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Intramuscular Neural Distribution of the Gastrocnemius for Botulinum Neurotoxin Injection: Application to Cosmetic Calf Shaping

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Purpose: Anatomical landmarks can provide vital information on the distribution of nerves in the gastrocnemius muscle. We aimed to provide an anatomical perspective on appropriate locations for botulinum neurotoxin (BoNT) injections in the medial and lateral parts of the gastrocnemius for calf shaping.
Materials and Methods: A modified Sihler's method was applied to both the medial and lateral parts of the gastrocnemius muscles (16 specimens). Intramuscular neural distributions were revealed by dissecting along a transverse line crossing the fibular head and superior margin of the calcaneal tuberosity.
Results: The intramuscular neural distribution for the medial and lateral parts of the gastrocnemius had the greatest arborized patterns in the 7/10–8/10 section of the medial head and 7.5/10–8.5/10 section of the lateral part of the gastrocnemius.
Conclusion: We propose that BoNT injections should be directed to the 7/10–8/10 section of the medial head and the 7.5/10–8.5/10 section of the lateral part of the gastrocnemius. Following our guidelines, clinicians can ensure satisfactory results with the use of minimal doses to limit adverse effects, such as gait disturbance, antibody production, and bruising, due to multiple injections. The results can also be altered and applied to electromyography.

Key Words: Clinical guideline, gastrocnemius, calf, cosmetic calf shaping, botulinum neurotoxin

INTRODUCTION

Botulinum neurotoxin (BoNT) limits neural communication by deterring the release of acetylcholine at the neuromuscular junction, which prevents muscle contraction.[1] In clinical settings, especially in aesthetics, BoNT is frequently used to de-

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activate the muscles contributing to facial expression for rhytid removal. Currently, expansion of the application of BoNT from more than managing wrinkles to shaping of the body in line with patient desires is increasing. Body shaping commonly targets the superior trapezius, deltoid, triceps brachii, quadriceps, and gastrocnemius muscles.[2-4] Generally, in women, muscularly developed medial and lateral gastrocnemius muscles can pose aesthetic concerns, and many women visit clinics to solve this problem.

Several conventional methods are available for the reduction of muscle bulk, including neurectomy. Myomectomy has been shown to be correlated with significant side effects, such as hematoma, hemorrhage, infection, nerve damage, and skin scarring.[5,6] Another option is liposuction, although it has limited efficacy in cases where the primary determinant of calf thickness is on the gastrocnemius muscle rather than subcutaneous fatty tissue.[3,7] Another method is ablative therapy using radiofrequency; however, this has been downgraded due to reports of fibrosis and significant contractures.[8] To avoid these issues, many patients tend to prefer minimally invasive therapies, such as BoNT injections. To date, however, no injection standardized protocols or guidelines have been determined for BoNT treatment of gastrocnemius muscular hypertrophy, with injecting depths and techniques varying depending on the practitioner's opinion and experience. Nonetheless, treatment with BoNT injections is recognized as safe and efficient.[9-12]

The results of BoNT vary in the presence of toxins at the presynaptic neurons at the motor-end-plates. Therefore, the injections should be targeted into intramuscular neural arborized areas.[1,13,14] The efficiency of using neural arborized-targeted BoNT injections has been proven to be effective in clinical trials in the biceps brachii and iliopsoas muscles. The study proved that neural-arborized targeting injections achieved much greater volume reductions than the control injections.[15,16] The downside of this practice is its short-term effects. Based on Bogari, et al.,[17] the effects of BoNT were not entirely insignificant at month 6 after treatment, and practitioners would normally recommend repeat treatment to preserve the desired contour. Another downside is the high expense of BoNT in large units needed for shaping, which has resulted in the reluctance of many patients to repeat the treatment after 6 months. Furthermore, overdosage and repeated treatment with BoNT can produce antibodies that affect treatment effects.[18-21] Many studies regarding intramuscular neural arborization of skeletal muscles have been published and adopted in clinical fields as guidelines.[22-26]

This study used a modified Sihler's staining technique, a whole-mount staining technique, aimed at revealing the intramuscular neural patterns without damaging the miniscule nerve branches. By revealing the distribution of intramuscular nerves, this study was able to suggest guidelines on effective and safe BoNT injection points along the gastrocnemius muscle for calf shaping.

