper lip. Even if excessive upper lip elongation results, it will benefit the patient.

Nasal tip

The tip of the nose plays an important role in nasal beauty. Aging process leads to the

drooping of the nasal tip accentuating any dorsal convexity of the nose. A ptotic or drooping nasal tip may result, in younger patients, from overactivity of the depressor septi nasi muscle and can be exacerbated by smiling. These patients usually present a drooping nasal tip and upper lip shortening when smiling, with a convex shaped face, prominent nose and underdeveloped chin. The patient should be evaluated according to the length of the upper lip and the nasal-labial angle before injected. Superficial landmarks are of the most importance. There are two ways of injecting the depressor septi nasi muscle: through the skin and intraorally [45,101-103]. The nasal area is quite sensitive so the use of topical anesthesia or ice bags to reduce the pain is recommended. The authors prefer the trans-cutaneous approach since the intraoral approach may present some difficulties to inject into the correct level. The injection points are marked at the base of the columella at the medial crural footplate. Two points are marked, one at each side of the medial crura (Figure 21). The injection should be superficial, inserting only the first third of the needle. The dose at each side is 1-2U of onabotulinumtoxinA or 4-6U of abobotulinumtoxinA [67]. The dose is adjusted based on desired degree and duration of correction, facial proportions, observed muscle action and adjacent muscle function.

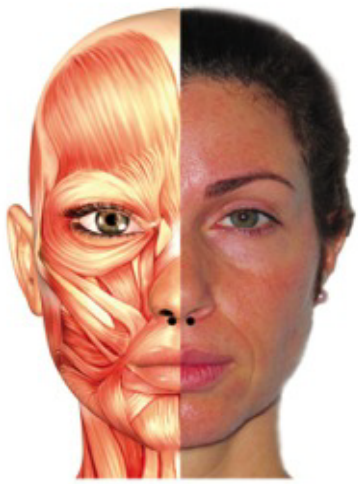

Figure 21: Injection points for nasal tip treatment.

Complications: Complications are rare if patients are selected properly. Pain is the adverse event most often reported. Over-blocking of the depressor septi nasi muscle may result in upper lip ptosis which may lead to the “joker” smile because of the action of the zygomaticus major muscle.

Perioral/lip rhytids

When aging the lip undergoes a typical series of changes includes the recession of the lateral lip, an increase in the upper white lip length, thinning of the vermillion bulk and dense vertical rhytids radiating around the mouth. These rhytids may also be called “smoker’s lines” and can result from environmental influence such as smoking and photodamage, hereditary factors and habitual hyperfunction of the orbicularis oris muscle sphincter (musicians)

[9,67]. Wrinkles of the upper lip are often treated by multiple modalities such as with fillers or resurfacing [104-106], but BTX-A can aid in improving the appearance of the perioral area, and becomes especially indicated when deep and static rhytids around the mouth and lips are present. The lip sphincter should be treated conservatively in order to efface deep oral wrinkles without compromising oral competence. The patient is asked to pucker and the wrinkles are marked. Care is taken to avoid injecting around the commissure because the toxin may diffuse and weaken the lateral lip elevator muscles, resulting in lip ptosis and drooling [98,107]. Injecting the midline should also be avoided to prevent effacement of Cupid's bow reducing the landmarks of a perfect lip. In general, four sites are treated, one or two injections per lip quadrant. Very small doses should be used in order to avoid dysfunctional mouth. The injections should be placed 5mm from the vermillion border in order to obtain a nice secondary effect of lip eversion. Inexperienced injectors should start injecting each lip quadrant with a total dose of 0.5U of onabotulinumtoxinA or 2U of abobotulinumtoxinA in an intramuscular plane to avoid complications of overtreatment (Figure 22) [67,98].

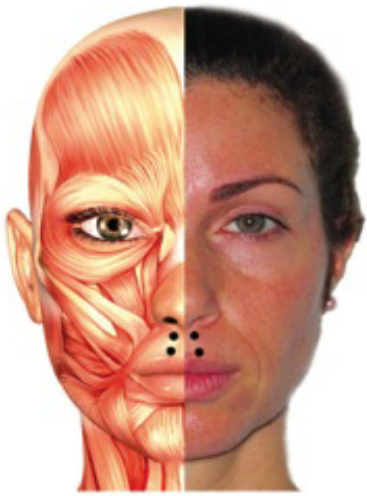

Figure 22: Injection points for the treatment of perioral rhytids.

