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*Research Article*

# Botulinum Toxin Treatment of Masseters at the Point of Neural Arborization with a Single-Entry Injection Technique

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## Abstract

Introduction: Botulinum Neurotoxin Type A (BoNT-A) is frequently used off-label to reduce masseter hypertrophy. Varied injection techniques and outcomes across populations highlight the need for a standardized, anatomical approach. We aimed to assess the safety and efficacy of a single-entry injection targeting the middle lower part of the masseter, which corresponds to the neural arborization zone of the masseteric nerve branches.

Methodology: This retrospective cohort study included 131 patients of Chinese, Caucasian and Middle Eastern/Asian ethnicity with benign bilateral masseteric hypertrophy treated with different types of BoNT-A at the point of neural arborization with a single-entry injection technique, by three different doctors. Clinical outcomes and adverse events (if any) during follow-up at 3- to 4-weeks and 3-months were noted.

Results: Dosages averaged 24 Botox units (BU), 27 BU and 28 BU for Botox®, Dysport®/Azzalure® and Xeomin®/Bocouture® respectively. Of 131 patients, 126 (96.2%) were female; mean age was 32.8 years. Ethnic distribution comprised 97 Chinese patients (74.0%), 22 Caucasian patients (16.8%) and 12 Middle Eastern/Asian patients (9.2%). Two minor complications (1.5%) were reported: mild asymmetry and lack of effect; no major complications such as infection or hematoma occurred.

Conclusion: The single-entry injection technique aimed at the point of neural arborization in the masseters is safe, effective and reproducible across ethnicities.

**Keywords:** Botulinum Toxin Type A; Masseter Muscle; Injection; Therapeutic Use

## Introduction

Masseter hypertrophy, which is usually benign with bilateral muscle involvement, can impact both facial aesthetics and function. While a slim jawline is commonly desired in East Asia, its popularity has surged in Western populations in recent years [1]. This has resulted in increased demand for non-surgical lower face contouring procedures, with botulinum neurotoxin type A (BoNT-A) as a key treatment modality [2]. BoNT-A injections into the masseter muscle offers a minimally invasive alternative to surgical reduction, effectively reducing lower facial width whilst also alleviating symptoms of bruxism and temporomandibular joint discomfort [3]. Despite its widespread use, there remains no consensus on the optimal injection protocol [4].

A 2013 Cochrane review concluded that while BoNT-A injections are effective in reducing masseter hypertrophy, methodologic limitations persist in existing studies [4]. Subsequent studies have further consolidated evidence supporting BoNT-A as a safe and effective non-surgical treatment for benign bilateral masseteric hypertrophy across various dosages and protocols [5]. A

systematic review by Rauso, et al., reported that both single- and multi-point injections yield comparable efficacy, achieving 26-31% masseter reduction at 3 months, suggesting that injection strategy may be optimized for patient comfort and procedural efficiency, instead of merely efficacy alone [5]. Supporting this, a recent Korean randomized crossover study demonstrated that a single-entry technique significantly reduced pain associated with the injections compared with three-point injection approaches, without compromising clinical outcomes [6].

The anatomical basis for optimizing BoNT-A delivery has also become a growing focus. Studies have highlighted the masseter's neural arborization zone, located in its deep posterior middle to lower third regions, as an optimal target for BoNT-A delivery due to its high density of motor endplates, allowing greater precision, lower dosing and reduced complication rates such as smile asymmetry and paradoxical bulging [7,8]. Building on this, Ng and Yang introduced a single-entry injection technique which uses consistent surface landmarks to access both deep and superficial muscle compartments through a single puncture, improving procedural comfort and minimizing diffusion to adjacent structures [9]. Despite promising early results, evidence across diverse populations remains limited. Therefore, this study evaluates outcomes in patients treated with this single-entry, arborization-targeted approach across multiple BoNT-A formulations and ethnic groups in both Asian and Western clinical settings, with the aim of assessing its safety and efficacy as a standardized anatomy-guided protocol for non-surgical masseter reduction.

