

Proposed Potential Anatomical Landmarks for Percutaneous Botulinum Toxin Injection in Anterocollis-typed Cervical Dystonic Patient: A Pilot Study Utilizing Thiel-embalmed Human Cadavers

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ABSTRACT

Objective: Botulinum toxin (BoTX) injection to the longus colli (LCo) muscle has been demonstrated to have a role in treating cervical dystonic (CD) patients. It can, however, cause critical complications and awareness of such complications is required. Currently, there is no substantial information regarding this novel procedure. This study aims to define the potentially safe method of injection based on assessment of anatomical measurements.

Materials and Methods: We examined distances between the puncture sites and adjacent structures in Thiel-embalmed human cadavers (n=20) to propose an alternative technique for BoTX injection. Parameters were examined for the medial and lateral approaches at the fifth and sixth cervical vertebral levels. We compared each variable between the two different vertebral levels and the two different approaches to evaluate statistical differences.

Results: Comparing distances between the puncture sites and neck anatomical structures in each of the two approaches, results were statistically significant. Similarly, we found using the medial approach statistical significance when comparing the measurements at the fifth with the sixth cervical vertebral level of the distances between the puncture sites and the thyroïdal arteries and recurrent laryngeal nerve ($p < 0.05$).

Conclusion: The present study results provide initial guidelines for the safe technique for BoTX injection into LCo. Our findings suggest that the medial approach at the C6 vertebral level is preferable with minimal injury to vital structures. Thus, it may provide an optional method and can be used as guidance to improve surgical practice. Ethical approval was not required for this study.

Keywords: Botulinum toxin (BoTX) injection; Cervical dystonia (CD); Longus Colli (LCo); Thiel-embalmed human cadavers (Siriraj Med J 2022; 74: 409-424)

INTRODUCTION

Cervical dystonia (CD) is the most frequent primary dystonia and is characterized by an involuntary contraction of neck muscle, causing abnormal head and neck motion and posture. The chemical de-innervation by intramuscular injection of botulinum toxin (BoTX) has been used as the

standard treatment to improve the quality of a patient's life by reducing accompanying symptoms, such as continuous pain or repetitive and uncontrollable muscle contraction.¹⁻³ Anterocollis type is one of the clinical spectrums of this condition presented with abnormal flexion deformity due to inevitable repeated contraction of anterior neck

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muscles. The longus colli muscle (LCo) is the pathogenesis of this clinical presentation. Botulinum toxin (BoTX) injection into LCo is ideal for treating a specific type of cervical dystonia, especially the treatment of anterocollis-typed dystonic patients.^{1,4} Localization of this muscle is crucial in clinical practice since this process serves as an essential step for achieving a desirable outcome. As part of the critical step, this process requires experience and accurate anatomical knowledge. A reliable and less harmful technique for targeting this muscle should be proposed and established to improve the accuracy of needle placement.⁵

Various techniques have been established to locate the targeted dystonic muscles for injections, such as the blind technique based on clinical examination. The ultimate goal of treatment for CDs is to completely de-innervate the causative muscles.⁶ Deep muscles, such as longus colli, can also be involved. Many studies have concentrated on the more accessible causative muscle, such as the sternocleidomastoid. In general, this muscle selection is preferable because it is superficially located in the cervical region, and it can be easily identified on clinical examination. In contrast, placing a needle into LCo, one of the causative muscles, is more challenging because this muscle is profoundly located in the pre-vertebral area within the cervical region.¹ The recommendation of imaging modality techniques, such as ultrasonography and electromyography, has been introduced for the BoTX injection because of the difficulty in locating this muscle in clinical practice, especially for the CD patient with abnormal flexion deformity.⁷⁻⁹ These techniques could reduce the misplacement of the needle and indicate the proper injection area.^{6,8,10,11} However, such techniques do not apply to deep cervical areas, especially the LCo. Under those circumstances, a blind technique becomes the method of choice. For it to be safe to conduct a blind intramuscular BoTX injection into this muscle, the recognition of cervical topographic anatomical variation is crucial in perceiving the depth from the anterior surface of the neck. Variation of posterior neck muscles has been documented, such as splenius capitis and semispinalis capitis.¹² Nonetheless, some remaining deep anterior neck muscles, for example, LCo, which are difficult to access from the anterior aspect of the neck, require more investigation.

We hypothesize that incomplete chemical de-innervation of the deep cervical muscle with BoTX injection may be one cause of treatment failures in patients with anterocollis. Most research being established to identify the safe position for BoTX injection in CDs is mainly focused on superficial and more robust cervical

muscles including sternocleidomastoid, splenius capitis, trapezius, semispinalis capitis, levator scapulae, and scaleni. However, complicated cases of anterocollis-type CDs who presented with deep neck muscle spasm, such as LCo, remain unexplored. LCo is not generally injected because there is scant research being conducted targeting this muscle to confirm the safest technique for BoTX injection. Furthermore, the reported clinical outcomes are usually based on a small sample size. It seems that guidance indicates safe and effective BoTX injections into this muscle should be established to reduce potentially harmful incidents.^{6,13}

LCo is located on the anterior surface of the C1-T3 vertebrae, and it is innervated by the anterior rami of the second (C2) to the sixth (C6) cervical spinal nerves. This muscle can be divided into three anatomic compartments: the superior oblique part, vertical intermediate part, and inferior oblique (the smallest element). LCo is gracile and is located adjacent to essential vital structures, such as great vessels, cranial nerves, trachea and oesophagus.¹⁴ LCo extends from the cervical region to the upper thoracic region. It is difficult to complete de-innervation this muscle using the conventional blind technique of BoTX injection. It can cause complications such as injury to the thyroidal artery or phrenic nerve. Dissemination of this toxin into nearby structures due to needle displacement can cause life-threatening adverse effects, including weakness of mistargeted muscles leading to dysphagia, dysphonia, or ptosis.¹⁵ This means that the degree of preferable treatment outcome is reduced. Therefore, considering a potential technique based on anatomical topographic landmarks to improve accuracy and minimize unfavourable complications of this procedure is essential, primarily when localizing the LCo.

There are two approaches based on a conventional blind technique to inject botulinum toxin into LCo, the medial and the lateral approach.^{6,16,17} The better approach can be selected after considering the position of the carotid artery in each patient. There is currently no substantial research comparing these approaches in cadaveric-based dissection studies. In the absence of a standard guideline regarding an anatomical landmark for this novel technique, BoTX injection into LCo was recommended at the anterior surface of the neck utilizing the sternocleidomastoid as a landmark at the C3 or C6 vertebral levels, known as the medial approach.¹⁸ This approach is preferable due to identifiable anatomical landmarks and avoids injury to the neurovascular bundle that locates more laterally. When comparing medial and lateral approaches, it is clear that great vessels and nerves located laterally have a high possibility of being injured by

needle puncture. As a result, it can be more challenging to access LCo via the lateral approach. However, in the anterocollis-type clinical setting, the accessible area for the medial approach could be diminished due to the flexion deformity in CD patients. Considering this problem, localization of LCo at the lateral area of the neck should be explored as an option for approaching this deep cervical causative muscle.

The medial approach using the blind technique for BoTX injection in anterocollis-typed CD patients at C3 and C6 vertebral levels was reported to be the clinically acceptable safe zones in most current publications.¹ Some critical remarks also question the superiority of medial approach in BoTX injection over the lateral approach in anterocollis-typed CD patients. It has to be taken into account that positioning of the needle for medial and lateral approaches in blind technique is performed according to palpable cervical anatomical landmarks to determine the precise needle insertion location. Observation from reported case studies mentioned the injury to external carotid arteries and sympathetic trunk when the needle placement using medial approach at the C3 level was performed. In addition, an increase in injury to structures occupied within the carotid sheath and an internal hemorrhage in the thyroid gland due to the injury of superior and inferior thyroidal arteries are mentioned when the needle placement using the medial approach at the C6 level was performed.