MATERIALS AND METHODS

This study was performed in accordance with the principles of the Declaration of Helsinki. All cadavers used in this study were legally donated to the Surgical Anatomy Education Center, Catholic University of Korea, College of Medicine (IRB approval code: MC23EISE0022). Appropriate consent and approval were obtained from the families of the cadavers before the dissection was performed. A total of 17 gastrocnemius muscles from Korean cadavers (6 male and 3 female patients; mean age at death, 73.6; range, 68–92 years) were stained using the modified Sihler's method to reveal intramuscular nerve arborization patterns. Prior to dissection, the gastrocnemius muscle was aligned anatomically. The gastrocnemius muscle was harvested from the transverse line of the fibular head and the calcaneal tuberosity (Fig. 1).

The arborizing patterns of the muscles were elucidated according to two lines: the gastrocnemius muscle was measured according to the transverse line along the fibular head (Line 1) and the transverse line along the calcaneal tuberosity (Line 0).

The gastrocnemius muscle was subjected to modified Sihler's staining as described by Liem and Douwe van Willingen.[27] The method involves several steps to obtain a visible representation

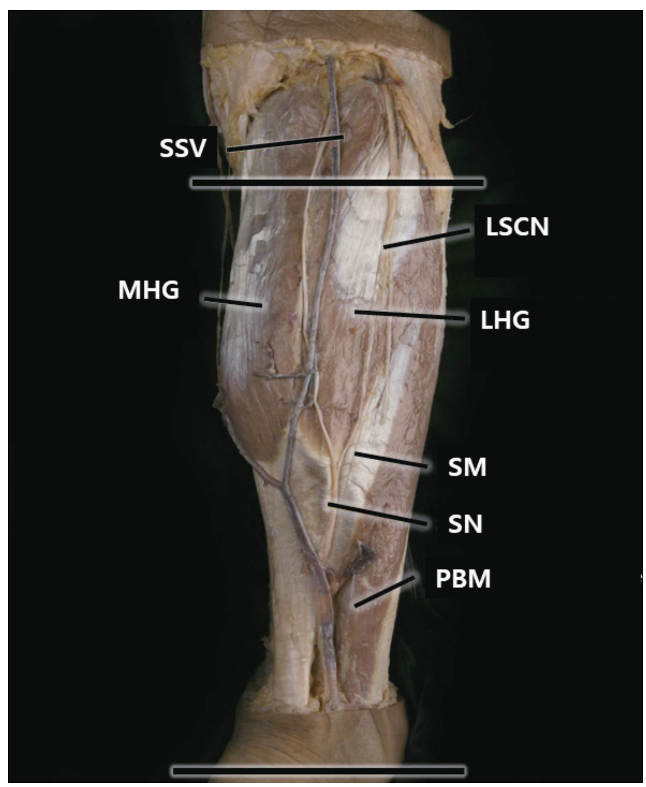

Fig. 1. The gastrocnemius muscle was harvested from the transverse line on the fibular head and the calcaneal tuberosity. The surrounding anatomical structures of the gastrocnemius muscle. SSV, small saphenous vein; MHG, medial head of gastrocnemius; LSCN, lateral sural cutaneous nerve; LHG, lateral head of gastrocnemius; SM, soleus muscle; SN, sural nerve; PBM, peroneus brevis muscle.

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of the intramuscular neural arborization patterns. After completing modified Sihler's staining, the intramuscular neural distributions of the gastrocnemius muscles were observed.

Modified Sihler's staining

The gastrocnemius muscle was sunk in non-neutralized formalin for 1 month for fixation (Fig. 2). After fixation, the specimens were placed in a solution composed of 3% aqueous potassium hydroxide (KOH) and hydrogen peroxide (H2O2), with an additional drop of 30% H2O2 added per liter of the 3% KOH solution. This process took 1 month to make the gastrocnemius specimen transparent. The transparent gastrocnemius specimens were then decalcified by placing them in a solution composed of 10% glycerin and 7% glacial acetic acid for a week. The decalcified gastrocnemius muscles were then stained in a solution of 10% glycerin and 10% Ehrlich's hematoxylin mixture for a week. The stained gastrocnemius muscles were immersed again in a solution of glycerin and glacial acetic acid until the stained nerves became visible. Finally, the gastrocnemius muscles were clarified and soaked in glycerin at concentrations ranging from 40% to 100% in increments of 20%.