**Methodology**

*Patient Selection*

A retrospective observational study was conducted on 131 patients treated by the authors for bilateral benign masseter hypertrophy between 2019 and 2025 across private clinics in both the United Kingdom and Singapore. All patients received bilateral BoNT-A injections specifically for aesthetic contouring or bruxism-related hypertrophy. Demographics including age, sex, ethnicity, toxin type and dose were recorded.

The cohort included 126 women and 5 men, with a mean age of 32.8 years. Patient ethnicities consisted of Chinese (n = 97), Caucasian (n = 22) and Middle Eastern/Asian (n = 12). Patients were offered BoNT-A in the absence of standard contraindications (i.e. neuromuscular disease, toxin hypersensitivity, prior adverse reaction and pregnancy/lactation). Exclusion criteria were patients who received BoNT-A injections but were excluded from analysis due to incomplete data or loss to follow-up. The study complied with international guidelines for retrospective chart review and all patients provided informed consent for both treatment and use of anonymized clinical photographs.

*Injection Protocol*

All injections were performed by experienced injectors using a standardized single-entry technique previously described by Ng and Yang [9]. The injection point was identified using a practical surface landmark method – the anterior border of the masseter represented by a line drawn from the tragus or earlobe to the oral commissure and the point of the most prominent bulge of the masseter located with the patient clenching [9]. This corresponds to the deep neural arborization zone of the masseter muscle, where the masseteric nerve (a branch of V3) enters the deep posterior portion of the masseter and distributes to motor endplates8 (Fig. 1).

Targeting the neural arborization zone minimizes off-target spread, lowers risk of smile asymmetry and improves safety. Further injections with this technique (but from the same single-entry point) were aimed more superficially at the superficial portion of the masseter to minimize the risk of paradoxical muscle bulging. Briefly, paradoxical muscle bulging is attributed to the presence of a deep inferior tendon (Fig. 2), which not only separates the deep and superficial muscle bellies of the masseter but can limit distribution of BoNT-A to each.

Anatomical guidelines by Yi, et al., also support mid-masseter targeting for consistent and effective results8. In this study, the mean doses administered per side were – Botox®: 24 BU, Dysport®/Azzalure®: 27 BU-equivalent, Xeomin®/Bocouture®: 28 BU-equivalent and ranges were – Botox®: 12-36 BU, Dysport®/Azzalure®: 8-40 BU, Xeomin®/Bocouture®: 23-50 BU.

Dilution ratios were standardized: Botox® = 100 units in 2.5 mLs saline, Dysport® = 500 units in 2.5 mLs saline, Azzalure® = 125

units in 1.25 mLs saline, Xeomin® / Bocouture® = 100 units in 2.5 mLs saline. BU conversions: Botox® = 1 unit, Dysport®/Azzalure® = 2.5 units, Xeomin®/Bocouture® = 1 units per BU.

*Follow-up*

Patients were evaluated at 3 to 4 weeks and 3 months post-treatment. Primary outcomes included clinical reduction in masseter bulk, assessed by standardized photographs compared with baseline and patient-reported self-satisfaction. Secondary outcomes included adverse events, specifically asymmetry, cheek hollowing, bulging, dysphagia and others.

