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ORIGINAL ARTICLE

Guidance in botulinum neurotoxin injection for lower extremity spasticity: Sihler's staining technique

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Abstract

Spasticity is a motor disease characterized by a velocity-dependent acceleration in muscle tone or tonic stretch reflexes linked to hypertonia. Lower limb spasticity has been successfully treated with botulinum neurotoxin; however, the injection sites have not been generalized. Sihler's stain has been used to visualize intramuscular nerve distribution to guide botulinum neurotoxin injection. Sihler staining is a whole-mount nerve staining technique that allows visualization of nerve distribution and mapping of entire nerve supply patterns in skeletal muscle with hematoxylin-stained myelinated nerve fibers. This study reviewed and summarized previous lower extremity spasticity studies to determine the ideal injection site for botulinum neurotoxin.

Keywords Sihler's staining · Intramuscular neural distribution · Botulinum neurotoxin · Spasticity

Introduction

The central nervous system is damaged, and spasticity plays a significant role in functional decline. Spasticity can be caused by a stroke, traumatic brain damage, multiple sclerosis, cerebral palsy, spinal cord injury, and other conditions affecting the central nervous system [2]. These individuals frequently have flexed hip and knee joints, an inverted forefoot or hindfoot, and restricted lower extremities, especially those with hemiplegic neurologic disabilities [7]. The lower extremity muscles have been targeted to reduce spasticity and aberrant gait patterns because spasticity in the lower limb causes instability and disturbance during gait. Botulinum neurotoxin (BoNT) injection has been regarded as one of the most secure and efficient treatments for spasticity [19]. The dosage of BoNT should be sufficient to provide appropriate toxin levels in the motor end plate zone of the targeted muscle, where it operates, because its effects rely on dosage [25]. BoNT can spread to nearby muscles if administered in excess, which could result in unintended paralysis [6, 8, 13].

Therefore, BoNT should be administered as close as feasible to the neuromuscular junction zones to reduce undesirable side effects and increase effectiveness. Numerous studies on the anatomical placement of motor end plate (MEP) zones in the targeted muscles have been published, as doing so is crucial for maximizing the therapeutic effectiveness of BoNT injections. Limitations include the inability to see the nerves and the potential for nerve injury in studies that mapped intramuscular nerve distribution to determine the BoNT injection locations [12, 15]. Additionally, other studies have noted challenges in precisely identifying neuromuscular connections [20].

The Sihler staining method, a whole-mount nerve staining approach that makes the muscle appear translucent while staining the nerves, can be used to overcome these constraints.

Sihler initially developed his staining technique using specimens of snake nerve dispersion and frog organs [21,

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24]. Wharton, who initially tested the procedure on human kidney, uterine, and ovarian organs [23], and Williams, who tested it on a human fetus, adapted it to human specimens. Many contemporary studies on nerve distribution patterns have used the modified Sihler approach, developed by Liem and Douwe van Willigen in the late twentieth century [14].

Sihler's approach has been modified for use with electromyography, injections of BoNT, lidocaine, and steroids to relieve pain. Since BoNT targets the most distal end of the motor neuron and Sihler's staining provides visualization of both the proximal and distal portions of these terminals, their most effective field is the distal portion of the motor nerve terminal.

Sihler's staining protocol and published articles on BoNT injections for treating spasticity in lower limb muscles, including the iliopsoas, hamstring, hip adductor, ankle invertor, peroneus longus, and brevis muscles, will be summarized in this review [10, 11, 18, 26–44].

Methods

Sihler's staining protocols

Sihler's staining technique entails several lengthy processes, with specimen preparation taking between 2 and 4 months depending on size. Another difficulty is that the staining process depends on the knowledge and abilities of conductors [14]. After harvesting, the specimens were stage-fixed in 10% formalin to begin the process. According to the size and thickness of the specimens, they are typically preserved in a 10% formalin solution for up to one or two months. When performing the fixation technique, a fresh formalin solution should be used to replace any hazy formalin. Maceration and depigmentation should be performed during the fixation phase.

