Advanced botulinum toxin techniques against wrinkles in the upper face

Giovanni Salti, MD\textsuperscript{a}, Ilaria Ghersetich, MD\textsuperscript{b},\textsuperscript{*}

\textsuperscript{a}“Florence” Surgical Center, Florence, Italy
\textsuperscript{b}Institute of Dermosciences, University of Florence, Florence, Italy

Abstract The use of botulinum toxin type A for facial rejuvenation is one of the most common procedures in esthetic medicine. Overall clinical and study experience with botulinum toxin type A for facial enhancement has confirmed that it is effective and safe even in the long term. The different injection techniques, the starting doses, the sex, the dilution, and the storing of the product, nevertheless, may influence the final result of the treatment. In this article we propose some recommendations including general principles as well as information about its specific use. Perhaps one of the most important perspectives is that, based on a well-established knowledge of the technique, individualized esthetic planning is the key to success. This depends on a precise understanding of the underlying anatomy and physiology of the individual muscles and their interactions, as well as on individual patient’s characteristics, including goals and expectations.

Botulinum toxin basic science

History of the cosmetic use of botulinum toxin

In 1990, Jean and Alastair Carruthers\textsuperscript{1} published their first report on the cosmetic use of type A botulinum toxin. They discovered this new indication of the drug by serendipity while treating patients for blepharospasm. Subsequently, they started expanding their research in the field and published further articles on the topic.\textsuperscript{2,3} Also, Blitzer and his group\textsuperscript{4} published extensively on the cosmetic use of the drug at the beginning of the 1990s. Later works widened the knowledge of the different commercial preparations of the drug\textsuperscript{5} and of the different types of toxins,\textsuperscript{6,7} leading to a widespread acceptance of the technique after 1995. A large body of scientific articles and textbooks are nowadays available for the cosmetic indication of botulinum toxins.

Basic pharmacology

Botulinum neurotoxin is the exotoxin produced by \textit{Clostridium botulinum}. Seven distinct serotypes of the neurotoxin, designated type A to G, have been identified. Type A is the most commonly toxin used for cosmetic purposes, although there are some reports about the use of type B toxin in aesthetics.

The toxin is a protein molecule consisting of a 50-kd light chain and a 100-kd heavy chain, linked by a disulfide bonding.\textsuperscript{8}

Botulinum toxin acts at the level of the neuromuscular plate and of other cholinergic synapses by a 3-step mechanism\textsuperscript{9}: after injection, the heavy chain rapidly binds to its target receptor on the cholinergic presynaptic
membranes, being internalized by endocytosis. Then, the disulfide bond is cleaved, and the light chain is liberated. The light chain acts as an endopeptidase with proteolytic activity, being able to cleave different plasma membrane proteins responsible for the exocytosis of acetylcholine. Serotype A leads to the cleavage of SNAP-25 (25-kd synaptosomal associated protein), whereas serotype B cleaves VAMP (Vesicle Associated Membrane Protein). When these proteins are lacking, no acetylcholine can be released and thus the synapse is blocked. The final effect is the clinical denervation of the muscle or of the innervated structure. Drug’s effect starts within 24 to 72 hours after injection, and its action is completed in about 2 weeks. The area of functional denervation depends on drug’s dosage and volume. To get a uniform and even effect, multiple injections over the target muscle are recommended. The effects of botulinum toxin on facial muscles persist for approximately 12 to 16 weeks. Because the blockade is not reversible, the remission of the effect occurs after regeneration, approximately 12 to 16 weeks. Because the blockade is not reversible, the remission of the effect occurs after regeneration (nerve sprouting) of motor axons and final reactivation of the blocked end plate.

**Dosage and toxicity, dilution and concentration, storage**

Type A is the most commonly available serotype of botulinum toxin. Several preparations are commercially available, although only one is presently approved for cosmetic use (Botox Cosmetic/Vistabel/Vistabex; Allergan, Irvine, CA), which we will refer to in this article.

When reading dosage data in the literature, it is mandatory to note which commercial preparation the dosing is referring to. The dosage is based on the biologic potency of the toxin and is expressed in units (1 U is the median lethal dose of the toxin when injected intraperitoneally in the rat). The lethal oral dose in humans is estimated at 3000 to 30,000 U. When maintaining an interval between doses of at least 10 weeks, a cumulative effect after repeated injections is not expected. A 100-U vial of the preparation contains 5 ng of toxin as lyophilized powder.

