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ORIGINAL ARTICLE

The five D's of botulinum toxin: Doses, dilution, diffusion, duration and dogma

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Abstract
The purpose of this review is to update cosmetic dermatologists and surgeons on the latest information about botulinum toxin injections for the treatment of the face and neck and to provide a practical guide to effective and safe technique. We review indications, recommended doses and dilutions, storage recommendations and injection techniques.

Key words: *Botulinum toxin, dynamic rhytids*

Introduction

The purpose of this review is to update cosmetic dermatologists and surgeons on the latest information about botulinum toxin injections for the treatment of the face and neck and to provide a practical guide to effective and safe technique. We critique some of the dogmas present in botulinum toxin literature. (The doses referred to in this article are specific to the botulinum toxin type A brand Botox; Allergan Inc.)

Anatomy

Successful injection technique requires a thorough understanding of the facial anatomy and interactions between the muscles. Physicians should be aware of the secondary compensation mechanisms that may occur when trying to paralyze or diminish the activity of a certain muscle or group of muscles.

Patient selection

Proper patient selection is essential to successful treatment with botulinum toxin. Patients should be instructed that only dynamics lines are likely to improve and that deep, static rhytids resulting from actinic damage or chronologic aging may not see improvement. Absolute contraindications for botulinum toxin therapy include the presence of infection at the proposed site of injections and hypersensitivity to any ingredient in the formulation (i.e. botulinum toxin, human albumin, and saline) (package insert). Relative contraindications are: (i) psychologically unstable patients or those who have unrealistic reasons and goals; (ii) people who are dependent on facial expression for their livelihood (e.g. actors and actresses); (iii) those afflicted with a neuromuscular disorder (e.g. myasthenia gravis, Eaton–Lambert syndrome); (iv) those taking certain medications that can interfere with neuromuscular transmission and amplify the effects of botulinum toxin (e.g. aminoglycosides, penicillamine, quinine, and calcium channel blockers); and (v) pregnant or lactating women (botulinum toxin is classified as a pregnancy category C drug). There are a few reports in women who became pregnant after the injections or who were injected during early and late pregnancy. These reports have not found any adverse effects to pregnancy or fetuses. Only one patient, who had experienced prior spontaneous abortions, suffered a miscarriage (1,2).

It should be known that botulinum toxin may interfere with neuromuscular blockade monitoring during general anesthesia because neuromuscular blockage is frequently monitored by facial nerve stimulation of the orbicularis oculi. Therefore, we suggest that patients should be told to inform their anesthesiologists if they have had botulinum toxin in the months anteceding surgery requiring this type of anesthesia. An alternative way to monitor neuromuscular blockage in these patients is by peripheral

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ulnar nerve stimulation of the adductor pollicis muscle (3).

Reconstitution technique

It was believed that botulinum toxin is a fragile toxin and that special attention should be paid in the reconstitution process. Early articles recommended that saline should be gently introduced into the vial to avoid formation of foaming during reconstitution. Also, the vial should not be shaken (4,5). Supposedly, bubbles associated with foaming could result in surface denaturation of toxin. Trindade de Almeida et al. (6) have reported that botulinum toxin (reconstituted with non-preserved saline) maintains its potency even if the vial is shaken and in the presence of foaming during the reconstitution process. Although this observation seems to agree with our clinical experience, further studies are needed to confirm these findings. Gentle reconstitution is best.

Storage

The manufacturer recommends that once reconstituted, botulinum toxin should be stored in the refrigerator (2–8°C) and used within 4 hours. A recent study showed that routine refrigerator storage of botulinum toxin reconstituted with preserved saline does not result in microbial contamination of the contents even after serial re-extraction of solution from the vials, and after handling of such vials by multiple personnel. Storage and subsequent reuse of botulinum toxin appears to be safe for at least 7 weeks after reconstitution (7). Hexsel et al. have demonstrated that botulinum toxin reconstituted with preservative-free saline may be injected up to 6 weeks after reconstitution without losing its effectiveness (8). However, they did not do bacterial assays to demonstrate the sterility of their solution. One must keep in mind that when using normal saline without preservative there is an increased risk of bacterial contamination and colonization of the solution the longer it remains unused. Anecdotally, the authors observed greater efficacy with freshly reconstituted botulinum toxin.

Dilution/diffusion

Each vial of botulinum toxin contains 100 U of toxin, 0.5 mg of human albumin and 0.9 mg of sodium chloride in a sterilized, vacuum-dried form without a preservative.