After the specimens were fixed, they were immersed in a maceration solution for four weeks and then rinsed with running water for 30 min to an hour. The maceration solution contains 0.2 ml of 3% hydrogen peroxide per 100 ml of a 3% aqueous potassium hydroxide solution. The maceration solution is periodically replaced every two days with a new one to keep the specimens transparent. The next stage is decalcification, where the specimens are placed in "Sihler's solution I." Sihler's solution I, which comprises 1/8 glacial acetic acid, 1/8 glycerin, and 6/8 of 1% aqueous chloral hydrate, needs to be changed every week.

After decalcification, the staining procedure is the next step. This staining procedure, which takes a month, is carried out by dipping the sample into "Sihler's solution II," which contains a solution of 1% aqueous chloral hydrate, 1/8 Ehrlich's hematoxylin, and 1/8 glycerin. Before proceeding to the destaining process, the staining state should be assessed; the best way to do this is with no light transmission. When only the stained nerve is visible, the destaining operation is stopped. This is carried out by dipping the sample into Sihler's solution I. At this point, the specimen must be carefully and frequently monitored to determine its status.

Except for the nerves, the muscle and other nearby components should be bleached. However, in most situations, complete bleaching is difficult. However, if the neuronal distribution is still discernible at this point, the processing of the specimen should proceed to the neutralization step. Following the destaining process, the specimens become acidic and are therefore neutralized for an hour in a solution of 0.05% lithium carbonate. Before the clearing stage, another hour of washing with running water is required. Multiple phases in the clearing stage increase the glycerin content from 40 to 100% at 20% intervals for a day. Overstained areas became more visible during the cleansing stage (Fig. 1).

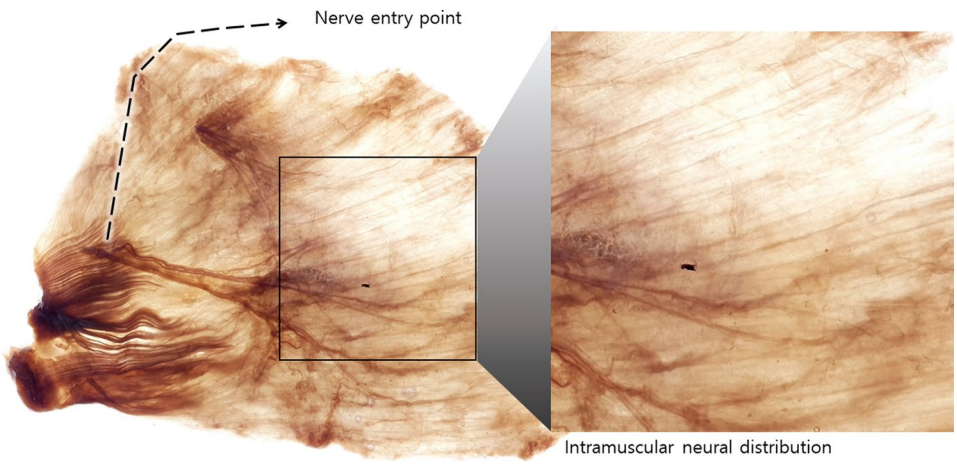

Fig. 1 The intramuscular neural distribution is revealed after the cleansing stage of Sihler's staining technique is conducted. The muscle is a sample of the teres major muscle

Results

Hip flexor (iliopsoas muscle)

The iliopsoas is the merged name of the iliacus and psoas major muscles. These two muscles are split cranially and merge distally to insert into the lateral trochanter of the femur. The anterior branch of the lumbar plexus at levels L1–L3 innervates the psoas major muscle, which is the primary hip flexor muscle. The iliacus muscle, which also functions as a hip flexor, is innervated by femoral nerve branches. Generally, hip flexion deformity and ongoing discomfort are caused by spastic contraction of the iliopsoas muscle in cerebral palsy. Patients with cerebral palsy typically receive intramuscular injections of BoNT to reduce stiffness.

