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Intramuscular neural distribution of the soleus for botulinum neurotoxin injection: application to spasticity

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Understanding the nerve distribution within the soleus muscle concerning anatomical landmarks is crucial for botulinum neurotoxin injections in managing spasticity. This study advocates an anatomically informed approach for administering botulinum neurotoxin injections in the soleus muscle. Utilizing a modified Sihler's method, investigation encompassed both soleus muscles (20 specimens), revealing the distribution of nerves within concerning a transverse line crossing the fibular head and superior margin of the calcaneal tuberosity. Intramuscular nerve distribution within the soleus muscle displayed prominent patterns in zone 5 for the lateral portion, zone 3 for the middle portion, and zone 5 for the medial portion. Our suggestion is to target botulinum neurotoxin injections towards the areas with the most prominent nerve distribution, specifically zone 5 in the lateral part, zone 3 in the middle, and zone 5 in the medial part of the soleus muscle. Following these guidelines, clinicians can ensure minimal doses and avoid adverse effects like gait issues, antibody production, and bruising due to multiple injections. These findings can also be adapted and utilized in electromyography.

Keywords Clinical guideline, Soleus, Calf, Cosmetic calf shaping, Botulinum neurotoxin

Botulinum neurotoxin (BoNT) functions by impeding the release of acetylcholine at the neuromuscular junction, thus inhibiting muscle contractions[1]. Spasticity treatment traditionally involves oral anti-spastic medications, phenol injections, surgeries, physiotherapy, or their combination. Intramuscular injections of botulinum toxin (BoNT) serve as an effective treatment option for managing spasticity, complementing these established therapies[2–4].

Following a stroke, the onset of spastic foot drop in post-stroke patients months later is highly debilitating. This condition, characterized by a decreased foot contact area, heightens the risk of balance loss, subsequently increasing the likelihood of ankle joint injuries and accidental falls[5].

Since 1912, tibial neurotomy has been effectively employed to decrease spasticity in the vicinity of the ankle joint[6,7]. Although neurotomy effectively reduces ankle joint spasticity, its frequency of performance has decreased significantly following the introduction of localized treatments like phenol blocks and BoNT injections, which act specifically on the affected areas[8–11].

The safest and most effective treatment for spasticity is BoNT therapy[12–15]. ts efficacy relies on targeting the BoNT precisely at the presynaptic neurons near the motor-end-plates, specifically into the intramuscular neural arborized area[1,16,17]. Clinical trials have established the effectiveness of neural-arborized targeted BoNT injections, showcasing substantial volume reduction compared to control injections, particularly in muscles like the biceps brachii and iliopsoas[18,19].

Additionally, excessive units of BoNT and frequent treatments can lead to the development of antibodies, diminishing the effectiveness of treatment[20–23]. Various studies focusing on the intramuscular neural arborization of skeletal muscles have been published and implemented as clinical guidelines in the field[6,24–28]. The research employed a modified Sihler's staining technique, a method designed to display the intricate neural pattern

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within muscles without causing harm to delicate nerve branches. Through this process, the study delineated the intramuscular neural distribution, offering specific recommendations for targeting BoNT injections in the soleus muscle to treat spasticity safely and effectively.

Materials and methods

This investigation adhered to the ethical standards outlined in the Declaration of Helsinki. All cadavers involved were lawfully donated to the Surgical Anatomy Education Center at the Catholic University of Korea, College of Medicine, following approval by the Institutional Review Board (IRB approval code: 21-003; approval date: February 15, 2021). The families of the cadavers provided explicit consent for dissection procedures. Twenty soleus muscles from deceased Korean individuals (7 males and 3 females; average age at death, 77.2 years; ranging from 64 to 89 years) were subjected to staining using the modified Sihler's method to visualize intramuscular nerve branching patterns. The dissection of the soleus muscle commenced along the transverse line spanning the fibular head to the calcaneal tuberosity. Subsequently, the muscle was vertically divided into lateral, middle, and medial segments of equal length. Muscular arborization patterns were delineated based on two reference lines: the transverse line of the fibular head and the transverse line of the calcaneal tuberosity. These lines further partitioned the soleus muscle into ten distinct zones, arranged from the bottom to the top (Fig. 1).