Recent researches focused on identifying the best method to target desirable muscle in CD patients by comparing the accurate needle placement between the blind injection method based on detectable anatomical landmarks and the image-facilitated method.⁵ It appears that image-guided injections may produce the favourable treatment outcome but did not appear to improve overall better accuracy for the anatomical needle placement to target LCo. This method might prevent unintended injury due to the misplacement of BoTX needle insertion. Current investigations suggested an image-facilitated method, such as EMG, is more suitable for localizing the contracting muscles during injection.¹⁹ This method is suitable for injecting muscles differing in size, and the deep-localized muscle requires a different approach compared to superficial ones. It can be noticed that the advantages and preferable treatment outcome of image-guided BoTX injection were reported, in most studies, were analyzed and interpreted utilizing a small sample size. In addition, most recommendations for anterocollis-type CDs regarding the use of device-guided injection to enhance anatomic precision are basically established with these selected muscles which are superficially located,

such as splenius capitis or sternocleidomastoid. For these reasons, this aspect should be considered when conducting treatment in CD patients using this technique in the more difficult accessible, gracile, or deeply located muscles such as the LCo.

Complications associated with BoTX injection into this muscle depend on the following criteria: mode of injection (medial or lateral approach), anatomical level of injection, posture, and sex differences. There have been no substantial studies or reports attempting to establish the adequate depth of the needle insertion for a practical approach into LCo without complication or injury to the adjacent structures. When a conventional blind technique is employed, the thickness of LCo musculature at different vertebral levels needs to be considered to ensure the accurate position of the needle and the specific cervical vertebral level to achieve the total de-innervation of the LCo. For this reason, appreciation of the depth of insertion at the various cervical vertebral levels becomes crucial to the safe conduct of the procedure. Indeed, this method is usually recommended at C3 and C6 levels in parallel with an imaging-assisted modality, such as ultrasonography or computerized tomography, to facilitate and enhance precision. However, research regarding needle placement for both medial and lateral approaches rather than those cervical levels has not been conducted. Variation of cervical and pre-vertebral anatomical contour, the difference in cervical soft tissue component, head and neck posture, cervical neurovascular variations, neck muscular dystrophy, and sex differences may also impact the risk of injury.²⁰⁻²⁶ Consideration should be given to sex differences in cervical anatomical structures and their effect on the treatment outcomes. Current literature has reported sex dimorphism and cervical muscle differences, both shape and size. Males tend to have larger cervical musculatures than females. It can suggest a close relationship between sexual dimorphism and the risk of injury to vital cervical organs.²⁷

This present study will concentrate on the accuracy of needle placement used to target the LCo by determining the safe anatomical landmark for BoTX injection using the conventional blind technique in Thiel-embalmed human cadavers. Medial and lateral approaches were used, from two different neck positions, flexion and supine, at the two different cervical vertebral levels, C5 and C6. We plan to propose the potential injection site associated with the topographic anatomy of the neck and related structures to reduce the risk of the Botulinum toxin injection into LCo. In addition, the effects of sex differences on the risk of adjacent vital structure injury will be examined.

MATERIALS AND METHODS

Experimental model and subject details

This study was conducted on 20 Thiel-embalmed human cadavers (male, n=10 and female, n=10). The study samples were cadavers used for educational purposes in the Department of Anatomy, Faculty of Medicine Siriraj Hospital, and approved by the Institutional Review Board from this faculty (Si 621/2020). All the cadavers have Thai origins, ranging from 60 to 70 years of age. The sex, cause of death and age at death were recorded. The cadavers were used by postgraduate surgical trainees for practical surgical sessions from June 2020 to February 2021. The structures of the neck region are intact and were not damaged by previous surgical practices.

Method details and rationale

This study used two approaches to define the most secure way for BoTX injection (Fig 1). First, the EMG needle (size 25 gauge, 25 mm.) was medially applied to the carotid sheath for the medial approach on the right side of the neck, passed through the thyroid gland, and then reached the LCo. Before performing the needle's puncture at the beginning of this study, each cadaver was set in the supine position. The anterior neck region was dissected to determine the needle placement in LCo (Fig 1). For the lateral approach, the needle was applied at the posterior border of the sternocleidomastoid muscle, traversed through the anterior scalene muscle and then reached LCo. In addition to this, we performed these two methods on different vertebral levels. We used the

superior border of thyroid cartilage as a landmark for C5 (Fig 2) and the inferior border of cricoid cartilage for C6 vertebral level (Fig 3). The cadaveric dissections were performed after the needle was placed and fixed using the spinal needle fixator. Then, each cadaver was set in a neck flexion position using the cadaveric fixator. Every step was performed on the left side of the neck resembling the protocol that had been previously performed on the right side of the neck to determine the effect of neck posture. Images were taken from the sample for a specific region of interest.

Subsequently, distances between the puncture sites and adjacent structures were measured to define the safest injection method for the longus colli (LCo) muscle. A sliding vernier caliper was used to measure distances on each side to determine the potential anatomical landmark for BoTX injection into LCo for the medial and lateral approaches at the two different cervical vertebral levels of the two positions (Fig 1). Six parameters were measured for the medial approach, including the distance from the skin to the anterior surface of LCo and the distances between the puncture sites and carotid sheath, trachea, superior thyroid artery, inferior thyroid artery, and recurrent laryngeal nerve. Three parameters were measured for the lateral approach, including the distance from the skin to the lateral border of LCo, the distances between the puncture sites and carotid sheath, and the distances between the puncture sites and phrenic nerve. Anatomical landmarks and measurements were described as shown in Table 1.

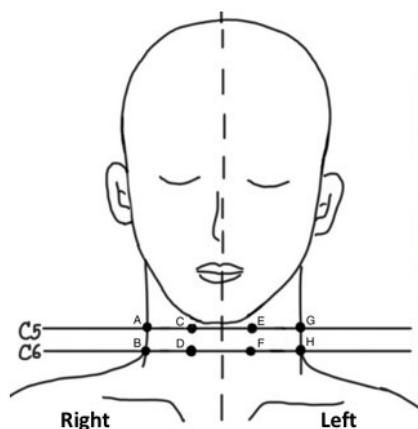


Figure 1.1

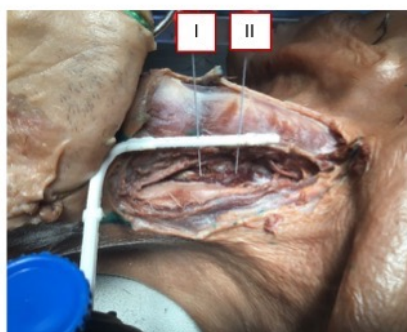


Figure 1.2



Figure 1.3

Fig 1. Anatomical landmarks represent puncture site in human cadaver. (1.1-Diagram illustrated landmarks of all approaches of botulinum toxin (BoTX) injection into longus colli (LCo) muscle in Thiel-embalmed embalmed cadavers (A- Lateral approach C5 supine position, B-Lateral approach C6 supine position, C-Medial approach C5 supine position, D-Medial approach C6 supine position, E-Medial approach C5 neck flexion position, F-Medial approach C6 neck flexion position, G- Lateral approach C5 neck flexion position, , and H-Lateral approach C6 neck flexion position, 1.2-Demonstrating medial approaches of BoTX injection into LCo in Thiel-embalmed cadaver in supine position (I-Medial approach, cervical vertebral level C5 and II-Medial approach, cervical vertebral level C6, and 1.3-Demonstrating lateral approaches of BoTX injection into LCo in Thiel-embalmed cadaver in supine position (I- lateral approach, cervical vertebral level C5 and II- lateral approach, cervical vertebral level C6).