According to a previous study, the intramuscular nerve arborization of the psoas major muscle is greatest from 2/5 to 4/5 of the distance between the posterior superior iliac spine (PSIS) (0/5) and the transverse process of the 12th thoracic vertebra (5/5) and the tendinous part of the muscle extends from 0/5 to 2/5 of the distance [34]. The iliacus muscle's arborization was greatest between 1/5 and 3/5 of the horizontal distance and 0 to 1/3rd of a longitudinal distance, as well as between 1/5 and 2/5 of a horizontal distance and 1/3 to 2/3 of a longitudinal distance, relative to the plane of the anterior superior iliac spine (ASIS) (0/0), PSIS (5/5), and lateral trochanter (3/3) (Fig. 2).

These findings imply that particular sites should receive BoNT injections in the iliac and psoas muscles. Additionally, because the injection location is above the PSIS, the posterior approach is the best strategy for focusing solely on the psoas major. However, based on the patterns of arborization, the proximal anterior approach is the best technique for treating both the psoas major and iliacus muscles [34].

Hip adductor (adductor longus and gracilis muscle)

Hip adductor spasticity can affect balance control during standing and walking, dressing, perineal hygiene, posture, and sitting [9]. According to Won et al., hip adductors are the best place to inject BoNT injections with regard to the pubic tubercle to medial epicondyle of the femur [25]. The adductor longus (Fig. 3) and gracilis (Fig. 4) normally receive their most intensive regions of innervation (6/10–8.5/10 and 6.5/10–7.5/10, respectively). These are recommended for injection point for the BoNT.

Hamstring muscle (semimembranous, biceps femoris, and semitendinosus)

Hamstring muscle spasticity causes increased knee flexion at first contact during walking and decreased knee extension during the terminal swing phase [1]. Hamstring spasticity

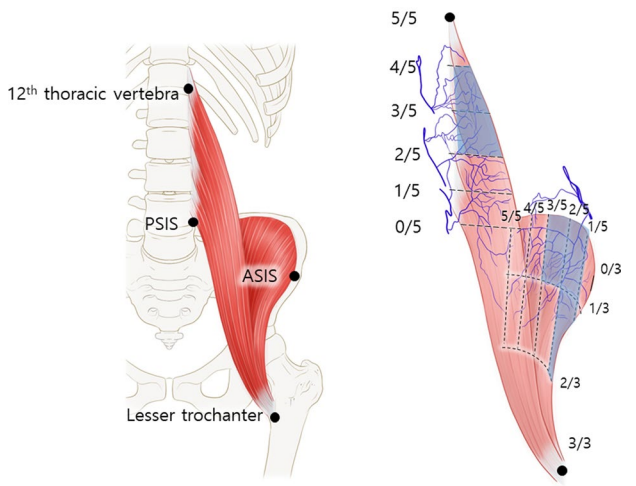

Fig. 2 The iliopsoas muscle is composed of the iliacus muscle and the psoas major muscle. The psoas major muscle's intramuscular nerve arborization was greatest from 2/5 to 4/5 of the distance between the posterior superior iliac spine (PSIS) and the transverse process of the 12th thoracic vertebra. The iliacus muscle's arborization was greatest between 1/5 and 3/5 of a horizontal distance and 0 to 1/3 of a longitudinal distance, as well as between 1/5 and 2/5 of a horizontal distance and 1/3 to 2/3 of a longitudinal distance, relative to the plane of the anterior superior iliac spine (ASIS), the PSIS, and the lateral trochanter. The recommended injection area are shaded in blue (colour figure online)

