





Intramuscular neural distribution revealed by Sihler's method: Application to botulinum neurotoxin injection in shoulder and arm contouring

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Intramuscular neural distribution revealed by Sihler's method: Application to botulinum neurotoxin injection in shoulder and arm contouring

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2014년 처음 해부학교실에 들어온 뒤 4년이라는 시간을 의학전문대학원에서 보낸 후 뒤늦게 다시 해부학을 공부하기 시작해서 학위논문을 작성하게 되었 습니다. 다시금 해부학의 길을 가게 된 것은 주변의 많은 분의 도움이 있었기 때문입니다.

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처음 해부학교실에 와서 지금까지 해부학 교실원들에게도 많은 조언과 도 움을 받았습니다. 해부학을 접하고 처음 쓰는 논문부터 많은 조언을 해주시고 정신적으로 지지해주셨던 이지현 선생님과 이강우 선생님, 업무적으로 부족한 부분을 항상 진심으로 도와주신 이규림 선생님, 항상 부족한 저를 적극적으로 도와주시고 관심을 가져주신 박현진 선생님과 배형규 선생님, 그리고 언제나 교실 업무에 많은 도움을 주셨던 박형수 선생님, 김수빈 선생님, 안혜련 선생 님께도 감사의 말씀을 드립니다. 항상 좋은 그림으로 같이 논문 작성을 도와 주신 허혜원 선생님께 큰 감사의 말씀을 전하고 싶습니다.

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Abstract

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The use of botulinum neurotoxin in body contouring is increasing with time. Body contouring usually targets the superior trapezius, deltoid, and lateral head of the triceps brachii, as hypertrophy in these muscles gives the arms and shoulders a "muscular" appearance, which raises cosmetic concerns, especially in women. We propose an ideal botulinum neurotoxin injection point for the superior trapezius, deltoid, and lateral head of the triceps brachii muscles for shoulder and arm aesthetic line contouring, describing the intramuscular nerve branching in these muscles, providing essential information for botulinum neurotoxin injection.

A modified Sihler's method was performed on the superior trapezius, deltoid, and lateral head of the triceps brachii muscles in 16, 14, and 16 specimens,



respectively. Intramuscular arborization of the superior trapezius muscles was examined regarding the external occipital protuberance superiorly and the horizontal line crossing the acromion of the scapula. Intramuscular arborization of the deltoid muscles was demarcated using the marginal line of the muscle origin, and the line connecting the anterior and posterior upper edges of the axillary region. Intramuscular arborization of the triceps brachii muscle of the lateral head was measured as the distance from the lateral epicondyle to the anteroinferior point of the acromion.

The intramuscular neural distribution had the greatest arborized patterns in the horizontal (1/5 - 2/5) and vertical (2/4 - 4/4) sections for the superior trapezius muscle, in the area between the horizontal 1/3 to 2/3 lines of the anterior and posterior deltoid bellies in the deltoid muscle, and 2/3 to the axillary line in the middle deltoid muscle. Intramuscular arborization patterns of the triceps brachii were observed at the lateral heads of 4/10 - 7/10. These areas, corresponding to the areas of maximum arborization, are recommended as the most effective and safe points for botulinum toxin injection.

Keywords: trapezius muscle, deltoid muscle, triceps brachii muscle, botulinum neurotoxin, injection point, intramuscular neural distribution



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I. INTRODUCTION

Botulinum neurotoxin restricts neuronal communication by inhibiting the release of acetylcholine at the motor end-plate, thereby disabling muscle contraction (Childers et al., 2004). Botulinum neurotoxin is frequently used to remove rhytids by inducing facial muscle deactivation. More recently, the application of botulinum neurotoxin has been extended to body contouring, and its use is in this field has been increasing. Body contouring usually targets the deltoid, superior trapezius, triceps brachii, quadriceps, and gastrocnemius muscles (Han et al., 2006). In general a hypertrophic deltoid muscle gives the arms and shoulders a "muscular" appearance, which raises cosmetic concerns in many people, especially women (Figure 1).

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Figure 1. Schematic image of a hypertrophied shoulder and arm. The hypertrophied superior trapezius, deltoid, and lateral head of triceps brachii muscles (A) and the aesthetic shoulder and arm line (B).