At present, the range of dilutions and injection volumes depends on the preference of the practitioner and the number of units to be injected. The subject is controversial. Some physicians advocate the use of more concentrated solutions to allow for more accurate placement and fewer side effects from diffusion into adjacent muscles. Their opponents on the other hand argue that concentrated solutions are difficult to work with and can lead to waste of material. Plus, some believe that diffusion is an advantage, allowing us to use fewer units in areas such as the frontalis muscle.

Dilutions reported in the literature have varied from 100 U/ml to 10 U/ml (equivalent to diluting with 1 ml to 10 ml, relatively). Most dermatologists dilute botulinum toxin with 1–3 ml saline (100–33.33 U/ml) when treating the face (4). Some studies have shown that dilutions between 100 U/ml and 10 U/ml appear to be equally effective and offer a similar duration of effect (5,9). One study examined the effect of injections in the forehead and found that higher dilutions were associated with a wider area of effect (10). On the other hand, Carruthers et al. showed that the more concentrated dose of botulinum toxin (5 U per 0.05 ml) was slightly more effective in reducing orbital rhytids than the more diluted dose (5 U per 0.25 ml), but the difference was small and likely non-significant (the study was too small in number to apply inferential statistics and determine significance) (11). Higher volumes (lower concentrations), however, seem to be associated with more pain (5).

Hsu et al. suggested that the ideal strategy may be to inject concentrated toxin at a low volume to target smaller muscle groups, while using a larger volume for larger, broad muscle groups, such as the frontalis muscle (10). They suggest the use of a short-needle, 30-gauge, 0.3-ml insulin syringe (Becton Dickinson) for more concentrated solutions because it allows the delivery of a precise number of units and limits the waste of toxin (the needle design allows no dead space in the needle hub). Flynn et al. reported that with this syringe, full depression of the plunger leaves less than 0.01 ml in the needle itself, a contrast with the 0.07 ml that is retained in the dead space in a traditional 30-gauge needle (12) At a 100 U/ml dilution, this would mean a loss of 7 U of botulinum toxin (12). The syringe comes with a silicone-coated 30-gauge needle, which easily penetrates the skin. The needle stays sharp for approximately four to six punctures.

Although the manufacturer of botulinum toxin recommends that it should be diluted with preservative-free saline, some studies have shown that this is not necessarily required. A few studies have reported that dilution with preserved saline is less painful and is as effective and safe as dilution with non-preserved saline for the treatment of upper-face dynamic lines (13) and essential blepharospasm (14). They attributed this effect to the anesthetic properties of benzyl alcohol contained in the preserved saline since the pH of both salines is similar (13,14). A recent study reported that dilution with 2% lidocaine chlorhydrate was as effective as

dilution with saline for axillary hyperhidrosis and was less painful (15).

Injection technique/doses

In general, injections should be done perpendicular to the skin and intramuscularly, directed to the belly of the muscle. Figures 1–13 show the injection patterns for the various groups of muscles. Each patient requires individualized injection patterns. Most patients have slight facial asymmetry at baseline. This should be noted and 'before' photographs should be taken. For areas that require special attention, specific instructions are provided below.

When considering dosing, the total number of units to be injected depends on the area, muscle mass and strength, and should be individualized. Generally, men require more units than women. Recommendations for specific areas are given below.

Frown lines

Injection technique

The procerus injection is in the midline at the upper nasal bridge. The muscle can be grasped between the thumb and index finger of one hand and the free hand is used to inject. For the corrugators, different injection points have been described. To avoid diffusion to the levator palpebrae superioris muscle, which can cause blepharoptosis, the injections should be placed at least 1 cm above the orbital rim (16). Press with one finger inferior to the injection point. This help prevents pain and lessens bruising. The eyebrow should not be considered the landmark for placing injections because the brow itself may be ptotic, plucked, shaped, tattooed, dyed, and otherwise modified. A typical injection pattern is shown in Figure 1.

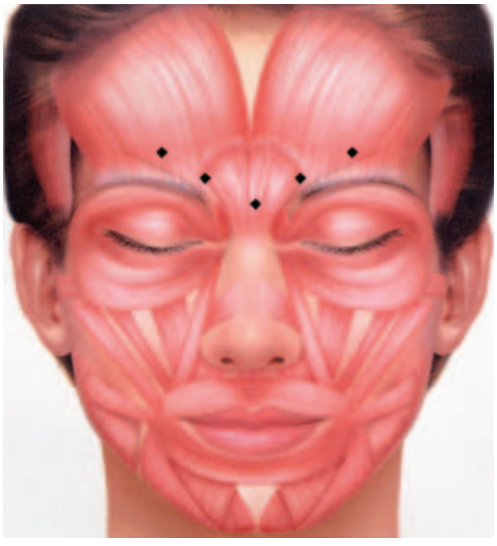

Figure 1. Injection points for frown lines.