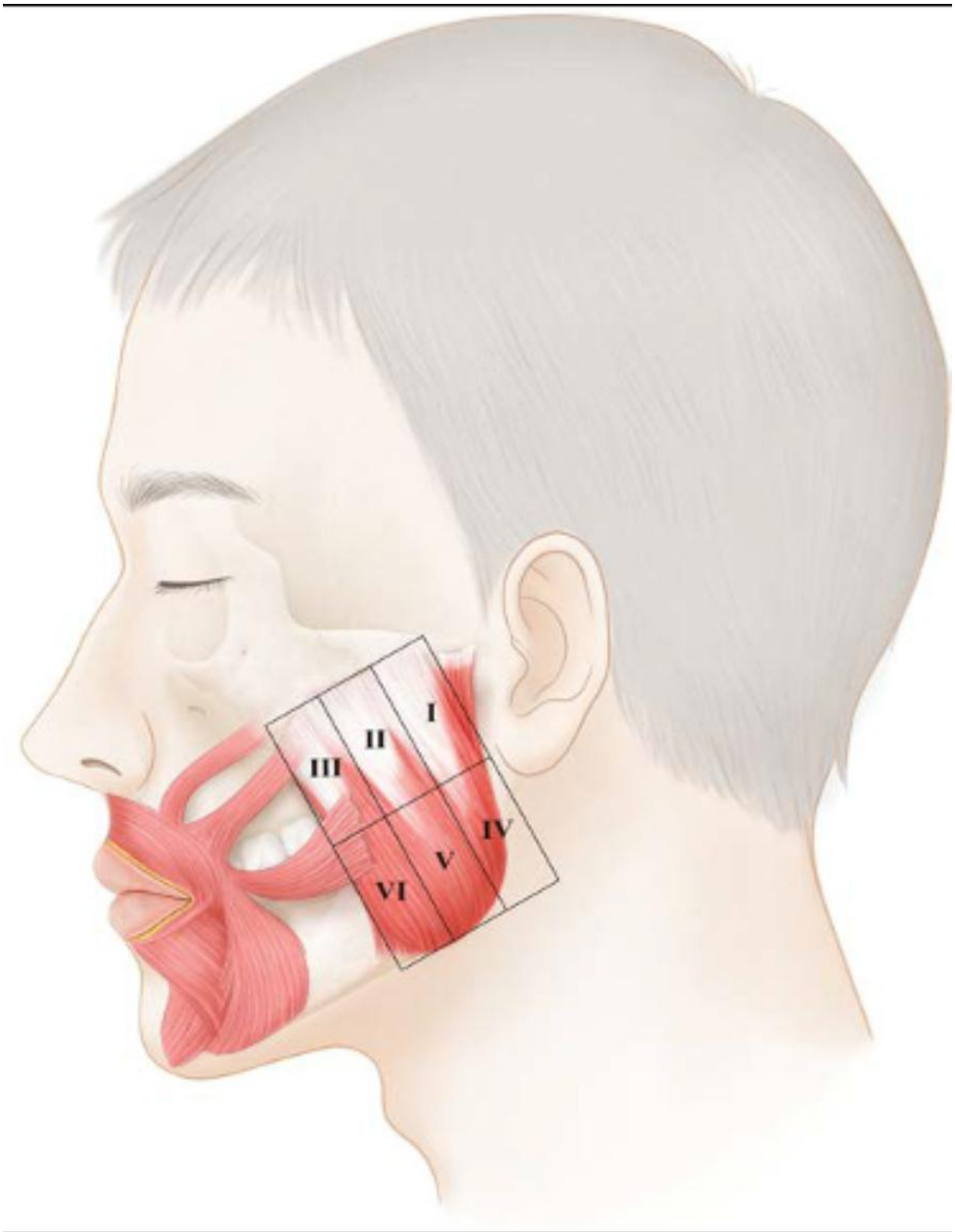


**Figure 1:** Anatomical relationship between the masseter and risorius muscles in the mid-lower face. Area V is the most arborized area for the botulinum neurotoxin injection site. Areas III and VI should be avoided because they overlap with the risorius muscle. Area VI should also be avoided as the facial artery passes immediately anterior to area VI. Areas I and II should not be injected to avoid damage to the masseteric nerve trunk and parotid duct and gland. Area IV should be avoided as the parotid gland overlaps the area. (Reproduced from Yi KH, Lee HJ, Hur HW, Seo KK, Kim HJ. Guidelines for Botulinum Neurotoxin Injection for Facial Contouring. Plast Reconstr Surg. 2022;150:562e-571e8; with permission).