The soleus muscle underwent modified Sihler's staining following the method outlined by Liem and Douwe van Willingen[29]. This technique involves a series of steps to visualize the intramuscular neural branching pattern. Upon completion of the modified Sihler's staining protocol, the distribution of intramuscular nerves within the soleus muscles was observed and analyzed.

Modified Sihler's staining

To reveal the neural arborization pattern of the soleus muscle, we employed the whole-mount staining technique known as "Sihler's staining," which allows visualization of intramuscular nerve distributions without causing damage to the nerves (Fig. 2). This method, adapted with some modifications, consists of seven steps. Here's a detailed description of the modified Sihler's staining process:

Fixation: The obtained soleus muscle was fixed in 10% unneutralized formaldehyde for three days.
Maceration and depigmentation: The fixed samples underwent maceration and depigmentation for two weeks using a 3% aqueous potassium hydroxide solution (with the addition of 1 mL of 3% hydrogen peroxide per 1000 mL).
Decalcification and whitening: Subsequently, the macerated samples were decalcified and whitened in a solution called "Sihler I solution" (a blend of 10% glacial acetic acid and 10% glycerin in distilled water) for three days.
Staining: After decalcification, the samples were stained in "Sihler II solution" (a mix of 10% Ehrlich's hematoxylin and 10% glycerin in distilled water) for one day.
Destaining: The stained samples were then destained using Sihler I solution for three hours.
Neutralization and blueing: Following destaining, the samples were neutralized in running tap water for an hour. Subsequently, the sample was immersed in 0.05% lithium carbonate for an additional hour to achieve blueing of the nerve fibers.
Clearing: Finally, the neutralized samples were cleared to achieve transparency. Fresh cadaver samples underwent clearing in 99% formamide, while samples from preserved cadavers were cleared in methyl salicylate.

Results

Intramuscular arborization patterns of the lateral belly of the soleus muscle

In the lateral belly of the soleus muscle, 15 out of 20 soleus muscles showed the most extensive arborization patterns in zone 5. Among 20 soleus muscles, three had their greatest arborization patterns in zones 4 and 5. Additionally, two out of 20 soleus muscles exhibited the greatest arborization pattern in zone 4.

Intramuscular arborization patterns of the middle portion of the soleus muscle

Regarding the middle portion of the soleus muscle, 14 out of 20 soleus muscles displayed the highest arborization patterns in zone 3. Among 17 soleus muscles, five had their greatest arborization patterns in zones 2 and 3. Furthermore, one out of 20 soleus muscles exhibited the greatest arborization pattern in zone 2 (Fig. 3).

Intramuscular arborization patterns of the medial portion of the soleus muscle

Concerning the medial portion of the soleus muscle, 16 out of 20 soleus muscles showcased the most extensive arborization patterns in zone 5. Additionally, three out of 20 soleus muscles displayed their greatest arborization patterns in zones 4 and 5. Moreover, one out of 20 soleus muscles had the greatest arborization pattern in zone 4.

Discussion

Hefter et al.[30] study aimed to compare the impact of administering BoNT injections into the soleus versus gastrocnemius muscles in post-stroke patients experiencing spastic foot drop. This research, conducted at a university hospital's BoNT clinic involving 24 adult patients new to BoNT treatment, demonstrated significant improvements in various measures related to spasticity, joint angles, and gait speed after 30 days for both injection approaches. However, there were no notable differences observed between the groups in terms of the modified Ashworth scale, gait speed, or ankle and knee joint angles. Although trends suggested potential improvements in active and passive ranges of motion favoring different muscle groups (soleus or gastrocnemius), the study found a significant difference in favor of the soleus group regarding active versus passive ankle extensions and