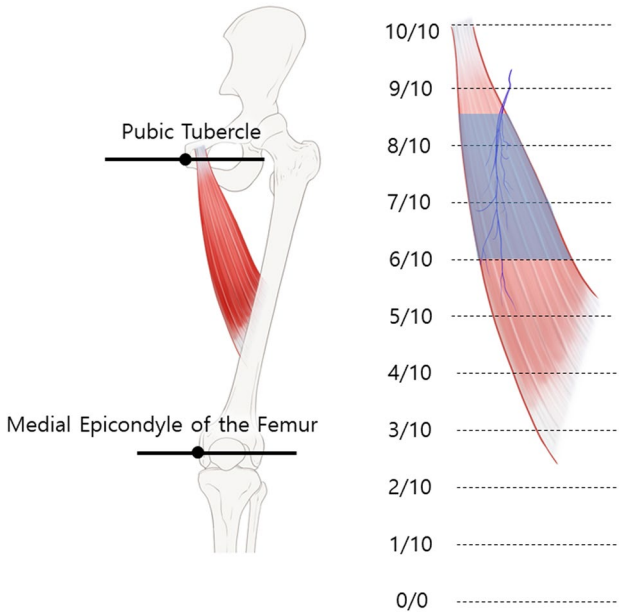

Fig. 3 The adductor longus received their most intensive regions of innervation 6/10–8.5/10, from the pubic tubercle (10/10) to medial epicondyle of the femur (0/0). These are suggested to be the injection point for the botulinum neurotoxin injection area (blue shaded) (colour figure online)

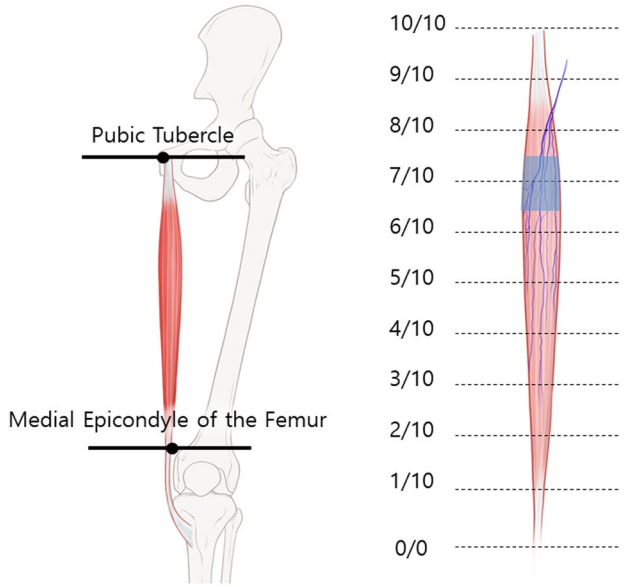

Fig. 4 The gracilis muscle received their most intensive regions of innervation 6.5/10–7.5/10 (blue shaded area), from the pubic tubercle (10/10) to medial epicondyle of the femur (0/0). These are suggested to be the injection point for the botulinum neurotoxin injection area (blue shaded) (colour figure online)