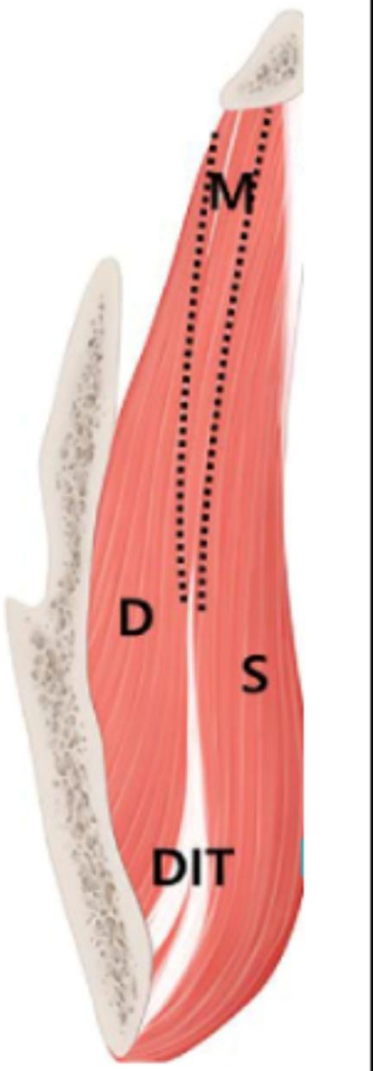


**Figure 2:** Coronal section of the masseter muscle. S, superficial layer of the masseter; M, middle layer of the masseter; D, deep layer of the masseter; DIT, deep inferior tendon. Guidelines for Botulinum Neurotoxin Injection for Facial Contouring. Plast Reconstr Surg. 2022;150:562e-571e8; with permission).

**Results**

*BoNT-A Doses*

Three commercially available BoNT-A formulations were used in treatment: Botox®, Dysport® (or its equivalent Azzalure®) and Boucouture® (or its equivalent Xeomin®). Botox® was administered in 51 patients at an average dose of 24 Botulinum toxin Units (BU) per side. Dysport® or Azzalure® was used in 64 patients, with an average dose of 67.5 Speywood units per masseter, corresponding to approximately 27 BU when using conversion ratios of 2.5:1, which is typically based on the treatment of glabellar lines [10,11]. Xeomin® or Boucouture® was used in 16 patients, with an average dose of 28 BU (Table 1).

*Outcomes*

Across the entire cohort, no major complications were observed (Fig. 3-5). One patient (0.8%) developed mild facial asymmetry following injection, while another patient (0.8%) reported no clinical improvement. Importantly, there were no cases of paradoxical masseter bulging, hematoma, ptosis, dysphagia or nerve injury. Overall, there was high patient tolerability and self-reported satisfaction at 3 months' follow-up in the current study.