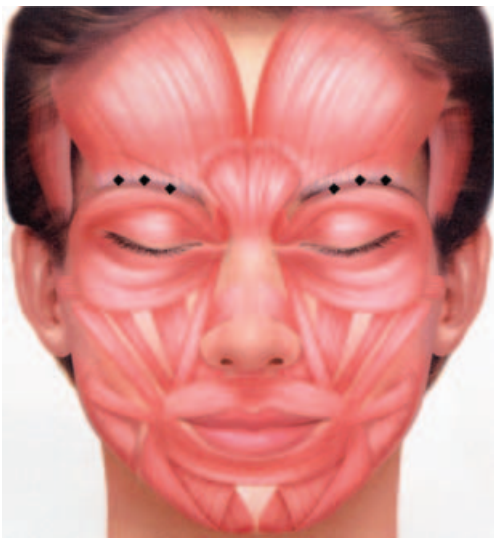

Figure 2. Additional points for frown lines.

After treatment of the glabella, some patients compensate the paralysis of the corrugators with enhanced contraction of the horizontal fibers of the orbicularis oculi muscle. The effects are: (i) increased vertical lines above the eyebrow and (ii) medial pulling of the upper eyelid. In this case, the authors find it helpful to inject the orbicularis oculi in a few points just above the orbital rim (Figure 2). We inject 1–2 U in each injection point without brow ptosis.

Doses for frown lines

Hankins et al. (5) found a threshold for a demonstrable response to botulinum toxin for glabellar folds to be between 5 and 12.5 U (1–2.5 per injection site, five injection sites), whereas an effective starting dose was between 12.5 and 20 U. In this study, dose did not affect the longevity of treatment.

Carruthers et al. (17) compared the efficacy, safety, and duration of effect of four doses of botulinum toxin (10, 20, 30 or 40 U) in the treatment of glabellar rhytids in females. A total of 20–40 U of botulinum toxin were significantly more effective and longer lasting than 10 U. There were no statistically significant differences among the three higher-dose groups (20, 30 or 40 U). Most individuals experienced benefits for 3–4 months; some individuals demonstrated an effect for up to 12 months. Among members of a consensus panel, 96% began treating women with either 20 or 30 U of botulinum toxin (18).

In men, a study comparing total doses of 20, 40, 60, or 80 U administered in the glabellar complex, found that 20 U was significantly less effective than the other doses (19). Among members of a consensus panel, 38% began treating men with 40 U and 30% frequently started with higher doses (45–120 U) (18).

Forehead rhytids

Injection technique

Before injecting the forehead the patient should be evaluated for brow and lid ptosis, which is especially important in elderly individuals. People with brow and lid ptosis try to correct and compensate the depressed position of their brow/lid by constant contraction of the frontalis. These patients should not be injected because any weakness of the frontalis may compromise their visual field.

In general, avoid injecting the frontalis without injecting the glabella. The unopposed action of the brow depressors (corrugators and procerus) could theoretically lead to medial brow ptosis. This is less evident in young patients.

When injecting the forehead it is important that all injections remain at least 1 cm above the orbital rim to reduce the potential for brow ptosis. The recent trend is to inject the frontalis quite high (at least 2 cm above the orbital rim) to maintain some brow movement and avoid a frozen look.

The pattern of injection of the forehead varies considering the desired brow shape. Usually, it is aesthetically more appealing for women to have an arched eyebrow and for men to have a flatter, horizontal eyebrow. For women desiring a more arched brow, inject the central portion of the frontalis and omit the lateral portion. These women should be warned that this pattern of injection can leave them with residual wrinkles above the lateral eyebrow. Sometimes it can result in an exaggerated lateral brow lift and a quizzical appearance known as 'Jack Nicholson's look'. If this happens, placement of 1–2 U of botulinum toxin placed about 2 cm above the eyebrow at the line of maximum eyebrow elevation usually corrects the problem. For men, the whole frontalis should be injected, including the lateral portions. The typical injection pattern is shown in Figure 3.

Doses for forehead rhytids

Levy et al. (20) reported that 5 or 10 U were equally effective for the treatment of forehead dynamic lines. Doses did not affect duration of effect. The authors emphasized the role of low doses of botulinum toxin injections to obtain good clinical results without the freezing aspect (20). Unfortunately, this study did not disclose whether patients were female, male or both.

Carruthers et al. compared three doses of botulinum toxin (16, 32 and 48 U) for female horizontal forehead rhytids. They found that higher botulinum toxin doses resulted in greater efficacy and longer duration of effect (21).