RESULTS

Intramuscular arborization patterns of the medial belly of the gastrocnemius muscle

Thirteen of the 17 gastrocnemius muscles had the highest arborization patterns in the 7/10–8/10 section (Fig. 3). Three out of 17 gastrocnemius muscles had 7.5/10–8/10 as the greatest arborization pattern. One out of the 17 gastrocnemius muscles had the greatest arborization pattern in the 8/10–9/10 section.

Intramuscular arborization patterns of the lateral belly of the gastrocnemius muscle

Fourteen out of the 17 gastrocnemius muscles had the greatest arborization patterns in the 7.5/10–8.5/10 section (Fig. 3). Two out of 17 gastrocnemius muscles had 7/10–8/10 with the greatest arborization pattern. One out of 17 gastrocnemius muscles had the greatest arborization pattern at the 8.5/10–9/10 section.

DISCUSSION

The gastrocnemius muscle has two separate heads, the medial and lateral heads.[28] The muscle originates from the bony surfaces of the medial and lateral condyles of the femur, and muscular fibers converge on the Achilles tendon, which inserts at the posterior part of the calcaneus. The gastrocnemius muscle is essential for the shape and shaping of the lower legs. Enlarged gastrocnemius muscles give the appearance of a muscular calf shape, which is commonly regarded as an indication of a manly like appearance. In contrast, an undesirable masculinized gastrocnemius muscle is a common aesthetic issue.[29]

A variety of injection locations have been suggested for shaping a hypertrophied calf; however, a consensus on a method of injecting BoNT into the calf muscle has not been achieved. Mostly, methods of muscle volumetric reduction are administered depending on the practitioner's experience and personal preferences. Previous studies have suggested that doses of BoNT vary from 30 to 200 units (U) per side of the gastrocnemius muscle. All injections are prepared by diluting a 100 U vial of onabotulinumtoxinA (ONA-BoNT/A) with 10 mL of normal saline to achieve a concentration of 1 unit/0.1 mL in the BoNT type A.

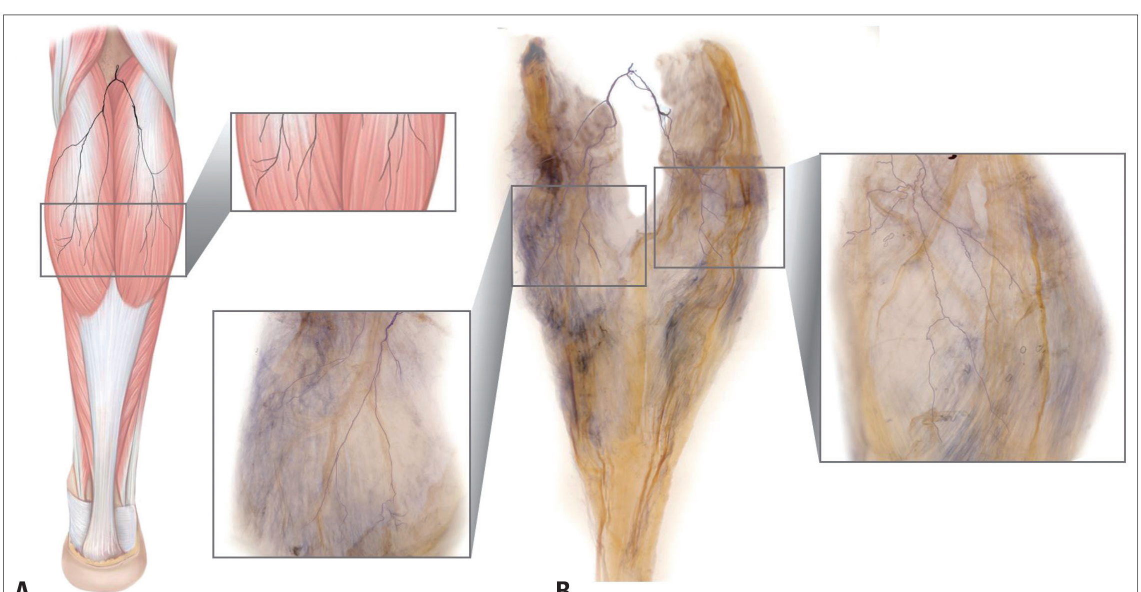

Fig. 2. Schematic image of the intramuscular neural distribution of the gastrocnemius muscle (A). Intramuscular distribution of the gastrocnemius muscle revealed after Sihler's staining (B). Enlarged panel represents the intramuscular nerve endings of the gastrocnemius muscle.