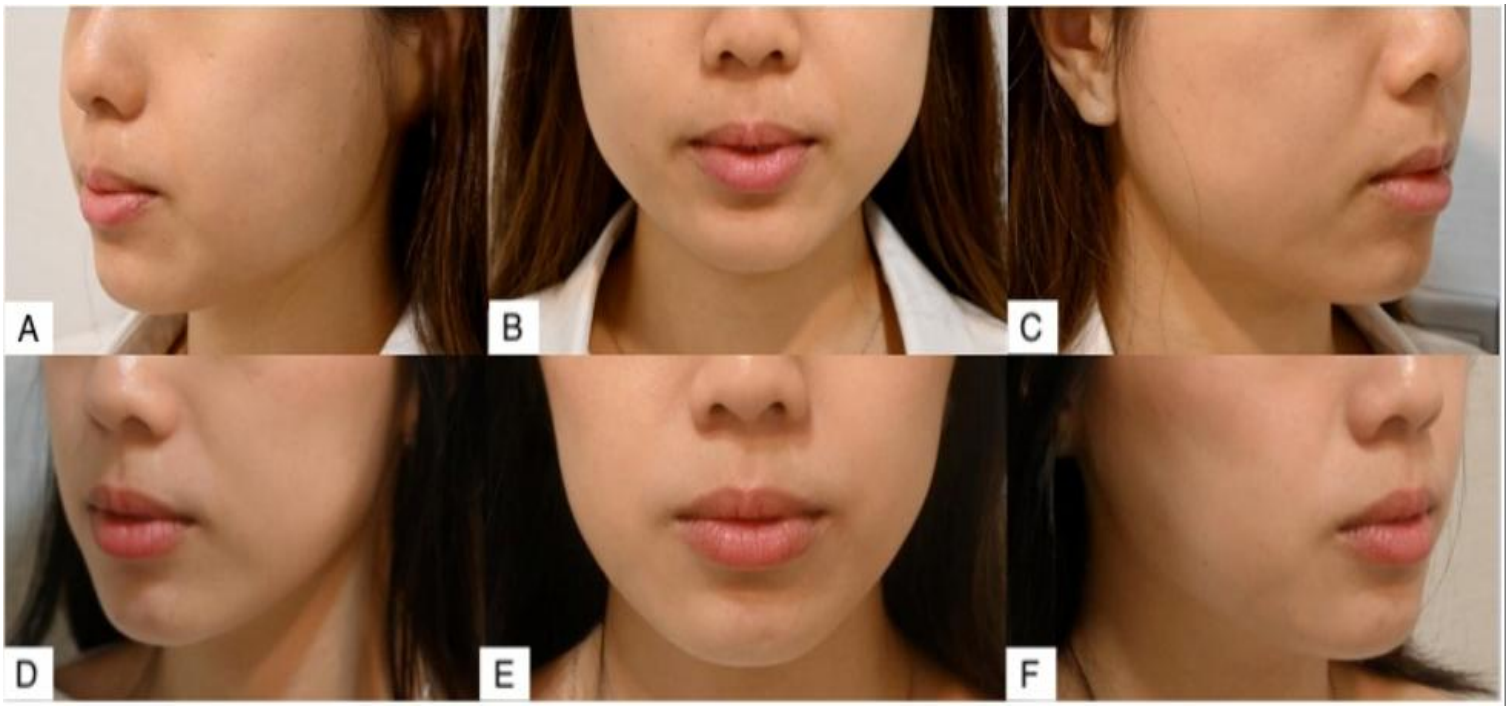


**Figure 3:** Representative patient (female, 26 years old, East Asian ethnicity) following treatment with Botox® for bilateral benign masseteric hypertrophy. 3A. Pre-procedure photograph in left oblique view; 3B. Pre-procedure photograph in anterior view; 3C. Pre-procedure photograph in right oblique view; 3D. Post-procedure photograph at 6 months' follow-up in left oblique view (after two treatments at 3-monthly intervals); 3E. Post-procedure photograph at 6 months' follow-up in anterior view. 3F. Post-procedure photograph at 6 months' follow-up in right oblique view.


**Figure 4:** Representative patient (female, 29 years old, East Asian ethnicity) following treatment with Dysport®/Azzalure® for bilateral benign masseteric hypertrophy. 4A. Pre-procedure photograph in left oblique view; 4B. Pre-procedure photograph in anterior view; 4C. Pre-procedure photograph in right oblique view; 4D. Post-procedure photograph at 6 months' follow-up in left oblique view (after two treatments at 3-monthly intervals); 4E. Post-procedure photograph at 6 months' follow-up in anterior view; 4F. Post-procedure photograph at 6 months' follow-up in right oblique view.

| Toxin | Patients (n) | Average Units Per Side/Masseter | BU Equivalent | Range Per Side (BU) |
|---|---|---|---|---|
| Botox® | 51 | 24 | 24 | 12 – 36 |
| Dysport®/Azzalure® | 64 | 68 | 27 | 8 – 40 |
| Xeomin®/Boucouture® | 16 | 28 | 28 | 23 – 50 |
| * BU = Botulinum Toxin Units | | | | |

**Table 1:** Summary of the breakdown of treatment formulations and dosages.


**Figure 5:** Representative patient (female, 32 years old, East Asian ethnicity) following treatment with Xeomin®/Bocouture® for bilateral benign masseteric hypertrophy. 5A. Pre-procedure photograph in left oblique view; 5B. Pre-procedure photograph in anterior view; 5C. Pre-procedure photograph in right oblique view; 5D. Post-procedure photograph at 6 months' follow-up in left oblique view (after two treatments at 3-monthly intervals); 5E. Post-procedure photograph at 6 months' follow-up in anterior view; 5F. Post-procedure photograph at 6 months' follow-up in right oblique view.

