# The Anatomy study of the Nasolabial fold region based on High-Frequency Ultrasound Investigation in Thai Subjects

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## Abstract

Objective: To study the variation of Nasolabial vessels in Nasolabial folds and the basic structure of the nasolabial area such as skin, subcutaneous, and SMAS by high-frequency ultrasound in Thai people. To enhanced safety in dermal filler injection and clinical practice. To identify the filler particles.

Methods: An observational cross-section study, a one-time examination by high-frequency ultrasound on the group who has received filler and never received filler to specify the depth and position of arteries and the structure of nasolabial folds.

Results: There were slight differences in the skin thickness, Subcutaneous layer, and SMAS in the filler injection group (n=8) and no filler group (n=25), the skin thickness of the non-filler group is slightly thicker than filler group, however the age, weight and the duration of the filler injection may affect the results. Moreover, there was a variation in the artery position in points A, B, and C of NLF in the same Thai population (n=33).

Conclusion: High-frequency ultrasound can visualize the basic structures of the Nasolabial folds region and can identify the position and the depth of the Facial artery in the NLF region.

**Keywords**: Nasolabial Fold, High-Frequency Ultrasound, Facial Artery, Piriform Fossa



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## Introduction

Facial aging is an intricate process involving interrelated changes to bone, muscle, fat, and skin (Swift et al., 2020). It is typified by skin tone and texture deterioration, deflation due to loss of bone and fat, descent of soft tissues due to loss of muscle tone and skin elasticity, and disproportion occurring in different facial areas at different rates and chronological times. These changes can lead to deleterious emotional, psychological, and social effects because they alter self-perception and may affect the interpersonal relations of an individual (Swift et al., 2020).

One of the regions on an aging face that people notice is the nasolabial folds. The exact causes of this include alterations in the bone of the orbital rim, bone reabsorption at the canine fossa, weakened malar and orbital ligaments, the descent of the subcutaneous malar fat pad of the cheeks with hypertrophy of the nasolabial fat compartments leading to a loss of midface volume and a deepening of the nasolabial folds (Swift et al., 2020).

Soft-tissue filler is the most popular procedure for re-volumization mid-face and nasolabial correction. However, the nasolabial area is also known as one of the hazard zones for fillers or any other surgery due to the facial artery and its anastomosis. This procedure has several drawbacks and consequences, including edema, erythema, lump formation, filler materials migration (Stefura et al., 2021), etc. Having thorough anatomic knowledge does not shield the expert from the possibility of creating an undesirable outcome. It is quite hazardous and may lead to blindness caused by skin necrosis and vascular occlusion. Understanding the precise depth and position of the artery within this area is crucial for performing safe and effective procedures, including plastic and reconstructive surgeries, and aesthetic procedures like dermal filler injections. This region's unique anatomy and vascularity make it a focus of interest for clinical and research purposes.

Ultrasound imaging technology has made significant advancements in recent years, enabling detailed visualization of anatomical structures in real time. High-frequency ultrasound, in particular, offers the potential to accurately determine the depth and position of arteries in the nasolabial region. This has immense clinical significance, as it can guide the administration of injectables such as botulinum toxin injection in masseter muscles, pre- and post-procedure of filler injection (Lee et al., 2020), and used to treat vascular complications, aid in surgical planning, and enhance the overall safety and efficacy of procedures.

While these studies provide valuable insights, there is a need for a comprehensive investigation focusing on the variable of the artery and the nasolabial structures within the nasolabial region. This research aims to fill this gap by utilizing advanced ultrasound imaging techniques to provide a detailed understanding of the artery's position, depth, and basic nasolabial fold structure variations in Thai population groups. The findings of this study can serve as a foundation for enhancing patient safety and optimizing aesthetic and dermatologic procedures in the nasolabial region. Furthermore, the outcomes of this research could contribute to learning new facts and educational resources for medical practitioners, making ultrasound a valuable tool for practice in the nasolabial area.

## Materials and Methods

### Subject for the study

The study was approved by the ethics committee of Mae Fah Luang University (Approval No. EC 232773-20). This is an observational cross-section study, a one-time examination by high-frequency ultrasound including 33 patients, a total of 66 nasolabial folds. The data collection was conducted after receiving the ethics approval certificate. Two doctors performed all the ultrasound evaluations, and the study duration is 4 weeks in March 2024. The inclusion criteria were as follows: (1) Healthy individuals; (2) Both male and female; (3)Age between 25-50 years old; (4) Volunteers who never received filler injection and have received filler before on nasolabial fold; (5)Volunteer with no other treatments like fat grafting; (6) Volunteers are required to sign consent documents outlining the benefits, risks, and publication of photographs; (7) Volunteers are required to be able to adhere to the study's instructions. The exclusion criteria were as follows: (1) History of facial operation; (2) Known Facial Vascular Malformation; (3) History of Thread Lifting; (4) Underlying diseases such as psychiatric diseases, HIV, and autoimmune diseases; (5) Pregnancy or lactation during a period of study; (6) Individuals had undergone previous trauma in the nasolabial region; (7) Individual who is allergic to ultrasound conducting gel. Outcome measurements included the depth and position of arteries, and the thickness of nasolabial fold structures such as skin, subcutaneous, the SMAS, and Bone.