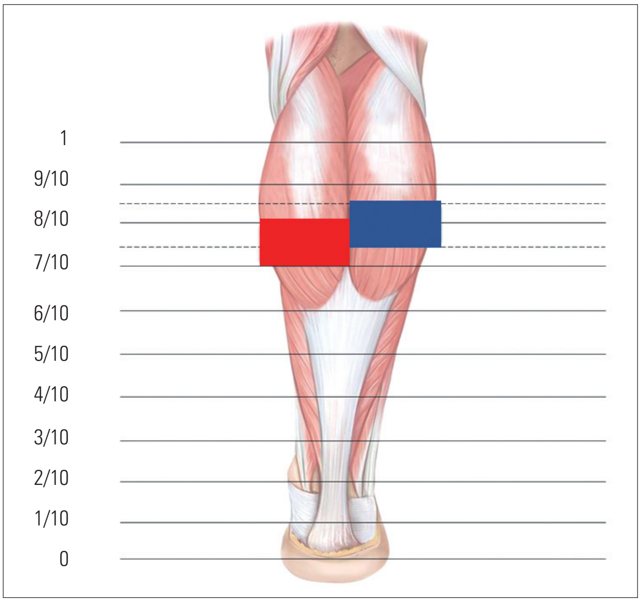

Fig. 3. Ideal injection points for botulinum neurotoxin injections in the gastrocnemius muscle. Line 1 represents the transverse line of the fibular head, and Line 0 represents the transverse line of the calcaneal tuberosity. The ideal injection points for BoNT treatment should be directed to the 7/10–8/10 section (red boxed) for the medial head and 7.5/10–8.5/10 section (blue boxed) for the lateral head.

BoNT injections for calf shaping was first suggested by Seo[2] injecting at five to six points along each of the heads of the gastrocnemius muscle. Before injection, it has been suggested to classify patients with mild, moderate, and severe hypertrophy and to administer dosages of 100, 150, and 200 U of BoNT per side, respectively. Han, et al.[3] defined BoNT injections into both the medial and lateral parts of the gastrocnemius muscle, with three points located along the lateral part and six injections at the medial part. Suh, et al.[30] comparably administered injections at three to six points along the most prominently bulged areas of the medial and lateral parts of the gastrocnemius muscle. In a study comparing dosages, researchers observed that reductions in calf circumference had not been dose dependent. The study results suggested that 160 U (80 U each leg) of BoNT resulted in a more general decrease in circumference, compared to 200 U (100 U each leg), at 6 months (15 mm vs. 10 mm). The 200 U group exhibited a faster preliminary reduction in circumference relative to that achieved with 160 U; however, decreases in calf circumference stopped at 3 months with 200 U, although they continued with 160 U. This suggests that treating only the gastrocnemius could indirectly cause hypertrophy of the soleus muscle for compensation or that injection locations should be personalized.

Lee, et al.[31] evaluated the outcomes of BoNT treatment in subjects with hypertrophied lower legs. The injection technique involved the participants being place in a prone position and injections being administered into the most bulging parts of the medial belly. Each injection was spaced 15–20 mm apart, and 23-gauge and 1-inch-long needles were used. BoNT was injected in the medial part of the gastrocnemius muscle, which is the most significant and practically less important muscle than the lateral head. The outcomes in terms of calf shaping were well retained for 6 months. Concerns were raised in terms of its negative effects, such as calf muscle dysfunction, which caused gait disorders and exhaustion after daily activity. Nonetheless, it is still the most effective and safe method of calf shaping.

