Department of Clinical Sciences, 2026

Upper airway stenosis

In relation to surgical technique, causation, and impact on quality of life

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Table of Contents

Abstract ... 3
Abbreviations ... 5
Thesis at glance ... 6
List of papers ... 7
Enkel sammanfattning på svenska ... 8
1. Introduction / Background ... 11
1.1 Upper airway stenosis and dyspnoea ... 11
1.2 Patient-reported outcome measures (PROMs) ... 12
1.3 Subglottic stenosis ... 13
1.3.1 Pathogenesis ... 15
1.3.2 Epidemiology and diagnosis ... 17
1.3.3 Treatment options and prognosis ... 18
2. Purpose and aims ... 20
2.1 Purpose ... 20
2.2 Aims ... 20
3. Material and methods ... 21
3.1 Study I ... 21
3.1.1 Participants and eligibility ... 21
3.1.2 Surgical procedures ... 21
3.1.3 Outcome measure ... 22
3.1.4 Statistical analysis ... 22
3.2 Study II ... 23
3.2.1 Participants and eligibility ... 23
3.2.2 Diagnostic confirmation ... 24
3.2.3 Surgical procedure ... 24
3.2.4 Patient-reported outcome measures ... 24
3.2.5 Statistical analysis ... 24
3.3 Study III ... 25
3.3.1 Participants and eligibility ... 25
3.3.2 Biopsy procedure ... 25
3.3.3 Nucleic acid extraction ... 26
3.3.4 Whole-genome sequencing (WGS) ... 26
3.3.5 Bioinformatic workflow ... 26
3.3.6 Comparative material ... 26
3.3.7 Statistical approach ... 28
3.4 Study IV ... 28
3.4.1 Study population and data sources ... 28
3.4.2 Definitions and classifications ... 29
3.4.3 Data extraction ... 29
3.4.4 Statistical analysis ... 30
4. Ethical considerations ... 31
5. Results ... 33

5.1 Study I 33
5.2 Study II 35
5.3 Study III 36
5.4 Study IV 36
6. Discussion 38
6.1 Principal findings of the thesis 38
6.2 Diagnostic challenges and disease definition 39
6.3 Surgical management and long-term outcomes 41
6.4 Patient reported outcomes and the lived experience 42
6.5 Biological considerations and disease persistence 42
6.6 Integrating the findings – a conceptual model 43
6.7 Clinical implications 44
6.8 Strength and limitations 45
6.9 Future directions 47
7 Conclusions and relevance 48
8 Acknowledgements 49
9 AI-Declaration 51
References 52

Abstract

Background

Subglottic stenosis (SGS) is a rare fibroinflammatory airway disorder characterized by progressive narrowing of the subglottic lumen. The condition predominantly affects middle-aged women and is associated with substantial morbidity, impaired health-related quality of life (HRQoL) and recurrent need for surgical intervention. Despite increasing clinical recognition, the pathogenesis of SGS remains incompletely understood.

Objective

To improve the understanding and clinical management of SGS through epidemiological characterization, evaluation of endoscopic treatment outcomes, assessment of patient-reported outcomes, and exploratory whole-genome sequencing (WGS) of stenotic tissue.

Methods

Four complementary studies were performed. Two retrospective cohort studies evaluated patients with subglottic and laryngotracheal stenosis in northern Sweden. Endoscopic treatment outcomes following balloon dilatation and CO_2 laser surgery were compared using surgery-free interval (SFI) as the primary endpoint. A prospective cohort study assessed symptom burden and HRQoL before and after balloon dilatation using the Dyspnea Index (DI) and Short Form Health Survey-36 (SF-36). In a second prospective cohort, stenotic tissue underwent high-depth shotgun WGS with bioinformatic taxonomic classification and sequence validation.