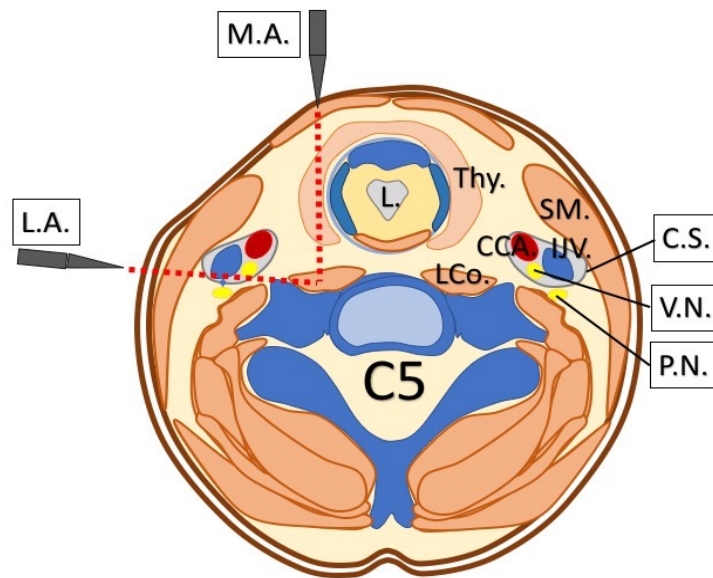


Fig 2. Schematic illustrated the topographic anatomy and related structures of the neck region at the C5 vertebral levels; red dot lines indicated the direction of the needle to target the longus colli muscle.

Abbreviations: M.A.; Medical approach, L.A.; Lateral approach, L.; Larynx, Thy.; Thyroid gland, SM.; Sternocleidomastoid muscle, C.S.; Carotid sheath, CCA.; Common carotid artery, IJV.; Internal jugular vein, V.N.; Vagus nerve, P.N. Phrenic nerve, C5; 5th cervical vertebral level

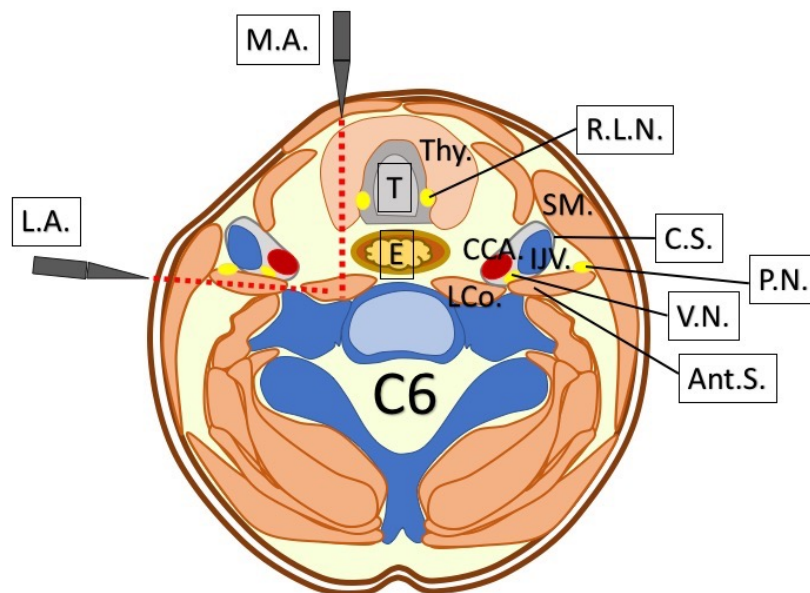


Fig 3. Schematic illustrated the topographic anatomy and related structures of the neck region at the C6 vertebral levels; red dot lines indicated the direction of the needle to target the longus colli muscle.

Abbreviations: M.A.; Medical approach, L.A.; Lateral approach, T.; Trachea, E.; Esophagus, Thy.; Thyroid gland, SM.; Sternocleidomastoid muscle, LCo.; Longus colli muscle, Ant.S.; Anterior scalene muscle, C.S.; Carotid sheath, CCA.; Common carotid artery, IJV.; Internal jugular vein, V.N.; Vagus nerve, P.N. Phrenic nerve, R.L.N.; Recurrent laryngeal nerve, C6; 6th cervical vertebral level

Statistical analyses

The mean and the standard deviation were calculated using the IBM SPSS version 27.0 for each measurement. A two-sample t-test was used to compare the outcomes between medial and lateral approaches in different vertebral levels, positions, and sexes. Comparison using one-way ANOVA was used to determine statistical differences

between each dataset with normal distribution. The median and the standard deviation were calculated for datasets with non-normal distribution. The paired stepwise comparison analysis was used to examine all the measured parameters to determine statistical differences between each subgroup. Such analysis included all variables that were systematically added and removed from the list of

TABLE 1. Description of anatomical landmarks and all measurements (N.A. = Not applicable).

Anatomical structures	Measurement (Distances from needle to)	
	Medial approach (M.A.)	Lateral approach (L.A.)
Carotid sheath (C.S.)	Medial border	Posterior border
Superior thyroid artery (Sup.Thy.A.)	Inferior border	Inferior border
Inferior thyroid artery (Inf.Thy.A.)	Superior border	Superior border
Recurrent laryngeal nerve (R.L.N.)	Lateral border	N.A.
Phrenic nerve (P.N.)	N.A.	Anterior border
Trachea (T.)	Lateral border	N.A.

measurement. The first variable was removed from the analysis after the initial comparison between the first and second variables from the list was completed. The remaining parameters were selected and subsequently compared. The Kruskal-Wallis test and post-hoc analysis were used to determine statistical differences between each dataset. A p -value of <0.05 was considered statistically significant.

RESULTS

The summary of anatomical landmarks and all measurement descriptions is presented in Table 1. Descriptive statistics of all measurements is presented in Table 2. Comparing the outcome between male and female human cadavers by paired student t -test showed no statistical differences between the two sexes in all adjacent anatomical structures to the injection site for both medial and lateral approaches (Table 3).

The distances between puncture sites and the carotid sheath and between medial and lateral approaches are presented in Fig 4. The median distances between the puncture sites and carotid sheath of all lateral approaches were 0.00 mm indicating the needle injury to carotid sheath. As shown in Fig 5.2, the lateral approach at C5 or C6 revealed no significant difference in the distances between the puncture sites and the carotid sheath in different subjects' positions. The paired comparison of distances between the puncture sites and the carotid sheath (Table 4) showed a statistically significant difference in all comparisons between medial and lateral approaches (except the comparison between C5-M-S and C5-L-F). Our findings suggest that the lateral approach may

cause injury to neurovascular structures adjacent to the sternocleidomastoid muscle, which clinicians should be aware of when performing BoTX injection with the lateral approach.

Comparisons of distances between the puncture sites and the vital structures among all medial approaches are presented in Fig 6, and comparisons of the distances between the puncture sites and the vital structure among all lateral approaches are presented in Fig 7. As shown in Figs 5.1 & 6.1, the medial approach at C5 or C6 revealed no significant difference in the distances between the puncture sites and the carotid sheath or trachea in different subjects' positions, respectively. However, we found that the distances between the puncture sites and the thyroid arteries were significantly greater in the neck-flexed position compared to the supine position (Figs 6.2 & 6.3). This also applies to the distances between puncture sites and the recurrent laryngeal nerve (Fig 6.4). Our findings suggest that the subjects' positions may affect the accuracy of the surface landmark on those neurovascular structures adjacent to the thyroid gland, which clinicians should be aware of when performing BoTX injection with the medial approach.