Bogari, et al.[17] intended to examine and establish the most effective method to correct the shape of the calf region and to determine which method produces maximum results to satisfy the needs of the participants and practitioners. They suggested a large dose and multiple injections of 96 U at 48 injection sites. However, the drawback of the study they reported was that participants experienced extreme pain during the procedure for multiple injections, even though topical anesthesia had been sufficiently treated. The pain visual analog scale score was an average rating of 5. The treatment method was time-consuming because 48 points on each leg were initially marked and measured well before injection. The control group that received a minimum dose and fewer injections of 72 U and 10 injections experienced only slight reductions in leg circumference, and the results of photographic analysis did not appear to satisfy the results of the post-treatment survey. In terms of pain, no participants reported grievances during the treatment, and the whole process less time-consuming. The treatments were performed under sterile conditions in a prone position using 1-inch, 25-gauge needles.

Sheverdin, et al.[32] conducted a study using Sihler's method to examine the distribution of nerve branches within and outside the triceps surae muscle. In all specimens examined, the medial gastrocnemius exhibited more extensive innervation, with thicker, more intensely stained branches and a greater abundance of ramification, compared to the lateral gastrocnemius. The region with the highest concentration of intramuscular branching within the gastrocnemius muscle was found to be between 20% and 30% of the calf length, approximately one segment below the area with the highest density of extramuscular branching. The arborization patterns observed in our study differ from the findings of the Sheverdin's study, as arborization was located in a higher position. Additionally, the previous study did not distinguish between the arborization patterns of the medial and lateral heads of the gastrocnemius muscle.

Currently, there is no generalized injection location or ideal method for BoNT treatment in the gastrocnemius muscle. In light of the results of this study, we recommend that clinicians use multiple injection sites according to intramuscular nerve distributions with a low dose and minimal injections of BoNT to avoid and minimize side effects. Areas of maximum arborization are recommended as the most effective and safe points for calf shaping with BoNT. We propose that BoNT treatments should be directed to the 7/10–8/10 section in the medial head

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and the 7.5/10–8.5/10 section in the lateral head, with respect to the transverse line of the fibular head (Line 1) and the transverse line of the calcaneal tuberosity (Line 0). This method is limited in that locating the intramuscular neural distribution area may not be precise; however, sonography-guided injection was superior to blind injection of the target muscle (Figs. 4 and 5). In addition, no study has clinically approved its effectiveness. Previous research exploring the dispersion of BoNT has recommended the utilization of multiple injections, as the toxin has a limited diffusion range of only a few centimeters from the site of injection.[13,33] Borodic, et al.,[33] in their study, discovered that when 10 U of toxin was injected into the longissimus muscle of rabbits, the diffusion of BoNT could extend up to 4.5 cm from the injection site. Our investigation makes significant contributions by identifying ideal injection sites in the gastrocnemius muscles to optimize the effectiveness of BoNT while minimizing the required dosage. Electromyography also could be adapted to our results.

To conclude, we propose that the most efficacious results for calf shaping with BoNT injection treatments could be achieved if practitioners consider the intramuscular neural distributions of the gastrocnemius muscle.

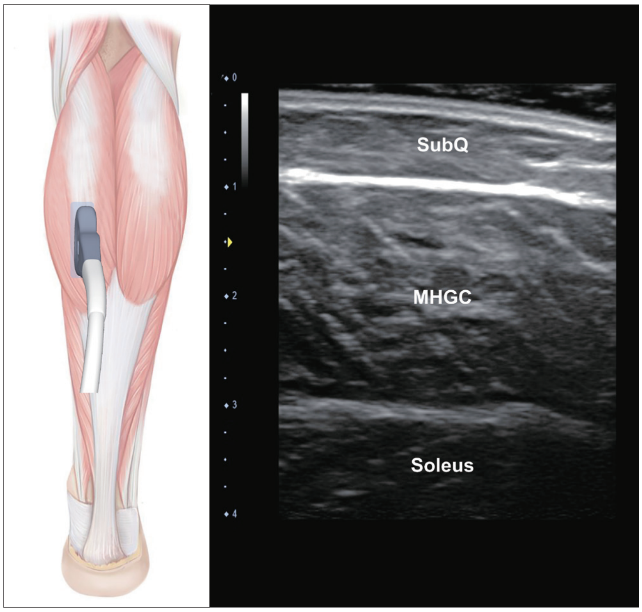

Fig. 4. Sonographic image of the medial head of the gastrocnemius muscle (MHGC) in a longitudinal view at the 7/10–8/10 section. SubQ, subcutaneous tissue.