Results

Balloon dilatation and CO_2 laser surgery demonstrated comparable long-term SFI, although balloon dilatation showed a transient early advantage. SGS was associated with marked impairment in HRQoL, with significant postoperative improvements in DI and multiple SF-36 domains. WGS identified consistent enrichment of *Cutibacterium Acnes* within stenotic tissue, suggesting a potential role in disease-associated inflammatory and fibrotic processes. In the broader laryngotracheal

stenosis cohort, unilateral vocal fold immobility was the most prevalent underlying diagnosis.

Conclusions

SGS remains a clinically challenging disorder with an incompletely defined pathogenesis. These studies provide novel insights into disease epidemiology, treatment outcomes, patient experience, and potential biological mechanisms. The findings support the integration of validated patient-reported outcome measures and molecular characterization into future SGS research and clinical practice.

Abbreviations

SGS	Subglottic Stenosis
ENT	Ear Nose and Throat
iSGS	Idiopathic Subglottic Stenosis
LTS	LaryngoTracheal Stenosis
PROM	Patient Reported Outcome Measure
COPD	Chronic Obstructive Pulmonary Disease
EMT	Epithelial-Mesenchymal Transition
ER	Estrogen Receptor
CTR	CricoTracheal Resection
LP	Laryngoplasty
SFI	Surgery Free Interval
WGS	Whole Genome Sequencing
EDI	Expiratory Disproportion index
DI	Dyspnea Index
SF-36	Short Form Health Survey 36
HRQoL	Health-Related Quality of Life
LTS	LaryngoTracheal Stenosis
UAS	Upper Airway Stenosis

Thesis at glance

	Study I	Study II	Study III	Study IV
Design	Retrospective observational, analytic case series	Descriptive Prospective cohort	Descriptive Prospective cohort	Descriptive Retrospective cohort
Disease studied	Subglottic stenosis	Subglottic stenosis	Subglottic stenosis	Upper airway stenosis
Sample collection	Consecutive	Consecutive	Consecutive	Consecutive
Timeframe	Jan. 1999-Dec. 2021	Sept. 2020-Aug 2023	Sept. 2020-Aug. 2023	Jan. 1999 Dec. 2019
Eligible	141	40	39	1441
Included	63	33	34	1071
Females	49 (78%)	30 (91%)	30 (88%)	447 (42%)
Males	14 (22%)	3 (9%)	4 (12%)	624 (58%)
Age (mean)	60.1	44.3	44.3	59.1
Year of publication	2023	2024	2026	2025
Results	No long-term difference in surgery-free intervals between balloon dilatation and CO_2 laser	Balloon dilatation resulted in significant postoperative improvements in patient-reported outcomes, as assessed by validated PROMs	Consistent enrichment of *Cutibacterium acnes* in stenotic tissue	Unilateral vocal fold paralysis is the most prevalent cause of upper airway stenosis

List of papers

This thesis is based on the four following papers, referred to in the text by their Roman numerals.

I. **Erlandsson A**, Werner M, Holm A, Schindele A, Olofsson K. Balloon dilatation versus CO_2 laser surgery in subglottic stenosis, a retrospective analysis of therapeutic approaches. *Acta Otolaryngol.* 2023;143(6):528-535. doi:10.1080/00016489.2023.2222756

II. **Erlandsson A**, Lundquist A, Olofsson K. Mapping quality of life after balloon dilatation in subglottic stenosis using Dyspnea index and Short Form Health Survey-36. *Eur Arch Otorhinolaryngol.* 2024;281(7):3701-3706. doi:10.1007/s00405-024-08667-0.

III. **Erlandsson A***, **Chorell S***, Tuiskunen Bäck A, Köhn L, Haney M, Olofsson K. Whole genome sequencing reveals a specific microbiota in subglottic stenosis - C. Acnes may contribute to inflammation. *Eur Arch Otorhinolaryngol* (2026). https://doi.org/10.1007/s00405-026-10630-0

IV. **Erlandsson A***, **Lundahl M***, Holm A, Olofsson K. A descriptive study on laryngotracheal stenosis in the Northern Sweden. *Acta Otolaryngol.* 2025;145(8):709-713. doi:10.1080/00016489.2025.2503000.