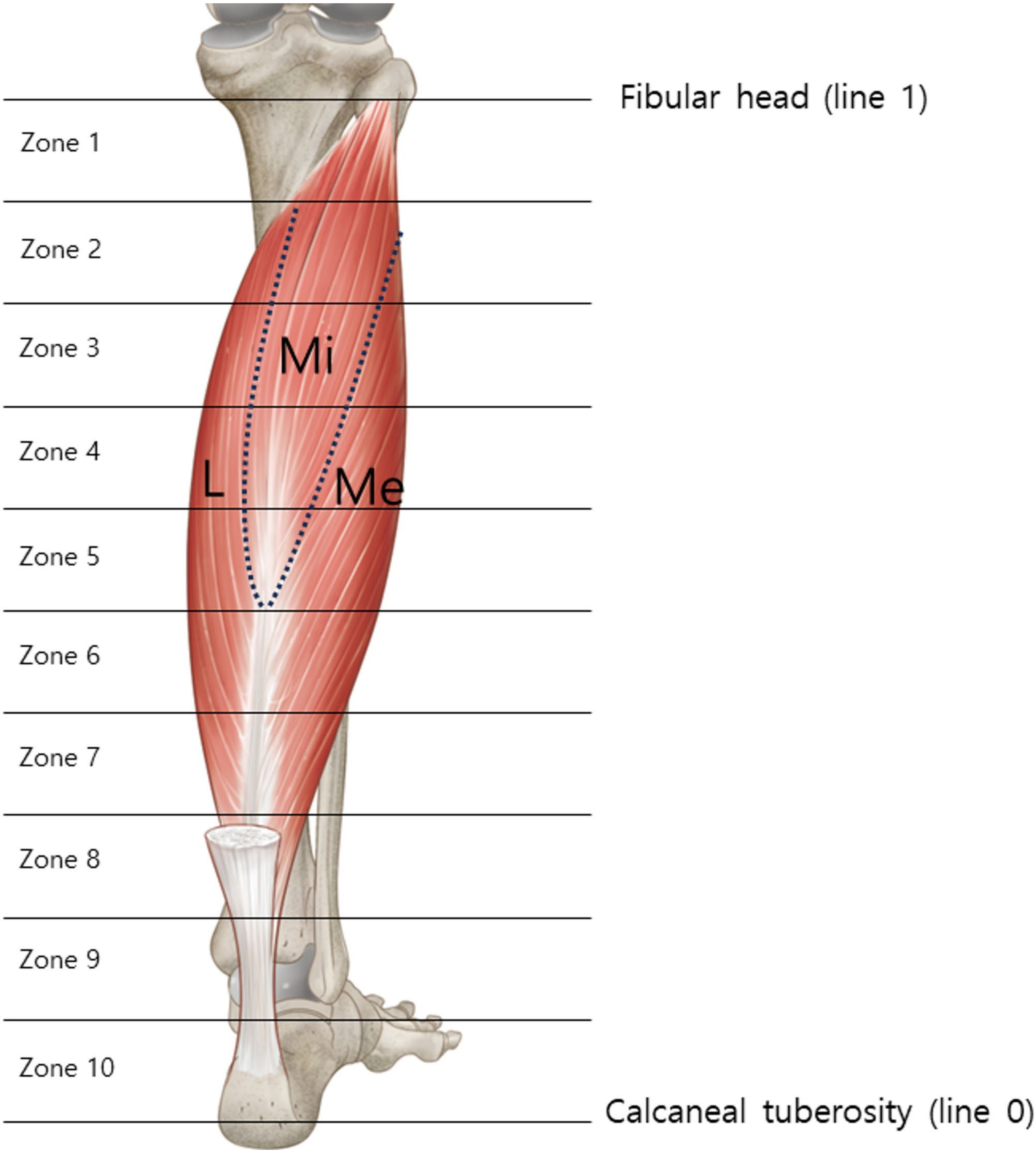

Fig. 1. The soleus muscle was divided into lateral (L), middle (Mi), and medial (Me) portions vertically, and the arborizing patterns were analyzed based on the transverse lines of the fibular head and calcaneal tuberosity. The muscle was further segmented into 10 zones from bottom to top.

knee flexions. Ultimately, while both injection methods proved effective, the study concluded that there were no clinically meaningful differences between selectively injecting BoNT into the soleus or gastrocnemius muscles.

