Advances in the Management of Facial Paralysis Sequelae

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Abstract

Background: Despite advances in the management of facial paralysis and its associated sequelae, therapies targeting the lower facial region remain underdeveloped. This thesis aimed to improve both the diagnostic and therapeutic modalities for facial paralysis, with a focus on the lower face.

Methods: This thesis employed anatomical and retrospective studies across three key areas. First, high-resolution ultrasound was evaluated for its ability to increase the precision of botulinum toxin injections in the treatment of facial synkinesis and gustatory hyperlacrimation (Paper I), as a preoperative tool to reduce surgical failures in lower lip depressor myectomies (Papers III–IV), and as a method for evaluating the platysma muscle in patients with facial paralysis (Paper V). Second, anatomical exploration was conducted to identify new potential nerve donors for reanimating the lower facial region (Paper II). Third, a novel classification system for facial nerve injuries was applied to a retrospective cohort to stratify patients and to propose a management algorithm for marginal mandibular nerve reconstruction (Paper VI).

Results: The use of high-resolution ultrasound significantly increased the accuracy of injections into the facial muscles and lacrimal gland (Paper I). High-resolution ultrasound also provided valuable preoperative information for depressor anguli oris myectomy (Paper IV) and allowed for the assessment of the platysma muscle in both the neck and face (Paper V). A literature review revealed a surgical failure rate of 21% for lower-lip depressor myectomies (Paper III). The ansa cervicalis nerve was established as an anatomically reliable nerve donor for selective marginal mandibular nerve grafting, although awareness of a common anatomical variant and the required modification of the surgical technique are crucial for surgical success (Paper II). A new classification system effectively stratified patients based on the severity of facial nerve injury, allowing for the creation of a management algorithm for marginal mandibular nerve reconstruction (Paper VI).

Conclusions: Application of the findings of this thesis may contribute to the improved management of patients with facial paralysis and associated sequelae, particularly with regard to the lower face.

Keywords: facial paralysis, facial palsy, facial synkinesis, synkinesis, facial contracture, facial nerve reconstruction, nerve transfer, nerve graft, microsurgery, myectomy, depressor anguli oris, depressor labii inferioris, platysma, mentalis, lacrimal gland, botulinum toxin, injection, ultrasound, sonography, ultrasonography, crocodile tears syndrome, gustatory hyperlacrimation, excessive tearing, literature review, review, classification system, management algorithm, plastic surgery, static reconstruction, dynamic reconstruction

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To my grandparents
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals. Reprints were made with permission from the respective publishers.


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<td>ACN</td>
<td>Ansa cervicalis nerve</td>
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<tr>
<td>CFNG</td>
<td>Cross facial nerve graft</td>
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<td>DAO</td>
<td>Depressor anguli oris muscle</td>
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<td>DLI</td>
<td>Depressor labii inferioris muscle</td>
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<tr>
<td>FA</td>
<td>Facial artery</td>
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<td>FN</td>
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Peripheral facial paralysis is a debilitating condition in which essential functions, such as speaking, eating, and expressing emotions, are compromised due to the loss of facial movement.\textsuperscript{1-3} However, the most profound ramifications may lie in the significant negative impact on social interactions and the psychological well-being of affected individuals.\textsuperscript{4,5} Individuals of all ages are affected, and its diverse etiology, which includes trauma, tumors, infections, congenital causes, neurological causes, and systemic causes, significantly influence prognosis and subsequent sequelae.\textsuperscript{5} In approximately 53\% of the cases, the cause of the paralysis remains unidentified. This subgroup is termed acute idiopathic peripheral facial paralysis, commonly known as Bell’s palsy.\textsuperscript{1}

The incidence of Bell’s palsy is estimated to be approximately 30 per 100,000 individuals,\textsuperscript{3,6,7} translating to around 3,000 people affected annually in Sweden. Among these, approximately 30\% experience some form of persistent sequelae, while 7–17\% experience moderate-to-severe sequelae, severely affecting the quality of life.\textsuperscript{3,8} In these cases, surgical treatment is mainly aimed at restoring facial movement and reducing the impact of sequelae, such as synkinesis\textsuperscript{9,10}. Although the management of facial paralysis has seen steady advancements in the last decades, several challenges remain. The objective of this thesis was to advance the available diagnostic and treatment options for the patient group in three key areas: refinement of existing surgical techniques, exploration of innovative surgical methods, and examination of new applications of contemporary technology for diagnostic and treatment purposes, all aimed at enhancing the outcomes for afflicted patients.

Facial nerve anatomy

The facial nerve is the 7th cranial nerve and primarily functions to innervate the facial expressive muscles.\textsuperscript{11} It also receives fibers that transmit taste from the anterior two-thirds of the tongue and provides parasympathetic innervation to the lacrimal, submandibular, and sublingual glands, thereby playing a role in tear and saliva production. Initially, the bilateral facial nerves are composed of two distinct roots: a motor root and a sensory root, the latter of which contains both sensory and parasympathetic nerve fibers. These motor, sensory,
and parasympathetic axons originate in different regions of the brainstem in the region of the pons. Before entering the facial canal, where these roots fuse to form the facial nerve, they enter the internal acoustic meatus of the temporal bone. Within the facial canal, three branches—the greater petrosal nerve, the nerve to the stapedius muscle, and the chorda tympani—diverge from the main facial nerve. The facial nerve then exits the cranium through the stylomastoid foramen and traverses the parotid gland tissue. Within the gland, it is subdivided into five branches, each of which innervates a different facial region.

1. The temporal (frontal) branch innervates the muscles of the forehead and is responsible for the movement of the forehead and eyebrows.
2. The zygomatic branch innervates parts of the orbicularis oculi muscle and is involved in eye closure.
3. The buccal branch innervates the midface and is involved in smiling (by raising the corners of the upper lip) and the movement of the nostril.
4. The marginal mandibular branch innervates the muscles involved in lower lip depression, and thus, expressions involving the lowering of the lower lip.
5. The cervical branch innervates the platysma muscle and is involved in lower lip depression.

Nerve interconnections are present among the three upper facial nerve branches, resulting in a functional overlap. By contrast, the marginal mandibular and cervical branches are more anatomically isolated. Consequently, injuries to these two branches have a lower likelihood of spontaneous recovery through axonal regrowth from adjacent nerve branches.\textsuperscript{12–15}

Facial nerve injury

The long pathway of the facial nerve makes it prone to injury.\textsuperscript{16} Multiple mechanisms underlie facial nerve damage, which, depending on factors such as the level of facial nerve injury, etiology, and time since denervation, lead to varying degrees of facial paralysis. The causes of facial paralysis can be classified into central etiologies, including cerebral injuries resulting from stroke or tumor, and peripheral etiologies resulting from injury to the facial nerve distal to the central nervous system.\textsuperscript{1,2} Peripheral facial paralysis, the primary focus of this thesis, has a variety of potential etiologies, including traumatic and iatrogenic injuries (14%), tumors (9%), herpes zoster (6%) or other infections (4%), and congenital causes (5%).\textsuperscript{1} However, the majority of cases (approximately 53%) are attributed to Bell’s palsy, for which the cause remains unidentified.
Classification of facial nerve injury

The severity of peripheral facial nerve injuries can be classified using the Seddon and Sunderland classification scales. The Seddon classification divides nerve injuries into three classes.

- **Seddon Class I.** Neuropraxia: injury to the myelin sheath with excellent prognosis.
- **Seddon Class II.** Axonotmesis: Class I + injury to axons with moderate-to-good prognosis.
- **Seddon Class III.** Neurotmesis: Class II + injury to the endoneurium, perineurium, and epineurium, with poor prognosis or no chance of spontaneous recovery.

In contrast, the Sunderland classification divides nerve injury into five classes (I–V), where Sunderland I correlates with Seddon’s neuropraxia and Sunderland V, complete nerve transection, correlates with neurotmesis. However, Seddon class II (axonotmesis) is further divided into three sub-categories in the Sunderland system: classes II–IV.

- **Sunderland Class I.** Injury to the myelin sheath only (corresponding to Seddon Class I).
- **Sunderland Class II.** Injury to the myelin sheath and axons.
- **Sunderland Class III.** Injury to the myelin sheath, axons, and endoneurium.
- **Sunderland Class IV.** Injury to the myelin sheath, axons, endoneurium, and perineurium.
- **Sunderland Class V.** Complete nerve transection (corresponding to Seddon Class III).

Different classes have different prognoses and clinical manifestations. Sunderland classes I and II can recover spontaneously. In class III, complete spontaneous recovery is possible, but unlikely, and endoneurial damage can lead to aberrant nerve regeneration and development of synkinesis. Classes IV and V are usually caused by trauma or iatrogenic transection during surgery and cannot recover spontaneously. In these cases, facial nerve reconstruction is necessary to regain facial function.

Symptoms and related sequelae

In most cases, peripheral facial paralysis presents with unilateral facial involvement, while bilateral paralysis is rare. The paralysis of the mimic muscles leads to difficulties expressing emotions and functional deficits in the oral
and ocular sphincters, with deleterious effects on one’s psychological well-being.\textsuperscript{4,5} Over time, further sequelae, such as facial synkinesis, may develop. Synkinesis manifests as facial contractions and involuntary movements in one part of the face when another part is moved voluntarily. This phenomenon typically occurs within 3 to 6 months following the onset of facial paralysis and is likely attributed to abnormal axonal sprouting.\textsuperscript{8,19} Gustatory hyperlacrimation, excessive tearing associated with gustatory stimulus, is another, although rarer, sequelae, impacting quality of life.\textsuperscript{21}

Importantly, many patients with etiologies such as Bell’s palsy, infectious causes, and mild mechanical injuries (neuropraxia) spontaneously undergo complete recovery. For example, 70\% of Bell’s palsy patients recover completely within 6 months.\textsuperscript{3} Early administration of prednisolone has been shown to further improve both the rate of recovery and reduce the incidence of sequelae; currently, it is the main therapy for Bell’s palsy.\textsuperscript{22,23} However, some patients do not recover facial nerve function despite medical treatment and rehabilitation. Thirty percent of Bell’s palsy patients suffer some form of sequelae: from persistent facial weakness and asymmetry to muscle contractions and facial synkinesis.\textsuperscript{3} More severe manifestations of synkinesis occur in 7–17\% of Bell’s palsy patients\textsuperscript{3,8} and is associated with facial asymmetry, impairment of social interactions, and functional deficits with reduced quality of life.\textsuperscript{24} In patients with persistent sequelae following peripheral facial paralysis, and after significant mechanical injury to the facial nerve (such as in complete transection along with regional tumors) where no chance of spontaneous recovery exists, surgical intervention is necessary to restore facial function.\textsuperscript{25}