Injection of botulinum neurotoxin to induce muscle inactivation is recognized as a safe and effective procedure (Brashear et al., 2002; Rosales et al., 2012). Generally, the outcomes of botulinum neurotoxin injection depend on the capture of botulinum neurotoxin by the presynaptic membranes of motor neurons at the motor end plate; therefore, injections should be administered into the intramuscular neural arborized area (Childers et al., 2004; Ramirez-Castaneda et al., 2013). The effectiveness of neural arborization-targeted botulinum neurotoxin injections has been proven in many clinical studies involving the biceps brachii and iliopsoas muscles. These studies showed that neural arborization-targeted injections demonstrated a much greater volume reduction than control injections (Gracies et al., 2009; Van Campenhout et al., 2013).



Moreover, overdosage and repeated treatment with botulinum neurotoxin induces antibody-mediated reactions that reduce the effects of treatment (Hsu et al., 2004; Kinnett, 2004; Lepage et al., 2005). Numerous studies regarding intramuscular neural arborization of skeletal muscles have been published (Rha et al., 2016; Yi et al., 2016, 2017; Yi et al., 2020; Yi et al., 2022).

To the best of our knowledge, no studies investigating the intramuscular neural distribution of the superior trapezius, deltoid, and lateral head of the triceps brachii muscles have yet been conducted. In addition, there are no conventional doses or guidelines for botulinum neurotoxin injection into these muscles. In the present study, we used the modified Sihler's staining technique, a whole-mount staining technique, to reveal intramuscular neural patterns (without damaging the nerve) in the superior trapezius, deltoid, and lateral head of the triceps brachii muscles. We aimed to thereby suggest effective and safe botulinum neurotoxin injection points in the deltoid, superior trapezius, triceps brachii muscle for arm and shoulder contouring.



I. MATERIALS & METHODS

This study was performed in accordance with the principles of the Declaration of Helsinki. All cadavers used in this study were donated to the Surgical Anatomy Education Center of Yonsei University College of Medicine. Appropriate consent and approval were obtained from the families of the cadaver subjects before dissection was initiated.

A total of 16 trapezius muscles from Korean cadavers (5 male and 3 female with a mean age at death, 71.4; range, 63–84 years) and 14 deltoid muscles from eight Korean cadavers (4 male and 4 female; mean age at death, 75.9; range = 73–84 years) were stained using the modified Sihler's method to reveal intramuscular nerve arborization patterns. Prior to dissection, each deltoid muscle was anatomically aligned . A total of 16 triceps brachii muscles from 10 Korean cadavers (5 male and 5 female; mean age at death, 78.2 years; range, 63–92 years) were dissected to harvest specimens and for the whole-mount staining process to detect intramuscular neural distribution.

Prior to dissection, each of the trapezius, deltoid, and triceps brachii muscles were aligned in the anatomical position. The muscles, including their origin and insertion points, were harvested. The trapezius muscle was harvested according to three landmarks: the external occipital protuberance superiorly, spinous process of the 12th thoracic vertebra inferiorly, and acromion of the scapula. The deltoid muscle was harvested from the posterior region of the spine of scapular, acromion, and clavicle, and inserted at the deltoid tuberosity. The triceps brachii muscle was harvested from the lateral epicondyle to the insertion at humerus.

The arborization patterns in the superior trapezius muscles were elucidated with



respect to the vertical and horizontal lines crossing the external occipital protuberance (EOP) superiorly, and the horizontal line crossing the acromion of the scapula (horizontal line 4/4 and vertical line 5/5) (Figure 2a). The deltoid muscle was elucidated with reference to two lines: the marginal line of the origin (OL) of the deltoid muscle and the line connecting the anterior and posterior upper edges of the axilla (AL) (Figure 2b). The deltoid muscle is divided into the posterior, middle, and anterior belly muscles. The lateral head of the triceps brachii muscle was measured as the total distance from the anteroinferior point of the acromion (line 0) and the lateral epicondyle (LE) (Figure 2c).