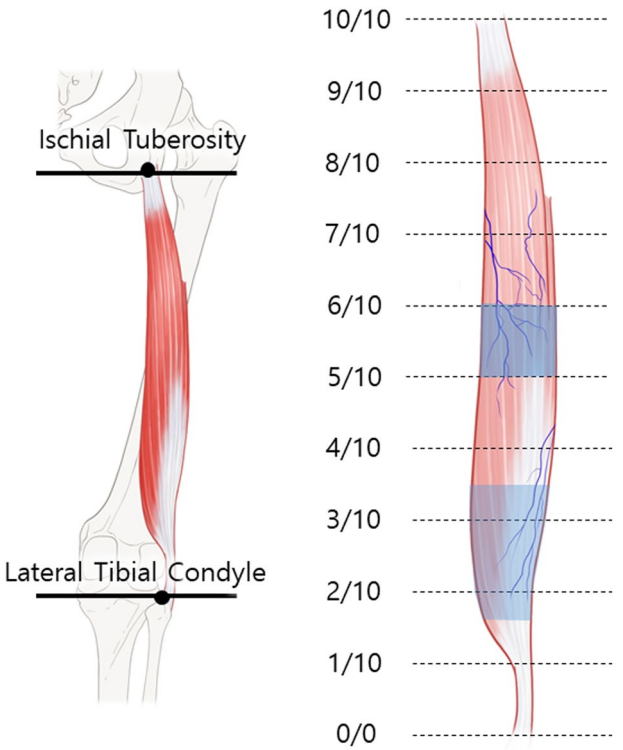

Fig. 5 The biceps femoris intramuscular arborization patterns were seen at 1.5/10–3/10 and 5/10–6/10 regarding the pubic tubercle (10/10) to lateral tibial condyle (0/0). These are the suggested injection points for the botulinum neurotoxin (blue shaded) (colour figure online)

and increased knee flexion during the stance phase of walking are connected [17]. Therefore, it has been targeted to address gait abnormalities as such spasticity results in atypical gait patterns, including the crouching and jump knee gaits [3]. During the stance phase of walking, BoNT injections into spastic hamstring muscles have been shown to reduce the popliteal angle and increase maximal knee extension [3].

The locations of the muscle origins, nerve entrance points, and intramuscular arborized regions were noted as percentages of the total distance from the line that crossed the medial and lateral tibial condyles (0/0) to the ischial tuberosity (10/10). For the biceps femoris, intramuscular arborization patterns were observed at 1.5/10–3/10 and 5/10–6/10 (Fig. 5); for the semitendinosus, at 2.5/10–4/10 and 6/10–8/10 (Fig. 6); and for the semimembranosus, at 2/10–4/10 (Fig. 7). According to this study, particular regions should receive BoNT injections to treat spasticity of the hamstring muscles. The most effective and secure injection sites are those where the arborization of intramuscular nerve branches is greatest [18].

Ankle flexors (flexor carpi hallucis muscle tibialis posterior muscle/flexor digitorum longus muscle)

Patients with lower extremity spasticity frequently have an inverted forefoot or hindfoot, restricted ankle dorsiflexion, and a spastic equinovarus foot, especially those with hemiplegic neurologic disabilities [4]. The ankle inverter muscles have been targeted to reduce spasticity and aberrant gait patterns because an equinovarus foot causes instability during the stance phase and poor foot clearance during the swing phase of gait [4, 16].

The distances between the most prominent point of the lateral malleolus from the lateral contour (0/0) and the fibular head (10/10) were used to calculate the portion of the muscle origins, nerve entrance sites, and intramuscular arborization regions [44]. For the flexor hallucis longus (2/10–5/10), tibialis posterior (7/10–8/10), and flexor digitorum longus (3/10–4/10), intramuscular arborization patterns were seen (respectively, Figs. 8, 9 and 10). These results imply that injections of botulinum toxin into certain muscles may be used to treat the stiffness of ankle invertors. The most effective and secure injection sites are recommended because they correlate with the areas of greatest arborization.

Discussion

Intramuscular BoNT is emerging as a successful and well-established choice for the treatment of spasticity following a stroke, cerebral palsy, and other central nervous system