Because the preparation is in powder form, it needs to be reconstituted before the injection. The recommended diluent is 0.9% saline without preservatives. It is usually recommended to gently inject the diluent into the vial, avoiding the formation of foam in the complex, which could result in toxin denaturation. More recent articles, however, have found no difference in efficacy between botulinum toxin not shaken and botulinum toxin shaken and foamed during reconstitution. Some authors proposed to add adrenaline to the solution to reduce the diffusion of the drug once in the tissues. Toxin concentration may vary depending on the medical and aesthetic goal. In the medical literature, concentrations of 100 U/mL (10 U/0.1 mL) to 10 U/mL (1 U/0.1 mL) are encountered, but there is a general consensus that 40 U/mL (4 U/0.1 mL) is the most versatile and useful concentration.

In general, high concentrations (low dilutions) mean low volume injections, with a more precise placement of the toxin and scarce diffusion, whereas low concentrations (high dilutions) may be helpful when a certain grade of diffusion is needed (eg, in the frontalis muscle). Using low concentrations, and hence small volumes, also reduces pain at the injection site. Because the range of transient denervation due to the spreading of the toxin is proportional to its dilution, lower dilutions reduce the risk of unwanted effects.

Most authors use concentrations of 40 or 50 U/mL, but a skilled use implies a fine tuning of dilutions and concentrations to perfectly treat each single muscle, in accordance with the desired degree of efficacy and toxin diffusion. Using varying concentrations of the toxin in different patients and/or in the same patient is the way to achieve the best results.

Once reconstituted, the drug must be stored at a temperature of 2°C to 8°C. After reconstitution, the drug should be used within 4 hours, as recommended by the manufacturer. This recommendation is for sterility purposes and not because of a loss of efficacy. The general practice indeed suggests that the drug still maintains its efficacy even over such a limited period. A study showed that no statistical differences were observed about toxin efficacy and duration of the effect in vials reconstituted at the time of injection, or at 2, 4, and 6 weeks before use. Any remaining solution must be inactivated by a 0.5% sodium hypochlorite solution.

**Injection technique**

There is no standard injection technique. Each patient’s individual characteristics must be taken into account because individualization of the treatment is the key to success. Independently of the technique chosen, any given region to be treated with botulinum toxin should be perfectly studied in its anatomical, static, and dynamic aspects.

Some rules apply when using botulinum toxin for cosmetic purposes. As with any drug, it is recommended to use the lowest efficacious dose tailored to every single patient to provide an effect lasting at least 12 weeks.

The right selection of drug concentration with respect to the area to be treated is mandatory to foresee efficacy and diffusion. The concentration also determines the volume of the injection.

Depending on the volume of reconstituted drug to inject, different types of syringes can be used.

The most commonly used syringes are 1-mL insulin syringes with removable 30-gauge, 13-mm needles. These syringes are marked with a 100-unit scale, allowing for a precise assessment of the number of units injected. The expert will often use the 0.3-mL Ultra-Fine II syringe equipped with a 30-gauge, 8-mm incorporated needle. This syringe allows for the best precision even with low volumes and very concentrated toxin; it is also provided with a fine, less painful needle and is discarded after only few injections, due to the small volume of solution loaded.
Apart from personal preferences, consensus exists about some general technical issues:

- Always examine the patient closely, when at rest and during mimics, taking pictures of him or her in both situations.
- Always mark out with a pencil every injection point before treatment.
- Always use nonalcoholic antiseptics because alcohol could be responsible for toxin inactivation.
- Always treat the patient in a sitting or semi-reclined position. This allows for a better visualization of the anatomy of the muscles to treat.
- Always inject into relaxed muscles.
- Always think in units and not volumes, to reduce confusion and possible over- or underdosing.
- Always note down treatment’s data (date of treatment, dilution of the drug, batch number of the drug, number of total units, units per point, pattern of injection).
- Always ask the patient not to manipulate the treated area for some hours after the injection.

Generally, the injection is not painful; however, some authors treat the target area with ice before treatment to prevent pain and bruising.

Botulinum toxin is usually injected intramuscularly. Subcutaneous or intradermal injections are also effective, although needing more injection sites to obtain maximum absorption. Intramuscular placement stings less and produces less local erythema but carries a higher risk of bruising.

Some of the different injection techniques will be reviewed hereafter.

**Contraindications, systemic effects, and immunogenicity**

Botulinum toxin should not be used in patients with neuromuscular transmission disorders (myasthenia gravis, Eaton-Lambert syndrome). Concomitant use of aminoglycoside antibiotics and spectinomycin should be avoided because
these drugs may potentiate the effect of the toxin. Patients with dysphagia should not be treated either. Because of a limited experience in the field, botulinum toxin therapy should be avoided during pregnancy and lactation, even if there are some anecdotal reports about its nonharmfulness. It should also not be injected in case of infection in the proposed site.

Relative contraindications are coagulation disorders and anticoagulant therapy.