Complications: Relative overtreatment may lead to functional impairment of the lips like inability to purse or maintain an embouchure, mild articulation difficulties with plosive consonants and difficulties in eating and drinking. Therefore, injections should be started with low doses and repeated as necessary to avoid oral incompetence or unwanted asymmetries [9].

Marionette lines

Marionette lines appear as deep folds, angled downward, that develop from the corners of the mouth and lower lips, which might give the total face an expression of being dissatisfied, sullen or even scornful. A combination of factors could lead to the formation of these folds like the loss of dermal collagen, fat atrophy and redundant or ptotic skin, all of which could be addressed by surgery or injectable fillers. In some cases these folds could also be deepen with time by the over-activity of the depressor anguli oris muscle due to its dermal attachments and

downward pull of the corners of the mouth. Botulinum toxin could be useful in weakening this muscle allowing the zygomaticus major and levator anguli oris muscles to lift the corner of the mouth back to a horizontal plane. In some patients the activity of the platysma muscle, which interdigitates with the depressor anguli oris muscle, may contribute to the deepening of the marionette lines; therefore, a treatment of both muscle groups can be helpful. The best result in selected patients is seen with the combination treatment of Botulinum toxin A and fillers, the first enhancing and prolonging the duration of the effect of the latter [107]. The patient is asked to grimace and show their bottom teeth in order to identify and palpate the depressor anguli oris muscle as well as additional platysmal bands. Usually two points per side are injected, one that targets the depressor anguli oris and the other one that targets the platysmal bands inserting at the lateral parts of the orbicularis oris muscle. Injection around the oral commissure or medial lower lip is avoided to prevent inadvertent weakening of the orbicularis oris or depressor labii inferioris muscles. It is recommended to keep a distance of at least 1cm from the corners of the mouth of the patient and the first point of injection can usually be found, after palpation, in the elongation of the naso-labial fold. Another injection point should be put more laterally in the area of the mandible in order to target the platysmal bands (Figure 23). Injection should be done in an intramuscular plane. Some authors place one single injection on each side no more than 1 cm above the inferior mandible rim in a line parallel with the oral commissure [9]. The recommended initial dose is 2-5U of onabotulinumtoxinA or 10U of abobotulinumtoxinA per injection point with adjustments made based upon each patient's muscle mass [9,67].

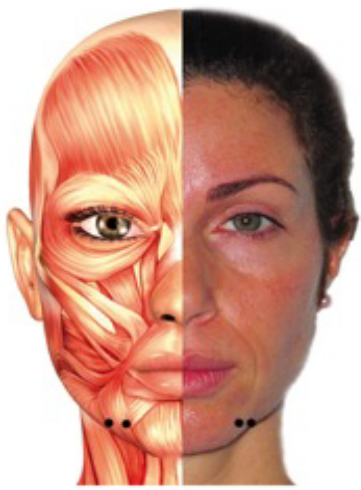

Figure 23: Injection points for Marionette Lines treatment.

Complications: Over treatment or injecting too close to the corner of the mouth may lead to asymmetry, oral incompetence such as drooling and articulation problems.

Cobblestone chin

Cobblestone chin, also known as pebbled, dimpled or golf ball chin develops when the

mentalis muscle, which inserts with several fibers in the dermis of this area, is contracted. A combination of processes contributes to the deepened chin convexity and orange-peel appearance of the skin seeing with aging, very similar to what happens with the perioral rhytids and the melomental fold. Weakening of the mentalis muscle can soften the mental crease and improve some of the skin dimpling not attributed to loss of dermal collagen. The patient is asked to contract the chin by pulling the lower lip down. The botulinum toxin A can be either injected in one single point or in two lateral points, one on the left and one on the right side, approximately 1 cm from the midline and just superior to the mental tubercle. No injections points should come closer than 1 cm of the lower lip. The mentalis muscle is the deepest muscle in this region, so intramuscular injection just above the periostium, as well as inferior to the crease, will avoid undesired oral incompetence and articulation problems from weakening of the oral sphincter and depressor functions. Some authors refer that, although the muscle is quite deeply located, superficial injections are fine and will lead to quite satisfactory results (Figure 24) [109]. The recommended total dose range is 2.5-8U of onabotulinumtoxinA and 2.5-20U of abobotulinum toxin A [9,67].