**Discussion**

This study supports the growing body of evidence that single-entry masseter injection, when precisely targeted to the neural arborization zone, provides highly predictable aesthetic and functional outcomes. Our results, demonstrating a 99.2% clinical response rate with only one case of mild asymmetry and one case of non-response to treatment, reinforce the value of this approach.

*Single-Entry Technique Efficacy*

Prior literature, including a meta-analysis of 28 studies with 748 patients treated with single-point injection, demonstrated a 26–31% masseter thickness reduction at 3 months, comparable to outcomes achieved with traditional multiple-point injection protocols [4]. This suggests that multiple puncture sites may be unnecessary when the toxin is delivered precisely into the neural arborization zone i.e. the key functional zone of the masseter. By injecting BoNT-A directly into this area which contains the highest density of motor endplates within the muscle, the portion of the muscle that contributes most significantly to hypertrophic bulk is addressed and even modest doses of BoNT-A may produce substantial functional weakening [7]. Another advantage of the single-entry technique is a more homogeneous pattern of denervation, as it allows layered delivery to both deep and superficial fibers, while avoiding spread to non-target structures. With multiple-point approaches, toxin distribution may vary depending on individual anatomy, such as the presence of the deep inferior tendon, potentially producing uneven muscle weakening and paradoxical bulging [12].

This technique also enhances the patient experience, arguably an equally important outcome in aesthetic practice. A 2024 Randomized-Controlled Trial (RCT) found significantly less pain with single-entry injections (VAS ≈ 3.3) versus three-point protocols (VAS ≈ 5.2) in 16 adults [6]. The combination of our findings with these data highlights not only the efficacy of single-entry injection, but also the patient-centered benefits, including reduced discomfort from fewer needle passes and lower overall complication rates. Reduced discomfort is especially relevant in populations seeking repeated treatments, where cumulative injection burden can influence adherence and overall satisfaction. These factors reinforce that the single-entry technique is not merely a procedural simplification but represents a purposefully devised anatomy-driven method that optimizes both clinical efficacy and patient acceptability.

*Implications for Safety in Practice*

Safety remains paramount in BoNT-A masseter treatments, especially because the muscle lies adjacent to important structures responsible for facial expression, mastication, airway protection and speech, where even minimal toxin diffusion can alter these functions. Targeting the neural arborization zone reduces the need for multiple superficial injections, minimizing the risk of inadvertently affecting adjacent facial muscles. Large-scale reviews by Yeh, et al., who evaluated published studies of BoNT-A injections into the masseter between 1994 and 2018 and Nishikawa, et al., whose series included 46,250 injections, have shown that masseteric BoNT-A has an excellent safety record, with most complications being mild, transient and self-resolving [13,14]. Kim, et al., then further quantified treatment related adverse events for the treatment of masseters at 1.07% [15]. Our minor complication rate of 1.5% – consisting of only one case of transient asymmetry and one non-responder – aligns closely with the existing data. The absence of off-target involvement of the other facial muscles resulting in adverse events such as flattening of the cheek contour, dysphagia etc. in our cohort underscores the protective effect of anatomically guided targeting.

*Muscle Function and Bite Force*

Functional preservation is a key consideration when treating masseteric hypertrophy, as the masseter contributes substantially to mastication and protective jaw reflexes. A 2023 RCT using Xeomin® (25 BU equivalent) per masseter demonstrated significant reductions in maximum bite force for up to three months [16]. Another clinical trial showed that 10 BU equivalent per side led to reduced bruxism symptoms, lower bite force and decreased muscle thickness at 4 weeks [17]. These reductions are a predictable pharmacological effect of chemodenervation and are generally transient, with full functional recovery observed as reinnervation occurs. Importantly, these studies with dosing protocols similar to ours have consistently demonstrated functional weakening without compromising activities of daily living including chewing, speech or oral competence. This available evidence supports that when BoNT-A is delivered precisely within the arborization zone at moderate doses, functional integrity of mastication is largely preserved. The controlled reduction in bite force is sufficient to achieve desired aesthetic and therapeutic benefits while maintaining oral function, highlighting the technique's suitability for sustained, repeated treatments.