### Ultrasound scanning

A high-frequency ultrasound device (Venue GE Healthcare, USA) with Power Doppler mode to visualize the facial artery. The hockey stick transducer L8-18i-Rs, footprint 11.1 × 34.8 mm, 2.5-16.8 MHz is placed along the Nasolabial line by focusing on three points, point A is at the lateral of the nose, point B the halfway between points A and C, point C is located 1-2 cm lateral to oral commissure (Figure 1). Then we used the Hockey-stick probe to scan the real-time image of the vessels of each volunteer on both sides of NLF and measure the basic nasolabial fold structure such as skin, subcutaneous, and SMAS layer in the group who never received filler and who have received filler.


**Figure 1:** Nasolabial folds created points A, B, and C both the right and left, hockey stick probe

Images of common facial tissue types, including the fascia, muscle, bone, and the dermis and subdermal layers. The blood vessels are anechoic and circular to oval in shape, fibrous tissue is hyperechoic streaks. The dermis is a small hypoechoic band. The muscle displays hypoechoic features because bone and fascia are dense, hyperechoic structures (Velthuis et al., 2021).

## Results

### Characteristics of the patients

Sixty-six nasolabial folds of 33 patients (7 men and 26 women, mean age, 33.79 years; range, 25-46 years) were evaluated. The volunteers who have received filler injections before were 8 and no filler was 25. None of them were taking any medication. The nasolabial severity score on both sides was mostly at level 3 (49.0%) which is moderate of the folds, followed by level 2 (36.0%) and level 4 or above (9.0%), respectively (Table 1).

**Table 1:** General characteristics (n=33)

| Characteristics | n (%) |
|---|---|
| Gender | |
| Male | 7 (21.0) |
| Female | 26 (79.0) |
| Age (years), mean±SD (min - max) | 33.79±6.46 (25 - 46) |
| History of filler | |
| Yes | 8 (24.0) |
| No | 25 (76.0) |
| Nasolabial severity score of both side | |
| 1 | 2 (6.0) |
| 2 | 12 (36.0) |
| 3 | 16 (49.0) |
| 4 | 2 (6.0) |
| 5 | 1 (3.0) |

Among 8 people who have received filler injections before, we found 6 people have remaining filler particles (Figures 2 and 3). However, the injection duration and the types of filler may play an important role in the tissue.


**Figure 2:** anechoic mass on the right nasolabial fold (point A), 30-year-old male volunteer after 3 days of HA filler injection


**Figure 3:** HA filler particles on the left side NLF, measured by Wide-band linear array probe on a 43-year-old female volunteer after a1-year filler injection



The Parameter measurement

(1) The thickness of each nasolabial layer (d1-d4) at point A left and right. At points B and C, the thickness of each layer (d1-d3) left, and right were measured.

(2) The position and depth of the facial artery at each point were measured.

The layer arrangement of the fascial planes of the face: skin (which has the epidermis and dermis), subcutaneous fat, musculoaponeurotic, comprised of the SMAS and facial muscles, loose areolar tissue or deep fat, periosteum and deep fascia (Whitney, 2024). The anatomical layer of the right-side nasolabial fold by high-frequency ultrasound of each point is shown in Figure 4.6.

- The measurement of the thickness of the Nasolabial folds is as follows (Figure 4):

- Skin thickness: from skin surface to subdermal

- Subcutaneous thickness: from subdermal to the subcutaneous

- The SMAS layer: from the skin surface to the SMAS layer
- The Bone: from the skin surface to the bone.