Treatment options

General considerations

Surgical correction of residual facial paralysis with associated sequelae can be categorized into two main groups. Reanimating procedures, in which facial nerve function is restored through nerve repair, is the treatment method of choice, as facial function can be reestablished.\textsuperscript{26–28} Deanimating interventions, on the other hand, aim to remove muscle function in parts of the face to achieve greater facial balance and reduce the impact of synkinesis and muscle contractions.\textsuperscript{29,30}

Reanimating surgical procedures

Dynamic reanimating procedures can restore movement and function of the paralyzed face.\textsuperscript{27,28} The treatment alternatives for facial nerve reconstruction
vary. Factors such as etiology, time since denervation, presence and size of the nerve gap between the proximal and distal parts of the facial nerve, adjuvant treatment such as radiation therapy, and age affect the choice of treatment. Direct nerve reconstruction is the treatment method of choice because it yields superior results. This requires both the proximal and distal nerves to be available without a nerve gap, which is not always the case. In the presence of nerve gaps, which are common in oncological resections, the use of nerve grafts or local nerve donors is necessary for the reanimation of distal nerves. Nerve grafts can bridge substantial nerve gaps, linking the distal facial nerve to the proximal facial nerve stump. Common donor nerves for these grafts include the sural and greater auricular nerves. If the proximal facial nerve stump is unavailable, cross-facial nerve grafting (CFNG) may be utilized. Here, axons from the contralateral healthy facial nerve are rerouted through a graft to the afflicted facial nerve, with the major benefit of spontaneous and synchronized facial expression. However, a drawback of using nerve grafts is that dual anastomoses result in a lower final axon count reaching the target, as an axon loss of 50% can be expected across each coaptation site. Furthermore, as axons regenerate at an average rate of 1 mm per day, it can take time for axons to reach their final target when required to travel long distances, such as in CFNGs, which can lead to muscle atrophy.

Local nerve transfers are an alternative and can be utilized for a higher axon load and dependable expressive power, as well as faster reinnervation times, owing to shorter distances for the axons to grow. The masseter nerve has become the preferred regional donor, while the hypoglossal-facial nerve transfer has had to undergo modifications to deal with problems of donor site morbidity. A problem, however, is that the resulting facial expressions are usually not spontaneous.

To overcome the issues of muscle atrophy when using nerve grafts and lack of spontaneity when using nerve transfers, the two methods (CFNGs and local nerve transfers) can be combined in what has been termed “babysitter” procedures, where both expressive power and spontaneity may result. With this technique, a local nerve transfer is rerouted to the target, together with the CFNG, to provide fast reinnervation while waiting for the axons of the graft to arrive, which prevents interim muscle atrophy. Following surgical correction, physical rehabilitation therapy is a cornerstone in the management of facial paralysis and related sequelae.

Deanimating interventions
Deanimating techniques aim to eliminate muscle function to restore symmetry, balance, and function of a paralyzed or synkinetic face. Myectomies, botulinum toxin injections, and neurectomies can be used in this regard.
The goal of myectomies is to surgically excise parts of a muscle to eliminate its function, while neurectomies achieve similar results by selectively removing the nerve supplying the muscle. On the other hand, botulinum toxin injections utilize the paralyzing effects of the neurotoxin, which occurs by blocking acetylcholine release at the neuromuscular junction. These injections need to be repeated regularly, as their effect is temporary.

These interventions are often employed on the contralateral non-paralyzed muscles that exert an excessive pull on the paralyzed side, resulting in facial asymmetry due to a lack of balancing power. These techniques can be used when nerve reconstruction is not possible or as a supplementary procedure following facial reanimation. Hyperkinetic or synkinetic muscles in the ipsilateral face, which cause contractures and synkinesis, are also commonly targeted.

Knowledge gaps

For this thesis, the following knowledge gaps were identified.

Lack of bedside assessment tools in the perioperative management of facial paralysis patients

Physical examination is currently the primary tool for evaluating patients with facial paralysis. Neurophysiological studies can also provide diagnostic information. Radiological examinations, on the other hand, are performed in the initial stage after the presentation of facial paralysis to verify the etiology. However, they are seldom used in preoperative planning or during treatment and interventions, mainly because the small structures involved cannot be evaluated easily. Recently, high-resolution ultrasound has shown potential in plastic surgery as an adjunct to pre-, peri-, and postoperative care. Nevertheless, its use has not been established, and its application in patients with facial paralysis must therefore be further explored. Potential areas of benefit identified include ultrasound-guided injections in the face and perioperative evaluation of the depressor anguli oris and platysma muscles to treat facial paralysis sequelae.

Complications and outcome variability following botulinum toxin injections

Regarding botulinum toxin injections, consistent outcomes are not guaranteed in the treatment of facial paralysis sequelae, such as facial synkinesis and gustatory hyperlacrimation. Botulinum toxin injection is considered the treatment
of choice for these conditions; however, the treatment effect and duration vary among patients, and complications such as diplopia, ptosis, and lagophthalmos commonly occur.\textsuperscript{47–49} When targeting the lacrimal gland to treat gustatory hyperlacrimation, complications occur in 21\% of the patients.\textsuperscript{47} More severe complications, such as ocular perforation with retinal damage, are continuously reported.\textsuperscript{50–56} The treatment methods must be refined to improve procedural outcomes and safety.

Challenges and unresolved questions surrounding outcomes in lower lip depressor myectomies

In patients with synkinesis or unilateral weakness of the lower lip depressor muscles, the depressor labii inferioris and depressor anguli oris, restoration of lower lip balance following facial paralysis is commonly achieved through lower lip depressor muscle myectomy.\textsuperscript{30,57,58} Myectomies of the depressor muscles are commonly performed in one of two scenarios: resection of the lower lip depressor muscles on the affected side in the case of hyperkinesis and synkinesis or, in instances of lower lip asymmetry due to unilateral weakness on the affected side, resection of the healthy muscles on the contralateral side. This procedure is considered effective and relatively simple to perform, with the benefit of lifelong results compared with deanimating botulinum toxin injections, which need to be repeated regularly.\textsuperscript{48} However, these myectomies commonly fail, forcing patients to undergo repeat surgery or alternative treatment.\textsuperscript{30,57,58} The incidence and underlying reasons for the common surgical failures remain poorly understood. Further exploration of the anatomy of the area is warranted to determine the reasons for the surgical failures, and the potential of perioperative ultrasound to reduce the risk of failure through perioperative sonographic analysis should be assessed.

Lack of methods for assessing the platysma muscle

Another muscle commonly affected by post-facial paralysis synkinesis and hyperkinesis is the platysma. Platysma synkinesis is generally associated with contractions in the neck,\textsuperscript{59,60} but the platysma can also be a less recognized cause of facial synkinesis, as the insertion height of the muscle in the face varies.\textsuperscript{61} Recent studies indicate that the platysma insertion height in the face lies more cranially than previously believed, but this data needs further corroboration.\textsuperscript{62,63} A more cranial insertion of the platysma in the face leads to a greater number of muscle fibers being recruited in the midface during synkinesis. Thus, the insertion height of the platysma muscle should affect the severity of facial synkinetic symptoms; however, this has not yet been proven. The platysma can be challenging to assess because of its thin structure, which is further amplified in elderly patients in whom age-related sarcopenia is
Apart from clinical examination, there is currently no established method to assess the platysma muscle in the neck or determine the insertion height in the face and, thus, its role in midface synkinesis. The absence of reliable methods for platysma examination poses challenges both clinically, where accurate diagnosis and treatment depend on precise assessment, and academically, where robust research is hindered by the inability to gauge muscle characteristics accurately. High-resolution ultrasound could provide a way to improve diagnostics and treatment in the management of platysma synkinesis by supporting the assessment of hypertrophy and active muscle contraction in the neck, guiding botulinum toxin injections, and determining the extent of the platysma in the midface to guide treatment strategies. Both age-related platysma thinning, and the muscle fibers being exceptionally thin cranially in the face, might affect ultrasound assessment adversely. The feasibility of locating these fibers sonographically must therefore be determined to employ ultrasound successfully as a general preoperative evaluation tool. The accuracy of ultrasound in evaluating the platysma muscle in the elderly and midface areas should be explored.

Lack of local nerve donors for reanimation of the lower lip

Regarding reanimation surgery, all patients with facial paralysis should ideally receive selective nerve reanimation across all five facial nerve branches. However, numerous circumstances preclude this comprehensive approach due to the lack of available donor nerves. In these cases, reinnervation is often prioritized for essential facial functions, such as eye closure and smiling. Consequently, the available nerve donors are expended on these high-priority targets, and because of this, the lower lip has frequently been neglected in facial reanimation. The available donor nerves for the marginal mandibular nerve are limited, and further research on reinnervation options for the lower face is imperative for optimizing facial nerve reconstruction.

Challenges regarding treatment selection and outcome comparisons for lower lip reanimation

Finally, a standardized approach for reanimation after an injury to the marginal mandibular nerve (MMN) is lacking. Dynamic reconstruction can yield restored facial function and optimal outcomes following facial paralysis, but a key issue is that patients with facial paralysis are a distinctly heterogeneous group. Clinical scenarios can vary greatly, with considerable differences in etiology, location of nerve injury, and availability of nerve donors. These differences demand tailored treatment plans and can make it difficult to compare surgical outcomes between patients. Therefore, further research is needed to determine how the clinical scenario influences the type of MMN
reconstruction method that can be used, and how the surgical outcome is affected. A standardized treatment algorithm for MMN reconstruction, based on distinct anatomical criteria, could standardize the care for these patients, facilitate decision-making when determining the optimal strategy for MMN reconstruction, and enable comparisons between groups of patients.
Aims

To address the knowledge gaps presented above, the overarching goal of this thesis was to advance the treatment of facial paralysis and its related sequelae, focusing particularly on the lower part of the face. The specific aims of each study were as follows.

Paper I
To examine the benefits of ultrasound guidance during botulinum toxin injections in the treatment of facial synkinesis and gustatory hyperlacrimation. This study aimed to compare ultrasound-guided injections with the conventional landmark-guided technique, with the hypothesis that the traditional landmark-based approach might lack precision and thus explain the common clinical complications. The primary aim was to determine whether ultrasound guidance enhanced the accuracy of these injections.