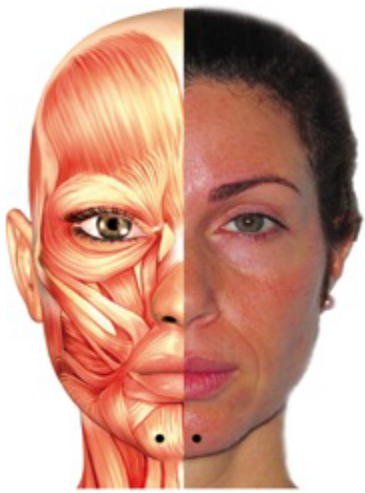

Figure 24: Injection points for Cobblestone Chin treatment.

Complications: Respecting the appropriate distance from the lower lip leads to no complications apart from bruising or hematoma. Injecting too close the lower lip may involve the depressor labii inferioris which would lead to a dysfunctional mouth with ptosis of the lower lip.

Platysmal Bands

Changes of the neck, when aging, can be caused by excessive skin laxity and loss of elasticity, jowl formation, lipodystrophy, submandibular gland ptosis and bone resorption [110,111]; the platysma muscle can become hyperkinetic, lose tone and be dehiscent in some

areas, all of which contribute to the so-called turkey neck appearance. In the anterior neck, the platysmal muscle separates from the contralateral side forming vertical bands that become more visible with forceful contraction, whereas in the lateral neck, additional bands develop around areas of weakened muscle tone and dehiscence [112]. Treatment with botulinum toxin A may soften the prominence of these bands when contracted. Furthermore, lateral cheek lines and marionette lines can be improved when reducing the strength of the platysmal bands. Patient's selection is very important in these kinds of patients; kinetic or hyperkinetic patients who contract the platysmal bands actively when speaking are best. Patients must be in a sitting position because this helps the active contraction of the platysma muscle. Patients are asked to grimace. Treatment follows the course of the contracted bands. Four to eight injections points (depending on the length of the band) are placed for each band approximately 1.5cm from each other. Grasping the band with the non-injecting hand might be helpful while injecting the contracted muscle in an intradermal plane (Figure 25). A conservative initial dose is recommended, using 2U of onabotulinumtoxinA or 5U of abobotulinumtoxinA per point [67]. Care must be taken to avoid deep injections or overdosing.

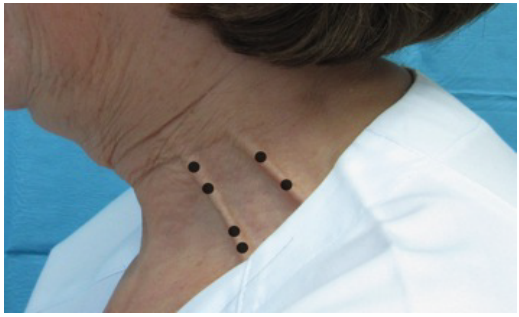

Figure 25: Injections points for platysma treatment.

Complications: Bruising is quite common also because pressure after the injection should only be applied carefully. The pharynx region should be spared from injecting because of the risk of diffusion of the toxin to the underlying muscles which may lead to difficulty swallowing, neck weakness and dysphonia [113,114].

BTX-A is not treatment for horizontal neck lines, other methods might be more appropriate, such as the combination of fillers and ablative procedures. Treatment of platysmal bands might become quite expensive due to the numerous injection points. Patients should be informed about this before starting the treatment [115,116].

Post-Treatment Care

Gentle pressure with or without cold compresses can be applied to the treated site immediately after each site is injected, if ecchymosis and erythema result as an immediate complication. Some authors suggest that the patient should be advised to actively contract the treated muscle in attempt to enhance toxin uptake by causing more rapid internalization of the toxin, but this has not been proved yet [11]. Patients are told to go back to normal life immediately after their session but are advised to avoid lifting, bending or straining for the 3

to 4 hours, particularly when the treated site is in the upper third of the face, to help prevent unwanted spread of the toxin. Flying and heat exposure do not have an adverse impact. Makeup is unrestricted. Non-aspirin products can be given if the patient complain of headaches and it's important to advise the patient not to take any drug or supplement that could interfere with coagulation in the 10 to 14 days after treatment in order to help prevent ecchymosis. Patients are told to return for a post injection examination after 2 weeks if they suspect a therapeutic failure or are not completely satisfied with the result. Otherwise, patients are advised to return after 3 to 4 months for additional treatment if they want to maintain the result.