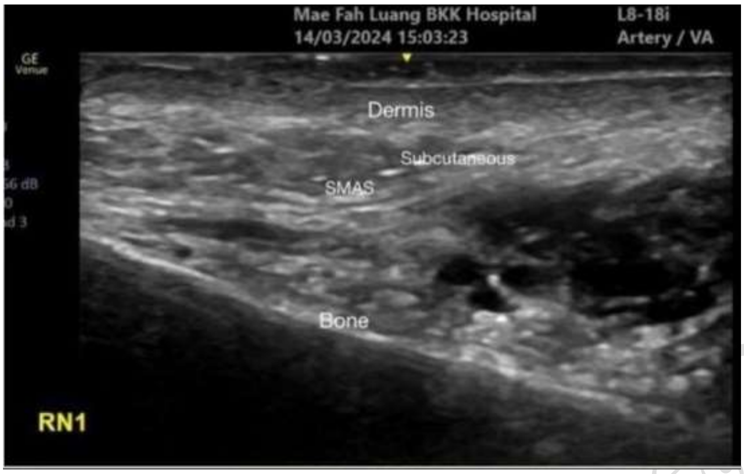


**Figure 4:** Anatomical layer of the right-side nasolabial fold by high-frequency ultrasound of each point A (RN1)

**Soft Tissue Thickness**

**Soft tissue thickness Point A:**

The thickness of each layer at point A of no filler inject (n=25) was as follows: skin 1.82±0.40 mm and 1.95±0.32 mm, Subcutaneous layer 3.08±0.84 mm and 2.93±0.79 mm, SMAS layer 4.98±1.07 mm and 4.84±0.98 mm. The thickness from the skin to bone was 9.72±1.87 mm and 10.56±1.99 mm, right and left respectively (Table 2).

The thickness of each layer in the filler group (n=8) was as follows: the skin 1.45±0.43 mm and 1.66±0.45 mm, the subcutaneous layer 2.84±0.61 mm and 3.24±0.39 mm, SMAS layer 4.48±1.11 mm and 4.85±0.56 mm, thickness from skin to bone 9.8±2.39 mm and 11.76±1.89 mm, right and left respectively (Table 2).

**Table 2:** Soft tissue thickness at point A (Lateral of the nose)

| Soft tissue thickness (mm.) | Right | | Left | |
|---|---|---|---|---|
| | mean±SD | min - max | mean±SD | min - max |
| **No filler group (n=25)** | | | | |
| Thickness of skin | 1.82±0.40 | 1–2.6 | 1.95±0.32 | 1.4-2.5 |
| Thickness from subdermal to subcutaneous | 3.08±0.84 | 1.6-5.1 | 2.93±0.79 | 1.7-4.8 |
| Thickness from skin to SMAS | 4.98±1.07 | 3.1-7.1 | 4.84±0.98 | 3.2-6.9 |
| Thickness from skin to bone | 9.72±1.87 | 6.3-12.9 | 10.56±1.99 | 6.8-14.7 |
| **Filler group (n=8)** | | | | |
| Thickness of skin | 1.45±0.43 | 0.9-2.0 | 1.66±0.45 | 1.0-2.3 |
| Thickness from subdermal to subcutaneous | 2.84±0.61 | 2.0-3.7 | 3.24±0.39 | 2.6-3.7 |
| Thickness from skin to SMAS | 4.48±1.11 | 3.1-6.5 | 4.85±0.56 | 4.3-5.8 |
| Thickness from skin to bone | 9.8±2.39 | 7.1-13.1 | 11.76±1.89 | 8.4-14.7 |

**Soft tissue thickness Point B**

The thickness of each layer of no filler was as follows: the skin 2.05±0.33 mm and 2.03±0.31 mm, the subcutaneous layer 3.11±0.60 mm and 3.52±0.64 mm, the SMAS layer 5.20±0.65 mm and 5.63±0.81 mm, right and left respectively.

The thickness of each layer of filler usage history was as follows: skin 1.81±0.35 mm and 1.99±0.35 mm, the subcutaneous layer 3.24±0.41 mm and 3.55±1.00 mm, the SMAS 5.03±0.57 mm and 5.45±1.12 mm, right and left respectively (Table 3).

**Table 3:** Soft tissue thickness at point B (Midpoint between points A and C)

| Soft tissue thickness (mm.) | Right | | Left | |
|---|---|---|---|---|
| | mean±SD | min - max | mean±SD | min - max |
| **No filler group (n=25)** | | | | |
| Thickness of skin | 2.05±0.33 | 1.5-2.8 | 2.03±0.31 | 1.1-2.5 |
| Thickness from subdermal to subcutaneous | 3.11±0.60 | 2.1-4.2 | 3.52±0.64 | 2.4-5.2 |
| Thickness from skin to SMAS | 5.20±0.65 | 3.9-6.6 | 5.63±0.81 | 3.4-7.0 |
| **Filler group (n=8)** | | | | |
| Thickness of skin | 1.81±0.35 | 1.4-2.4 | 1.99±0.35 | 1.4-2.8 |
| Thickness from subdermal to subcutaneous | 3.24±0.41 | 2.7-3.9 | 3.55±1.00 | 2.7-5.8 |
| Thickness from skin to SMAS | 5.03±0.57 | 4.1-5.6 | 5.45±1.12 | 4.1-7.3 |

**Soft tissue thickness Point C**

The thickness of each layer of no filler was as follows: the skin 1.84±0.34 mm and 1.82±0.41 mm, the subcutaneous 3.03±0.67 mm and 3.48±0.73 mm the SMAS layer 4.92±0.83 mm and 5.04±1.24 mm, right and left respectively.