Paper II
To expand the limited availability of donor nerves in the lower face by exploring the anatomical technical feasibility of using the ansa cervicalis nerve as a donor for reinnervation of the marginal mandibular nerve through anatomical dissection.

Paper III
To identify the surgical failure rates of lower lip depressor myectomies through a literature search, and to revisit the anatomy of the lower lip depressor muscles through cadaver dissection. The primary aim of this study was to optimize the surgical technique to reduce the high rate of surgical failures.
Paper IV
To further explore the high failure rates in lower lip depressor muscle myectomy and enhance the surgical technique. This study aimed to investigate the depressor anguli oris (DAO) muscle using high-resolution ultrasonography and anatomical dissection. The main objective was to identify the potential reasons for the common surgical failures and determine the reliability of sonographic measurements in this area.

Paper V
To establish the capability of high-resolution ultrasound to assess the platysma muscle in the neck and determine the insertion height in the face in an elderly cohort. This could provide further information on ultrasound as a tool to aid in the diagnosis and treatment of synkinesis of the platysma muscle, and on the role of the platysma in midface synkinesis.

Paper VI
To stratify patients undergoing reconstruction of the marginal mandibular nerve using a new three-level classification system for facial nerve injuries in a retrospective study. The main goal of this study was to determine whether the classification system could enable more robust comparisons of surgical outcomes and simplify treatment selection by proposing a management algorithm for marginal mandibular nerve reconstruction.
Ethical considerations

Papers I–V
Anatomical data were collected in accordance with the local ethical guidelines at the Division of Anatomy, Center for Anatomy and Cell Biology, Medical University of Vienna, Austria. According to these guidelines, studies supervised by a medical specialist in anatomy and involving body donors who have provided signed consent for the donation of their bodies for medical research and pre- and postgraduate teaching are covered by general ethical approval; thus, a separate approval from the local institutional ethics committee is not required. This general ethical approval is applicable to Papers I, III, IV, and V and the anatomical components of Paper II. In addition to this general ethical approval, supplementary ethical applications were submitted and approved by the local institutional ethics committee for Papers I, II, IV, and V for additional evaluation (EC numbers: 1375/2020, 1402/2020, 1403/2020).

Paper II and Paper VI
The retrospective clinical study in Paper VI, and the clinical case study in Paper II, were approved by the ethical institutional committee in Sweden (Dnr:2020-03492 with amendment 2022-04681-02 and 2023-01156-02). Signed letters of informed consent were collected from patients for participation in the study, and additional oral and written consent regarding the publication of photos and/or video material was recorded and collected.
Statistical analysis

Paper I
A sample size calculation was performed based on a power of 80% and statistical significance level of 5%. The independent chi-square test was used to compare variables at the group level and in different subgroups. Fischer’s exact test was used if the chi-square test was not considered valid. All tests were two-sided, with a significance level of $\alpha = 0.05$. Statistical analyses were performed using SAS 9.4.

Papers II, III, IV, and V
Continuous variables were assessed for normality to guide descriptive statistics. Means and standard deviations summarize the central tendencies and dispersion of the data. Subgroup analysis was performed when applicable. Data processing, statistical analyses, and graphical representations were conducted using R (versions 3.6 to 4.3)\textsuperscript{67} in the Tidyverse ecosystem.\textsuperscript{68}

Paper VI
The assumptions of normality were tested on the residuals using QQ plots, and the assumptions of homoscedasticity were tested using Levene’s test. As a result of the small cohort and some variables displaying non-homoscedasticity, all variables were analyzed using the non-parametric Kruskal-Wallis test regarding the differences between the outcome parameters based on the categorized level of injury. Dunn-Bonferroni post-hoc tests were used for pairwise comparisons of the subgroups. All tests were two-sided, with a significance level of $\alpha = 0.05$. To quantify the magnitude of the observed differences, effect sizes were calculated using eta-squared ($\eta^2$) based on the Kruskal-Wallis H-statistic, where commonly interpreted values are 0.01–0.06 (small effect), 0.06–0.14 (moderate effect), and $\geq 0.14$ (large effect). All data processing, statistical analysis, and graphical representations were performed using R (version 4.3.0 (2023-04-21))\textsuperscript{67} in the Tidyverse ecosystem.\textsuperscript{68}
Methods

Paper I

A randomized split-face study was performed, in which common botulinum toxin targets were injected with blue ink using either high-resolution ultrasound or landmark guidance in 26 hemifaces. The following structures were injected: the lacrimal gland, orbicularis oculi muscle, depressor anguli oris muscle, and mentalis muscle (one injection in the gland and two in each muscle; injection sites marked with crosses, shown in Figure 1). The injection accuracy was verified through dissections where the targets were exposed, and the following assessments were performed for each target using a binary yes or no approach:

- Is most of the ink (>50%) inside the intended target (as opposed to outside)?
- Is all the ink (100%) inside the intended target?
- Is the correct target colored (any ink in the target)?
- Were any unintended structures colored?
Figure 1. Illustration showing the lacrimal gland being injected from the lateral side under ultrasound guidance in Paper I. The four targets studied are highlighted (lacrimal gland, orbicularis oculi, depressor anguli oris, mentalis) with the crosses representing the injection sites.

Paper II
Anatomical part

In the anatomical part of this study, 24 hemifaces from adult human body donors were dissected. The ansa cervicalis nerve (ACN) was located either by identifying the hypoglossal nerve and following the ACN caudally, or by locating the branches in the omohyoid muscle and following it cranially. First, the maximal harvestable length of the ansa cervicalis nerve (ACN) was
measured from its anatomical macroscopic origin at the junction with the hy-
poglossal nerve, as distally as possible to where it looped behind the internal
jugular vein. Second, the nerve was transected distally, and the transfer to the
marginal mandibular nerve (MMN) was simulated (Figure 2).

Figure 2. Illustration of the anatomy and surgical method in Paper II.
a. Normal anatomy of the marginal mandibular nerve (MMN) and the ansa cervicalis
nerve (ACN). The black arrow marked with number 5 corresponds to the site where
the transection of the ACN was performed. 1: MMN; 2: Hypoglossal nerve; 3: Supe-
rior root of ACN; 4: Inferior root of ACN; 5: ACN loop; 6: Branches from ACN to
the infrahyoid muscles.
b. Illustration of the surgical procedure: selective reinnervation of the MMN using
ACN.

Clinical part
In the clinical part, a 28-year-old male underwent ansa cervicalis to marginal
mandibular branch transfer after parotidectomy for carcinoma during which
the MMN was sacrificed and was presented as a case report. The clinical out-
come was evaluated 12 months postoperatively using the modified Terzis’
Lower Lip Grading Scale with 25 observers and photogrammetry.
Paper III

In **Paper III**, dissections were performed in ten hemifaces from adult human body donors. The depressor anguli oris (DAO) and depressor labii inferioris (DLI) muscles were exposed by dissecting the overlying skin and adipose tissue. The following measurements were performed at the height at which myectomies are generally performed: muscle width and distance from the midline of the mandible to the lateral borders of each muscle. Furthermore, the intraoral distance from the lateral part of the mandibular canine to the lateral border of the DAO was measured (Figure 3). A literature search was performed to study the failure rates of lower lip depressor myectomy.

Figure 3. Illustration of anatomical measurements performed in **Paper III**. 1: Width of DLI; 2: Width of DAO; 3: Distance from midline to lateral DLI; 4: Distance from midline to lateral DAO; 5: Intraoral distance from lateral canine to lateral DAO.
Paper IV

The anatomical features of the depressor anguli oris muscle and the surrounding anatomy were examined using high-resolution 22 MHz ultrasound. The depressor anguli oris (DAO) muscle was evaluated in relation to the surrounding anatomical features in 38 hemifaces in 19 non-embalmed cadavers. Measurements were performed by a radiologist and included the DAO muscle width, distance from the mandibular midline to the lateral DAO border, muscle depth, and spatial relationship between the lateral DAO border and the facial artery (Figure 4). Furthermore, both the DAO and depressor labii inferioris (DLI) muscles were assessed for muscle fiber continuity with the surrounding muscles (the platysma, orbicularis oris, and mentalis). Dissections were performed to expose the area, and the measurements were repeated. The sonographic measurements were compared with dissection measurements.

Figure 4. Example image from the dissections in Paper IV. The depressor anguli oris (DAO) muscle can be observed with the facial artery lying lateral to it. The dotted lines mark the measurements performed: the distance from the midline of the mandible to the lateral DAO border, the DAO width, and the distance between the lateral DAO border and the medial wall of the facial artery.
The platysma muscle was assessed using high-resolution 22 MHz ultrasound in 38 hemifaces from 19 non-embalmed cadavers of advanced age (83.5 ± 8.7 years (range 67–100 years)). The most superior insertion point of the platysma muscle in the face was determined using ultrasound within a coordinate system, relative to a line from the angle of the mandible to the inferolateral corner of the orbit (referred to as the angulo-orbital line, comprising the y-axis; Figure 5). First, the angulo-orbital line was measured. The platysma muscle in the neck was then studied. If detectable, it was followed into the face to determine the facial insertion height, which was marked on the skin. The markings were measured in relation to the coordinate system, generating two measurements along the x- and y-axes, respectively. Dissections were performed to verify the insertion height of the muscle in a similar manner.