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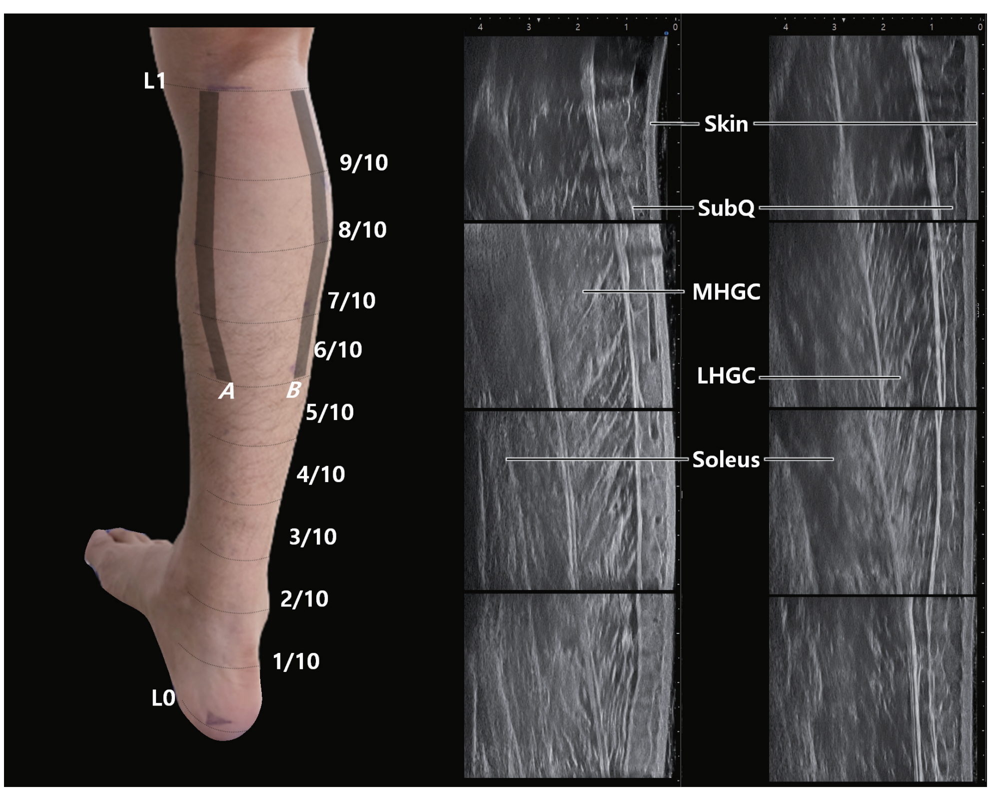

Fig. 5. Ultrasonographic image of the medial head of the gastrocnemius muscle (MHGC) (A) and the lateral head (LHGC) (B). The probe has been moved from the transverse line crossing the fibular head (L1) to the superior margin of the calcaneal tuberosity (L0). SubQ, subcutaneous tissue.

AUTHOR CONTRIBUTIONS

Conceptualization: Kyu-Ho Yi and Hyun Jin Park. Data curation: Kyu-Ho Yi and Hyun Jin Park. Formal analysis: Jin-Hyun Kim and Seon-Oh Kim. Funding acquisition: Gwahn Woo Cheon and Min Ho An. Investigation: Hyung-Jin Lee and Ji-Hyun Lee. Methodology: Gwahn Woo Cheon and Min Ho An. Project administration: Hyung-Jin Lee and Ji-Hyun Lee. Resources: Hyung-Jin Lee and Ji-Hyun Lee. Software: Hyung-Jin Lee and Ji-Hyun Lee. Supervision: Gwahn Woo Cheon and Min Ho An. Validation: Gwahn Woo Cheon and Min Ho An. Visualization: Hyung-Jin Lee and Ji-Hyun Lee. Writing—original draft: Kyu-Ho Yi and Hyun Jin Park. Writing—review & editing: Hyung-Jin Lee and Ji-Hyun Lee. Approval of final manuscript: all authors.

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