The thickness of each layer of filler usage history was as follows: the skin 1.63±0.33 mm and 1.71±0.27 mm, the subcutaneous layer 3.43±1.08 mm and 3.93±1.21 mm, the SMAS layer 4.95±1.30 mm and 5.54±1.18 mm, right and left respectively (Table 4). Display Bar graph and error bar showing soft tissue thickness at points A, B, and C on the right and left side, including a history of filler injection is shown in Figure 5.

**Table 4**: Soft tissue thickness at point C (1-2cm near the corner of the mouth)

| **Soft tissue thickness (mm.)** | Right | | Left | |
|---|---|---|---|---|
| | mean±SD | min - max | mean±SD | min - max |
| **No filler group (n=25)** | | | | |
| Thickness of skin | 1.84±0.34 | 1.2-2.4 | 1.82±0.41 | 1.2-2.8 |
| Thickness from subdermal to subcutaneous | 3.03±0.67 | 1.9-5.0 | 3.48±0.73 | 2.1-4.9 |
| Thickness from skin to SMAS | 4.92±0.83 | 3.5-6.6 | 5.04±1.24 | 0.7-7.1 |
| **Filler group (n=8)** | | | | |
| Thickness of skin | 1.63±0.33 | 1.0-2.0 | 1.71±0.27 | 1.4-2.1 |
| Thickness from subdermal to subcutaneous | 3.43±1.08 | 2.2-5.0 | 3.93±1.21 | 2.5-5.9 |
| Thickness from skin to SMAS | 4.95±1.30 | 3.3-6.8 | 5.54±1.18 | 3.7-6.9 |

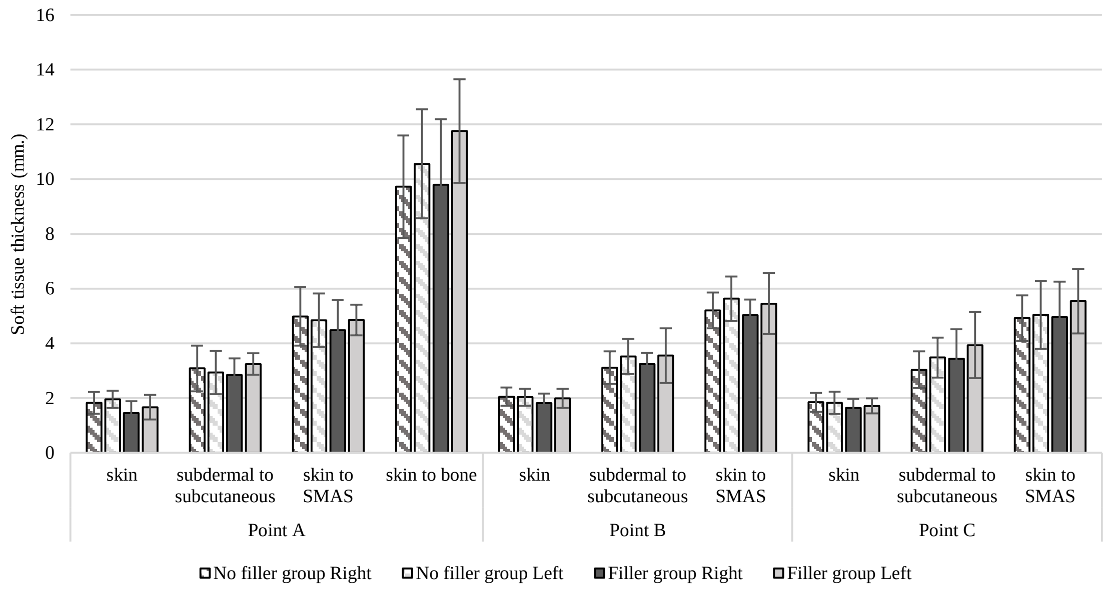


**Figure 5:** Display Bar graph and error bar showing soft tissue thickness at points A, B, and C on the right and left side, including a history of filler injection

Depth and position of the nasolabial vessel

Nasolabial vessels and Position

The facial artery splits off from the external carotid artery and courses the inferior border of the mandible. It curves upward to the lateral aspect at the pre-masseteric notch beneath the platysma. Located within the buccal space, there exists a tortuously rising structure with a diameter measuring roughly 2.14mm (Isaac et al., 2023). The facial artery follows its route in the nasolabial fold, where it divides into the inferior and superior labial branches, and lateral nasal branch and forms anastomoses with the septa and alar branches, the dorsal nasal branch. It ultimately concludes its course as the angular artery.