Paired stepwise comparison in Table 5 shows statistically significant differences in the distances between the puncture sites and the superior thyroid artery between C5 and C6 vertebral levels. These findings indicate that the needle placement at the C5 level using a medial approach tends to injure the superior thyroid artery without effect from the neck position.

There were statistically significant differences in distances between the puncture sites and the inferior

TABLE 2. The summary of descriptive statistics of all measurements, n = 20 (C5 = C5 vertebral level, C6 = C6 vertebral level, N.A. = Not applicable, S.D. = Standard deviation).

Parameters	Supine position								Neck flexion							
	Medial approach				Lateral approach				Medial approach				Lateral approach			
	C5		C6		C5		C6		C5		C6		C5		C6	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Carotid sheath	3.84	2.06	4.64	2.07	1.56	2.23	0.73	1.16	4.82	2.82	5.01	2.13	1.44	1.94	0.73	1.36
Trachea	6.14	2.97	5.43	3.30	N.A.	N.A.	N.A.	N.A.	7.60	2.77	6.61	2.18	N.A.	N.A.	N.A.	N.A.
Superior thyroid a.	4.22	7.09	11.77	5.57	N.A.	N.A.	N.A.	N.A.	3.15	3.15	10.06	5.27	N.A.	N.A.	N.A.	N.A.
Inferior thyroid a.	21.28	7.25	12.19	5.86	N.A.	N.A.	N.A.	N.A.	13.15	6.79	8.77	12.14	N.A.	N.A.	N.A.	N.A.
Recurrent laryngeal n.	7.41	2.63	8.93	11.42	N.A.	N.A.	N.A.	N.A.	6.57	2.31	5.20	1.91	N.A.	N.A.	N.A.	N.A.
Phrenic n.	N.A.	N.A.	N.A.	N.A.	1.26	1.49	1.67	1.92	N.A.	N.A.	N.A.	N.A.	2.37	1.71	1.94	2.02

TABLE 3. Comparisons of the outcome between male (n = 10) and female (n = 10) human cadavers (C5 = C5 vertebral level, C6 = C6 vertebral level, n. = nerve, a. = artery, N.A. = Not applicable, S.D. = Standard deviation).

Parameters	Male (n=10)								Female (n=10)							
	Supine position								Supine position							
	Medial approach				Lateral approach				Medial approach				Lateral approach			
	C5 (C)		C6 (D)		C5 (A)		C6 (B)		C5 (C)		C6 (D)		C5 (A)		C6 (B)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Carotid sheath	3.45	2.44	4.31	2.40	1.14	1.81	0.98	1.45	4.23	1.62	4.97	1.75	1.97	2.62	0.47	0.77
Trachea	6.65	3.41	5.40	3.31	N.A.	N.A.	N.A.	N.A.	5.62	2.54	5.46	3.48	N.A.	N.A.	N.A.	N.A.
Superior thyroid a.	3.15	3.43	10.57	4.64	N.A.	N.A.	N.A.	N.A.	5.29	9.58	12.97	6.39	N.A.	N.A.	N.A.	N.A.
Inferior thyroid a.	22.19	7.90	12.37	7.09	N.A.	N.A.	N.A.	N.A.	20.37	6.83	12.00	4.72	N.A.	N.A.	N.A.	N.A.
Recurrent laryngeal n.	8.38	2.94	7.24	3.03	N.A.	N.A.	N.A.	N.A.	6.43	1.95	10.62	16.12	N.A.	N.A.	N.A.	N.A.
Phrenic n.	N.A.	N.A.	N.A.	N.A.	0.94	1.67	1.20	1.81	N.A.	N.A.	N.A.	N.A.	1.58	1.30	2.14	2.00

Parameters	Neck flexion								Neck flexion							
	Medial approach				Lateral approach				Medial approach				Lateral approach			
	C5 (E)		C6 (F)		C5 (G)		C6 (H)		C5 (E)		C6 (F)		C5 (G)		C6 (H)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Carotid sheath	4.53	3.16	4.89	2.22	1.99	2.18	1.07	1.48	5.10	2.57	5.13	2.14	0.89	1.59	0.38	1.20
Trachea	6.95	2.58	6.33	2.04	N.A.	N.A.	N.A.	N.A.	8.24	2.94	6.89	2.39	N.A.	N.A.	N.A.	N.A.
Superior thyroid a.	3.60	3.47	11.81	6.45	N.A.	N.A.	N.A.	N.A.	2.70	2.92	8.30	3.18	N.A.	N.A.	N.A.	N.A.
Inferior thyroid a.	11.05	6.47	10.13	16.68	N.A.	N.A.	N.A.	N.A.	15.24	6.76	7.40	5.38	N.A.	N.A.	N.A.	N.A.
Recurrent laryngeal n.	6.57	2.15	5.15	2.05	N.A.	N.A.	N.A.	N.A.	6.56	2.58	5.25	0.59	N.A.	N.A.	N.A.	N.A.
Phrenic n.	N.A.	N.A.	N.A.	N.A.	1.92	1.73	1.44	1.29	N.A.	N.A.	N.A.	N.A.	2.82	1.66	2.44	2.53

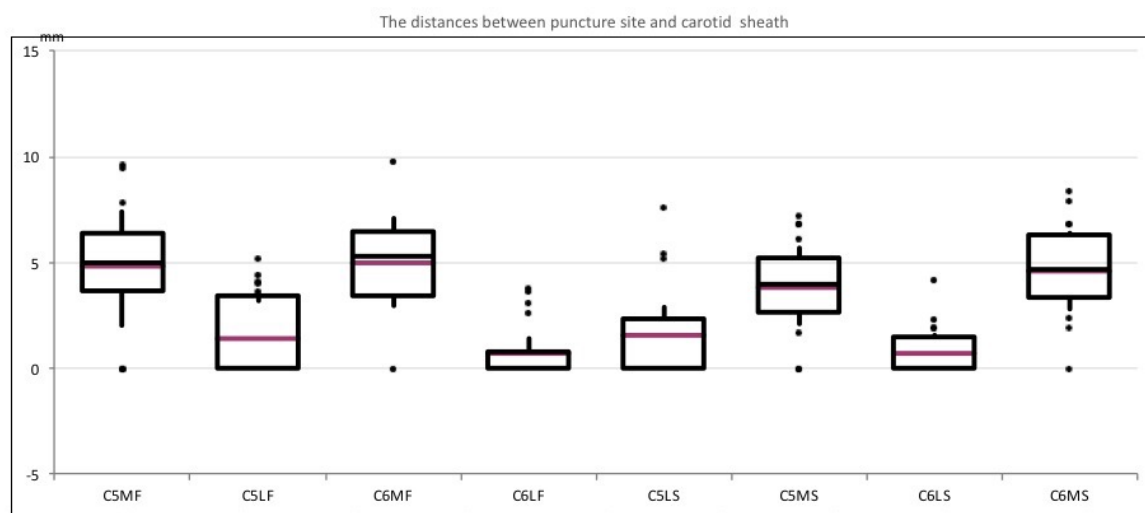


Fig 4. Distances between puncture site and carotid sheath of medial and lateral approaches in different vertebral levels and positions represented as boxplots. Each boxplot summarized all measurements taken per one anatomical landmark as shown in Fig 1.1, $n = 20$. (Medial approach C5 neck flexion position = C5-M-F, Lateral approach C5 neck flexion position = C5-L-F, Medial approach C6 neck flexion position = C6-M-F, Lateral approach C6 neck flexion position = C6-L-F, Lateral approach C5 supine position = C5-L-S, Medial approach C5 supine position = C5-M-S, Lateral approach C6 supine position = C6-L-S, and Medial approach C6 supine position = C6-M-S). Important distribution information, such as means (solid red lines within box), median (solid black lines within box), interquartile ranges (boxes), and outliers (solid black dots) are presented. Error bars represents means \pm SD.