*Shared first authorship

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Enkel sammanfattning på svenska

Tänk dig att du är en medelålders kvinna som får allt svårare att andas trots den astmamedicin din hälsocentral har förskrivit. Du tycker det är konstigt att inget hjälper mot den fysiska och psykiska utmattningen du upplever. Du har blivit stresskänslig över din lufthunger, dåliga sömn och oförmåga att prestera på jobbet, i din familj och med dina vänner. Du får slutligen gehör för dina funderingar hos en uppmärksam distriktsläkare som ordnar med en remiss till öron-, näsa-, och halskliniken. Efter en snabb undersökning får du veta att du har en förträngning precis under stämbanden. Din luftväg är nu 7 x 7 mm på platsen för förträngningen, men borde egentligen vara 12 x 12 mm. Denna förträngning benämns subglottisk stenos.

Subglottisk stenos (SGS) är en förträngning av luftvägen precis nedom stämbanden i struphuvudet. Stenosen ger både in-, och utandningssvårigheter, mödosamma hostningar och ibland röstpåverkan. Stenosen kan imitera andra förträngningar längre ner i luftvägen ex. astma. Nittiofem procent av patienterna är kvinnor och sjukdomen orsakar 0.49 fall / 100 000 individer och år. Det spekuleras om att våldsamma och långdragna intubationer i luftvägen vid operation, sura uppstötningar och systemsjukdomar kan bidra till utveckling av SGS. Det finns en undergrupp som benämns idiopatisk (okänd orsak) SGS. Detta avhandlingsarbete vill föreslå en större tillämpning av den terminologin då vi i dagsläget saknar kunskap om orsak till SGS, diagnostiska algoritmer och därmed grund för indelning i undergrupper. Detta återspeglas i svårigheter att rapportera hur utbredd SGS är inom en population vid en specifik tidpunkt och hur stor andelen är av nya SGS fall i befolkningen under en specifik tidsperiod. Vårt kunskapsgap kring orsak återspeglas också genom den stora variationen i behandlingsarsenal som finns att tillgå. Den vanligaste behandlingsmetoden är endoskopisk d.v.s. operationen sker i halsen och inte genom ingrepp utifrån genom huden. Den endoskopiska kirurgin består vanligen av en ballongvidgning samt en injektion av kortison i det trånga området. Denna teknik utgör minst risk för skada på stämbanden, rösten och luftvägen men kräver oftast upprepad behandling. Den öppna kirurgiska teknik där operatören genomgår huden på halsen och lyfter ut

det trånga luftrörspartiet innebär längre vårdtid, och större risker att permanent påverka röst men behöver sällan upprepas. Idag finns studier som visar att andelen kirurgiska behandlingar minskar med sjukdomstid, vilket kan tolkas som en minskad sjukdomsaktivitet i framtiden för patienten och därmed blir ett val av kirurgisk metod med så låg risk som möjligt att föredra vid behandling av okomplicerade mjuka SGS.

Avhandlingens övergripande syfte är studera orsak till subglottisk stenos, utvärdera tillståndets påverkan på livskvalitet och behandlingseffekt vid ballongvidgning samt att förbättra diagnostik av subglottisk stenos.

Vi har inte påvisat någon skillnad i antal behandlingar vid 10 års uppföljning då vi jämfört två olika behandlingsalternativ: ballongvidgning och CO_2 laser som användes under två olika tidsperioder. I en studie på 34 fall har vi publicerat data om signifikant låg livskvalitet innan operation och hög livskvalitet efter ballongvidgning med hjälp av livskvalitetsformulär. På samma patientmaterial analyserade vi färskfryst vävnad med hjälp av helgenomsekvenseringsteknik (WGS). Det innebär att vi läser av en organisms hela DNA-sekvens (hela genomet) och kan på så vis kartlägga vilka virus, bakterier och svampar som finns i SGS. Metoden ger en enorm mängd data som kräver avancerad analys och tolkningskompetens. Vi hittade DNA-genomet för *Cutibacterium acnes* i 28/34 prover. Denna bakterie är associerad med ärrbildning och fibros i hud, akne, bröstimplantatskirurgi och viss ortopedisk kirurgi. Vi rapporterar också att den vanligaste orsaken till högt andningshinder är godartad och ligger i stämbandsnivå så kallad ensidigt stillastående stämband.