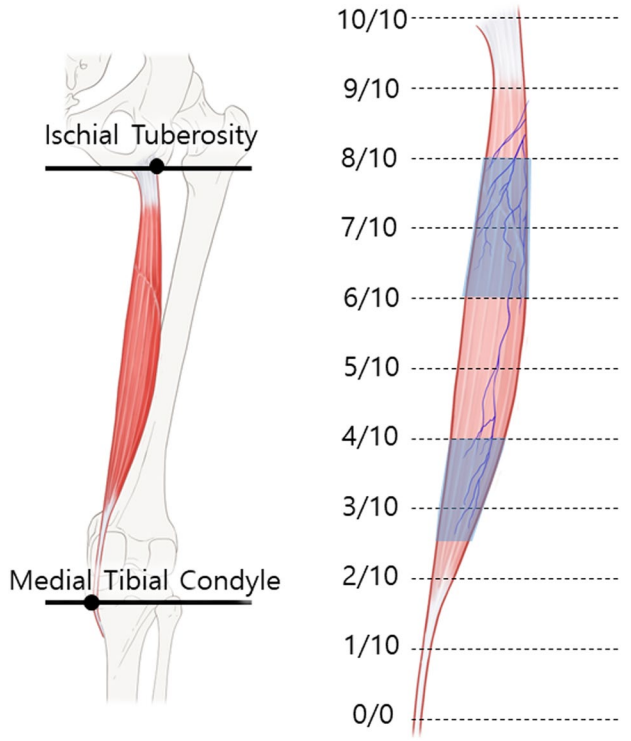

Fig. 6 The semitendinosus intramuscular arborization patterns were seen at 2.5/10–4/10 and 6/10–8/10 regarding the pubic tubercle (10/10) to medial tibial condyle (0/0). These are the suggested injection points for the botulinum neurotoxin (blue shaded) (colour figure online)

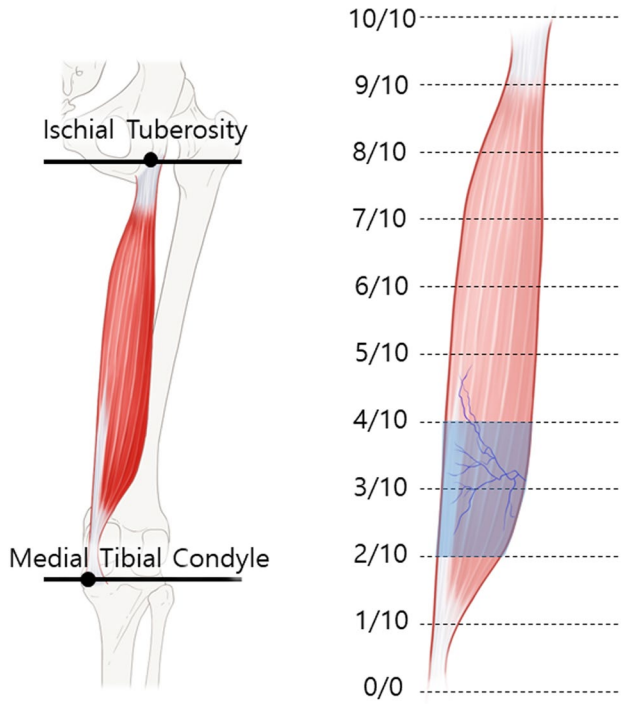

Fig. 7 The semimembranous intramuscular arborization patterns were seen at 2/10–4/10 regarding the pubic tubercle (10/10) to medial tibial condyle (0/0). These are the suggested injection points for the botulinum neurotoxin (blue shaded) (colour figure online)

injuries. Lower limb spasticity can be treated with the goal of reducing muscle tone, making care easier, relieving discomfort, improving hand cleanliness, and improving motor function. Patients with lower limb spasticity may have an improved ability to walk, lessen painful toe clawing, and use orthoses. If the BoNT injection was administered sparingly, the patient tolerated it well. Another constraint is high BoNT expenses. Intramuscular injections with directed neural distribution may assist in improving the efficacy of this therapy. Clinical trials on the biceps brachii and psoas major muscles have demonstrated the efficacy of arborized area-targeted injection, which is administered in the region where we anticipate the majority of neuromuscular connections to be located [5, 22]. In these investigations, injections specifically directed at the neuromuscular junction caused a significantly greater reduction in muscle volume than conventional injection techniques [5, 22].