Depth of nasolabial artery in all subjects (n=33)

At Point A, the mean depth of the nasolabial vessel (the angular artery) at Point A on the right and left sides was found to be 5.84±2.37 mm and 6.42±2.22 mm, respectively. The right side was found the present of the artery in 30 subjects, while the left side was found in 32 subjects

At Point B, the mean depth of the Facial artery at point B on the right and left sides was found to be 5.05±1.76 mm and 5.58±2.45 mm, respectively. The right side was found the present artery in 25 subjects, while the left side was found in 23 subjects.

At Point C, the mean depth of the Facial artery at point C on the right and left sides was found to be 6.03±1.79 mm and 6.69±1.77 mm, respectively (Table 5). The right side found the presence of the artery in 32 subjects, while the left side was found in 33 subjects.

**Table 5:** Depth of the nasolabial vessel at points A, B, and C

| **Depth of nasolabial vessel (mm.)** | **Right** | | | **Left** | | |
|---|---|---|---|---|---|---|
| | **n** | **mean±SD** | **min - max** | **n** | **mean±SD** | **min - max** |
| **Point A (Lateral of nose)** | **30** | **5.84±2.37** | **2.4-13.7** | **32** | **6.42±2.22** | **2-10.3** |
| **Point B (Midpoint between points A and C)** | **25** | **5.05±1.76** | **2.8-9.5** | **23** | **5.58±2.45** | **2.5-11.4** |
| **Point C (1-2cm near the corner of the mouth)** | **32** | **6.03±1.79** | **1.5-11.2** | **33** | **6.69±1.77** | **2.2-9.7** |

Depth of nasolabial artery in Split Group Filler and Non-filler

The participant with no filler usage history had a mean depth of the nasolabial vessel at point A: 5.55±1.69 mm and 6.08±2.24 mm, at point B: 5.02±1.84 mm and 5.37±2.41 mm, at point C: 6.15±1.75 mm and 6.34±1.75 mm, right and left respectively (Table 6).

The volunteer with filler usage history had a mean depth of the nasolabial vessel at point A: 6.63±3.72 mm and 7.61±1.81 mm, at point B: 5.15±1.66 mm and 6.17±2.71 mm, at point C: 5.68±1.99 mm and 7.79±1.43 mm, right and left respectively (Table 6).

The comparison of depth of the nasolabial vessel between no filler and filler usage history (Table 6) at points A, B, and C was found not to be statistically significantly different at the 0.05 level on the right and left sides (P>0.05).

**Table 6:** Depth of the nasolabial vessel

| Depth of nasolabial vessel (mm.) | No filler group (n=25) | | | | | | Filler group (n=8) | | | | | | Right | Left |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Right | | | Left | | | Right | | | Left | | | | |
| | n | mean±SD | min - max | n | mean±SD | min - max | n | mean±SD | min - max | n | mean±SD | min - max | p-value | p-value |
| Point A | 22 | 5.55±1.69 | 3.1-9.6 | 25 | 6.08±2.24 | 2.0-10.2 | 8 | 6.63±3.72 | 2.4-13.7 | 7 | 7.61±1.81 | 5.1-10.3 | 0.282 | 0.107 |
| Point B | 19 | 5.02±1.84 | 2.8-9.5 | 17 | 5.37±2.41 | 2.8-11.4 | 6 | 5.15±1.66 | 3-8.1 | 6 | 6.17±2.71 | 2.5-9.9 | 0.875 | 0.507 |
| Point C | 24 | 6.15±1.75 | 3.3-11.2 | 25 | 6.34±1.75 | 2.2-9.6 | 8 | 5.68±1.99 | 1.5-7.6 | 8 | 7.79±1.43 | 5.8-9.7 | 0.528 | 0.053 |

Point A: lateral of the nose; Point B: midpoint between points A and C; Point C: 1-2cm near corner of the mouth.