Combination Treatments

In common practice Botulinum toxin A is usually injected alone, although it could be used in combination with other treatments, during the same or susequent session, to treat the different layers of the skin and various conditions. In the last few years one of the most important changes in facial rejuvenation has been a shift from a two-dimensional focus on hyperdynamic facial lines and immobilization of corresponding muscle to an increased comprehension and appreciation of the three-dimensional aspects of facial aging, particularly the loss of volume and its effect on treatment approaches [117]. This has changed the way Botulinum Toxin A is used in clinical practice. Practitioners now tend to treat not only one single area of the face but multiple areas to provide a more natural and relaxed look [118,119]. Moreover, BTX-A is everyday more often used in combination with other modalities, including dermal fillers. When BTX-A is used in combination with fillers, it becomes possible to address facial rejuvenation from a three-dimensional rather than a two-dimensional approach, providing more pleasing, longer-lasting aesthetic outcomes [107]. When botulinum toxin is combined with filler, it tends to prolong the longevity of the filler by decreasing the metabolism in the surrounding tissue. BTX-A may be injected into the frontalis muscle on the upper part of the forehead (1 to 2cm above the brow) and a filler can be injected in this lower area of the forehead in order to efface the residual static lines of the entire forehead [120]. The same can happen when treating deep glabellar lines; a combination of toxin and fillers may be necessary to achieve an optimal result [121]. When treating the orbicularis oris muscle, the better aesthetic outcome of treating the vertical lip lines or the lip itself with fillers in combination with BTX-A become immediately evident. It is said to be the perfect treatment for male patients because of the depth of the male wrinkles [107]. Botulinum toxin and fillers can be safely used at the same time because one procedure does not interfere with the other because both are injected into different layers. Usually, the toxin is injected first and the filler soon after [116]. When botulinum toxin is combined with laser resurfacing, it has the potential to enhance the effect by improving collagen reorganization while the skin is paralyzed [122]. Great results are seen when botulinum toxin is used in combination, but not simultaneously (the injection of the toxin should be performed 1 or 2 weeks before the procedure), with laser resurfacing of the lower lid or the lip [123-125]. Regular postoperative injections every 4 to 6 months prolong and expand and refine the laser resurfacing procedures [126]. Botulinum toxin can be used together with chemical peels, especially in patients with photodamaged skin due to excessive sun exposure [122]. While the toxin treats the dynamic wrinkles, the chemical peels treat superficial skin wrinkling and pigmentation. BTX-A treatment is also successfully used in combination with various surgical procedures such as surgical brow lift allowing for greater stability of brow elevation, upper and lower eyelid blepharoplasty and rhytidectomy enhancing the results and prolonging the aesthetic outcome.

Complications: Botulinum toxin is a very safe drug when used appropriately. As the effects of the toxin begin to wear off in 10-12 weeks post-treatment, any undesired results are ultimately self-limited [127]. Irreversible medical complications are not known. There are three general categories of complications: local, regional and systemic.

The most common transient local complications are self-limited adverse reaction at the injection site that typically subsides within a few days without treatment. These include injection site pain, bruising, swelling, erythema, edema, ecchymosis, tenderness, headache and short term hyperestesias [72,73,128]. Headaches are usually mild and last a few hours and are attributed to the initial muscle spasm caused by the toxin in the first 12 hours, followed by muscle weakness for months thereafter [9].

Regional complications are adverse events caused by local diffusion of the toxin into areas not meant to be treated. Some of those have been previously described in previous sections. One of the most feared regional complications is blepharoptosis which is due to the diffusion of the toxin into the levator palpebrae muscles after treatment of glabellar lines or periorbital rhytids [90,93,129]. Ptosis can occur as early as 48 hours or as late as 7 to 10 days post-treatment. Proper technique is the best means to avoid this complication, injecting 1cm superior to the orbital rim and use of the non-dominant index finger to support the desired muscle mass without deep injection through or near the orbital septum [45]. Apraclonidine, an alfa2-adrenergic agonist, can be used to decrease the severity of lid ptosis by stimulating the Mueller's muscle; an elevation of 1 to 3mm can be obtained in this manner [77-80,116]. Brow ptosis is another complication that can occur more often in older patients and can be avoided with proper technique [9,67]. Ectropion can be caused by injections placed around the lower lid affecting the function of the orbicularis oculi muscle [95]. Strabismus have been described after misplaced lateral (crow's feet) or medial (bunny lines) periorbital treatment due to the diffusion of the toxin to the rectus lateralis muscle [55,91]. Other regional complications include dysphagia from platysma muscle injection or loss of facial expression (mask-like face) from excessive paralysis [111,113,114].