Among members of a consensus panel, 94% of panel members recommended a total starting dose of 10–20 U for women, with 61% recommending the lower dose. For men, 32% recommended starting with 20 U, and 46% recommended starting with 30 U. The calculated typical starting dose was 15 U for women and 20 U for men (18).

Eyebrow lift

Injection technique

Two common techniques for achieving an eyebrow lift are: (i) injecting the glabella alone and (ii) injecting the lateral orbicularis oculi (vertical fibers), lateral to the mid-pupillary line. Some perform injections for the lateral orbicularis oculi in only one point at the temporal fusion line (Figure 4).

The rationale for injecting the glabella alone can be explained in two ways. First, weakening of the muscular depressors of the brow (medial and lateral orbicularis oculi, procerus, corrugator, and depressor supercilii) may allow for unopposed action of the primary brow elevator (frontalis muscle) and result in brow elevation (22). Second, it may be due to diffusion of botulinum toxin into and partial

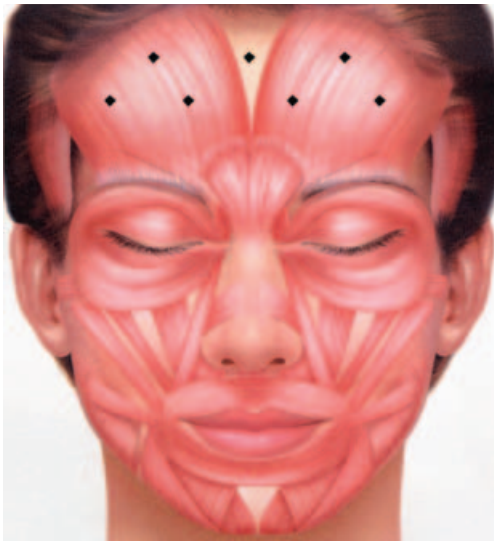

Figure 3. Injection points for forehead rhytids.

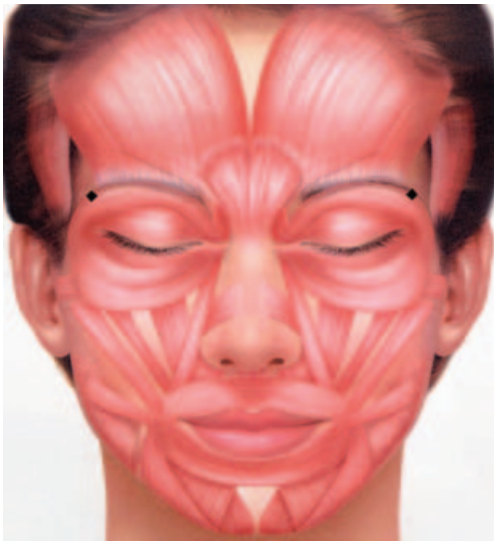

Figure 4. Injection points for eyebrow lift.

inactivation of the medial fibers of the frontalis, with resulting increased muscle tone in the lateral and superior muscle fibers of the frontalis (23).

Doses for eyebrow lift

One study showed that injections of 20–40 U botulinum toxin into the glabella alone (with the most lateral injection at the mid-pupillary line) led initially to a dramatic lateral eyebrow elevation, which was followed by a central and medial eyebrow elevation that peaked at 12 weeks after treatment (23). Interestingly, too little botulinum toxin (10 U) resulted in a mild brow ptosis and a brief fall in the eyebrow position. According to the authors, brow ptosis with low doses of botox could be caused by partial weakening of the frontalis without corresponding weakening of the strong depressor muscles (23).

Ahn and coworkers (24) demonstrated a statistically significant elevation of the brow after injection of 7–10 U of Botox into the lateral orbicularis oculi muscle lateral to the mid-pupillary line.

Crow's feet

Injection technique

Treatment for crow's feet is directed to the lateral orbital portions of the orbicularis oculi muscle. Injections should be superficial (because the orbicularis is very thin and superficial), kept lateral (approximately 1–1.5 cm from the orbital rim) and directed 'outside' the orbital rim to avoid diffusion to extra-ocular muscles and palpebral portion of the orbicularis oculi which can cause strabismus and lid ptosis. Injecting the area below the zygomatic arch should be avoided because diffusion to the zygomaticus major muscle can lead to lip and cheek ptosis, resulting in an appearance of peripheral facial paralysis. A typical injection pattern is shown in Figure 5.