Data were analyzed with an independent t-test

* Statistically significant at the 0.05 level (α=0.05)

Position of the vessel in all subjects (n=33)

The facial artery at position A (lateral of the nose) on the right side was mainly found in the muscle layer at 34.0%, followed by the supra-periosteal layer at 30.0%, the subcutaneous layer at 24.0%, and the subdermal layer at 3.0%, respectively. On the left side, mainly found were the muscle layer at 46.0% was mainly found, followed by the supra-periosteal layer at 30.0%, the subcutaneous layer at 18.0%, and the subdermal layer at 3.0%, respectively.

The facial artery at position B (midpoint between points A and C) on the right side was mainly found in the muscle layer and subcutaneous layer each at 35.5%, and the subdermal layer at 3.0%, respectively. On the left side, mainly found were the muscle layer at 34.0%, followed by the subcutaneous layer at 27.0%, and the subdermal layer at 9.0%, respectively.

The facial artery at position C (1-2cm near the corner of the mouth) on the right side was mainly found in the muscle layer at 79.0%, followed by the subcutaneous layer at 15.0%, and the subdermal layer at 3.0%, respectively. On the left side, mainly found were the muscle layer at 91.0% (Figure 6), followed by the subcutaneous layer at 6.0%, and the subdermal layer at 3.0%, respectively.

However, the facial artery was absent at position A on the right and left sides in 9.0% and 3.0% of subjects, respectively. It was absent at position B on the right and left sides in 24.0% and 30.0% of subjects, respectively. Only 3.0% of subjects were absent at position C on the right side (Table 7).

**Table 7:** Position of facial artery in all subjects (n=33)

| **Position of facial artery (n=33)** | Right, n(%) | Left, n(%) |
|---|---|---|
| Point A (Lateral of nose) | | |
| Subdermal layer | 1 (3.0) | 1 (3.0) |
| Subcutaneous layer | 8 (24.0) | 6 (18.0) |
| Muscle layer | 11 (34.0) | 15 (46.0) |
| Supra-periosteal layer | 10 (30.0) | 10 (30.0) |
| Absent artery | 3 (9.0) | 1 (3.0) |
| Point B (Midpoint between points A and C) | | |
| Subdermal layer | 1 (3.0) | 3 (9.0) |
| Subcutaneous layer | 12 (35.5) | 9 (27.0) |
| Muscle layer | 12 (35.5) | 11 (34.0) |
| Absent artery | 8 (24.0) | 10 (30.0) |
| Point C (1-2cm near corner of the mouth) | | |
| Subdermal layer | 1 (3.0) | 1 (3.0) |
| Subcutaneous layer | 5 (15.0) | 2 (6.0) |
| Muscle layer | 26 (79.0) | 30 (91.0) |
| Absent artery | 1 (3.0) | 0 (0.0) |


**Figure 6:** Location of facial artery in a muscular layer on the left side NLF (point C; 1-2cm near corner of the mouth)

## Discussion

The Nasolabial fold region is a very vulnerable area in the face, which can cause people to appear old and unapproachable. As people get older, the nasolabial folds can occur and can lead to further aggravation of depression, so correction is recommended. The nasolabial region consists of bone, muscle, fat, and skin. The facial bone offers stability and framework for the attachment of surrounding soft tissue. As we age, the bones shrink and remodel, which causes soft tissue that lies on the top to recede and change position. The malar fat pad may descend from loss of support of bone and the weakening of the orbital and malar ligament. Moreover, the repetitive contraction of muscles and changing muscle tone led to a deepening of the folds.

However, nasolabial filler injection carries a high risk due to the complex structure and vessel anastomosis in the area. It is important to have a thorough understanding of the distribution and properties of the soft tissue at each layer in the nasolabial region, especially in terms of depth. While cadaver anatomy helps demonstrate the intricacies of the tissue, it is not precise in estimating tissue depth. To accomplish this, we can use ultrasonic exploration. The layers of the nasolabial fold that we can identify by high-frequency ultrasound are dermal thickness, subcutaneous, SMAS, and bone.