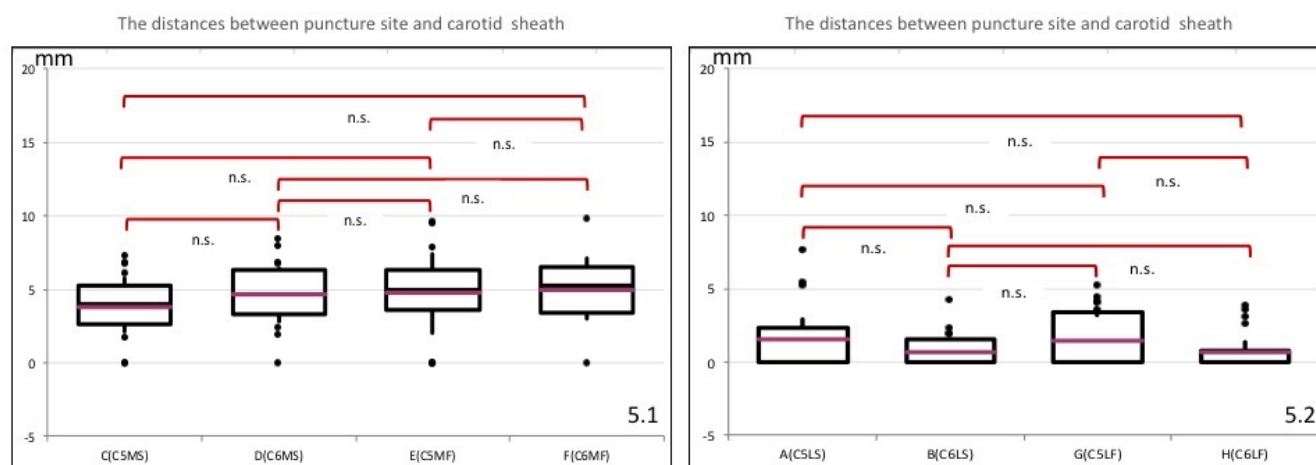


Fig 5. Distances between puncture site between puncture site and carotid sheath from the medial approach (5.1) and from the lateral approach (5.2) between two different vertebral levels and positions represented as boxplots. Each boxplot summarized all measurements taken per one anatomical landmark referred Fig 1.1, $n = 20$. Important distribution information, such as means (solid red lines within box), median (solid black lines within box), interquartile ranges (boxes), and outliers (solid black dots) are presented. Error bars represents means \pm SD. Red bars represented comparison between subgroups. The n.s. above boxplots under significant bar mark measurements showed no statistical significances, according to one-way ANOVA tests performed at a 0.05 significance levels. (n.s. = no statistical significance; Medial approach C5 supine position = C5-M-S, Medial approach C6 supine position = C6-M-S, Medial approach C5 neck flexion position = C5-M-F, Medial approach C6 neck flexion position = C6-M-F, Lateral approach C5 supine position = C5-L-S, Lateral approach C6 supine position = C6-L-S, Lateral approach C5 neck flexion position = C5-L-F, and Lateral approach C6 neck flexion position = C6-L-F).

TABLE 4. Summary of stepwise comparison of distances between the puncture sites and the carotid sheath according to one-way ANOVA tests performed at a 0.05 significance levels. (Medial approach C5 supine position = C5-M-S, Medial approach C6 supine position = C6-M-S, Medial approach C5 neck flexion position = C5-M-F, Medial approach C6 neck flexion position = C6-M-F, Lateral approach C5 supine position = C5-L-S, Lateral approach C6 supine position = C6-L-S, Lateral approach C5 neck flexion position = C5-L-F, and Lateral approach C6 neck flexion position = C6-L-F).

Paired stepwise comparison		P-value	Paired stepwise comparison		P-value
C5-M-S (C)	vs C6-M-S (D)	0.98	C5-M-F (E)	vs C6-M-F (F)	1
	vs C5-M-F (E)	0.94		vs C5-L-S (A)	< 0.01
	vs C6-M-F (F)	0.85		vs C6-L-S (B)	< 0.001
	vs C5-L-S (A)	0.09		vs C5-L-F (G)	< 0.001
	vs C6-L-S (B)	< 0.01	C6-M-F (F)	vs C6-L-F (H)	< 0.001
	vs C5-L-F (G)	0.058		vs C5-L-S (A)	< 0.001
	vs C6-L-F (H)	< 0.01		vs C6-L-S (B)	< 0.001
C6-M-S (D)	vs C5-M-F (E)	1	C5-L-S (A)	vs C5-L-F (G)	< 0.001
	vs C6-M-F (F)	0.99		vs C6-L-F (H)	< 0.001
	vs C5-L-S (A)	< 0.01		vs C6-L-S (B)	0.97
	vs C6-L-S (B)	< 0.001	C6-L-S (B)	vs C5-L-F (G)	1
	vs C5-L-F (G)	< 0.01		vs C6-L-F (H)	0.97
	vs C6-L-F (H)	< 0.001		vs C5-L-F (G)	0.98
C5-L-F (G)	vs C6-L-F (H)	0.98		vs C6-L-F (H)	1

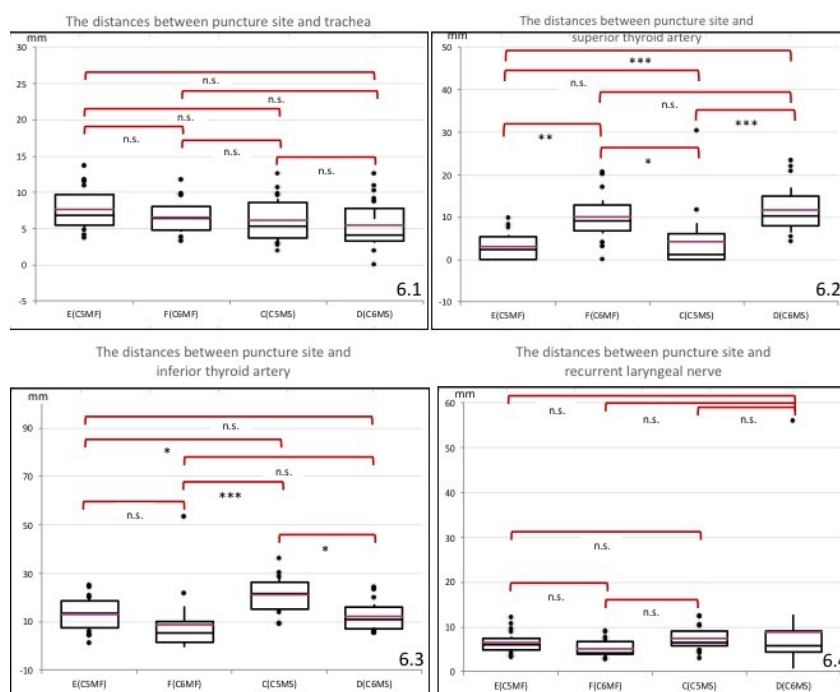


Fig 6. Distances between puncture site between puncture site and trachea (6.1), superior thyroid a. (6.2), inferior thyroid a. (6.3), and recurrent laryngeal nerve (6.4) from the medial approach between two different vertebral levels and positions represented as boxplots. Each boxplot summarized all measurements taken per one anatomical landmark as shown in Fig 1.1, n = 20. Important distribution information, such as means (solid red lines within box), median (solid black lines within box), interquartile ranges (boxes), and outliers (solid black dots) are presented. Error bars represents means \pm SD. Red bars represented comparison between subgroups. Asterisks above boxplots under significant bar mark measurements show statistical significances, according to one-way ANOVA tests performed at a 0.05 significance levels. (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and n.s. = no statistical significance; Medial approach C5 supine position = C5-M-S, Medial approach C6 supine position = C6-M-S, Medial approach C5 neck flexion position = C5-M-F, and Medial approach C6 neck flexion position = C6-M-F).