The superior trapezius, deltoid, and lateral head of the triceps brachii muscles were stained using a modified Sihler's staining technique (Liem and Douwe van Willingen, 1988). This technique was performed in several steps to obtain a visible representation of the intramuscular neural arborization pattern.





Figure 2. Division of the muscles when determining the arborization pattern of the specimens. Specimens were harvested with reference to the anatomical landmarks of each superior trapezius muscle (A), deltoid (B) and triceps brachii (C) muscles. EOP, external occipital protuberance; AC, acromioclavicular joint; OL, origin line of deltoid; AL, line connecting the anterior and posterior upper edges of the axilla; AIA, anteroinferior point of the acromion; LE, lateral epicondyle



The Modified Sihler's staining

The superior trapezius, deltoid, and lateral head of the triceps brachii muscle specimens were fixed by soaking in non-neutralized formalin for a period of one month. During this fixation stage, formalin was replaced whenever the tissue became cloudy. After one month of fixation, the specimens were soaked in a mixture of aqueous potassium hydroxide and hydrogen peroxide solutions. This process lasted for approximately one month to ensure that the superior trapezius, deltoid, and lateral head of the triceps brachii muscle specimens became transparent. Transparent specimens were decalcified by placing them in a mixture of glycerin, glacial acetic acid, and aqueous chloral hydrate solutions for four weeks. The decalcified superior trapezius, deltoid, and lateral head of the triceps brachii muscles were then stained by soaking in a mixture of glycerin, Ehrlich's hematoxylin, and aqueous chloral hydrate solutions for 5 weeks. The stained superior trapezius, deltoid, and lateral head of the triceps brachii muscles were re-soaked in a mixture of glycerin, glacial acetic acid, and aqueous chloral hydrate solution until the stained nerves became visible. Finally, the aspects of the superior trapezius, deltoid, and lateral head of the triceps brachii muscles were displayed (fibers were made transparent) by soaking the muscles in increasing concentrations of glycerin solution (40%, 60%, 80%, and 100%) (Figure 3).





Figure 3. The trapezius muscle underwent modified Sihler's method. The method consists of stages of fixation (A), maceration and depigmentation (B), decalcification (C), staining, and clearing (D).



Ⅲ. RESULTS

Intramuscular neural arborization patterns in the superior trapezius muscle

Fifteen of the 16 superior trapezius muscles had two regions with the greatest arborization patterns in the horizontal 1/5 - 2/5 and vertical 2/4 - 4/4 regions. One of the 16 superior trapezius muscles had one region of the horizontal 1/5 - 2/5 and vertical 3/4 - 4/4 (Figure 4).



Figure 4. Intramuscular distribution of the superior trapezius muscle. EOP, external occipital protuberance; AC, acromioclavicular joint.



Intramuscular neural arborization patterns in the deltoid muscle

In 13 of the 14 posterior deltoid muscle bellies, the arborization patterns were concentrated in the 1/3 - 2/3 regions (Figure 5); the pattern was denser in the 2/3-axillary line region in the remaining posterior belly. Ten of the 14 middle deltoid bellies showed the greatest arborization pattern in the 2/3 - axillary line regions; in the remaining four middle bellies, the arborization pattern was greatest in the 1/3 - 2/3 regions. Twelve out of the 14 anterior deltoid bellies had the greatest arborization pattern in the 1/3 - 2/3 regions two, arborization was concentrated in the 2/3 - axillary line region.



Figure 5. The intramuscular distribution of the deltoid muscle. OL, Origin line of deltoid; AL, Axillary line



Intramuscular neural arborization patterns in the lateral head of triceps brachii muscle

Of the 15 lateral heads of the triceps brachii muscles that had intramuscular branching, 14 had the most nerve arborization, located at the lateral part of 4/10 - 7/10 (Figure 6), and one had the most nerve arborization located at the lateral part of 3/10 - 5/10.



Figure 6. The intramuscular distribution of the triceps brachii muscle.



W. DISCUSSION

The superior trapezius, deltoid, and lateral head of the triceps brachii muscles are key muscles that contribute to the contour of the shoulder and upper arm. The hypertrophied superior trapezius, deltoid, and lateral head of the triceps brachii muscles create muscular shoulder and arm contours, which are regarded as symbols of masculinity. However, for many women, such demarcated deltoids result in an undesirable masculine appearance of the arms and shoulders, raising esthetic concerns (Shin et al., 2021).