Hesse et al.[31] conducted a study to investigate the effects of BoNT treatment on ankle muscle activity during walking among patients experiencing severe extensor spasticity post-stroke. Involving twelve chronic hemiparetic outpatients, the study administered BoNT injections in specific leg muscles and assessed changes in ankle spasticity alongside complex gait analysis, including kinematic electromyography (EMG), before and after the treatment. The results showed that nine patients experienced reduced spasticity, improved gait ability, and a more normalized muscle activity pattern, notably a significant reduction in premature plantar flexor muscle activity. Among these patients, a specific EMG pattern (type I), linked to increased stretch-reflex excitability,

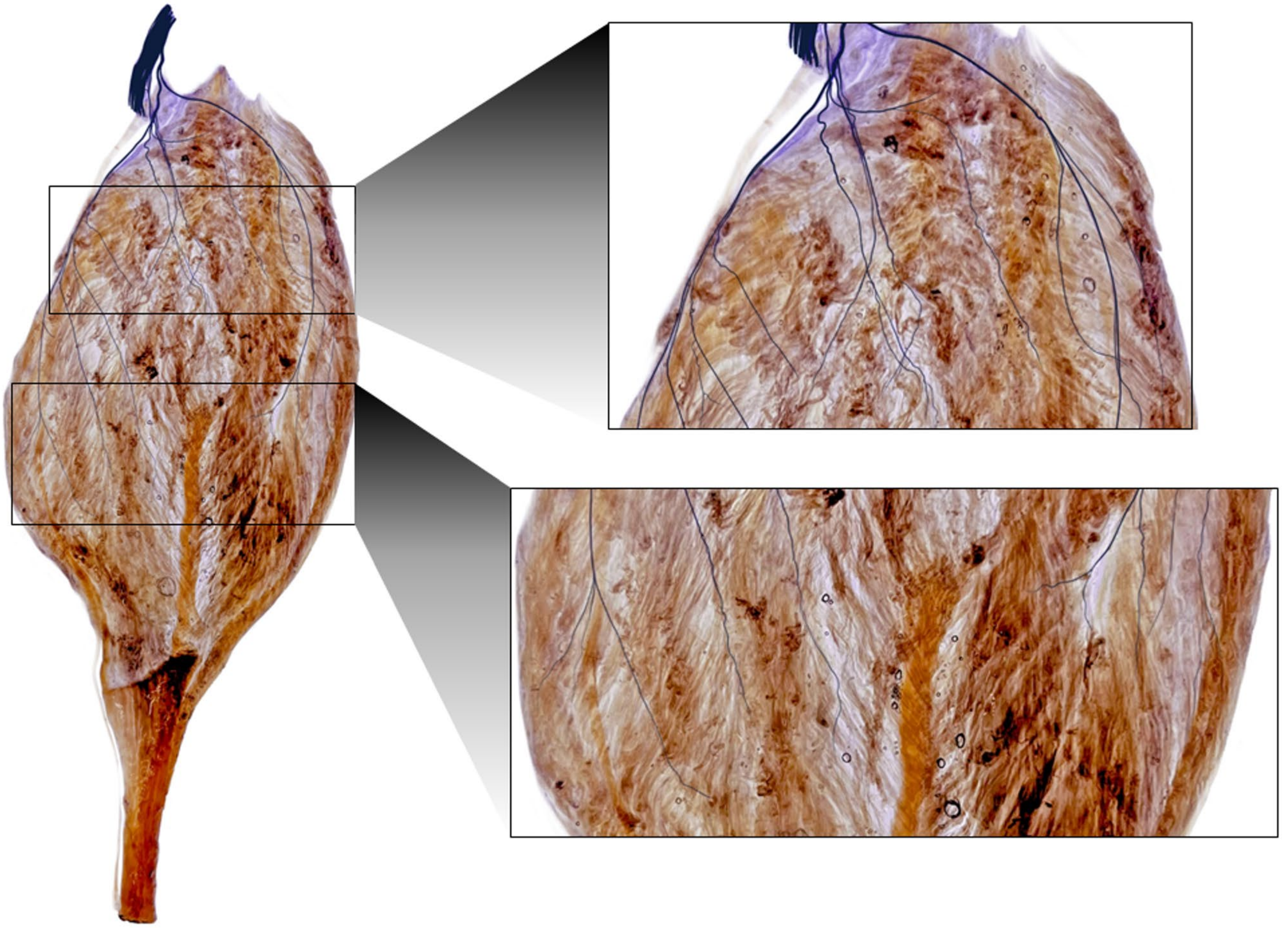

Fig. 2. This figure illustrates the intramuscular neural distribution of the soleus muscle obtained after applying Sihler's staining technique. An enlarged panel showcases the nerve endings within the soleus muscle.

was identified as a positive predictor for favorable treatment outcomes. Conversely, three patients did not benefit from the treatment, with no changes or worsening in muscle tone, gait ability, and muscle activation. This study underscores the positive impact of BoNT in managing lower limb extensor spasticity after stroke, highlighting the association between decreased muscle tone, improved gait parameters, and a more normalized EMG pattern, particularly reduced premature plantar flexor muscle activity. Moreover, the identified qualitative EMG pattern (type I), signaling increased stretch-reflex excitability, was noted as a favorable predictor for treatment success.