Measurement at points A to C in the no filler injection group showed that the thickness of each layer from the left and right sides was not much different. At point A, the skin thickness on both sides was 1.82±0.40 mm and 1.95±0.32 mm and the subcutaneous thickness was 3.08±0.84 mm and 2.93±0.79 mm. The SMAS thickness from the skin surface were 4.98±1.07 mm and 4.84±0.98 mm, and the bone were 9.72±1.87 mm and 10.56±1.99 mm. At point B, the skin thickness was 2.05±0.33 mm and 2.03±0.31 mm, the subcutaneous layer was 3.11±0.60 mm and 3.52±0.64 mm, the SMAS was 5.20±0.65 mm and 5.63±0.81 mm right and left respectively. At point C, the skin thickness was 1.84±0.34 mm and 1.82±0.41 mm, the subcutaneous was 3.03±0.67 mm and 3.48±0.73 mm, the SMAS was 4.92±0.83 mm and 5.04±1.24 mm right and left respectively. Current research by Salvia et al., 2023, about Ultra-High-Frequency Ultrasound as an Innovative Imaging Evaluation of Hyaluronic Acid Filler in Nasolabial Fold Region, Italy. He measured the dermal thickness of the nasolabial region at the baseline was 2.04mm $(\pm\mathbf{0.09}\boldsymbol{mm})$ and 2.05 mm $(\pm\mathbf{0.1}\boldsymbol{mm})$ for right and left respectively. By comparing our study with this study, the skin thickness in our study non-filler group was slightly less than the skin thickness at baseline measured by Salvia et al., 2023. However, the measurement was not much different. Another study by Qiao et al., 2019, Long-Term Follow-Up of Longevity and Diffusion Pattern of Hyaluronic Acid in Nasolabial Fold Correction through High-Frequency Ultrasound, he measured the dermal thickness by ultrasound before the filler treatment was 1.45 ± 0.16 mm (minimum, 1.03 mm; maximum, 1.84 mm). The skin thickness determined by Qiao et al., 2019 and our investigation in the group without filler do not differ either.

In addition, the measurement at points A to C in the filler injection group (n=8) showed that the thickness of each layer from the right and left side of point A: the skin thickness was 1.45±0.43 mm and 1.66±0.45 mm, the subcutaneous was 2.84±0.61 mm and 3.24±0.39 mm, the SMAS was 4.48±1.11 mm and 4.85±0.56 mm, the bone was 9.8±2.39 mm and 11.76±1.89 mm. At Point B of the filler group, the skin thickness was 1.81±0.35 mm and 1.99±0.35 mm, the subcutaneous was 3.24±0.41 mm and 3.55±1.00 mm, and the SMAS was 5.03±0.57 mm and 5.45±1.12 mm. At Point C, the skin thickness was 1.63±0.33 mm and 1.71±0.27 mm, the subcutaneous was 3.43±1.08 mm and 3.93±1.21 mm and the SMAS was 4.95±1.30 mm and 5.54±1.18 mm right and left respectively. The mean of dermal thickness after filler injection by Salvia et al., 2023 was 2.28mm $\pm\mathbf{15}\boldsymbol{mm}$ on the right and.27 mm $\pm\mathbf{0.15}\ \boldsymbol{mm}$ on the left. If we compare these two studies, the dermal thickness after filler injection by Salvia et al., 2023 was thicker than the filler injection group (n=8) of our study. Moreover, if we compare the filler injection group (n=8) and no filler group (n=25), the skin thickness in the non-filler group is slightly thicker than the filler group. Nonetheless, the BMI, the age, the duration of the filler injection, and the types of filler may affect the measurement.

Blood vessels represent the primary risk while receiving filler injections. Non-ischemic complications and ischemic complications such as arterial occlusion, followed by ischemia, with the necrosis of skin or vision loss, were associated with the injection of dermal filler in the nasolabial region. Lee et al.,2020, used DUS to visualize the artery anatomy position of the nasolabial fold in 80 cases of the same Korean background: detect the artery present in the subdermal layer 13%, the subcutaneous layer 29%, the muscle layer 24%, and submuscular layer 4%, also 31% located lateral to nasolabial fold. In this investigation, we detected variances in artery position at each point A-C in the same Thai population (n=33). In point A, the depth of the angular artery was 5.84±2.37mm and 6.42±2.22mm, right and left respectively. We found the variation position of the angular artery in the subdermal layer 3% both right and left, the subcutaneous layer 24% and 18%, the muscular layer 34% and 46%, the supra-periosteal layer 30%, and the absent 9% and 3% in right and left respectively. At point B, the depth of the facial artery was 5.05±1.76mm and 5.58±2.45mm right and left. The position of the artery in the subdermal layer was 3% and 9%, the subcutaneous layer 35.5% and 27%, the muscular layer 35.5% and 34%, and the absent 24% and 30% left and right side respectively. At point C, the depth of the artery was 6.03±1.79mm and 6.69±1.77mm right and left. The position of the artery in the subdermal layer was 3% on both sides, the subcutaneous layer was 15% and 6%, the muscular layer was 79% and 91% on the right and left respectively, and the absent artery was 3% on the right side of the nasolabial fold. The absence of the artery means the artery could not be detected near the nasolabial lines or on the line. In point B, we confirm by moving the probe to the lateral and medial side and we count the presence of the artery only on the line of the nasolabial folds.