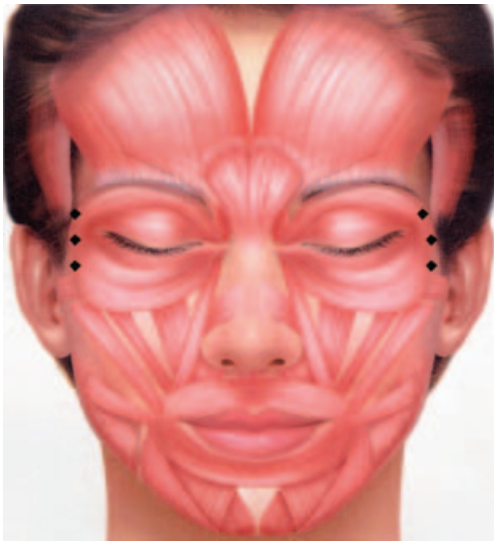

Figure 5. Injection points for crow feet.

It is important to distinguish between lines caused by orbicularis contraction and those produced by the zygomaticus muscles. These muscles push the cheek up towards the periorbital region, contributing in some patients to crow's feet. The wrinkles produced by the zygomaticus action can only be treated if the zygomaticus complex is injected, which is risky and can lead to facial droop and an asymmetrical smile. There is a good way to distinguish between the wrinkles produced by the orbicularis and the ones produced by the zygomaticus. When evaluating the crow's feet, instead of asking the patients to smile, ask them to close their eyes as hard as they can. The wrinkles produced by this movement are more likely to be due only to orbicularis oculi action and therefore be improved with botulinum toxin.

Doses for crow's feet

Lowe et al. (25) found that 6, 12 or 18 U of botulinum toxin (per side) were significantly superior to placebo for the appearance of crow's feet (men and women were included in this study). They did not find a clear dose–response relationship.

In another dose-finding study (that compared 3, 6, 12 and 18 U (per side)), greater doses had a better and longer duration of effect. A clear difference between the 12 U and 18 U was not seen. The authors concluded that 12 U was the optimal dose (26).

Among members of a consensus panel, 96% started treating crow's feet with 8–16 U per side in women and 89% used 12–16 U per side in men (18).

Infraorbital folds/opening of the eye

Injection technique

Injecting the lower eyelid can correct wrinkles in the area and cause an opening of the eyes. Careful selection of these patients is essential. Patients with lower eyelid laxity and those who have undergone previous eyelid surgery are more prone to complications, particularly ectropion. Patients with pre-existing fat protrusion are not good candidates because when the musculature is weakened this condition can be aggravated (27). Other contra-indications are patients with Sjogren's syndrome, other conditions associated with dry eyes (which can be worsened) and patients who have lower scleral show.

Injections should be directed to the pretarsal portion of the orbicularis oculi. The needle should be inserted from a lateral position, oriented horizontal to the ground and tangential to the lower eyelid to avoid puncture of the orbit if the patient moves during the procedure. Usually, one or two sites are injected, the first one being a point 3 mm below the ciliary margin in the mid-pupillary line and the

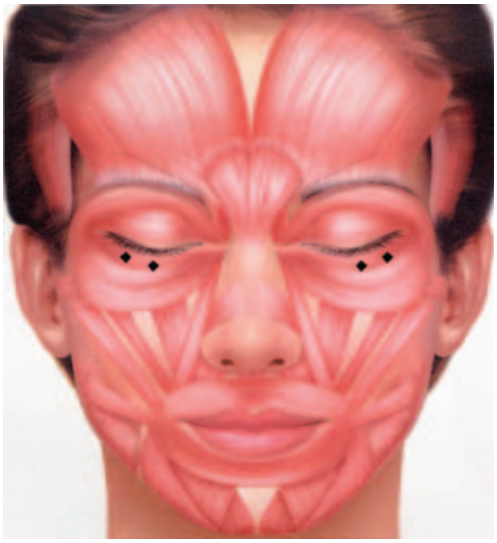

Figure 6. Injection points for infraorbital folds and opening of the eye.

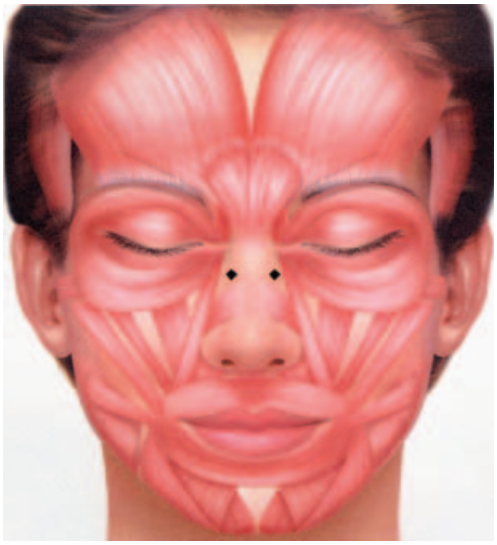

Figure 7. Injection points for bunny lines.

second being halfway beneath the mid-pupillary line and the lateral canthus (3 mm inferior to the ciliary margin) (Figure 6). In this area, injections should be kept superficial (subdermal) to avoid diffusion to the inferior oblique muscle (which can result in diplopia).