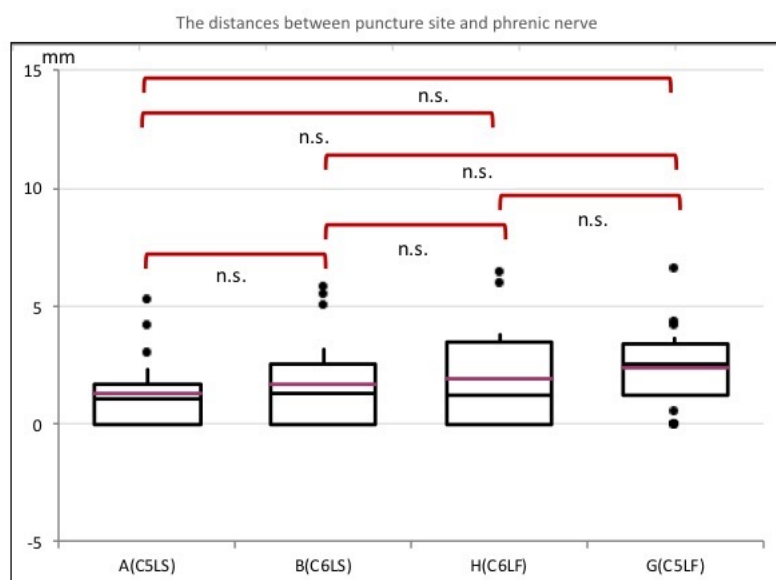


Fig 7. Distances between puncture site and phrenic nerve from the lateral approach between two different vertebral levels and positions represented as boxplots. Each boxplot summarized all measurements taken per one anatomical landmark as shown in Fig 1.1, $n = 20$. Important distribution information, such as means (solid red lines within box), median (solid black lines within box), interquartile ranges (boxes), and outliers (solid black dots) are presented. Error bars represents means \pm SD. Red bars represented comparison between subgroups. The n.s. above boxplots under significant bar mark measurements showed no statistical significances, according to Kruskal-Wallis tests performed at a 0.05 significance levels. (n.s. = no statistical significance; Lateral approach C5 supine position = C5-L-S, Lateral approach C6 supine position = C6-L-S, Lateral approach C5 neck flexion position = C5-L-F, and Lateral approach C6 neck flexion position = C6-L-F).

TABLE 5. Summary of stepwise comparison of the trachea, superior thyroid artery, inferior thyroid artery and recurrent laryngeal nerve according to one-way ANOVA tests performed at a 0.05 significance levels. (Medial approach C5 supine position = C5-M-S, Medial approach C6 supine position = C6-M-S, Medial approach C5 neck flexion position = C5-M-F, and Medial approach C6 neck flexion position = C6-M-F).

Parameter	Paired stepwise comparison	P-value	Parameter	Paired stepwise comparison	P-value
Trachea	C5-M-S (C) vs C6-M-S (D)	0.89	Superior thyroid a.	C5-M-S (C) vs C6-M-S (D)	< 0.001
	vs C5-M-F (E)	0.45		vs C5-M-F (E)	0.94
	vs C6-M-F (F)	0.96		vs C6-M-F (F)	< 0.05
	C6-M-S (D) vs C5-M-F (E)	0.13		C6-M-S (D) vs C5-M-F (E)	< 0.001
	vs C6-M-F (F)	0.63		vs C6-M-F (F)	0.8
	C5-M-F (E) vs C6-M-F (F)	0.75		C5-M-F (E) vs C6-M-F (F)	< 0.01
Inferior thyroid a.	C5-M-S (C) vs C6-M-S (D)	< 0.05	Recurrent laryngeal n.	C5-M-S (C) vs C6-M-S (D)	0.89
	vs C5-M-F (E)	< 0.05		vs C5-M-F (E)	0.98
	vs C6-M-F (F)	< 0.001		vs C6-M-F (F)	0.72
	C6-M-S (D) vs C5-M-F (E)	0.98		C6-M-S (D) vs C5-M-F (E)	0.67
	vs C6-M-F (F)	0.65		vs C6-M-F (F)	0.29
	C5-M-F (E) vs C6-M-F (F)	0.44		C5-M-F (E) vs C6-M-F (F)	0.91

thyroid artery in medial approaches (except the comparison between C6-M-S vs C5-M-F, C6-M-S vs C6-M-F, and C5-M-F vs C6-M-F) from paired stepwise comparison in Table 5. There was no statistically significant difference in the distances between the puncture sites and recurrent laryngeal nerve (Table 5). Unlike the medial approach at the C5 level, these findings indicate that the needle placement at the C6 level using the medial approach tends to injure the inferior thyroid artery without any effect from the neck position. It means that the needle placement using a medial approach either at C5 or C6 level can cause an injury to the recurrent laryngeal nerve. Neck position may also alter the risk of injury to this structure.

We found that the distances between the puncture sites and the thyroid arteries were significantly greater in the neck-flexed position compared to the supine position (Figs 6.2 & 6.3). Our findings suggest that the subjects' positions may impact injury to thyroidal vessels, which clinicians should be aware of when performing BoTX injection with the neck flexion deformity CD patients.

There were no differences of the median between distances from the puncture sites and carotid sheath using the lateral approach (C5-L-S, C6-L-S, C5-L-F and C6-L-F) were 0.00 mm, as shown in Fig 4). These findings indicate that the needle placement using a lateral approach at either cervical level can cause an accidental injury to the carotid sheath. This was also the pattern showed when comparing the medians of the distances between the puncture sites and the phrenic nerve between two cervical vertebral levels in both neck positions. There was no statistically significant difference in the distances between the puncture sites and the vital structures among all lateral approaches (Figs 5.2 & 7). Our findings suggest that the subjects' positions may not alter the risk of injury to the carotid sheath and phrenic nerve, which clinicians should consider when performing BoTX injection using the lateral approach in CD patients.

DISCUSSION

This study provides anatomical references to the longus colli (LCo) muscle. To reduce complications, it proposes the potential alternative technique for BoTX injection to LCo in terms of an anatomical-based approach using human cadavers compared with the current technique. Results from different injection approaches with various parameters are compared at the fifth and sixth cervical vertebral levels.²⁸

Although most current publications recommend that the injection should be performed at the C3 and C6

vertebrae levels, we chose C5 and C6 vertebral levels for this present study.^{1,6} The objective in selecting these two different vertebral levels is to avoid the bifurcation of the common carotid artery and reduce the possibility of accidental injury to the vertebral artery.⁵ These two landmarks also have the palpable bony landmark to facilitate the identification of the precise cervical vertebral level.^{29,30} The authors could not find any information about the recommendation regarding the specific site for complete chemical de-innervation of LCo at the C5 vertebral level. Concerning anatomic compartments of the LCo, this muscle may have distinct functions because it has three distinctive parts, representing the unique function of the neck biomechanical property. Oblique parts may act as the lateral neck flexor, while the inferior oblique part functions as the neck rotator. These functional aspects should be a consideration regarding the treatment outcome in patients with cervical dystonia (CD) and applying BoTX injections into this muscle.