Figure 5. Example image from the dissections in Paper V. The solid black line marks the distance from the mandibular angle to the inferolateral corner of the orbit (angulo-orbital line). The arrow marks the highest point of platysma muscle fiber insertion detected in the hemiface. The ‘y’ designates the distance along the designated line (the y-axis) to the maximal insertion height of the platysma muscle. The ‘x’ represents the horizontal distance from this line to the muscle’s highest point of insertion along the x-axis. In the presented case above, the value for ‘x’ is negative, indicating that the highest point of platysma insertion is dorsal to the line.
This retrospective single-center study was conducted at the Department of Plastic and Maxillofacial Surgery, Uppsala University Hospital, Sweden, on patients who underwent marginal mandibular nerve (MMN) reconstruction between January 2010 and September 2022. Patient records were reviewed for demographic characteristics and data, such as etiology of facial paralysis, surgical method, timing of reinnervation, complications, and outcome measures, such as Sunnybrook scores. The level of facial nerve injury was defined in each case according to a novel three-level classification system (Levels 1, 2, or 3; Figure 6). Surgical outcomes were evaluated using the following objective, subjective, and quality of life measures. Postoperative photographs and videos were examined using photogrammetry (Emotrics) and the Sunnybrook Facial Grading System. Fourteen independent observers graded each patient using the Terzis’ Lower Lip Grading Scale. Surviving patients were contacted for symptom and quality of life assessments using the Facial Disability Index (FDI) and Facial Clinimetric Evaluation (FaCE) scales. The outcome measures were analyzed based on the level of injury, with the goal of proposing an algorithm for a systematic management of marginal mandibular nerve reconstruction.
Results

Paper I

In total, 182 injections were administered in 26 hemifaces (91 using ultrasound guidance and 91 using landmark guidance). With ultrasound guidance, most of the ink (>50%) was found within the correct target in 88% of the cases, compared to 50% with landmark guidance (p<0.001). The difference was most pronounced in injections involving the lacrimal gland (62% vs. 8%), depressor anguli oris muscle (100% vs. 46%), and the mentalis muscle (100% vs. 54%) (all p<0.05). Moreover, ultrasound guidance led to all the ink being deposited within the correct target in 65% of the cases, compared to 29% with landmark guidance (p<0.001). The injection accuracy, if any ink was found inside the target, was 100% with ultrasound guidance compared to 83% with landmark guidance (p<0.01). Twenty-three percent of the landmark-guided injections aimed at the depressor anguli oris muscle inadvertently stained the facial artery, although this finding was not statistically significant (p=0.22) (example from dissections in Figure 15). The results are presented in Table 1. Images from the ultrasound-guided injections of the four targets with their corresponding anatomical translations are shown in Figure 7.

Table 1. Table showing all analyzed data comparing the differences in ink location between ultrasound-guided and landmark-guided injections. Numbers in bold indicate statistical significance.

<table>
<thead>
<tr>
<th></th>
<th>Most ink inside target</th>
<th>All ink inside target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ultrasound-guided</td>
<td>Landmark-guided</td>
</tr>
<tr>
<td>All targets</td>
<td>88% (46/52)</td>
<td>50% (26/52)</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>62% (8/13)</td>
<td>8% (1/13)</td>
</tr>
<tr>
<td>Orbicularis oculi</td>
<td>92% (12/13)</td>
<td>92% (12/13)</td>
</tr>
<tr>
<td>Depressor anguli oris</td>
<td>100% (13/13)</td>
<td>46% (6/13)</td>
</tr>
<tr>
<td>Mentalis</td>
<td>100% (13/13)</td>
<td>54% (7/13)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Correct target colored</th>
<th>Unintended structure colored</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ultrasound-guided</td>
<td>Landmark-guided</td>
</tr>
<tr>
<td>All targets</td>
<td>100% (52/52)</td>
<td>83% (43/52)</td>
</tr>
<tr>
<td>Lacrimal gland</td>
<td>100% (13/13)</td>
<td>62% (8/13)</td>
</tr>
<tr>
<td>Orbicularis oculi</td>
<td>100% (13/13)</td>
<td>100% (13/13)</td>
</tr>
<tr>
<td>Depressor anguli oris</td>
<td>100% (13/13)</td>
<td>85% (11/13)</td>
</tr>
<tr>
<td>Mentalis</td>
<td>100% (13/13)</td>
<td>85% (11/13)</td>
</tr>
</tbody>
</table>
Figure 7. Example images from the ultrasound-guided injections in Paper I. Sonographic images to the left, with corresponding anatomical translations in the middle. The suggested probe position for each target is shown to the right, where the black lines indicate the suggested probe positions, and the aligned black dots indicate the direction of the probe marker and thus the insertion direction of the needle used in this study. Ant=anterior (direction), Lat=lateral, Inf=inferior, LG=lacrimal gland, OO=orbicularis oculi, DAO=depressor anguli oris, FA=facial artery, MM=mentalis muscle.

Row 1: Left lacrimal gland injected from the lateral direction. The blue area at the top of the image represents ultrasound gel.
Row 2: Left orbicularis oculi injected from the inferior direction.
Row 3: Left depressor anguli oris injected from the lateral direction.
Row 4: Left mentalis injected from the medial direction.
Paper II

Anatomical part

In the 24 hemifaces studied, tension-free coaptation between the ACN and MMN was possible in 67% of the cases (mean harvestable ACN length in this subgroup: 100 ± 12 mm). In the remainder, a clinically significant anatomical variant (“short ansa”) was encountered, which precluded tension-free coaptation to the MMN due to a length of only 37 ± 12 mm. A workaround was explored, where tensionless coaptation was possible in this subgroup only when using a modification of the surgical technique, with an infrahyoid muscle nerve branch as an extension that provided an additional 62 ± 15 mm. The mean harvestable length of all nerves, when including the extension in the subgroup, was 100 ± 12 mm. An example photograph from the dissections is presented in Figure 8. The data are presented in Table 2.

Table 2. Data from dissection measurements in Paper II. The first three columns present the data in millimeters (mean ± standard deviation), and the last three in percentages. ACN: superior root of the ansa cervicalis nerve. MMN: marginal mandibular nerve. DAO: depressor anguli oris muscle.

<table>
<thead>
<tr>
<th>Harvestable length of ACN (excluding the extension in the ‘short ansa’ cases)</th>
<th>Additional length gained in ‘short ansa’ cases</th>
<th>Harvestable length of ACN (including the extension in ‘short ansa’ cases)</th>
<th>Tensionfree coaptation to MMN at crossing with facial vessels</th>
<th>Tensionfree coaptation to MMN at lateral DAO border</th>
<th>Prevalence of ‘short ansa’ variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>79±33 mm</td>
<td>62±15 mm</td>
<td>100±12 mm</td>
<td>100% (21/21)</td>
<td>100% (21/21)</td>
</tr>
</tbody>
</table>

Clinical part

Analysis of the clinical case showed improvement in Terzis’ Lower Lip Grading Scale from 1.44 to 3.24 at the 12-month follow-up. Clinically, improved tone, symmetry, and function of the lower lip were observed (Figure 9), which was corroborated by photogrammetry analysis, where improved symmetry was seen in all parameters during depressor activation. Asymmetry during commissure excursion improved from 18.0 to 0.6 millimeters, commissure height improved from 4.3 to 4.2, upper lip height from 3.5 to 1.2, lower lip height from 5.3 to 4.4, and smile angle from 11.4 to 8.8 (smile angle unit in degrees). The postoperative period was uneventful, and there were no complications. An intraoperative photograph is shown in Figure 8.
Figure 8. Left image: Image from dissections in Paper II showing the normal anatomy, with the superior root of the ansa cervicalis nerve (ACN) leaving the hypoglossal nerve caudally. Right image: Intraoperative image of the clinical case, showing facial nerve reconstruction using triple facial nerve transfer: 1) the proximal facial nerve trunk was connected to the temporal and upper zygomatic branches (with nerve grafts) for forehead and eye reanimation; 2) the masseter nerve was connected to the lower zygomatic and buccal branches for smiling; and 3) the marginal mandibular nerve was reinnervated with the selective ansa cervicalis nerve end-to-end transfer for reanimation of the lower lip.

Figure 9. Photographs illustrating the outcome of the clinical case in Paper II, a patient undergoing facial nerve reconstruction with triple facial nerve transfer (see Figure 8 caption for description). The patient was asked to show three expressions (from left: neutral, lower lip depression, and smile) at follow-up 1 month after surgery and at the 12-month follow-up.
Paper III

In the 10 hemifaces studied, the widths of the depressor labii inferioris (DLI) and depressor anguli oris (DAO) at the height where myectomies are generally performed were 20 ± 4 mm (15–30) and 14 ± 3 mm (10–18), respectively. The distances from the midline to the lateral border of the muscles were 32 ± 4 mm (25–35) and 54 ± 4 mm (50–60). The distance from the lateral side of the canine to the lateral border of the DAO was 24 ± 2 mm (20–25 mm). The literature search included three studies and revealed a mean recurrence rate of 21% in myectomies of the lower lip depressor muscles (Table 3).

Table 3. An overview of the results from the literature search, presenting the recurrence rate of lower lip depressor myectomies, and the muscles resected in the different studies.

<table>
<thead>
<tr>
<th>Article</th>
<th>Recurrence rate (n)</th>
<th>Muscles resected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hussain et al (2004)</td>
<td>17 % (7/42)</td>
<td>DLI</td>
</tr>
<tr>
<td>Chen &amp; Tang (2007)</td>
<td>24 % (8/33)</td>
<td>DLI + DAO</td>
</tr>
<tr>
<td>Lindsay et al (2011)</td>
<td>33 % (1/3)</td>
<td>DLI</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>21% (16/78)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Paper IV

In the 38 hemifaces, the ultrasound assessment revealed the mean width of the DAO muscle to be 16.2 ± 2.9 mm, while the distance from the midline of the mandible to the lateral DAO border was measured at 54.4 ± 5.7 mm. According to the dissection measurements, the width was found to be 14.5 ± 2.5 mm and the distance to the lateral border 52.3 ± 5.4 mm (Figure 10).

The location of the facial artery was found at a mean of 1.0 ± 2.6 mm lateral to the lateral DAO border in the cohort using ultrasound (n=38, range: -4 to +9). In 5 of the 38 hemifaces (13%), the lateral DAO fibers completely covered the facial artery, and the mean distance between the lateral muscle border and the artery in this subgroup was -2.2 ± 1.3 mm (range: -4 to -1). In 18 of the 38 cases (47%), the facial artery was located lateral to and in direct contact with the lateral DAO border (0 mm between the artery and the lateral border) (Figure 11). Significant muscle fiber continuity was present between the DAO and the surrounding muscles in 5% of the cases, while continuity between the
depressor labii inferioris and the surrounding muscles was considerably more common and more pronounced.