Genom att kartlägga sambandet mellan subglottisk stenos (SGS) och bakterier som drivande faktorer till den inflammation och ärrbildning som karakteriserar tillståndet, kanske vi kan modifiera behandlingen. Idag behandlas patienterna med luftrörsvidgande kirurgi, i framtiden kanske vi kan erbjuda tilläggsbehandling. Den ökade kunskapen om patienternas sänkta livskvalitet och momentana effekt av den

endoskopiska vidgningen av luftvägen utgör viktig information till patienten, en del av vårdsäkerheten samt bidrar med utmärkt underlag för fortsatta studier. Kunskapen om att nedsatt stämbandsrörlighet utgör den vanligaste orsaken till högt andningshinder förväntas minska diagnostiska fel kring högt andningshinder.

Vi behöver adressera detta forskningsområde utifrån ett omfattande personligt lidande, höga vårdkostnader och som ett tack till våra forskningspersoner som deltar till fördel för andra än sig själva.

1. Introduction / Background

1.1 Upper airway stenosis and dyspnoea

The inability to breathe properly is a severe disabling symptom with high impact on quality of life. Dyspnoea can be caused by diseases in the cardiovascular system, lungs and the upper as well as the lower airway and is among the most anxiety-provoking symptoms encountered in medicine, as it is directly perceived as a threat to survival. Unlike pain, which may be localized or intermittent, the sensation of impaired breathing is immediate and unavoidable, often eliciting a strong emotional response in affected individuals [1,2].

From the patient's perspective, dyspnoea is not only a physical limitation but a profound psychological burden. The sensation of airflow restriction may lead to fear of suffocation, loss of control, and heightened vigilance toward bodily symptoms. Episodes of acute or exertional dyspnoea may provoke panic-like reactions, further amplifying symptom perception and distress[3]. Chronic respiratory symptoms are well known to be associated with anxiety and depressive symptoms, and this interaction is bidirectional. Psychological stress may exacerbate the subjective experience of dyspnoea, while ongoing breathing difficulties impose a constant cognitive and emotional strain on the individual [4].

Many people are familiar with common causes of dyspnoea, such as asthma and chronic obstructive pulmonary disease (COPD), both of which have well-established pathophysiological mechanisms and evidence-based treatment options. In contrast, structural causes of upper airway obstruction are less frequently considered in clinical practice. The overlap in symptomatology may lead to misdiagnosis and delayed recognition, prolonging patient suffering and increasing the risk of psychological sequelae associated with persistent unexplained dyspnoea [5]. Upper airway stenosis is a broad and inclusive term describing pathological narrowing of the respiratory tract extending from the nasal cavity to the trachea, encompassing the pharynx and larynx.

Laryngotracheal stenosis (LTS) refers to a pathological narrowing of the airway at any level from the supraglottic level to the trachea. Anatomical classification of LTS further defines subglottic stenosis as a stenotic area in the subglottic region within the larynx. Although less common than lower airway disease, upper airway obstruction is frequently associated with disproportionate symptom burden relative to the degree of anatomical narrowing[6].

1.2 Patient-reported outcome measures (PROMs)

Evaluation of surgical success is a process that must involve both the physical aspects of the surgery but perhaps even more important is to evaluate the patient experience and hopefully improvement in quality of life. Introduced in 1992, Short Form Health Survey 36 (SF-36) [7] has been used for many years in evaluation of different medical and surgical treatments. The instrument provides a robust measure of treatment effectiveness and serves as an important tool for assessing improvements or deterioration in patients' quality of life. Covering 8 specific health issues it presents a broad picture of the patient's quality of life.