In anterocollis-typed CD, the abnormal posture in anterior neck flexion can affect the anatomical relation. This study indicates that the different methods of BoTX injection into LCo in Thiel-embalmed cadavers led to different outcomes. Two percutaneous approaches were used to collect the data: medial and lateral. We examine the possible technique to target LCo to avoid damaging adjacent structures at C5 and C6 vertebral levels (Figs 2 & 3). Selection of the cadaveric position, supine and neck flexion, replicates the patient's clinical presentation and follows most research scenarios. By accessing the LCo from the medial approach, we are utilizing the bony landmark of the thyroid cartilage, which is well known for indirectly indicating the structures of the anterior cervical region. The thyroidal arteries are located anterolateral to the LCo at the C5 and C6 vertebral levels (Fig 8). They are at risk of being injured using this approach in neck flexion and supine positions. It should be considered that the possibility of superior thyroid artery injury is higher in the medial approach at the C5 vertebral level than at the C6 vertebral level (Fig 8.1). The inferior thyroid artery was more likely to be injured at a C6 vertebral level than a C5 vertebral level from the medial approach in the same neck position. Locating the cricoid cartilage at C6 and inserting the needle immediately next to the trachea can avoid an injury to the carotid artery within the carotid sheath. Awareness of the potential possibility of neurovascular complications is considered. However, our observation has not detected such injuries from the medial approach.

Previous studies described a medial or lateral approach relative to the carotid sheath when targeting

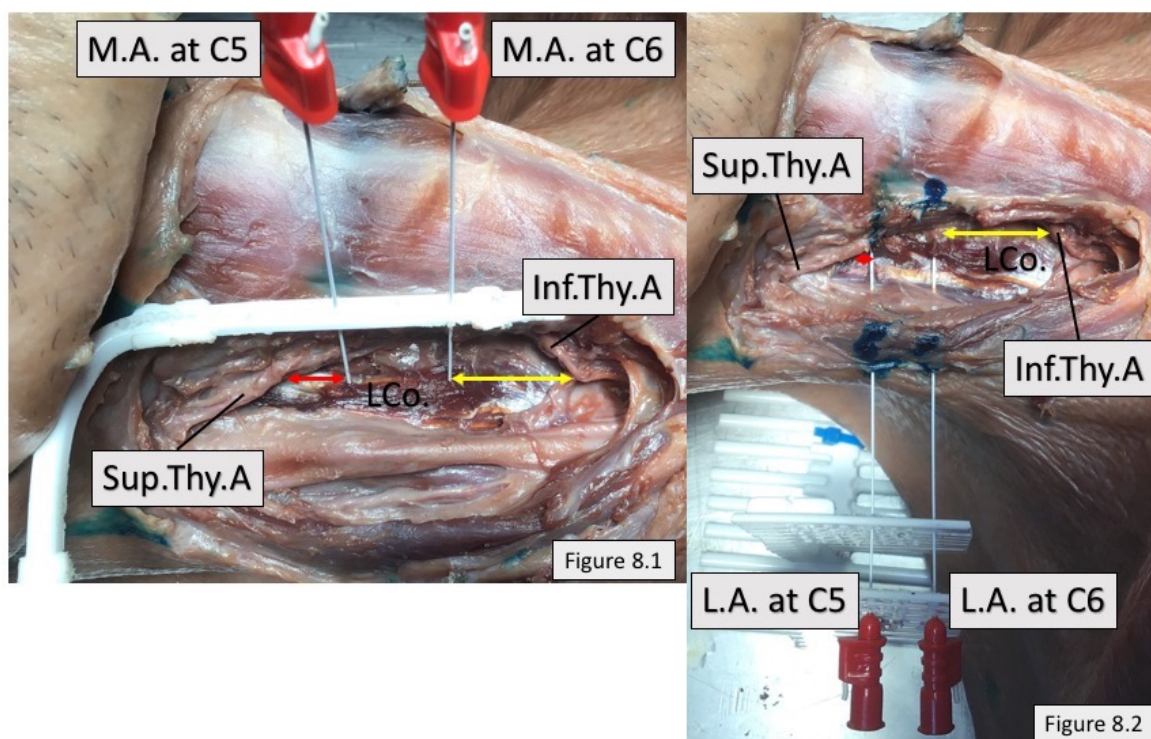


Fig 8. Measurements of distance between the needle placement into longus colli muscle in Thiel-embalmed cadavers and thyroidal arteries. (8.1-Medial approach and 8.2-Lateral approach; M.A.at C5 = Medial approach at C5, M.A. at C6 = Medial approach at C6, L.A.at C5 = Lateral approach at C5, L.A. at C6 = Lateral approach at C6, LCo. = Longus colli muscle, Sup.Thy.A. = Superior thyroid artery, Inf.Thy.A. = Inferior thyroid artery, Double-headed red arrow = Measurement from the needle to inferior border of superior thyroid artery, and double-headed yellow arrow = Measurement from the needle to superior border of inferior thyroid artery)

the LCo muscle using the position of the patient's neck as an anatomical reference. The disadvantage of the medial approach is that the needle is inserted into and penetrates the thyroid gland, with an increased risk of puncturing the thyroid arteries. The lateral approach has the disadvantage of the needle passing through a narrow space between the anterior tubercle and carotid sheath (Figs 2 & 3). As a result, the lateral approach is more likely to cause injury to the carotid sheath than the medial approach. In addition, puncture through the anterior scalene muscle is unavoidable and may increase the tendency of phrenic nerve injury. An additional limitation of both selected approaches at C5 and C6 levels is that they only allow injection of the most distal portion of LCo. This means that the cephalic part of this muscle may only be incompletely de-innervated.

Our proposed lateral approach at C5 and C6 can avoid penetrating injury to the thyroid gland and reduce the potential risk of accidental injury to thyroidal vessels. However, injury to the carotid sheath is inevitable with this approach at both cervical levels. We were performing needle insertion at the C5 and C6 levels; at either level, the needle trajectory provides access to the LCo (Fig 8.2). After performing cadaveric dissection, it was realized

that needle placement for BoTX injection using a lateral approach at C5 was close to the superior thyroid artery. The needle placement below the C6 level should not be done due to the position of LCo being deeply located in the upper thoracic region. This approach is more likely to cause injury to the carotid sheath than the medial one. This study successfully targeted the muscle with the blind technique for medial and lateral approaches in supine and neck flexion positions. During the cadaveric dissection study, direct measurements were made, providing the confirmation that this is indeed the LCo. Localization of LCo in the lateral approach was analyzed following the identification of the posterior border of the sternocleidomastoid muscle, which accounts for its usefulness in the actual clinical circumstance and may reduce the degree of uncertainty regarding our protocol.

Publications about the complications of BoTX injection into LCo are sparse regarding procedures using tools not designated for medial and lateral approaches at C5 and C6 vertebral levels. None of the previous studies reported differences in structural complications between the medial and lateral approach at C5 and C6 vertebral levels concerning sex dimorphism or neck posture, confirming the necessity of this present research. Various complications,

such as pain, hoarseness of voice or dysphagia, have been reported, without mentioning a recommendation of a safe position to avoid those complications. It can be noticed that the lateral puncture may contribute to more severe complications than the medial approach, and the advancement of the needle to approach LCo may be more traumatic for the neurovascular bundle than the currently recommended technique, the medial approach at C3 and C6 vertebral levels. In this study, we did not observe any significant laryngeal damage when performing cadaveric dissection following the needle insertion. However, on cadavers, hemorrhage or structural swelling cannot be observed, and therefore only significant noticeable damage such as accidental puncture or laceration could be observed and documented. Nevertheless, using the proposed technique of introducing a needle into the LCo through the skin at the C5 level, any complications should be similar to those of the recommended method, except the injury to the superior thyroid artery. Moreover, this technique maybe practised on a human cadaver to enhance these clinical skills for BoTX injection.