Clinicians must be cautious about several issues when administering BoNT injections. A greater concentration of BoNT may induce the toxin to spread to nearby muscles, resulting in unfavorable palsy [6, 8]. Additionally, frequent and heavy doses of BoNT injections have led to the development of antibodies that lessen its effectiveness. Thus, BoNT injection must be administered directly into the neuronal

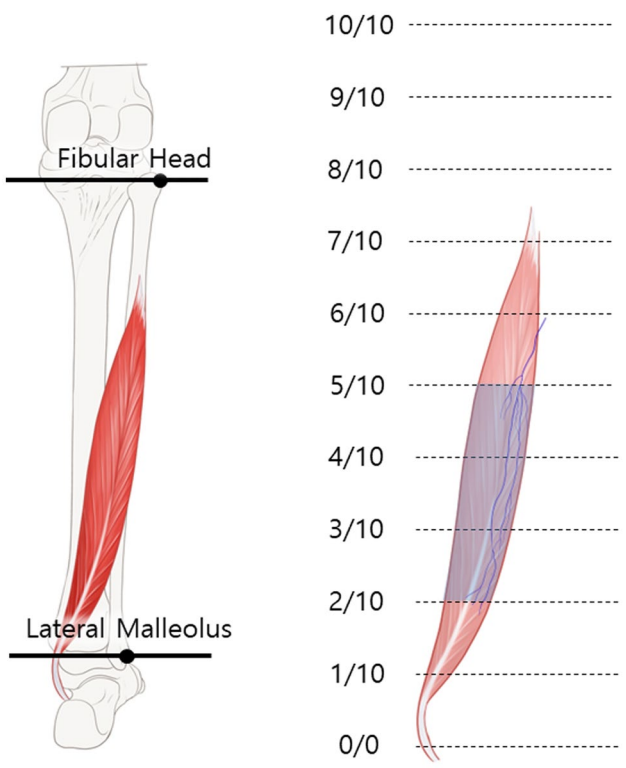

Fig. 8 The flexor carpi hallucis was analyzed in distances between the most prominent point of the lateral malleolus from the lateral contour (0/0) and the fibular head (10/10) and had most intramuscular arborization at region of 2/10–5/10 (shaded in blue) (colour figure online)

arborized regions to increase its efficacy and reduce its side effects.

Studies have focused on locating the neuronal arborized regions inside muscles to pinpoint the BoNT injection site. Furthermore, prior research has documented neurologic impairment caused by mechanical injury when injectable therapies are administered at the nerve trunk, where the nerve is punctured.

The advantages of Sihler's staining approach include an efficient way to observe how intramuscular neural distribution is distributed, even with tiny nerve ends. Because staining is performed at the myelinated sheath, which does not cover the neuromuscular junction, this may not represent the motor end plate; nevertheless, this distance of a few micrometers may be disregarded.

Manual dissection was not necessary because Sihler's approach uses a whole-mount nerve staining technique. Since latex was injected vascularly, the staining process displayed not only the intramuscular neural distribution pattern but also the arterial distribution. The neuronal distribution makes it difficult to distinguish between sensory and motor neurons, despite the merits of this technique. Although it is known that sensory neurons have less myelination and may be somewhat more discolored than motor neurons, it is still difficult to distinguish them. Tracking the nerve close to where it will be stained or harvested reveals the nerve's makeup. Specimens obtained from females, with less mass, are suitable for the staining method because the results depend on the technician's experience and staining limitations, resulting in thick and large specimens.