The trapezius muscle originates from the occipital protuberance, nuchal ligament, and supraspinous ligament at C8-T12, and inserts into the lateral third of the clavicle, acromion, and spine of the scapula. The muscles can be divided into the superior, middle, and inferior parts. The muscles maintain their posture and scapular movements. The main motor component is supplied by the spinal accessory nerve. The deltoid muscle has three distinct bellies: the anterior, middle, and posterior deltoids (Sakoma et al., 2011), which originate from the clavicle, acromion, and spine of scapula, respectively. The deltoid muscle has an extensive origin converging as the muscle fiber runs downwards along the deltoid tuberosity of the humerus. The triceps brachii muscle is a thick muscle consisting of three heads, each of which has a different role in upper limb movement with different origins. The long head originates from the infraglenoid tubercle of the scapula; therefore, the muscle acts as an elbow extendor and a shoulder adductor. The medial head originates from the dorsal humerus, is distal to the radial groove, and is connected to the intermuscular septum. The lateral head originates from the dorsal humerus proximal to the radial groove, where it fuses with the intermuscular septum. The lateral head is known to be a target for body contouring.

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Zou et al. evaluated the aesthetic outcomes of a single botulinum neurotoxin injection in terms of bilateral superior trapezius hypertrophy (Zhou et al., 2018). They concluded that a single botulinum neurotoxin injection had satisfactory results in terms of body contouring; however, 7% of the participants had no obvious improvement, which may be explained by improper injection points and low dosages. Bae et al. further reported a significant reduction in the thickness of the superior trapezius muscle after a single botulinum neurotoxin injection (Bae et al., 2018). Additionally, many studies have reported that the majority of patients with cervical dystonia show significant improvements in posture, pain, and quality of life after treatment with botulinum neurotoxin injection enhances the elasticity of the trapezius muscle and that the relieving effects of botulinum neurotoxin can be observed immediately after injection (Evidente and Pappert, 2014; Nilesh and Mukherji, 2013).

A previous anatomical study proposed injection points by dissecting and tracing nerve endings with the naked eye (Lee et al., 2017). However, their suggestion of injecting 2/5 - 3/5 (converted to our measure) of the superior trapezius muscle differed from our results of 1/5 - 2/5. We believe that injections of 2/5 to 3/5 have the risk of damaging the main branch of the accessory nerve. There have been case reports of significant paralysis caused by injective treatment, damaging the main branch of the spinal accessory nerve supplying the trapezius muscle (Jankovic, 2001). Additionally, botulinum neurotoxin injection into the trapezius muscle has been reported to lead to subacromial impingement syndrome following botulinum neurotoxin overdose, or damage to the main branch of the spinal accessary nerve (Abbott and Richardson, 2007). Further studies are therefore needed to investigate the use of botulinum neurotoxin doses at our injection points



A previous study conducted clinical research on botulinum neurotoxin injections in hypertrophied deltoid muscles (Shin et al., 2021). They evaluated the efficacy and safety of intramuscular botulinum neurotoxin injections for the treatment of deltoid muscle hypertrophy. Ten patients were enrolled in their study who were treated with a total of 25 units of botulinum neurotoxin per deltoid belly. They used ten injection points in the most prominent area (on palpation) of the deltoid muscle. During follow-up with ultrasonography, the thickness of the deltoid muscles was reduced significantly from week 2 to the 4th month following after injection. The authors therefore concluded that a single botulinum neurotoxin injection had satisfactory results in terms of ameliorating body contouring. Another study performed MRI evaluations of the effect of botulinum neurotoxin injections on arm contouring in ten patients (Seo, 2017). Three months after botulinum neurotoxin injections, the deltoid muscle volume decreased by 17% and 34% at 50 U and 75 U, respectively. In their study investigating the dose efficacy relationship of botulinum neurotoxin, muscle weakness following 75 U injection significantly reduced the ability of the patients to raise their arms; thus, the recommended dose at the end was 50 U on each arm. However, they used 17 points over the deltoid muscle, with four points at the anterior, four points at the posterior, and five points at the middle deltoids.