Doses for infraorbital folds/opening of the eye

One study reported the use of 2 U for the lower eyelid with mild improvement. In this study, better results were attained when the lower eyelids were treated in conjunction with the crow's feet (28). Another study demonstrated a dose–response curve in the degree of improvement when 2, 4, or 8 U were used, with greater units showing better results (29). However, increasing the number of units was associated with more side effects. Five of eight patients in the 8-U study group were not happy with their treatment because of bothersome side effects such as photophobia, incomplete lid sphincter, and lower eyelid edema (29). Also, the authors reported that 8 U often produced an undesirable degree of increase in palpebral aperture (with patients reporting a 'sad' look and exhibiting a good degree of scleral show) and an excessive lateral rounding of the lower lid (29). They suggest that 2 U should be used initially in the lower lid, alone (for eye opening) or along with the crow's feet (for periorbital wrinkles), and the patient re-evaluated in 2 weeks. If no undesirable effects are noticed and further improvement is desired, another 2 U can be injected (29).

Bunny lines

Injection technique

Injections should be on the lateral aspect of the nasal wall above the nasofacial groove (Figure 7). Massaging should be generally avoided, particularly in a downward direction, because diffusion to the levator labii superioris alaeque nasi muscle can cause ipsilateral lip ptosis.

Doses for bunny lines

Among members of a consensus panel, the usual total starting dose was 2–5 U. Men may receive a dose that is slightly higher by 1 U (18).

Perioral rhytids

Injection technique

Injections are done superficially at or above the vermillion border in the area of muscle contraction adjacent to the vertical creases (Figure 8). The corners of the mouth are not injected to prevent

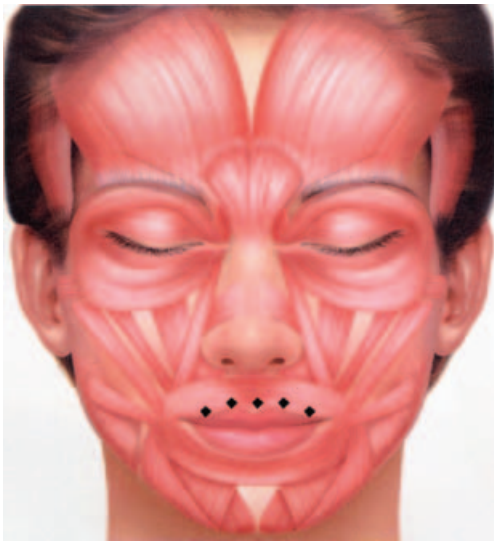

Figure 8. Injection points for perioral rhytids.

weakness of the lateral lip elevators, which can lead to drooping of the lateral lip and drooling (30). Common side effects include difficulty whistling, saying 'p' and 'b', kissing, eating soup with a spoon and drinking through a straw. For this reason musicians or public speakers should not receive treatment in this area. Also, patients with a very thin/atrophic upper lip or a long columella-to-vermillion distance should not be injected. These patients may experience an even thinner lipped appearance because of the slight lengthening of the lip (31).

Doses for perioral rhytids

The goal is to inject as few units as possible to obtain good cosmetic results while preserving functionality of the mouth. Among members of a consensus panel, the average starting dose for the perioral area was approximately 5–6 U. Most panel members recommended 1–2 U per injection point (18).

It has been reported that perioral rhytids return more quickly than forehead and glabellar lines and patients usually need retreatment every 2–3 months (31).

Elevation of the corner of the mouth

Injection technique

Patients are usually asked to pull down the corners of their mouths or to show their inferior teeth to aid in the location of the depressor anguli oris muscle before the injection. The site of the injection is usually a point where the trajectory of nasolabial fold meets the jaw line. One injection in each side is the rule (Figure 9). It is important to localize the muscle before the injection; if the injection is too medial it can cause an ipsilateral weakness of the depressor labii, if it is too high it can compromise the sphincter function of the orbicularis oris (30).