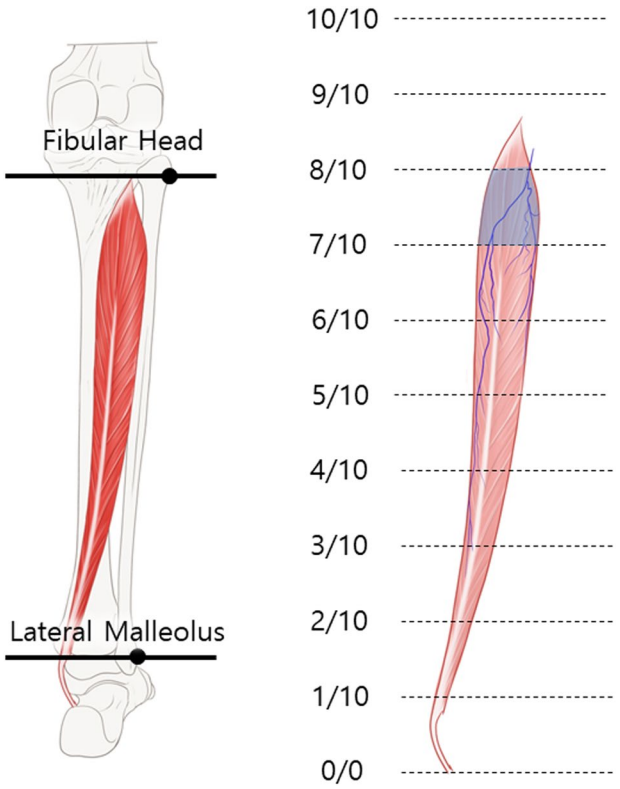

Fig. 9 The tibialis posterior had the most arborization of intramuscular neural distribution at 7/10–8/10 regions (shaded in blue) (colour figure online)

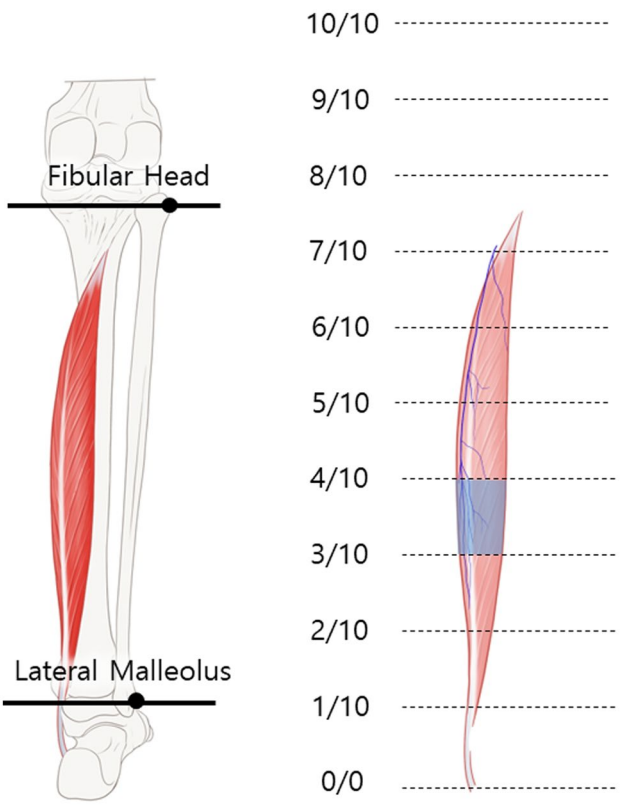

Fig. 10 The flexor digitorum longus muscle had the arborization was greatest between at region 3/10–4/10 (shaded in blue) (colour figure online)

Conclusion

In this review, we have identified and summarized the ideal BoNT injection sites for the lower extremity muscles for spasticity using Sihler's staining technique to reveal the intramuscular distribution.

Acknowledgements This study was conducted in compliance with the principles set forth in the Declaration of Helsinki. Consent was received from the families of the deceased patients before beginning the dissections. The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge that can then improve patient care. Therefore, these donors and their families deserve our highest gratitude.

Author contributions All authors have reviewed and approved the article for submission. K-HY, H-WH, and H-JL were responsible for conceptualization; K-HY and MHA prepared the original draft; K-HY and H-WH contributed to writing—reviewing and editing; H-WH was involved in visualization; and K-HY and H-JL took part in supervision.

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Data availability The data are available on request.

Declarations

Conflict of interest I acknowledge that I have considered the conflict of interest statement included in the "Author Guidelines." I hereby certify that, to the best of my knowledge, no aspect of my current personal or professional situation might reasonably be expected to significantly affect my views on the subject I am presenting.

Ethical approval This study was conducted in compliance with the principles set forth in the Declaration of Helsinki. Consent was received from the families of the deceased patients before beginning the dissections.

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