Electromyographic studies revealed minor variations in nontarget muscles, suggesting that low amounts of the drug enter the systemic circulation, without exerting a clinically relevant effect.\textsuperscript{22,23}

Neurologic patients can develop neutralizing antibodies.\textsuperscript{24} It seems that these antibodies are mainly produced in response to the injection of the toxin at high dosage and/or at frequent intervals.\textsuperscript{25} The latest batches of drug contain a purer toxin and are less prone to induce antibody’s production.\textsuperscript{18} To date, there are no reports of antibody formation after injection for cosmetic purposes; it is recommended to use the lowest effective dose of the drug, to avoid treatment repetition before 12 weeks have elapsed, and to avoid short-term booster injection.\textsuperscript{18,26}

**Patient evaluation**

When approaching a cosmetic patient, the physician should be aware that a good result means a pleased patient, and that a patient is pleased when the physician meets her or his expectations. The physician should not simply understand patient’s needs and suggest the best option to address the problem, but he should also identify unrealistic expectations. Each target area should be discussed in detail with the patient to share with her or him a common idea for an optimal correction.

In particular, the degree of paralysis should be carefully clarified with the patient for the determination of treatment dosage. The glabellar area evokes mostly negative feelings (anger, worry, thoughtfulness) and can be completely...
paralyzed, whereas other areas express positive feelings and need to keep their ability to move. So, in these areas, it is important to obtain only a partial effect, eliminating wrinkles at rest while leaving a residual mimic capacity.

Probably the most important cosmetic preoperative evaluation is the analysis of preexisting asymmetries. Almost nobody is perfectly symmetrical, and all asymmetries, even minor, should be pointed out to the patient because after the treatment, she or he will look at her or his face almost constantly, finding out any asymmetry and considering the treatment responsible for it.

Advanced concepts in botulinum toxin treatment of the upper face

Browlift and brow reshaping

Besides classic indications, browlift is the first most requested treatment. Browlift patients are often different from classic wrinkle patients because they are usually younger and do not ask for a rejuvenation treatment but for a pure cosmetic effect.

The ideal patient for this type of treatment is young, with good skin elasticity, and with no static wrinkles because the range of brow elevation that can be achieved with botulinum toxin is limited to 1 to 3 mm. A pretreatment evaluation should take into account some important features, such as the shape of the eyebrow, the presence of preexisting asymmetries, the presence of a supraciliary wrinkle, and the desired modifications. The original shape of the eyebrow must be respected, thus limiting the chances to modify its aspect. A supraciliary wrinkle is another important limiting factor because the elevation of the brow tail could worsen it. In this case, a complete elevation of the eyebrows is better than a rebalancing of the shape.

The balance between elevator and depressor muscles should be studied before performing any treatment because only the frontalis muscle elevates the eyebrows, whereas the corrugator supercili, procerus, and orbicularis ones move...
Downward and medially the eyebrows (Figs. 1 and 2). Corrugator supercilii and depressor supercilii muscles are attached to the periosteum in the glabellar region under the frontalis muscle, deep in the medial aspect. The muscles then continue laterally and superficially to insert in the skin above the eyebrows. The corrugator is usually treated in 2 points: medially at its bone attachment, and laterally at its skin insertion. The medial corrugator injection can be performed deeply or superficially; a deep plane injection acts only on the corrugator muscle, whereas a superficial injection acts on the frontalis too. The consequence is that a deep injection leaves the frontalis unaffected and able to elevate, whereas a superficial injection targets also the frontalis, causing a relaxation of the head of the eyebrow. Thus, to achieve a rebalancing effect, the exotoxin should be applied also superficially in the medial aspect of the corrugator muscle; whereas to obtain a medial elevation of the glabellar complex, it should be applied only deeply (Figs. 3-6). Sometimes it is useful to obtain only a rebalancing of the shape by using the bascule movement of the brow when the medial part is depressed and the lateral part is elevated by the upward traction of the frontalis.

The arched brow is the classic feminine eyebrow shape. To create or enhance this shape, the injections should be concentrated in the medial and central area of the brow. Leaving the lateral brow untreated helps to create the downward slope at the lateral edge of the brow, accentuating the arch. A single toxin injection in the orbicularis reduces its action in the central brow area and leaves the frontalis unopposed. To evaluate this injection site, it is useful to place a finger under the brow in the midpupillary line, asking the patient to squeeze the eyes as hard as she or he can. The outer edge of the orbicularis will pull down the finger, indicating the point where the brow depressor has to be weaken (Figs. 7-9).

Nowadays, a flared brow is widespread considered very fashionable. To obtain this type of shape, we inject the lateral section of the orbicularis oculi starting from the midpupillary line and we also treat the crow’s-feet area to release the pulling force of the orbicularis at the lateral brow edge. We do not treat the procerus because our objectives are a medial lowering and a lateral lifting.