Systemic reactions can include nausea, fatigue, malaise, flulike symptoms, distant rashes, respiratory arrest and death. Botulinum toxin is not expected to be present in peripheral blood at measurable levels following intramuscular injection when therapeutic doses are used. Generalized adverse events are reported very rarely in aesthetic medicine, where only very low doses of the toxin are given. In fact, most of the literature on complications is from non-cosmetic sources. Depending on the dose and the number of muscle injected, the onset of systemic reactions typically occurs within 1 week and they continue for 1-2 weeks. Typical effects of systemic action would include dry mouth, red eyes, accommodation disturbances and gastrointestinal symptoms [59,67,77,79,80,130]. The patients at greatest risk are children treated for muscle spasticity disorders, such as cerebral palsy, even in doses comparable to those used to treat cervical dystonia [9].

Immunogenicity

Immunologic complications include acute type I reactions and may be attributable to human serum albumin. These may occur in patients treated with large volume of toxin since the development of IgG neutralizing antibodies against the toxin seems to correlate with an increasing number of injections and the total cumulative dose thus leading to a decreased efficiency due to an inactivation of the toxin itself [132]. In aesthetic medicine, where usually

very low doses are used, the problem of antibodies seems to be of little concern [67].

Nonaesthetic Uses for Botulinum Toxin

The principal therapeutic aim of any kind of condition involving treatment with botulinum toxin is to reduce undesired or excessive contraction of striated or smooth muscles. Since its very first successful experiments on animals in ophthalmology [133], the use of botulinum toxin has increased exponentially throughout the spectrum of medical specialties. It is used with success for the treatment of many neuromuscular conditions and it's recognized to be a first-line therapy for focal dystonias [134,135]. In the (Table 2) there are some examples of the numerous applications for botulinum toxin [136-151]. Many other potential uses of botulinum toxin in medicine are yet to be discovered.

Partial List of the Use of Botulinum Toxin in Medical Terapy			
Achalasia	Dental procedure	Hyperhidrosis	Reduced appetite
Anal Fissure	Esophagel stricture	Inner ear disorder	Spinal cord injury
Back pain	Essential tremore	Neck pain	Strabism
Bening prostatic hypertrofy	Following Mhos microsurgery repair	Massateric muscle hypertrofy	Temporomandibular joint disfunction
Blepharospasm	Facial spasm	Overactive bladder	Teet grinding
Brest reconstruction and augmentation	Gustatory sweating (Frey's syndrome)	Poststroke limb spasticity	Vasospastic disorders Vocal cord disorders
Cerebral palsy	Facial nerve disorder	Parotid fistulas	Tourette's syndrome
Cervical dystonia	Hedaches	Pressure ulcers	Wound healing

Table 2: Non cosmetic applications for Botulinum Toxin in medicine [11].

Conclusion

Minimally invasive aesthetic procedures have grown exponentially in number in the last several years. Botulinum toxin A, is a mainstay of facial rejuvenation and its use is an 100% effective procedure. It is absolutely safe when used at therapeutic dosage. A very important point that has to be perfectly understood is that botulinum toxin A formulations are not interchangeable, therefore the practitioners must learn how to use each product properly to provide optimal and safe outcomes for their patients. The new concepts of aging and rejuvenation have moved towards an appreciation of the three dimensional aspects of aging, including the contribution of volume loss to appearance. For this reason the overwhelming trend is toward increased combined use of botulinum toxin A and hyaluronic acid fillers as well as other modalities such as resurfacing, to provide a more balanced and harmonious aesthetic result and at the same time increasing the longevity of the outcomes. The primary aesthetic use of botulinum toxin remains in the face and neck, being the treatment of glabellar lines the only use approved and all the other uses considered off-label. The potential uses of botulinum toxin in aesthetic and non-aesthetic medicine are numerous and in continuous development.

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