Figure 10. Boxplots comparing the ultrasound measurements and dissection measurements of the “Width of depressor anguli oris” and “Distance from the midline to the lateral border of depressor anguli oris” in Paper IV. Each point represents a single measurement for a single hemiface. Black crosses represent the mean values, and horizontal black lines represent the median values.
Figure 11. Bar chart showing the relative distribution of the distances between the facial artery and the lateral border of the depressor anguli oris muscle in the hemifaces in Paper IV. The darker columns, which constitute approximately 60% of the cases, indicate the number of hemifaces, where the distance was at or below 0 mm; thus, the artery was either directly adjacent to, or completely covered by, the lateral DAO muscle fibers. Theoretically, these cases might have an increased risk of inadequate DAO resections and, thus, surgical failure.
Paper V

Using high-resolution ultrasound, the platysma muscle could be visualized in the neck in 34 of the 38 hemifaces (89.5%), while it was not discernible in 4 hemifaces. The platysma could be followed into the face up to a mean height of $2.1 \pm 0.8$ cm above the mandibular angle ($n=34$, range = 0.3–3.6) along the angulo-orbital line (the $y$-axis). The dissections revealed the insertion height to lie $2.9 \pm 1.0$ cm above the mandibular angle ($n=38$, range = 0.8–5.2) (insertion heights visualized in Figure 12, box plots with comparisons shown in Figure 13). The superior-most platysma muscle fiber insertion site was found at a mean of $2.3 \pm 1.1$ cm ventrally to the angulo-orbital line along the $x$-axis when using ultrasound ($n=38$, range = 0.4–4.6), compared to $1.3 \pm 0.8$ cm in the dissection measurements ($n=38$, range = 0–3). The four cases that could not be detected using ultrasound were thin and hypoplastic during dissection.

Figure 12. Illustration of the platysma muscle inserting into the midface in Paper V. The bony landmarks used for measurement are visible (mandibular angle and inferolateral corner of the orbit). The small crosses mark the maximal insertion heights of all individual hemifaces from the dissection measurements.
Sixteen patients who underwent MMN reconstruction were included (7 female, 9 males; mean age 46.5 ± 20.6 (range 6–71)). The etiologies of facial paralysis included tumors (n=13), trauma (n=2), and structural neurovascular conflict (n=1). The surgical techniques used were nerve repair (n=1), nerve transfer using either the cervical branch of the facial nerve (n=4), ansa cervicalis nerve (n=4), or hypoglossal nerve (n=6), or vascularized nerve grafts (n=1). Further summary statistics are presented in Table 4. Surgical outcomes were evaluated according to the level of facial nerve injury. All the results and statistical analyses are shown in Figure 14. As shown in the figure, Level 3 injuries yielded the best outcomes, followed sequentially by Level 2 and Level 1 injuries, a trend consistent across all outcome measures. Statistically significant differences between injury levels were observed across all outcome variables except for the FaCE scale analysis, which yielded a p-value of 0.055. Post-hoc analysis examining the inter-level differences within the subgroups indicated that the most pronounced variations in outcomes were between levels 1 and 3. A post-hoc analysis was also conducted on the FaCE scale results because of the variable’s borderline significance, revealing a significant difference between Level 1 and Level 3 in these measures. In the photogrammetry analysis, a significant difference was observed between Levels 1 and 2.
Table 4. Descriptive statistics of patients included in **Paper VI**. “All Levels” include the full cohort (all patients from Levels 1, 2, and 3), and then each Level is presented separately.

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Summary statistics by level of injury</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Level of Injury</strong></td>
<td>All Levels</td>
</tr>
<tr>
<td>Number of patients</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
</tr>
<tr>
<td>Right Sided Paralysis</td>
<td>7</td>
</tr>
<tr>
<td>No Complications</td>
<td>10</td>
</tr>
<tr>
<td>Length of Follow-Up ± SD (Months)</td>
<td>27 ± 16</td>
</tr>
<tr>
<td>Age ± SD (Years)</td>
<td>46 ± 21</td>
</tr>
<tr>
<td><strong>Etiology</strong></td>
<td></td>
</tr>
<tr>
<td>- Tumor</td>
<td>12</td>
</tr>
<tr>
<td>- Trauma</td>
<td>2</td>
</tr>
<tr>
<td>- Structural</td>
<td>1</td>
</tr>
<tr>
<td><strong>Surgical method</strong></td>
<td></td>
</tr>
<tr>
<td>- Direct nerve repair</td>
<td>1</td>
</tr>
<tr>
<td>- Cervical branch</td>
<td>4</td>
</tr>
<tr>
<td>- Ansa cervicalis</td>
<td>1</td>
</tr>
<tr>
<td>- Hypoglossal</td>
<td>4</td>
</tr>
<tr>
<td>- Nerve graft</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 14. Boxplots of the outcome variable scores in Paper VI, based on the level of facial nerve injury (red: Level 1; blue: Level 2; green: Level 3), with each score marked by a circle. Note that the photogrammetry analysis represents the mean “asymmetry between facial halves”; therefore, lower scores are better. For all other scores, higher scores are better. P-values are shown below the name of each outcome parameter (underscored) with post-hoc subgroup analyses below (bold if significant). η²: effect size. Outliers marked with their corresponding patient ID.
Discussion

Peripheral facial paralysis can afflict previously healthy patients without discernible cause, and the sequelae can be conspicuous and life-altering. Despite significant progress in treatment alternatives over the last few decades, many patients are still left with nonfunctioning faces with a loss of physiological function and great psychological distress. The aim of the research performed in this thesis was to improve the outcomes after facial paralysis and hopefully lessen the lasting burden on these patients. Several findings of this work have the potential to positively impact outcomes after facial paralysis treatment. In this thesis, we have:

1. Shown that standard landmark-guided botulinum toxin injections to treat facial paralysis sequelae have a low accuracy, explaining the common complications and variable treatment results of today. We further demonstrated that ultrasound guidance significantly improves injection accuracy and can thus potentially improve safety and clinical outcomes.
2. Explored and presented a new local nerve transfer donor, the ansa cervicalis nerve, for selective reinnervation of the lower face, an area that has not been prioritized previously in facial reanimation due to a lack of donor nerves.
3. Presented new anatomical data on symmetry-restoring lower lip myectomies, which will hopefully reduce the common surgical failures of the procedure.
4. Demonstrated that ultrasound can be easily implemented as a preoperative evaluation tool to acquire important preoperative information. We showed that ultrasound examination of the lower lip depressor muscles might reduce surgical failures. Furthermore, we showed that platysma evaluation can reveal valuable information for both the diagnosis of platysma involvement in facial disfigurement and as an aid in the choice of treatment.
5. Proposed and implemented a novel classification system for facial nerve injuries to facilitate outcome comparisons between groups of patients with facial paralysis. We also proposed a treatment algorithm based on the classification of the level of facial nerve injury for reconstruction of the marginal mandibular nerve to reanimate the lower face.
The use of ultrasound in the management of facial paralysis sequelae

The use of bedside, provider-performed, ultrasound has grown exponentially over the last few years across many medical specialties due to technological developments leading to improved image quality, smaller and more portable devices, and reduced purchasing costs. However, these developments have not led to ultrasound being broadly adopted in plastic surgery. In the limited areas explored, ultrasound has nonetheless shown promise. Some authors have provided an overview of ultrasound’s current role in plastic surgery. For flap harvesting, ultrasound was shown to be able to supplement, or even supplant, the gold standard tools to map vascular trees used today, further corroborated by a recent randomized controlled trial. Ultrasound is used increasingly for lymph vessel mapping prior to LVA surgery. In the management of facial paralysis, basic uses of ultrasound are being explored. Quantification of muscles in facial paralysis patients was shown to be reliable. Evaluation of synkinesis in facial paralysis patients demonstrated that ultrasound could quantify DAO hyperkinesis by looking at the muscle thickness. The facial nerve has also been shown to be assessable and thus aid in the prognostication of Bell’s palsy patients. However, the comprehensive exploration of the potential benefits of ultrasound in the field of facial paralysis is still in its early stages.

Ultrasound probe choice and image resolution

Choosing the correct equipment and an optimal ultrasound probe is essential for the successful application of ultrasound in clinical practice. When using clinical ultrasound, resolutions are commonly divided into three categories: conventional, high, and ultrahigh. The boundaries between these categories are not universally agreed upon, but are generally defined as follows: conventional frequencies range from approximately to 2–12 MHz, high-resolution frequencies exceed 10–20 MHz, and frequencies exceeding 30 MHz are categorized as ultra-high-resolution ultrasound. Higher resolutions result in higher detail, but at the cost of lower penetration. High-resolution ultrasound can provide submillimeter resolutions, differentiating structures below 100 microns, while at the extreme end of ultra-high-frequency ultrasound, 70 MHz probes can differentiate structures down to 30 microns. The latter, however, limits the scanning depth to below 10 mm, thus restricting its clinical usefulness in many scenarios. An optimal balance between image resolution and penetration must be found for each application. In Papers I, IV, and V, a 22 MHz hockeystick ultrasound probe was used. We found that this probe provided sufficient detail to successfully visualize the facial structures with
high accuracy. When examining thicker muscles, such as the DAO, where high detail is not crucial, lower resolutions can also be adequate. However, we found that thinner muscles, such as the depressor labii inferioris, were not reliably visible using an 18 MHz probe, and the interconnections between the muscles of the face in Paper IV only became apparent when using the 22 MHz probe. Thus, for a detailed analysis of the facial anatomy, we recommend frequencies of 22 MHz or more. A hockeystick probe is preferred for the face because of its small footprint, allowing for an examination of difficult-to-access areas, such as visualizing the lacrimal gland in the eye socket. Because the structures of the face are shallow, higher resolutions can provide even more visual details. Future studies should assess the optimal resolution for evaluating the structures relevant to the management of facial paralysis.

Learning curves of clinician-performed ultrasound

Many studies have shown that the learning curves of clinical ultrasound use are manageable if the scope of learning is constrained to basic applications and simple questions, as is the goal of bedside, clinician-performed, ultrasound. Basic ultrasound applications, such as needle guidance for procedures, can be learned quickly by inexperienced users with only hours of training. Ultrasound-guided peripheral line placement, which is similar to ultrasound-guided botulinum toxin injections, requires between 5 to 25 observed placements to achieve basic competency. Surgeons without pre-existing ultrasound experience have been shown to, with one hour of ultrasound training, achieve a high-degree of accuracy in gallbladder ultrasound examinations when compared to radiologists. In the emergency department, surgeons generally perform well when using ultrasound to assess abdominal pain, leading to further clinical benefits, such as increased diagnostic accuracy, decreased admission frequency, fewer ordered tests, and an earlier decision regarding the need for surgery. The ultrasound procedures and examinations suggested for facial paralysis management in the current thesis can all be considered relatively simple. With targeted practice, plastic surgeons should be able to learn the relevant techniques in a relatively short amount of time.