*Female Predominance in BoNT-A Injection for Masseter Hypertrophy*

The marked female predominance within this cohort likely reflects underlying aesthetic demand influenced by sociocultural preferences. Benign bilateral masseteric hypertrophy is commonly observed among Asian populations and reduction of lower facial width with BoNT-A is frequently sought to achieve a slimmer, more tapered facial contour [18]. In many cultural contexts, particularly East Asia, a narrower lower face is perceived as a desirable feminine aesthetic, which may explain why female patients disproportionately pursue this treatment for concerns such as lower facial fullness. Conversely, male patients may be less likely to seek masseter reduction, as a broader mandibular angle is often culturally associated with masculinity and masseter contouring may therefore be perceived as feminizing. The gender distribution observed in this study is therefore likely reflected of sociocultural aesthetic preferences and treatment-seeking behaviour rather than biological sex differences in the prevalence of masseter hypertrophy.

*Cost-Efficiency of Dysport® in Masseteric Hypertrophy*

Because the typical cost-per-unit of Dysport® is less than or similar to Botox®, Dysport® may offer a more cost-effective option to patients for long-term treatment of masseteric hypertrophy. This mirrors findings from comparative pharmacological studies in experimental, therapeutic and aesthetic applications of BoNT-A [19-21].

A randomized, double-blind, contralateral split-face study by Nestor and Albon highlighted that the clinical duration of action of Dysport®, was significantly longer than Botox® when treating the frontalis [22]. Kassir, et al., also demonstrated similar

outcomes when treating the glabellar and crow's feet areas [23]. Interestingly, both studies also reported earlier time to action. Michaels et al. then conducted a cost comparative study between Dysport® and Botox® and concluded that the former proffered significant cost savings with comparable efficiency [24].

In the context of masseter hypertrophy, where repeated treatments are typically required every 4 to 6 months, even small differences in unit efficiency and cost may have significant cumulative financial implications. Patients and clinicians may therefore benefit from considering Dysport® as a cost-conscious choice, particularly when long-term aesthetic maintenance or bruxism management is planned.

*Comparison of Botulinum Toxin Formulations: Clinical and Practical Insights*

BoNT-A formulations are widely used in both functional and aesthetic medicine, with onabotulinumtoxinA (Botox®), abobotulinumtoxinA (Dysport®/Azzalure®) and incobotulinumtoxinA (Xeomin®/Bocouture®) being the most prominent commercially available products. Although all three share the same 150 kDa neurotoxin core, they differ in manufacturing processes, excipients and clinical characteristics, which contribute to differences in efficacy, diffusion, immunogenicity and patient preference [25,26].

While all BoNT-A products contain the same active neurotoxin, their composition varies significantly in terms of complexing proteins and formulation matrices. Dysport®/Azzalure® contains a complex of the neurotoxin with accessory proteins and requires higher dosing by unit count to achieve equivalent clinical effects compared to Botox®. Clinical practice commonly applies a conversion ratio of approximately 2.5:1 (Dysport:Botox) in aesthetic treatments [10,11,20,21]. Dysport®/Azzalure® has also been noted to produce a broader diffusion pattern, which may be advantageous when treating large, fan-shaped muscles such as the masseter [19,25]. This broader spread may contribute to improved clinical outcomes with fewer injection sites and associated discomfort.

In contrast, Xeomin®/Bocouture® is formulated to include only the purified 150 kDa neurotoxin, omitting complexing proteins. This is achieved through a proprietary manufacturing process designed to reduce antigenic load and potentially lower the risk of neutralizing antibody formation [20].

Such immunogenicity is a critical consideration for patients requiring repeated BoNT-A treatments over time. The presence of complexing proteins and inactive bacterial fragments in some formulations has been associated with an increased risk of neutralizing antibody development. This may lead to secondary treatment failure in a small subset of patients [25,26]. Xeomin®/Bocouture®, due to its purified nature and lack of accessory proteins, may thus carry a lower immunogenic potential and has been recommended for patients at higher risk for antibody formation or those requiring frequent injections [25,26]. Nevertheless, for most patients undergoing aesthetic treatment at standard intervals and doses, clinically significant immunogenicity remains rare.