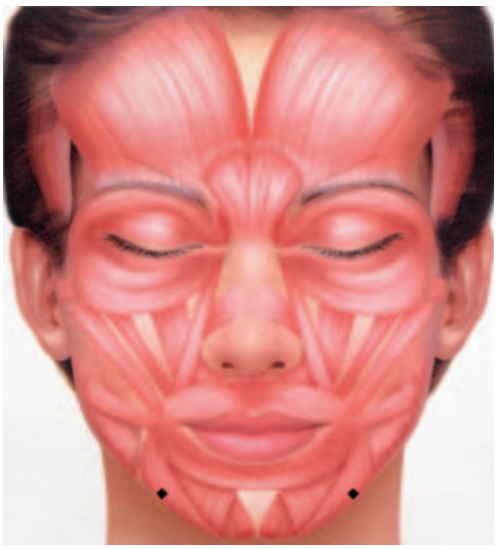

Figure 9. Injection points for elevation of the corner of the mouth.

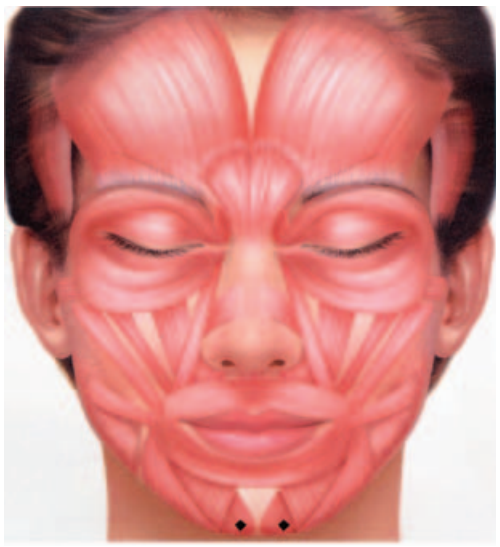

Figure 10. Injection points for dimpled chin.

Doses for elevation of the corner of the mouth

In the depressor anguli oris muscles, low doses are used: 2–5 U in each site (18).

Dimpled chin

Injection technique

Injections should be placed deep because of the reasonable amount of fat that exits in the chin area. One injection in the midline or two (one in each side of the point of the chin) (Figure 10) are used in the mentalis at the most distal point from the orbicularis oris – the prominence of the chin. This injection site is chosen to avoid complications from oris orbicularis weakening (30).

Doses for dimpled chin

Among members of a consensus panel, the typical total starting dose was 5–6 U, with some experienced users going up to 12 U. In general, they found that 5–10 U should be adequate. Dosing is similar in women and men; occasionally, men who are treated may require a dose 2–3 U greater than that used for women. The total dose is divided evenly among sites if more than one injection point is used (18).

Elevation of the tip of the nose

Injection technique

The injection point is at the base of the columella (Figure 11). Caution should be taken with individuals who already have a long columella-to-vermillion

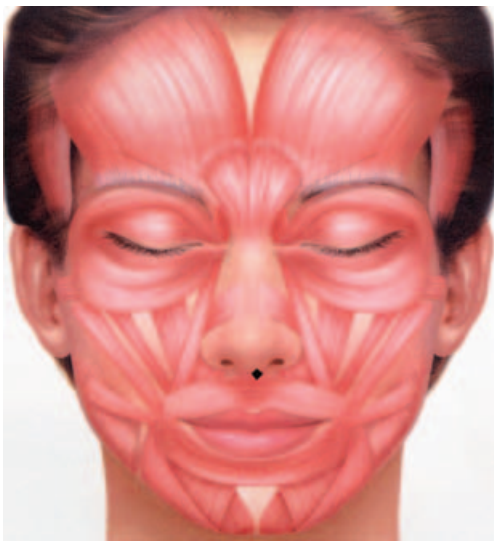

Figure 11. Injection point for elevation of the tip of the nose.

distance because there is a significant risk of lip ptosis with the procedure.

Doses for elevation of the tip of the nose

A total of 2–3 U are typically used in this area (30).

Excessive gingival show ('gummy' smile)

Injection technique

Injections are placed bilaterally in the nasofacial groove into each levator labii superioris alaquae nasi (Figure 12). The injection site can be localized by placing a fingertip on the pyriform aperture just inferior to the nasomaxillary groove.

Doses for excessive gingival show

One unit per site is usually used.