Barriers to implementation of clinician-performed ultrasound

There are drawbacks and barriers to the clinical implementation and use of ultrasound. Apart from the required training, where busy surgical practices might impede the opportunity for learning, the required ultrasound equipment
can be expensive and space-consuming. Currently, however, the cost of small handheld ultrasound devices has decreased drastically, minimizing the effect of hardware acquisition on ultrasound implementation. Next, the application of ultrasound can increase the time needed to perform clinical assessments or treatments, such as botulinum toxin injections. With training, however, the time needed for ultrasound application decreases, and the use of clinician-performed ultrasound has been shown to save both time and money while simultaneously improving patient satisfaction. The drawbacks of using ultrasound need to be weighed against the potential benefits of each application.

Addressing the clinical challenges of botulinum toxin injections in the face

Regarding the use of ultrasound in the management of patients with facial paralysis, an important area that has been identified in need of attention was the complication rate and varying treatment effect and treatment duration of botulinum toxin injections for facial synkinesis and gustatory hyperlacrimation. The injections are currently performed using landmark and palpation-based techniques, and complications are common. Botulinum toxin injections into the lacrimal gland cause complications in 21% of the cases, ranging from ptosis, diplopia, rectus muscle dysfunction, conjunctivitis, and hematoma. In a double-blinded and placebo-controlled study, injections around the eye were shown to cause lagophthalmos in 53%, and exposure keratitis in 47% of the cases. Muscle injections for treating facial synkinesis can also lead to complications such as lagophthalmos, exposure keratitis, diplopia, ptosis, and oral incompetence. Troublingly, despite being transient and relatively benign, patients have declined further treatment because of these adverse outcomes. Even more serious complications have been reported when targeting peri-ocular muscles and structures, with retinal damage following unintended globe perforation and intra-ocular injections. Awareness of these complications, together with variances in treatment effect and duration, has led to an ongoing debate on whether the injections should be performed transcutaneously or transconjunctivally for optimal outcomes. The results have been conflicting, with no definitively preferred method. Treatment dosages have also been discussed, where a potentially increased accuracy in transconjunctival injections has been hypothesized to enable lower injection doses, which, in turn, can lead to fewer complications.

The use of ultrasound guidance when performing botulinum toxin injections in other fields has been shown to improve injection accuracy and treatment
results and reduce the risk of complications.\textsuperscript{136–142} To provide a definitive means to increase accuracy and reduce complications in the face, we compared landmark-guided and ultrasound-guided injections in a randomized setting and found clear differences between the groups in Study I. For example, in the lacrimal gland, we found that 38\% of the landmark-guided injections completely missed the target, whereas 92\% of the injections misplaced most of the ink in the surrounding tissue outside the intended target. The surprisingly low injection accuracy of landmark-guided injections may explain the clinically observed complication rates. In comparison, with ultrasound guidance, no injections missed the target, and only 38\% misplaced most of the ink outside the target (both with $p < 0.05$). Significant differences were also found when comparing injections into the facial muscles. With landmark guidance, 15\% of the injections into the depressor anguli oris and mentalis completely missed the target, whereas 54\% and 46\% of the injections, respectively, misplaced most of the ink outside the intended target. Using ultrasound, none of the injections missed the targets ($p = 0.480$ in both cases), and most of the ink was placed inside the intended target in all cases (both $p < 0.05$). Interestingly, when ultrasound guidance was used, all the ink was found inside the intended target in 100\% of the cases, with no ink found outside, compared to only 31\% in the landmark-guided group ($p < 0.05$), demonstrating significantly improved accuracy in the ultrasound group (Table 1). In addition to the generally poor accuracy when using landmark guidance, an important finding was that 23\% of the landmark-guided injections aimed at the depressor anguli oris muscle accidentally stained the facial artery (an example image from dissections is shown in Figure 15). Many landmark-guided DAO injections were aimed too far laterally. Many studies have assessed the anatomy of the DAO to improve botulinum toxin injections, and the main anatomical risk is often described as unintentional injection of adjacent muscles, such as the depressor labii inferioris, platysma, or risorius.\textsuperscript{143–146} The risk of vessel injection, as demonstrated in Paper I, has not been described previously. The increased accuracy provided by ultrasound will hopefully be translatable to a reduction in clinical complications and improved safety in the intended patient population, together with potentially more consistent treatment effects and duration.
Figure 15. Demonstration of one of the cases where the landmark-guided injection technique led to an accidental staining of the facial artery when aiming at the depressor anguli oris muscle in Paper I (right side). The injection was placed too far laterally.

Refining lower lip depressor myectomies to reduce surgical failures

In Papers III and IV, the lower lip depressor muscles were studied to elucidate the anatomical factors contributing to the failure rates of lower lip depressor myectomy. First, in Paper III, a literature search was performed regarding the surgical failure rate. The literature search revealed a surprisingly high rate of surgical failure (21%) (Table 3). It has been hypothesized that these failures could partially be attributed to the difficulty in delineating the lateral muscle fibers during surgery, leading to residual lateral muscle fibers and preserved muscle function. Therefore, the aim of Paper III was to describe the relevant anatomical features of the depressor muscles and provide further information on the location of their lateral borders to reduce surgical failure. Detailed anatomical descriptions and measurements of the lower lip depressor muscles and their surrounding anatomy were provided. We showed that the lateral border of the depressor anguli oris was located 54 ± 4 mm (50–60) and the depressor labii inferioris 32 ± 4 mm (25–35) from the midline of the mandible, which should be considered during surgery to ensure complete muscle resection. However, this study was not designed to elucidate the underlying causes of surgical failure, which is a conundrum that continues to
elude surgeons. Therefore, in Paper IV, the depressor anguli oris muscle (DAO) was revisited using high-resolution ultrasonography.

The aims of Paper IV were twofold: 1) to further explore the anatomical reasons for the common surgical failures in lower lip depressor myectomies using ultrasound and dissections and 2) to study the use of ultrasonography as a preoperative assessment tool to reduce failures in lower lip depressor myectomies. The application of high-resolution ultrasound allowed us to examine the lower lip area in detail, with structures undisturbed by dissection. First, the anatomical data in the larger cohort in Paper IV corroborated the measurements from Paper III regarding the required lateral resection distance for the DAO (the distance from the midline to the lateral DAO border was $52.3 \pm 5.4$ mm (44–64). Next, the exact relationship between the problematic lateral DAO border and the facial artery was examined, revealing that the facial artery bordered, or was covered by, the lateral border of the DAO in 60% of the cases (Figure 11 and Figure 16). Based on these observations, we hypothesize that surgeons may consider the facial artery as the lateral boundary of the surgical field to mitigate the risk of vessel injury. Given the complexity of delineating the lateral fibers because of the connective tissue in the area,\textsuperscript{30,57} this approach could result in incomplete resection with preservation of the lateral DAO fibers overlying the artery. Consequently, close proximity of the lateral DAO border to the facial artery may lead to residual muscle function and immediate surgical failure. In such cases, efforts should be made to include muscle fibers lying above and lateral to the facial artery during resection.
Figure 16. An example of an ultrasound image showing the lateral depressor anguli oris (DAO) muscle fibers covering the facial artery in a living person. This overlap might lead to inadequate resections and residual lateral muscle fibers and could thus be an explanation for the common surgical failures. The facial artery is visible with red/blue Doppler flow in the right part of the image. The DAO is hypoechoic (darker) and surrounded by hyperechoic (brighter) connective tissue, and the white crosses mark the medial and lateral DAO borders. The mandible can be observed as a sloping hyperechoic white line in the middle of the image.

Lastly, continuity between the muscle fibers of both the DAO and depressor labii inferioris with the surrounding muscles was common, especially in the latter (Figure 17). This continuity could lead to residual muscle function, despite adequate resection due to muscle function takeover.
Both phenomena (muscle fibers overlapping the artery and muscle continuity), which may contribute to surgical failure, can be identified through a concise, targeted preoperative ultrasound examination conducted by the surgeon. The 21% surgical failure rate highlighted in the literature review of Paper III encompassed both early and late failures.\textsuperscript{30,57,58} The findings of Paper IV have the potential to diminish the occurrence of early failures by enhancing the precision of muscle resections and facilitating alternative treatments for patients with pronounced muscle interconnections. However, they will not influence the incidence of late failures, which are likely attributed to scarring and tissue reconnection, requiring alternative management strategies.\textsuperscript{147}
Addressing the lack of tools for assessing the platysma muscle

In Paper V, high-resolution ultrasound was used to evaluate the platysma muscle. The rationale for this study was that no effective methods exist for evaluating the platysma muscle, apart from palpation and visual inspection. The muscle can be difficult to assess because of its thinness, especially in older patients with platysma sarcopenia. Furthermore, the thin fibers of the platysma inserting into the face can give rise to midface synkinesis; however, there is no way to determine whether the platysma is involved in midface synkinesis, apart from treating the condition using botulinum toxin or myectomy and evaluating the outcome. In this study, high-resolution ultrasound was applied to determine its usefulness in anatomical mapping of the muscle in an elderly cohort, both in the neck and midface.

The dissections showed that the platysma inserted at 2.9 ± 1.0 cm above the mandibular angle, corroborating the scarce literature available on the facial portion of the platysma muscle, stating that the muscle inserts higher up in the face than previously believed. High-resolution 22 MHz ultrasound could trace the platysma muscle in the neck in most hemifaces (34/38) and could follow the muscle fibers in the face up to 2.1 ± 0.8 cm above the mandibular angle before disappearing (Figure 18). Thus, the platysma was easily visualized in the neck, indicating that ultrasound can be used to assess the platysma thickness, diagnose, and quantify hyperkinesis and hypertrophy, and guide botulinum toxin injections in this area. All the four hemifaces where the platysma was not detected had significant sarcopenia (two cadavers aged 85 and 100 years at the time of death).

Regarding the midface insertion height, an important question arises: Does the 0.8 cm mean discrepancy between the ultrasound and the dissection measurements indicate that high-resolution ultrasound is unsuitable for evaluating the facial portion of the platysma muscle? Such a conclusion is likely premature. As mentioned previously, the fine fibers tapering into the face become increasingly difficult to locate using ultrasound, as they become thinner cranially (demonstrated in Figure 18). In the current study, an elderly cohort was examined, with a mean age of 83.5 ± 8.7 years (range 67–100 years) and with sarcopenia of several muscles in the face, including the platysma muscle, further complicating the assessment. Despite these circumstances, high-resolution ultrasound could visualize an average of 72% of the facial portion of the platysma. In comparison, the platysma is often hypertrophied on the affected side in patients with platysma synkinesis. The thicker muscles in these patients, as well as in younger patients without sarcopenia, should be more easily assessed using ultrasound than the thin muscles of the cohort in Paper V. An
even more accurate assessment is expected in these patients; however, clinical studies are needed to confirm this.