In our cohort, Dysport®/Azzalure® was the most commonly used formulation, reflecting its effectiveness, particularly in populations with broader masseter morphology – a pattern consistent with prior research in East Asian patients [18,27]. Although it requires more units numerically, Dysport®/Azzalure® is often considered cost-effective due to its favorable pricing per unit and broad muscle coverage with fewer injection points. Botox® was the second most used, largely due to brand recognition and consistent results. Xeomin®/Bocouture®, while theoretically advantageous for long-term immunogenic safety, was less frequently selected and often required higher dosing to achieve similar clinical outcomes, making it a less popular choice in practice.

Although diffusion is influenced by a variety of factors including dose, injection volume and technique, some clinicians suggest that Dysport®/Azzalure®'s formulation may allow for more efficient distribution across target musculature [28]. While direct comparative diffusion studies are limited, this anecdotal pattern is reflected in both practitioner experience and select comparative reports [29,30].

**Limitations and Future Directions**

There are several limitations that warrant consideration in this study. First, the retrospective design restricts the ability to control for confounding variables such as previous BoNT-A exposure or bruxism severity. It was also not possible to determine whether all patients were first-time recipients of this treatment, as prior injection history was not consistently volunteered or documented in their clinical records. Consequently, we were unable to ascertain whether patients who had previously received BoNT had been treated using a single- or multiple-point injection technique. This limits our ability to control for the potential influence of prior treatment exposure to outcomes. However, dosing in the present cohort was guided by established clinical experience to ensure consistency in administration.

Secondly, injection-related pain was not formally recorded, as this was not a pre-defined outcome measure. The absence of systematically collected pain scores limits objective assessment of tolerability, although the procedure is generally associated with minimal discomfort. Moreover, the study lacks a comparison group treated using a multiple-point injection technique, which restricts comparative conclusions. However, all authors consistently utilize single-point technique in routine practice, allowing for the procedure to be standardized across the cohort and strengthening internal validity of the findings. The absence of objective quantitative measurements, such as imaging-based (e.g. MRI or ultrasound) volumetric analysis or assessment of bite force, limits the precision of evaluation of efficacy of our single injection technique. Standardized, blinded photograph evaluation or 3D scanning would further strengthen aesthetic outcome assessment, but again, was not the primary outcome of this study conducted within private practice settings. Finally, toxin choice was not randomized, instead, it reflected clinician preference and patient factors, which may introduce selection bias in comparative interpretations. Future prospective randomized controlled trials with longer follow-up, imaging-based quantitative outcome measures as well as validated patient-reported scores are required to better characterize the long-term safety and superiority of facial contouring using toxins for bilateral benign masseter hypertrophy via a single-entry technique in the neural arborization zone, including potential side effects such as localized firmness, compensatory temporalis hypertrophy or long-term irreversible atrophy.

**Conclusion**

Our experience confirms that a single-entry, arborization-targeted BoNT-A injection is a safe, effective and reproducible method for masseter reduction across ethnicities regardless of toxin type. These results are consistent with current evidence and support the further adoption of simplified, anatomy-guided protocols for aesthetic and functional treatment of masseter hypertrophy.

**Conflict of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Weiguang Ho and Nicole Ellen James contributed equally to the work and should be considered co-first authors.

**Data Availability Statement**

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Ethical Statement**

The project did not meet the definition of human subject research under the preview of the IRB according to federal regulations and therefore was exempt.

**Informed Consent Statement**

Written informed consent has been obtained from the patients whose pictures are included in this paper.

**Authors' Contributions**

This work has been presented, in part, by Mr Weiguang Ho, at the Annual Scientific Meeting of the British Association of Aesthetic Plastic Surgeons (BAAPS), London, 28-29 September 2023 where it was shortlisted for the Abstract Prize; and will be

presented by Miss Nicole Ellen James at the Joint Meeting of the European Society of Plastic, Reconstructive and Aesthetic Surgery (ESPRAS) & of the Swiss Society of Plastic, Reconstructive and Aesthetic Surgery, Bern, 5 September 2026.

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