PROMs specific for upper airway-related dyspnoea are scarce. A systematic review detected only three PROMs suitable for evaluation of upper airway dyspnoea [8]. And only one of these, Dyspnea Index (DI), was developed de novo for evaluation of upper airway related symptoms whereas the other two were adapted from PROMs primarily developed for lower airway disease. DI was established and validated for the specific use in evaluation of diseases affecting the upper airway providing a useful tool in evaluation of these patients and were recently also translated to Swedish[9,10]. In our research area most validated and robust PROMs are based on the broader construct of upper airway stenosis rather than the specific diagnostic category of laryngotracheal stenosis (LTS). Therefore, for methodological reasons and to ensure consistency with the available outcome measures, the more inclusive term *upper airway stenosis* is reflected in the title of this thesis.
Given the recurrence rate of the disease, long-term management strategies must balance durability of airway improvement with

treatment morbidity. Importantly, anatomical measures alone do not fully capture treatment success. Patient-reported outcomes are effective in tracking the patient's quality of life, pain levels, and physical function directly from their own perspective without the researcher's interpretation. PROMs empower patients by making their voices the central focus of clinical care, driving value-based medicine and shared decision and as such are essential components in assessment of SGS research.

1.3 Subglottic stenosis

Subglottic stenosis (SGS) is a soft tissue narrowing of the airway just below the vocal folds, often without an identifiable cause (Figure 1).

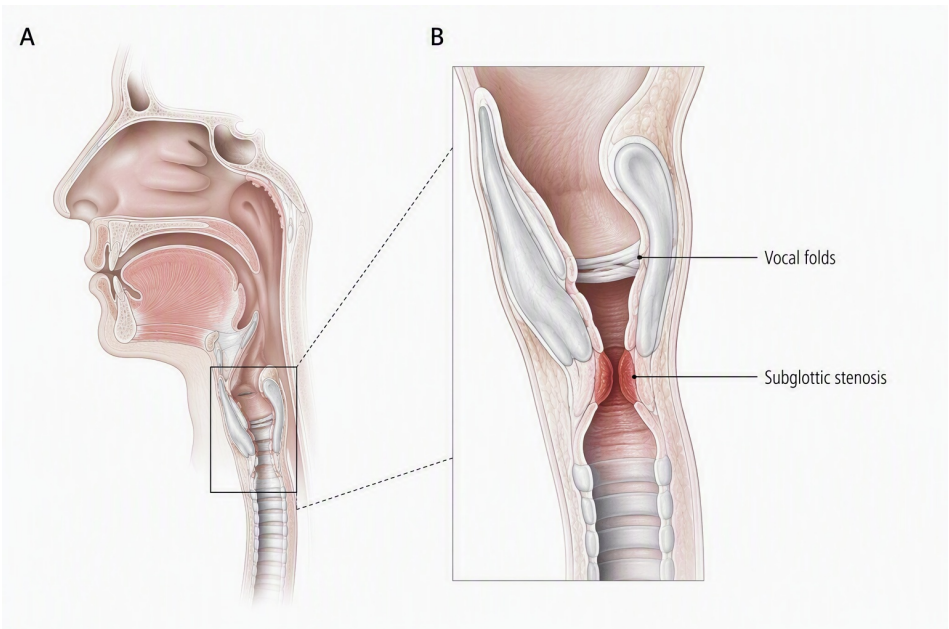

Fig 1: Anatomical location of subglottic stenosis (SGS).
(A) Sagittal overview of the upper airway showing the location of the subglottic region. (B) Magnified view of the larynx demonstrating subglottic stenosis immediately below the vocal folds. Figure generated with AI.

The nomenclature of SGS and LTS can be somewhat confusing. LTS is a specific form of upper airway stenosis involving pathological narrowing of the larynx and/or trachea. Within this group, SGS constitutes a

distinct anatomical subtype characterized by narrowing of the airway immediately below the vocal folds in the larynx and above the trachea [11,12](figure 2).