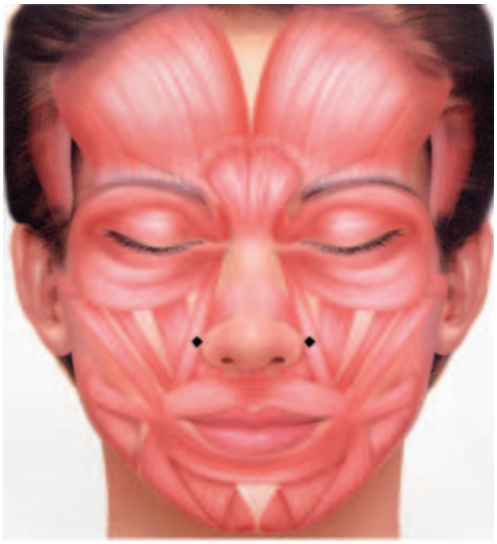

Figure 12. Injection points for excessive gingival show.

Platysmal bands

Injection technique

To identify properly the platysmal bands, patients are asked to forcefully contract their necks by clenching their teeth. Each band is grasped individually and held firmly between the thumb and index fingers. Injections are placed directly into the platysmal band at 1.0- to 1.5-cm intervals along the band, starting at the jawline and descending all the way to the clavicular border (32). Adverse reactions include neck weakness (manifested by difficulty lifting the head off a pillow from the decubital position), dysphonia and difficulty swallowing (18).

Doses for platysmal bands

In the published literature, a wide range of doses has been used to treat platysmal bands. Brandt and Boker (32) and Matarasso et al. (33) agree that most patients need a total of 50–100 U to achieve optimal correction, while Kane (34) uses doses between 10 and 40 U. Among members of a consensus panel, doses were also quite variable, with total doses of 6−40+ U per band (usually two bands are treated in a session) (18). The authors have success using 2 U per injection site spaced 1–2 cm apart along the length of the bands. Not all patients respond regardless of the dose.

Redefinition of the jaw line/mini neck lift

Injection technique

This is a new technique the authors have been using with good results for patients with mild laxity of the neck and poor definition of the jawline. We inject six points evenly distributed along the jawline (Figure 13).

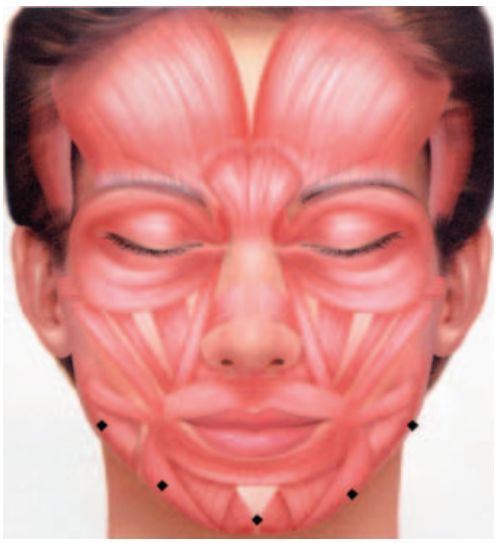

Figure 13. Injection points for mini neck lift.

Doses for redefinition of the jaw line/mini neck lift

Three to four units are used in the four lateral points of injection and 2 U in the central ones.

Other recommendations

Massaging the points of injection is a common practice. It helps to smooth the 'bumps' after the injection and some believe it helps with the diffusion of the toxin. To our knowledge, there are no studies looking at this particular subject. One should be cautious, however, when massaging areas where diffusion can be a problem such as the procerus and bunny lines. An alternative technique to massaging is the 'press and hold' technique, which helps with the 'bumps' and possibly with bruising.

It has been long taught that after injection of botulinum toxin, patients should exercise the affected muscles for up to 4 hours to enhance cellular uptake. There is no published data to document the need for this prolonged exercise. Data suggest that cellular uptake takes only 32–64 minutes in the nerves of actively contracting muscles (35). This suggests at most patients should exercise their injected muscles for up to 1 hour after treatment, not 4 hours.

Similarly, it is recommended by some physicians that patients should avoid bending their heads to reduce the chance of unwanted diffusion on the toxin. No controlled studies have been conducted to clarify whether bending of the head actually influences diffusion. On a consensus panel published in 2004, 71% of the members did not recommend to their patients to avoid bending their heads (18). If one chooses to do so, the recommendation of staying upright should also be limited to 1 hour, since, as mentioned above, the toxin binding process takes only 1 hour (36).

Conclusion

Botulinum toxin injections are the most common aesthetic procedure performed in the United States. Our understanding and knowledge about the toxin has grown tremendously since it became available, which has led to an expansion of its use and indications. As new data became available, dogmas were left behind. A thorough understanding of the facial anatomy, the injection technique as well as proper dilution and storage are essential to a successful outcome without complications. This review of the 5 D's is a practical up-to-date guide to safe and effective technique.

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