In the realm of aesthetic treatments, BoNT is increasingly utilized for body contouring to meet individual patient preferences. This method often targets various body areas such as the superior trapezius, deltoid, triceps brachii, quadriceps, and gastrocnemius muscles[32–34]. Typically, well-developed soleus muscles in women can result in a more muscular appearance in the lower part of the calf, which is a common aesthetic concern. As a result, many women seek solutions for this issue by visiting clinics. The research by Jung et al.[35] involved 30 patients treated with BoNT therapy to reduce ankle circumference without impacting functionality over a six-month period. Their method included administering Botulinum toxin injections to shape the ankle by targeting the soleus muscle. They employed a procedure of sterilizing the skin and injecting 50 units of botulinum toxin mixed with 1.2 mL diluent on both sides of each soleus muscle, totaling 100 units per ankle. Clinical photographs were taken while patients stood with relaxed legs on the floor, and follow-up assessments were conducted at 2, 4, and 6 months post-procedure. Ankle circumference was measured using a digital camera and tape measure positioned 5 cm above the lateral malleolus. This method has shown promise for ankle correction and highlights the significance of reducing calf circumference by targeting the soleus muscle, not solely for ankle correction but also for overall aesthetics.

The study suggests that for BoNT treatment in the soleus muscle, multiple injection sites should be used based on intramuscular nerve distribution, utilizing low doses to minimize side effects. It recommends targeting areas of maximum arborization for BoNT injections to ensure effectiveness and safety. However, it acknowledges limitations, indicating that while zone-based injections can locate intramuscular neural distribution, sonography-guided injections may be more precise. Clinical validation of this approach is pending. The study proposes directing BoNT treatments to zone 5 in the lateral portion, zone 3 in the middle portion, and zone 5 in the medial portion concerning specific anatomical lines. It emphasizes the need for electromyography adaptation