According to a previous study on the lateral head of the triceps brachii muscle, botulinum neurotoxin should be injected linearly into the lateral bulging area when standing at attention or into the upper bulging area when raising the arms horizontally (Seo, 2017). This study recommended that 20 - 30 units of botulinum neurotoxin per side is required for the lateral head of the triceps brachii muscle. At present, there is no consistent point for botulinum neurotoxin injection into the triceps brachii muscle. However, lack of effectiveness and low patient satisfaction are common reasons why one-fifth of patients abandon botulinum neurotoxin



treatment (Comella, 2008). These limitations can be avoided by using accurate injection points at lower dosages. Precise injection was based on our suggestion that a lower dose can be used.

Currently, there are no standardized injection points for botulinum neurotoxin injection in the superior trapezius, deltoid, or lateral head of the triceps brachii muscles. Based on the findings of this study, we recommend that clinicians use multiple injection sites (following the intramuscular neural pattern in the superior trapezius, deltoid, and lateral head of the triceps brachii muscles) with a low dose of botulinum neurotoxin to avoid side effects and to achieve maximum efficacy.

According to a previous study, the optimal dilution concentration of botulinum neurotoxin for body contouring is 2 U/0.1 ml, which can be obtained by reconstituting 100 U of botulinum neurotoxin with 5 ml solvent (4.8 ml of normal saline + 0.2 ml of epi-lidocaine) (Seo 2016). A low concentration of botulinum neurotoxin diffused well into the voluminous muscles, and a 0.2 ml mixture of epi-lidocaine (1:100,000 epinephrine) was added to prevent botulinum neurotoxin from being easily rinsed off into intramuscular vessels because these muscles have a high vascular supply. One study demonstrated that botulinum neurotoxin diffuses through a diameter of up to 4.5 cm from the point of injection, in the case of a 10 U injection into the rabbit longissimus dorsi muscle, and that a 1 U injection has a diffusion gradient ranging from 1.5 cm to 3 cm (Borodic et al., 1994).

In the case of the superior trapezius muscle, we propose that botulinum neurotoxin treatment should be directed to the horizontal line 1/5 - 2/5 and vertical 2/4 - 4/4 sections of the superior trapezius muscle (Figure 7).





Figure 7. Injection guideline for the superior trapezius muscle. We propose that botulinum neurotoxin treatments should be directed to the horizontal line 1/5–2/5 and vertical 2/4–4/4 sections of the superior trapezius muscle. The most neural arborized area is the red shaded areas. EOP, external occipital protuberance; AC, acromioclavicular joint;

For the deltoid muscle, we propose that botulinum neurotoxin injections should be administered at the area between lines 1/3 to 2/3 of the anterior and posterior deltoid bellies and 2/3 to axillary line in middle belly (Figure 8); taking surface anatomical structures the marginal line of the muscle origin and the line connecting the anterior and posterior upper edges of axillar region as references.





Figure 8. Injection guidelines for the deltoid muscle. We propose that botulinum neurotoxin injections should be administered at the area between lines 1/3 to 2/3 of the anterior and posterior deltoid bellies, and 2/3 to axillary line (AL) in the middle deltoid belly. Injection should be conducted in the red shaded areas. OL, origin line of deltoid; AL, line connecting the anterior and posterior upper edges of the axilla

Deltoid intramuscular injections, such as vaccines and trigger point injections, our results. Lastly, for the lateral head of the triceps brachii muscle, injection at 4/10 - 7/10 would treatment of hypertrophy of the muscles (Figure 9). These areas, corresponding to the areas of maximum arborization, are recommended as the most effective and safe points for botulinum toxin injection.





Figure 9. Injection guideline for the lateral head of the triceps brachii muscle. Botulinum neurotoxin injection at the 4/10 - 7/10 The most neural arborized area are the red shaded areas. AIA, Anteroinferior point of acromion; LE, Transverse line crossing lateral epicondyle

The limitation of this study is that the vertical percentage could precisely locate the intramuscular neural distribution area; however, sonography-guided injection is superior to blind injection of the target muscle (Figure 10). Additionally, no study has clinically approved its effectiveness.