For a stronger flare, as preferred by fashion models, we may also consider to treat the frontalis in the central part.
forehead area to further drop the medial brow and accentuate the lateral arch. It is also possible to give more or less elevation to the brow tail depending on the dosage of the drug. For a softer flare with a downward lateral edge, we suggest not to treat the crow’s-feet area (Figs. 10-12).

The horizontal brow is the natural shape of the brows in most of the women who have not cosmetically correct them. Injecting the brow symmetrically at the medial and lateral edges and concomitantly releasing the procerus muscle will produce a “more open-eyed look.” The objective is an unopposed elevation at the medial and lateral ends of the brow (Figs. 13-15).

**Periocular techniques**

The lateral canthal lines begin to appear around 20 to 25 years of age. These lines are firstly only dynamic wrinkles, but later they become fixed and present even at rest. These lines are caused by the contraction of the lateral aspect of the orbital portion of orbicularis oculi muscle. This is a very superficial muscle constituted by fibers originating inferiorly on the anterior aspect of the infraorbital rim and superiorly on the supraorbital rim, and inserting in the lateral canthal tendon. Because there are different patterns of periocular wrinkles (diffuse, mostly superior, mostly inferior, and diffuse fine) (Figs. 16-19), different techniques of injection can be used in this area. The same patient can have one or multiple patterns of wrinkles so the treatment should be individualized for each single periocular area. Dynamic wrinkles respond well while static wrinkles are not completely amenable to botulinum toxin injections, although repeated treatments greatly reduce static wrinkles too.

The classic 3-spot technique can be reserved to a diffuse pattern of lines.
In case of localized lines (superior or inferior), the wheal-and-mash technique may be more indicated (Fig. 20). This is a very simple way to administer the drug. One or two wheals of drug are gently massaged upward, downward, and laterally, resulting in a diffuse spreading of the drug without the risk of injuring the numerous vessels in this area using repeated injections.

In case of diffuse fine lines, a multispot technique (up to 8 injections) may be indicated (Fig. 21). For this type of injections, a strictly intradermic puncture is mandatory to obtain a limited shift of the pharmacologic solution and to avoid bruising.

In case of mostly inferior lines, a single injection may also be used (Fig. 22). This technique of “kebabing” the orbicularis muscle with a single tangential puncture causes less discomfort to the patient, but it should be carried out by a skilled and experienced operator because the use of a longer needle is suggested and the continuous retrograde injection requires the maintenance of a constant cutaneous plane as well as a constant pressure on the plunger (Figs. 23 and 24).

Avoiding mephisto

The frontalis muscle is the only elevator muscle in the upper face. Its complete treatment causes a flaccid paralysis with brow ptosis: it is therefore necessary to avoid injecting some areas of this muscle to leave some mobility, useful to get a lifting effect. Sometimes, when too much lateral activity is left, the tail of the eyebrows is excessively pulled upward, resulting in an unnatural look that has been well described as “mephisto.”

The lateral extension of the frontalis muscle ends at the level of the temporal fusion line (Fig. 25) so that it is not necessary to inject laterally to it.
The area medial to the temporal fusion line and lateral to the midpupillary line can be classified in 3 different patterns:\textsuperscript{34}:

- **Type 1:** no wrinkles on contraction in the lateral part of the frontalis above the lateral edge of the eyebrow.
- **Type 2:** wrinkles on contraction in the upper area of the lateral part of the frontalis not extending toward the lateral edge of the eyebrows.
- **Type 3:** wrinkles on contraction all over the lateral part of the frontalis, from the scalp to the eyebrow.

**Type 1** is found in young patients with good skin elasticity, whereas **type 3** is found in older patients with diminished skin elasticity.

This small area of the frontalis muscle is responsible for the mephisto side effect and must be carefully studied, both at rest and on contraction, because our goal is the determination of a cosmetically pleasant elevation of the brow tail without wrinkling.

In case of type 1 pattern, there is no need to inject laterally to the midpupillary line because, here, the frontalis muscle will pull upward the eyebrow without wrinkling.

In case of type 2 pattern, it is necessary to inject a small quantity of toxin (1-2 U) over the most protruding area of the frontalis, between the temporal fusion line and the midpupillary line. This will paralyze the wrinkling area without affecting the elevation of the eyebrow that will be moderately lifted.

In case of type 3 pattern, 1 U should be injected in the upper part of the lateral frontalis and 1 U in the lower area of the lateral frontalis, between the temporal fusion line and the midpupillary line, taking care to keep the injection site about 0.5 to 1 cm above the lowest supraciliary wrinkle. This is the most critical case because these injections will remove wrinkling from the area, but they will also limit the activity of the frontalis muscle with no elevation of the eyebrow; it is better not to treat old patients who use their frontalis to elevate the eyebrow and eyelids.

### References