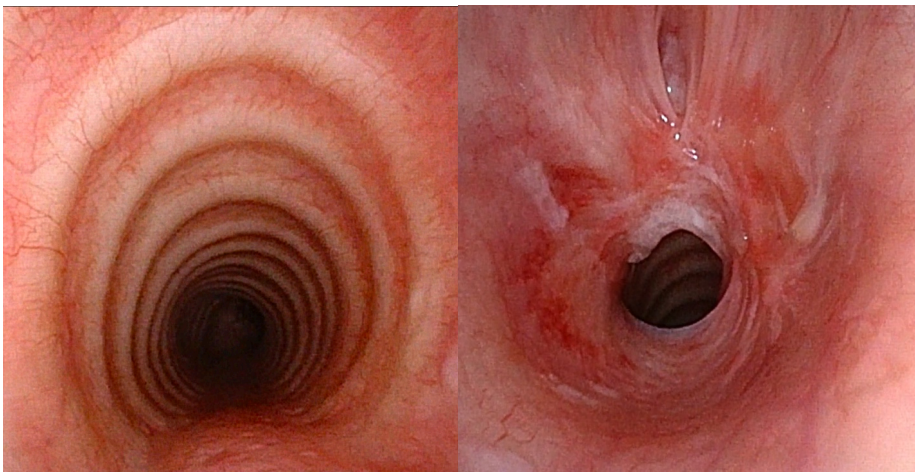

Fig 2: On the left normal trachea in the subglottic space. To the right the same anatomic location with a stenosis. Image reproduced with permission from Professor Katarina Olofsson.

Although the underlying aetiology of SGS remains incompletely understood from a broader pathophysiological perspective, the condition has been stratified into several subgroups based on presumed aetiology. The largest of these subgroups is idiopathic subglottic stenosis (iSGS), a diagnosis assigned when no identifiable cause can be established. For the purposes of this thesis, subglottic stenosis (SGS) is strictly based on anatomical localization of the stenotic area and not according to presumed etiological factors[13].

SGS has also been associated with trauma during intubation or prolonged intubation as well as inflammatory diseases such as Granulomatous Polyangiitis (GPA). Definitions of iSGS vary between studies, often incorporating criteria related to prior intubation history and coexisting systemic disease. This variability complicates interpretation of epidemiological data and may contribute to an underestimation of both disease incidence and prevalence. Irrespective of etiological classification, SGS is a progressive soft tissue airway

disorder characterized by gradual constriction of the subglottic lumen, leading to increasing airflow obstruction. If left untreated, disease progression may result in significant respiratory impairment, with the potential for critical airway compromise and life-threatening consequences.

Misdiagnosis is frequent in SGS, with patients often initially treated for asthma or other obstructive pulmonary disorders. The gradual onset of symptoms and the partial, transient response to bronchodilator therapy may further obscure the underlying diagnosis[14].

1.3.1 Pathogenesis

Although clinical awareness of SGS is growing, its underlying pathogenesis is still insufficiently elucidated, highlighting a distinct and unmet research need. Previous literature aims to separate idiopathic from non-idiopathic forms of SGS. We advocate a more holistic and less debated terminology for SGS, based on the anatomical location of the soft tissue airway narrowing rather than its presumed pathophysiological aetiology. This approach aims to facilitate study design, improve the consistency and reliability of prevalence and incidence estimates, and enhance comparability between studies employing similar methodologies.

Current evidence suggests that SGS is driven by chronic inflammation and dysregulated wound healing rather than a purely mechanical or traumatic process[15-17]. Several inflammatory pathways have been implicated in SGS. Upregulation of the interleukin-17A (IL-17A) and interleukin-23 (IL-23) axis has been demonstrated, indicating involvement of adaptive immune responses[18,19]. Transforming growth factor-β (TGF-β), a cytokine with pivotal roles in tissue repair, fibrosis, and cellular differentiation, may serve as a key mediator of the fibrotic scar formation characteristic of SGS[18]. Studies have also revealed increased expression of PD-1 and PD-L1, a pathway relevant in the development of pulmonary fibrosis [20].