Figure 10. Ultrasonographic image of the superior trapezius (A), deltoid (B), and lateral head of the triceps brachii (C) muscles. Sonography-guided injection is superior to blind injection of the target muscle. SP, spinous process; AC, Acromioclavicular joint; OL, Origin line; AL, line connecting the anterior and posterior upper edges of the axilla; LE, Transverse line crossing lateral epicondyle



V. CONCLUSION

At present, there is no consensus regarding the optimal point for botulinum neurotoxin injection in the deltoid, superior trapezius, and triceps brachii. In this study, we recommend that clinicians use multiple injection sites with a low dose of botulinum neurotoxin to avoid side effects and to achieve maximum benefits. In our investigations, we used the Sihler's staining method, which offers a possible solution for resolving the restrictions of manual dissection. The application of Sihler's staining to the superior trapezius, deltoid and triceps brachii muscles will provide precise and thorough insights into their neural distribution.



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Abstract (in Korean)

설러 방법을 통한 근육 내 신경 분포에 관한 연구: 어깨 및 위팔 윤곽에 보툴리눔 신경독 주사에 적용

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이규호

피부과 및 성형외과에서 보툴리눔 신경독은 안면 근육 비활성화를 통한 주름 제거 에 자주 사용된다. 현재 보툴리눔 신경독의 적용 분야는 신체 윤곽의 교정으로 확장 되고 있으며, 이 분야에서 보툴리눔 신경독의 사용은 늘어나는 추세이다. 신체 윤곽의 보툴리눔 신경독의 적용은 일반적으로 등세모근의 윗부분, 어깨세모근 및 위팔세갈래 근의 가쪽갈래를 대상으로 한다. 일반적인 여성의 경우 등세모근의 윗부분, 어깨세모 근 및 위팔세갈래근의 가쪽갈래의 비대는 팔과 어깨를 울퉁불퉁하게 만들어 미용적인 문제로 보툴리눔 신경독 주사를 맞게 된다.

본 연구는 어깨 및 팔 윤곽 등 미용적인 목적을 위한 등세모근의 윗부분, 어깨세모 근 및 위팔세갈래근의 가쪽갈래의 이상적인 보툴리눔 신경독 주입 지점을 제안한다. 이 연구는 등세모근의 윗부분, 어깨세모근 및 위팔세갈래근의 가쪽갈래의 근육내 신 경 분포를 설명하여 보툴리눔 신경독 주사에 대한 정보를 제공한다.

설러 염색 방법을 통하여 각각 16, 14, 16개의 등세모근의 윗부분, 어깨세모근 및 위팔세갈래근의 가쪽갈래에 대한 염색을 진행하였다. 등세모근의 윗부분의 근육내 신 경의 분포는 위쪽으로 바깥뒤통수뼈융기, 어깨뼈 봉우리를 기준으로 설명하였고, 어깨 세모근의 근육내 신경 분포는 근육이 시작하는 뼈의 능선과 겨드랑이가 접히는 앞과 뒤쪽 포인트를 연결하는 선을 기준으로 하여 구분하였으며, 위팔세갈래근의 경우 팔 꿈치머리의 중간점에서 어깨뼈 봉우리까지의 총 거리를 기준으로 하였다.

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등세모근의 윗부분의 근육내 신경 분포는 수평 1/5-2/5 그리고 수직 2/4-4/4에서 가장 큰 수목 패턴을 보였다. 어깨세모근의 근육내 신경 분포는 어깨세모근의 빗장부분과 가시부분의 수평 1/3과 2/3 사이 영역에서 봉우리부분은 2/3과 겨드랑이선 사이에서 가장 큰 수목 패턴을 보였다. 위팔세갈래근의 가쪽갈래의 경우 근육내 신경의 수목 패턴은 4/10-7/10에서 관찰되었다. 최대 수목 부위에 해당하는 이 부위는 보툴리눔 신경독 주사를 위한 가장 효과적이고 안전한 부위로 추천된다.

핵심 되는 말 : 등세모근, 어깨세모근, 위팔세갈래근, 보툴리눔 신경독, 주사점, 근육내 신경 분포