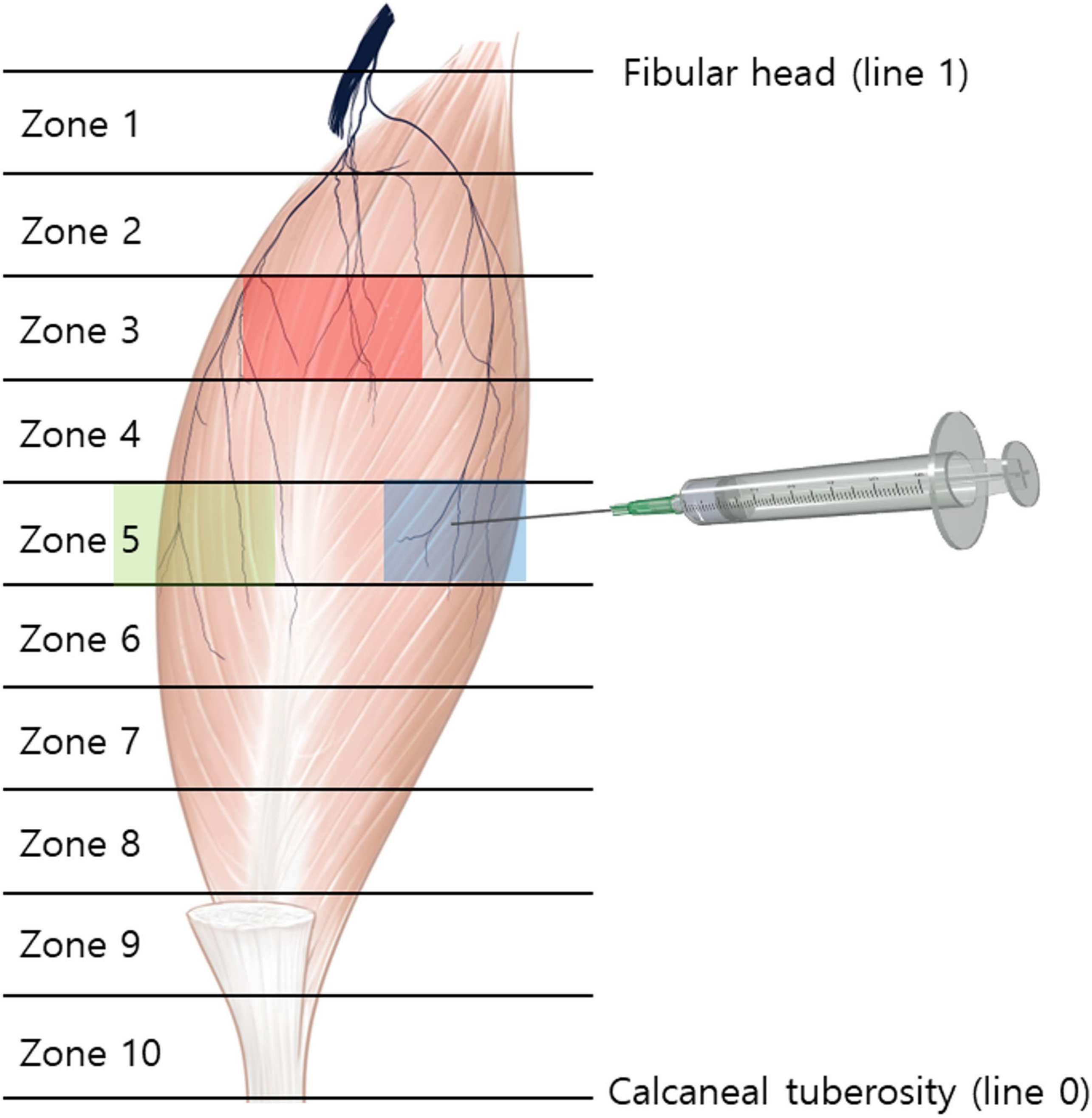

Fig. 3. A schematic representation indicating the suggested injection sites for botulinum neurotoxin (BoNT) treatment in the soleus muscle. Line 1 marks the transverse line of the fibular head, and line 0 marks the transverse line of the calcaneal tuberosity. The ideal injection points for BoNT treatment are recommended in zone 5 for the lateral portion (green shaded), zone 3 for the middle portion (red shaded), and zone 5 for the medial portion (blue shaded).

to optimize results and concludes that a comprehensive understanding of soleus muscle intramuscular neural distribution is crucial for achieving the most effective outcomes in BoNT treatments.

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Author contributions

All authors have reviewed and approved the article for submission. Conceptualization, Kyu-Ho Yi, Ji-Hyun Lee, Hye-Won Hu Writing—Original Draft Preparation, Kyu-Ho Yi, Hye-Won Hu, Sung-Oh Hwang Writing—Review & Editing, Ji-Hyun Lee, Hye-Won Hu Visualization, Hyung-Jin Lee, Hye-Won Hu Supervision, Kyu-Ho Yi, Hyung-Jin Lee.

Declarations

Competing interests
The authors declare no competing interests.

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