Over several decades, there has been constant improvement and expansion in the use of ultrasound for medical diagnosis. High-frequency ultrasound enables the real-time detection of tissue motion and blood vessel dynamics. Lately, numerous studies have recommended the use of Ultrasound for detecting the facial artery and the use of guided filler injection or lipo-filling. In our study, we concentrated on the real-time analysis of the position and depth of the facial artery and soft tissue structures. Unfortunately, we could not distinguish every anatomical layer of the nasolabial folds, particularly the separation of the muscle layer from the SMAS layer. Additionally, the depth of vessels below the skin, as measured by ultrasound, could vary if excessive pressure was applied with the probe. Furthermore, the relationship between the nasolabial folds and the anatomic layers, depth, and position of the artery was the main emphasis of this investigation rather than the course.

This study has some limitations. First, high-frequency ultrasound requires a significant learning curve, making it less accessible to practitioners without specialized training. Second, high- frequency ultrasound is limited in its ability to distinguish every layer within the nasolabial folds. Future research should aim to delineate the specific arrangement of facial soft tissue layers, particularly in the nasolabial fold region. Additionally, studies should investigate the pathway of the facial artery to develop definitive protocols for the use of ultrasound in visualizing facial soft tissue structures.

## Conclusion

Ultrasound has been widely used in dermatology due to its advantages of non-invasiveness and real-time imaging. High-frequency ultrasound can visualize facial soft tissue and measure the thickness of the nasolabial fold from the skin to the bone. Additionally, its power Doppler mode can identify the facial artery at each mapped point. Using ultrasound devices prior to filler injection might be a good planning strategy. Identifying anatomical layers and blood vessels is essential for safe filler treatments and lipo-filling in nasolabial correction.

## References

Isaac, J., Walker, L., Ali, S. R., & Whitaker, I. S. (2023). An illustrated anatomical approach to reducing vascular risk during facial soft tissue filler administration – a review. *JPRAS Open, 36,* 27–45. https://doi.org/10.1016/j.jpra.2022.09.006

Lee, W. (2023). Hyaluronic acid filler injection guided by Doppler ultrasound. *Archives of Plastic Surgery, 50*(04), 348–353. https://doi.org/10.1055/s-0043-1770078

Qiao, J., Jia, Q.-N., Jin, H.-Z., Li, F., He, C.-X., Yang, J., Zuo, Y.-G., & Fu, L.-Q. (2019). Long-term follow-up of longevity and diffusion pattern of hyaluronic acid in nasolabial fold correction through high-frequency ultrasound. *Plastic & Reconstructive Surgery*, *144*(2). https://doi.org/10.1097/prs.0000000000005848

Salvia, G., Zerbinati, N., Manzo Margiotta, F., Michelucci, A., Granieri, G., Fidanzi, C., Morganti, R., Romanelli, M., & Dini, V. (2023). Ultra-high-frequency ultrasound as an innovative imaging evaluation of hyaluronic acid filler in nasolabial folds. *Diagnostics, 13*(17), 2761. https://doi.org/10.3390/diagnostics13172761

Stefura, T., Kacprzyk, A., Droś, J., Krzysztofik, M., Skomarovska, O., Fijałkowska, M., & Koziej, M. (2021). Tissue fillers for the nasolabial fold area: A systematic review and meta-analysis of randomized clinical trials. *Aesthetic Plastic Surgery, 45*(5), 2300–2316. https://doi.org/10.1007/s00266-021-02439-5

Swift, A., Liew, S., Weinkle, S., Garcia, J. K., & Silberberg, M. B. (2020). The facial aging process from the "Inside out." *Aesthetic Surgery Journal, 41*(10), 1107–1119. https://doi.org/10.1093/asj/sjaa339

Velthuis, P. J., Jansen, O., Schelke, L. W., Moon, H. J., Kadouch, J., Ascher, B., . . . Cotofana, S. (2021). A guide to doppler ultrasound analysis of the face in cosmetic medicine. part 2: Vascular mapping. *Aesthetic Surgery Journal, 41*(11). https://doi.org/10.1093/asj/sjaa411

Whitney, Z. B. (2024, January 30). *Anatomy, skin, superficial musculoaponeurotic system (SMAS) fascia.* StatPearls [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK519014/

Zhao, Y., Huang, X., Fu, Z., Zhang, L., Jin, T. T., Pan, L., Lai, F., Wang, J., Wu, S., & Chen, C. (2023). The Anatomy Study of Temporal Region based on ultrasound investigation: A spatial structure study. *Journal of Craniofacial Surgery, 34*(5), 1570–1574. https://doi.org/10.1097/scs.0000000000009236