Figure 18. A comparison of the sonographic visualization of the platysma muscle in Paper V. In the face (top image), where it is thinner, and, in the neck (bottom image), where it is thicker and more easily visualized. In the face, in the top image, the platysma is 0.5 millimeters thick caudally and lies at a depth of 5 mm, while the cranial part above the mandible is approximately 0.25 millimeters thick, tapering into the face. In the bottom image of the neck, the platysma muscle is thicker and easier to visualize. In the bottom image, the muscle was approximately 1 mm thick, lying at a depth of 5 mm.
Exploration of a novel nerve donor for selective reanimation of the lower face

In Paper II, we showed for the first time that the use of the ansa cervicalis nerve (ACN) as a selective donor for the marginal mandibular branch of the facial nerve (MMN) is anatomically feasible. This procedure was demonstrated in a case report that presented an achievable outcome. A literature search showed that some studies have reported the use of ACN to reinnervate the facial nerve. In these studies, ACN has only been used for reinnervation of the main facial nerve trunk, whereas selective reinnervation of single branches of the facial nerve has not been reported (demonstrated by the column marked in gray in Table 5). The results of these studies have been inconsistent,\textsuperscript{149–155} which explains why the nerve donor has not been clinically adopted. A likely explanation for the unpredictable results is the substantial size mismatch between the ACN and the main facial nerve trunk, with the ACN providing only 20\% of the fascicular surface area of the proposed recipient\textsuperscript{156} and a diameter difference of 1:3.\textsuperscript{152} Doubts have been raised as to whether the ACN can provide enough axons for the trunk, which has an axon count of 6,000–7,000.\textsuperscript{157–161} However, the use of ACN to reinnervate a single branch of the facial nerve, such as the MMN with an axon count of 1,603 ± 849,\textsuperscript{65} should be consistently reliable.

In Paper II, we showed that the gross diameters of the ACN and MMN matched (Figure 8). A central discovery was that tension-free coaptation of ACN with MMN was possible in only 67\% of the hemifaces. In the remaining 33\%, a clinically relevant anatomical variant termed “short ansa,” in which the ACN nerve is shorter than usual,\textsuperscript{162,163} precluded tension-free coaptation to the MMN. In this study, none of the “short ansa” cases reached the MMN without tension (Table 2). Therefore, solutions were explored. ENT surgeons have used the nerve branches from the ACN to the infrahyoid muscles to extend the reach of the ACN when transferring it to the recurrent laryngeal nerve.\textsuperscript{163–166} When applying this technique to the selective ACN-MMN transfer, an additional 62 ± 15 mm was gained, and all “short ansa” ACN donors reached the MMN without tension. Thus, this workaround overcomes the lack of length in the subgroup, enabling the use of ACN-MMN transfer in a broad group of patients.
Table 5. An overview of the literature search from Paper II, presenting studies in which the ansa cervicalis nerve was used for reinnervation of the facial nerve. The third column, marked in gray, shows that no studies exist on selective innervation of the peripheral facial nerve branches, only on reinnervation of the entire facial nerve trunk. The outcome varied, as demonstrated in the last column to the right.

<table>
<thead>
<tr>
<th>Authors and country</th>
<th>Journal</th>
<th>Recipient nerve for ansa cervicalis</th>
<th>Anatomical study?</th>
<th>Number of clinical cases</th>
<th>Additional procedures?</th>
<th>Follow-up (months)</th>
<th>Reported outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vejbrink Kildal et al 2022 (Sweden)</td>
<td>Current study</td>
<td>Marginal mandibular nerve (selective)</td>
<td>Yes</td>
<td>1</td>
<td>Yes</td>
<td>12</td>
<td>Improvement in photogrammetry and subjective evaluation.</td>
</tr>
<tr>
<td>Liang et al 2015 (China)</td>
<td>Zhonghua Yi Xue Za Zhi</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>14</td>
<td>NA</td>
<td>Mean 24.6</td>
<td>91.7% HB II-IV</td>
</tr>
<tr>
<td>Leong et al 2013 (UK)</td>
<td>Annals of Otology, Rhinology &amp; Laryngology</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>9</td>
<td>Yes</td>
<td>At least 24</td>
<td>Best achieved: 44% HB IV</td>
</tr>
<tr>
<td>Gidley et al 2010 (USA)</td>
<td>The Laryngoscope</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>1</td>
<td>Yes</td>
<td>NA</td>
<td>Best achieved: HB VI</td>
</tr>
<tr>
<td>Schipper et al 2005 (Germany)</td>
<td>Der Chirurg</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>8</td>
<td>NA</td>
<td>Mean 16.5 (5-28)</td>
<td>50% HB II 25% HB III 25% HB IV</td>
</tr>
<tr>
<td>Arndt et al 2004 (Germany)</td>
<td>Otolaryngology - Head and Neck surgery (poster abstract)</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>11</td>
<td>Yes</td>
<td>At least 12</td>
<td>55% HB II 36% HB III 9% HB IV</td>
</tr>
<tr>
<td>Conley et al 1979 (USA)</td>
<td>Plastic &amp; Reconstructive Surgery</td>
<td>Facial nerve trunk</td>
<td>No</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>‘... uniformly required additional procedures to support the face’</td>
</tr>
</tbody>
</table>

In the clinical case report, an improved tone of the lower lip was evident after MMN reconstruction, resulting in improved symmetry in the resting state and during movement, with clinically verified contraction of both the depressor anguli oris and the depressor labii inferioris (Figure 9). Swallowing was not necessary to achieve this, indicating that the lower lip depression was released from the previous ACN function. The postoperative course was uneventful, and no donor site morbidity or clinically significant complications were observed.

Based on this combined anatomical and clinical study, we believe that selective ACN transfer to the distal facial nerve branches expands the available options for the reanimation of the lower face. In retrospective Paper VI, additional patients who underwent ACN-MMN transfer were included and analyzed.
A management algorithm for marginal mandibular nerve reconstruction based on a novel classification of facial nerve injuries

A significant challenge facing facial paralysis surgeons is individual patient variability, which makes the choice of the optimal treatment method demanding. Factors such as the etiology of paralysis, location of the nerve injury, impact of the resection area on the availability of surrounding local donor nerves, the quality of the wound bed (which is important to consider both at the time of surgery and for potential future radiotherapy), age, and patient preference all influence the available operations. We hypothesized that these factors, intrinsic to the clinical scenario, rather than the surgical reconstruction method alone, would influence the surgical outcomes after MMN reconstruction. For instance, in patients with complete unilateral facial paralysis, MMN reconstruction may yield poorer outcomes due to potentially longer nerve gaps, more intricate reconstructions, and a greater focus on rehabilitation directed toward the prioritized upper and midface, rather than lower lip movement. Therefore, comparisons of surgical outcomes based solely on reconstruction method may be misleading.

Currently, there are no established systems for the classification of facial nerve injuries. A classification system for facial nerve injuries would allow clinicians to stratify patients with various types of facial paralysis into uniform groups. This could facilitate surgical planning regarding treatment choices and enable more robust comparisons of outcomes between and within the distinct groups. Therefore, in Paper VI, a new, simple, three-level classification system for facial nerve injuries was explored (Figure 6) and applied to a subgroup of patients who had undergone MMN reconstruction in a retrospective study. The goal was to study whether differences in surgical outcomes after MMN reconstruction could be detected, depending on the level of facial nerve injury. Another goal was to determine whether the classification system could be used to stratify patients with facial paralysis to facilitate the choice of treatment and provide natural groups where outcome comparisons could be performed.

Analysis of the surgical outcomes based on the level of facial nerve injury revealed clear and consistent differences between the levels, as shown in Figure 14. A pattern was seen across all outcome parameters, where distal Level 3 injuries resulted in the best outcomes, whereas the worst outcomes were consistently seen in proximal Level 1 injuries. These differences were statistically significant for all parameters, except for the quality-of-life FaCE scale, with a p-value of 0.055. The clinical outcomes across the different injury levels are shown in Figure 19.
The worse outcomes in the quality-of-life assessors’ FaCE and FDI in Level 1 injuries compared to more distal Level 2 and 3 injuries can probably be explained by the more extensive facial paralysis in Level 1 patients. Optimal surgical outcomes can be difficult to achieve in patients with complete hemifacial paralysis who undergo more complicated reconstructions, resulting in lower patient satisfaction and quality of life. The same reasoning can be applied to the Sunnybrook assessment. The scale evaluates the whole facial function, including facial synkinesis. Thus, worse scores can be expected in proximal injuries with complete hemifacial paralysis, which was also observed in our study. Interestingly, in all the quality-of-life measures, the Level 1 group had clearly worse scores, while the scores of patients with Level 2 and 3 injuries were similar. In comparison, in the Sunnybrook assessment, Levels 1 and 2 seemed more closely related. This indicates that patients with Level 2 injuries have a higher quality of life, resembling that of Level 3 patients, despite having Sunnybrook scores that are similar to those of Level 1 patients. The lower lip-specific parameters of Terzis’ lower lip grading scale and the lower lip parameters of the photogrammetry evaluation more closely resemble the score differences of the quality-of-life scales. Thus, lower lip function after
MMN reconstruction in the current study showed better function and less asymmetry with more distal facial nerve injury.