One of the main concerns regarding BoTX injection into the LCo is the recurrent laryngeal nerve injury, which may lead to laryngeal complications such as the weakening of the laryngeal muscles. Laryngeal injury has occasionally happened following the blind technique. However, due to the possibility of severe disability, risk using this technique should be determined. The medial approach at the C5 vertebral level has a higher chance of injuring the recurrent laryngeal nerve than the medial approach at the C6 vertebral level. However, this complication is not observed in the present study. Using our technique, the tip of the needle was placed above the C6, avoiding the recurrent laryngeal nerve. We also determine the injury to the phrenic nerve regarding lateral approach at two different levels. In the present study, injury of the phrenic nerve was not detected in two different cervical vertebral levels.

Inserting the needle at the lower part of the neck increases the likelihood of traversing the thyroid gland during the injection; in our study, we used C5 and C6 vertebral levels.¹⁷ However, previous research recommended the injection of BoTX at the C3 level. In patients with severe neck flexion deformity with a limited area to insert the needle, this recommended approach at the C3 vertebral level is inapplicable. We have not observed any complications related to the thyroidal vessels when attempting the medial approach for this neck posture at the C6 vertebral level. In contrast, attempting to insert the needle through the thyroid gland at the C5 level showed a high tendency to injure the superior thyroidal

artery (Figs 8.1 & 8.2). Publications have reported various degrees of anatomical variation of the superior thyroid artery, either the location where this structure originates from the external carotid artery or their branches and the relation between this vessel and thyroid cartilage.²⁵ Despite this study, the needle penetrated the thyroid gland in all cases and there was no association with vascular puncture. However, it should be noted that this present study, primarily at the C5 level, was conducted utilizing human cadavers. As a result, visible hemorrhage from these vessels and within the thyroid gland may be obscured.

Determination of sex differences and their effect on the variation of cervical anatomical landmarks for the BoTX injection should be considered in clinical practice.³¹ Current literature reports observe the anatomical variation of cervical structures between the two sexes.^{12,26,32-34} Although we examined all parameters from cadaveric samples, it was clinically relevant to discuss previous studies on the effects of musculoskeletal conditions and therapeutic interventions. The LCo acts as a local spine stabilizer and facilitates neck flexor and contralateral rotator. From a clinical perspective, the anatomical orientation, musculature and robusticity of this muscle, functional component and zonal innervation of LCo muscle should be further explored for its clinical relevance in individuals with CD. Based on the present results, the different injection techniques can alter the outcomes and risk of complications after interventions. Such complications may alter the functional compartment of this muscle and affect neck motion after BoTX injection. The present study also validated the sex difference concerning the location of the newly proposed landmark for BoTX injection and demonstrated no effects of sex differences on anatomical-related complications. Previous studies reported differences of subcutaneous tissue between the two sexes that may impact on the depth from the skin to approach the LCo. The present study was conducted on Thiel-embalmed cadavers where subcutaneous fat is not well preserved and usually dissolves over time. Despite the distances measured from the skin to the LCo, we decided to exclude these variables from statistical analyses due to the reason previously mentioned. For all of these reasons, the results being observed and determined in this study regarding the relationship between sex dimorphism and cervical structures might be inapplicable to the general human population. Future research should examine the optimization between subject-specific study samples in the present study and the actual clinical situation regarding sex dimorphism and cervical musculature in the CD patient.

Thiel-embalmed cadavers were used in this present study to preserve the biomechanics of the soft tissue and the joint mobility of the human body, unlike normal embalmed cadavers that have a limitation on tissue integrity and joint flexibility.^{35,36} Thiel's cadaveric-based experiments in terms of anatomical significance are recognizable for their life-like preservation. This cadaveric type provides favourable conditions for the image-facilitated procedure and excellent tissue preservation and is also found to help explore anatomical structures.³⁷ This embalming method provides the advantage of tissue plasticity and flexibility that cannot be achieved in formalin-embalmed cadavers.³⁸ In addition, such a method of embalming has been reported to reduce the potential risk of biosafety compared with studies conducted on a fresh frozen cadaver.³⁹ Furthermore, the Thiel-embalming process can lend itself to preliminary surgical skills training in a highly realistic and life-like setting.⁴⁰ Coupled with the challenge of reducing serious complications and undesirable consequences of BoTX injection, rehabilitation practitioners are confronted with a requirement to exercise and develop their skills. Thiel-embalmed cadavers meet these demands utilizing a highly anatomically accurate training model. Current studies highlighted the benefit of training courses, such as the training of prevertebral BoTX injection utilizing this type of human cadaver. This particular type of human cadaver provides life-like tissue quality, muscular consistency, and sensible perception of anatomical landmarks, which are ideally suitable for training courses similar to fresh frozen cadavers.

This proposed technique provides reliable, consistent access to a difficult-to-target muscle and is helpful for CD patients who experience treatment failure after a BoTX injection into superficial neck muscles has been administered.

This technique demonstrates some limitations and shows the clinicians who perform these techniques following our results should exercise caution. Further investigations are required. First, it was conducted in Thiel-embalmed cadavers; therefore, the anatomical distances could be different when performed in humans. Additional research should be conducted to establish the cervical anatomy in real clinical situations, especially further studies in humans using an imaging modality. It was challenging to conduct a study utilizing imaging modality in Thiel-embalmed cadavers because of the difficulty locating vital structures precisely. Second, recent research conducted in a small group of human cadavers on injections of deeply located muscles where the approach is challenging, the longus colli, has led to different methodologies being proposed. Although most

results obtained were statistically significant, the literature surrounding the treatment of CD is sparse, possibly because CD itself is relatively rare and the utilization of BoTX to treat this clinical condition remains controversial. As clinical trials were only established in the late 1990s, the fact that this study lacks randomized-controlled clinical trials may limit the dependability of the results. Third, the majority of the human cadavers in our department is mainly confined to the elderly aged group. As a result, the effect of age and the outcome utilizing our proposed technique has not been examined. Future research being conducted across the age range should be considered to determine this aspect. Last, there were no intra- and inter-observer technical measurement errors in this present study, limiting the generalizability and application of BoTX injection in patients with different characteristics.

Our approach provides some beneficial aspects compared to previously described approaches-first, the possibility of utilizing the thyroid cartilage as landmark to identify the LCo. Second, the neck position in supine and neck flexion impacts the risk of injury to the neurovascular bundle. Third, variation of the thyroidal vessel, especially the superior thyroidal artery and its branch, should be considered when inserting the needle using a medial approach at C5 and C6 vertebral levels. Last, the medial approach tends to injure the recurrent laryngeal nerve, while the lateral approach can cause injury to the phrenic nerve. For these reasons, the medial approach at the C6 vertebral level would be the potential safest landmark for BoTX injection to target the LCo muscle in the anterocollis-type CD patients.

CONCLUSION

Combining anatomical-based knowledge and clinical practices utilizing cadaver simulator training is the key to performing accurate botulinum toxin (BoTX) injections without direct visualization into the longus colli (LCo), especially when the standard guidelines remain unavailable. This study proposes an alternative approach for injecting the LCo. We conclude that the medial approach at the C6 vertebral level is more precise, safer and demonstrates a more beneficial outcome in surgical practices than the lateral approach. This study also emphasises the importance of the topographic anatomical dimension of the neck region using a cadaveric approach, allowing proper planning for practical skill development utilizing a human cadaver and preventing life-threatening injury to the neurovascular bundle.

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