**Paper VI** demonstrated the difficulty of comparing the surgical outcomes of patients undergoing the same surgical reconstruction method but with different initial clinical scenarios and levels of injury. For example, patients who underwent hypoglossal to MMN transfer in the Level 2 group achieved consistently better outcomes than those who underwent the same procedure in the Level 1 group (See Table 2 in **Paper VI**). The proposed classification system could be useful for stratifying patients with facial paralysis and should enable more robust comparisons of surgical outcomes in patients with facial paralysis by comparing homogenous patient subgroups. Based on the delineating differences in surgical outcomes between the injury levels and differences in the available donor nerves, a management algorithm for MMN reconstruction was proposed based on the new classification system (Figure 20). This algorithm can facilitate the selection of an optimal reconstruction method in complex cases involving lower lip paralysis.
Figure 20. Proposed management algorithm for the reconstruction of the marginal mandibular branch of the facial nerve in Paper VI. Start by assessing the level of facial nerve injury and the availability of the proximal MMN stump to determine the possibility of direct nerve repair. The algorithm can then be used to determine the available options for MMN reconstruction. Available donor nerves are shown in the top-right corner.
Limitations

Cadaveric studies

Cadaveric studies have several inherent limitations stemming from their methodology and the nature of the study subjects. A primary concern is the lack of diversity within cadaveric donor populations. **Papers I–V** included a predominantly elderly cohort, which raises questions about the generalizability of the findings to other, more diverse populations, given that the anatomical structures can undergo age-related changes. The unknown medical history of the body donors might also potentially influence the anatomical assessments. Furthermore, the geographical spread of the cohort was probably limited, as potential body donors are generally found locally, which leads to challenges in extrapolating the conclusions to a more diverse population.

Another significant limitation of cadaveric studies is that the anatomy of body donors may not necessarily reflect that of living tissue, depending on the preservation method used. In **Papers I–V**, cadavers were preserved using the fresh-frozen method, which minimizes alterations to anatomical structures. However, the lack of tissue movement, dynamic muscle evaluations, and pulsatile blood vessels makes physical examination more challenging. This might have led to a lower injection accuracy in the landmark-guided group in **Paper I**, increasing the difference between cohorts. However, the same factors also complicate ultrasound imaging; for example, vessel examinations are significantly facilitated using Doppler imaging. Importantly, palpating the lacrimal gland in a live patient is similar to palpation in cadavers, and the lacrimal gland injections in **Paper I** are, therefore, likely to be comparable to injections in live patients. This is noteworthy, as the biggest difference in injection accuracy in **Paper I** was observed in this target.

Another limitation is the measurement bias introduced when measuring distances using measuring tape and rulers. To reduce this effect, all measurements were performed by two examiners who agreed to the exactness of each measurement. Ideally, all measurements should have been performed multiple times by different examiners and averaged.

The minimum sample size required for descriptive anatomical studies is not universally agreed upon. In **Paper III**, only 5 cadavers were included, which
can be considered a rather small cohort. In Paper IV, apart from the ultrasound assessments, the same measurements were repeated in a further 19 cadavers with similar results, thus corroborating the results of Paper III.

Ultrasound studies

The ultrasound studies have their own set of limitations that should be acknowledged. In Papers I, IV, and V, a radiologist conducted the ultrasound injections and assessments to establish a gold-standard result. However, it is important to note that the ultimate goal of these studies is for plastic surgeons to perform the ultrasound-guided procedures themselves. Therefore, the use of a radiologist in these studies does not answer the critical question of whether plastic surgeons can achieve similar levels of accuracy. In Paper I, the use of an expert radiologist performing the ultrasound-guided injections potentially led to a higher injection accuracy than if they had been performed by plastic surgeons. To assess the applicability of ultrasound guidance in the hands of plastic surgeons, future studies should focus on evaluating the learning curves for plastic surgeons in adopting this technique.

In Paper I, operator bias might have influenced the results, as a single operator performed the ultrasound-guided injections, while another performed the landmark-guided injections. Ideally, several operators with different surgical experience levels would have participated, with the same operator first performing the landmark-guided injections unilaterally, and then applying ultrasound for injections in the contralateral hemiface. In Papers IV and V, ultrasound and dissection measurements were compared. Tissue movement following the dissections, exacerbated by looser skin in older individuals, might have amplified the differences between these measurements and thus confounded the results.

A strength of Paper I is the partially blinded nature of the study, in which the hemifaces were first randomized to the injection technique. Measures were then taken to blind the individuals performing injections, dissections, and assessments to the injection technique. However, owing to a lack of skilled dissectors and time constraints, the same plastic surgeon who performed the landmark-guided injections also performed some of the dissections of the hemifaces. Several measures were taken to limit the possibility of this individual recognizing the hemifaces (covering parts of the face to avoid recognition, waiting 24 hours before dissections to reduce the chance of remembering, randomizing the order of dissections, the plastic surgeon only performing some of the dissections, while the majority were performed by other, truly blinded examiners). Unfortunately, because of this, the study was not fully blinded.
Retrospective study with a small sample size

The case study in Paper VI had limitations inherent to retrospective studies. Furthermore, the small sample size is problematic, especially when studying specific injury levels, with some cases only represented by a small number of patients and surgical methods. However, the significant differences in outcome among the injury levels ensured statistically significant results for most outcome parameters. Future studies should determine the influence of the injury level on surgical outcomes in a larger population with facial paralysis. As this was a retrospective study, the patients were not randomized to the received treatments. This might result in allocation bias, in which patient characteristics may differ between injury level groups because of the underlying factors. As two patients were excluded due to death and thus a lack of analyzable photo and video material, survivorship bias may also have influenced the results.

Subjectivity in outcome assessment after facial reanimation

One of the challenges in evaluating the outcomes of facial paralysis surgery is the subjectivity of the assessment process. This subjectivity may have impacted the findings presented in Papers II and VI. Subjective assessment tools are already abundant, while novel tools for objective assessment are continuously being proposed. Photogrammetry is used to analyze facial asymmetry, machine learning is used to mark the facial features automatically, and 3D-cameras are used to visualize the patient’s features completely. However, a common drawback in many of these objective tools is that they often rely on subjective decisions made prior to each objective assessment. For instance, the choice of which image to analyze can significantly influence the perceived outcome. Additionally, the instructions given by the photographer to the patient, as well as the patient’s understanding of these instructions and their incentive to cooperate, can influence the facial movements captured in the images or recordings. During successive follow-up appointments, it is challenging for patients to activate the same specific facial muscles or expressions repeatedly in a consistent manner. Especially when these visits are spaced several months apart, patients may unintentionally convey a different facial expression during a follow-up session compared with their previous visit. In situations where there appears to be a perceived deterioration in a patient’s clinical progress when relying on objective assessment tools, this can frequently be attributed to one of these factors. These concerns underscore the pressing need within the field for standardized and genuinely objective tools that can be employed to evaluate outcomes following facial reanimation.
procedures. Establishing such tools would contribute to a more accurate and unbiased assessment of patient outcomes and surgical results.\textsuperscript{169,170}
Conclusions and future directions

This thesis has contributed to the field of facial paralysis and its associated sequelae, with a particular emphasis on the lower facial region. This was accomplished by exploring novel approaches to the treatment, diagnosis, and classification of facial nerve injuries. The key findings and conclusions drawn from the papers included in this thesis are summarized as follows, together with suggested directions for future research.

Paper I

This randomized anatomical study showed that the current landmark-guided injections in facial structures have low injection accuracy, potentially explaining the high complication rates described in the literature. Ultrasound guidance significantly increased the injection accuracy and reduced the amount of injectate lost to the surrounding tissue, which might result in fewer complications clinically. Randomized, controlled, and properly blinded clinical trials in patients with facial paralysis sequelae, with plastic surgeons performing the injections, are needed to explore the effects of ultrasound guidance on complications, treatment outcomes, and treatment duration.

Paper II

The selective transfer of the ansa cervicalis nerve to reinnervate the marginal mandibular nerve is anatomically feasible. A modification of the surgical technique was required to achieve tension-free coaptation between the nerves in 33% of the cases, where a clinically relevant anatomical variant of the ansa cervicalis nerve was found (“short ansa”). A clinical case report was presented in which a patient underwent the proposed transfer with significant improvements in the symmetry and function of the lower lip. Further studies are required to confirm the reliability and success rate of the proposed technique in terms of symmetry, function, and spontaneity. Comparison of the results between patients with and without the surgical modification used in “short ansa” cases should be performed. Furthermore, potential donor site morbidity should be studied in patients with facial paralysis without pre-existing vocal sequelae,
as earlier ENT studies only involved patients with preceding laryngeal nerve injury.

**Paper III**
Detailed measurements of the anatomy of the lower lip depressor muscles were presented to reduce the common surgical failures in lower lip depressor myectomies. As the lateral borders of the depressor muscles can be difficult to define during surgery, the presented measurements should be considered in order to ensure the resection of the full lateral width of the muscles.

**Paper IV**
Preoperative high-resolution ultrasonographic assessment of the depressor anguli oris can provide important anatomical information prior to myectomy. Two potential explanations for the common surgical failures were proposed. Overlap of the lateral depressor anguli oris muscle fibers over the facial artery might prevent surgeons from extending the muscle resection to the full lateral length of the muscle. Muscle fiber continuity between the depressor anguli oris and the surrounding muscles might uphold muscle function despite adequate depressor resection. Ultrasound can reveal both preoperatively, which may allow for the modification of the surgical plan to reduce common surgical failures. Future clinical studies should be performed to assess the feasibility and clinical value of preoperative ultrasound prior to the procedure.

**Paper V**
This study provides information on the possibility of assessing the platysma muscle using high-resolution ultrasound, demonstrating that it can be assessed in both the neck and midface in elderly patients, despite sarcopenia in older individuals. Thus, the use of high-resolution ultrasound for diagnostic and treatment purposes of the platysma muscle in patients with facial paralysis can be further explored. Clinical studies should be carried out in which high-resolution ultrasound is used to assess the platysma thickness and guide botulinum toxin injections in the neck to explore the clinical benefits in the diagnosis, prognostication, and treatment outcomes. Furthermore, the cranial facial insertion height should be determined in patients with midface synkinesis to assess both the degree of midface synkinesis and the effect of synkinesis treatment, depending on the insertion height.
Paper VI

The surgical outcome after marginal mandibular nerve reconstruction was significantly influenced by the level of the facial nerve injury. Thus, the proposed classification system effectively stratified patients with facial paralysis, allowing for more robust comparisons of the surgical outcomes within distinct groups. A management algorithm for marginal mandibular nerve reconstruction based on the proposed classification system was presented, offering clinicians a structured approach to manage marginal mandibular nerve paralysis. Additional studies, preferably multicenter and involving larger cohorts, should be conducted to further validate and evaluate the proposed classification system and management algorithm.
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To begin with, I would like to thank the patients who took part in the research studies forming this thesis. Your involvement and shared experiences have been crucial. I also extend my deepest gratitude to those who have selflessly donated their bodies to science, without whom the work within would not have been possible. I genuinely hope that the contributions of this thesis will positively impact current and future patients.

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