More recently, epithelial–mesenchymal transition (EMT) has been proposed as a potential mechanism in SGS pathophysiology. EMT is a process by which epithelial cells acquire mesenchymal characteristics, including increased migratory capacity and resistance to apoptosis [21-24]. Dysregulated EMT has been implicated in fibrotic disease across multiple organ systems and may represent a unifying mechanism linking inflammation to persistent stenosis in SGS.

The female predominance has over the years resulted in several studies investigating the connection between female hormones/hormone receptors and SGS. Interestingly a marked female predominance is also evident in cases with other causative factors present such as Granulomatous Polyangiitis (GPA) making the connection between females and SGS even more interesting. Previous studies have been able to detect expression of oestrogen receptors (ER) in the subglottic space of non-SGS cases as well as in SGS affected patients when investigating biopsies [25-27]. Oestrogen generally promotes faster healing with improved scar quality, while low oestrogen levels (such as during menopause) can lead to slower healing, thinner skin, and more noticeable, sensitive, or rigid scar tissue [28]. However, to our knowledge, no study has been able to reveal a clear connection between female hormonal regulation and development of SGS. Interestingly, recent studies on higher levels of testosterone in SGS patients have been presented, further highlighting the importance of research regarding hormonal pathways [29].

Although chronic inflammation and dysregulated wound healing are central features of SGS, these mechanisms do not fully explain disease onset, female predominance, or the high rate of recurrence following technically successful surgery. Previous studies have suggested that microbial factors may contribute to the local inflammatory milieu in SGS, including reports of bacterial DNA within the lamina propria and development of bacterial biofilms[21,30,31]. Traditional methods such as culture or targeted PCR, however, have limited ability to characterise the full microbial community and may overlook low-virulence or slow-growing organisms.

Whole-genome shotgun sequencing (WGS) enables hypothesis-free, high-resolution identification of microbial DNA directly from tissue and allows robust discrimination between true intralesional microbiota and environmental contaminants [32]. As such, WGS represents a powerful exploratory tool for investigating potential biological contributors to SGS pathophysiology and may offer insight into mechanisms that sustain chronic inflammation and fibrosis. WGS has been investigated in swab samples from laryngotracheal stenosis (LTS) patients with and without tracheostomy revealing a significant decrease in microbial diversity [33]. To our knowledge WGS has not been performed on biopsies from SGS lesions.

1.3.2 Epidemiology and diagnosis

SGS is a rare disease, with reported incidence varying depending on inclusion criteria and definition. Idiopathic SGS is estimated to occur at an annual rate of approximately 0.2–0.25 per 100,000 individuals[34], while inclusion of all cases increases the reported incidence. A striking epidemiological feature of SGS is its strong female predominance, particularly among middle-aged women of Caucasian origin[35].

Although rare, SGS imposes a substantial burden on affected individuals and healthcare systems. Patients are often of working age and may require repeated interventions over many years, leading to cumulative morbidity, reduced quality of life, and significant healthcare costs per patient comparable to those of chronic diseases such as COPD and diabetes mellitus[36,37].

Patients with undiagnosed SGS suffer from a significant diagnostic delay, with the median time to diagnosis being 24.5 months. Commonly patients are treated as if they had asthma [5,14] causing prolonged suffering for patients with a potential fatal outcome if left untreated[14].

Because of the challenges associated with identifying these patients in primary care several attempts have been made to utilize parameters derived from widely available spirometry measurements[38]. The expiratory disproportion index (EDI) has been proposed as a promising

diagnostic parameter, and its utility has been supported by several studies. However, despite this evidence, it does not yet appear to be widely implemented in routine clinical practice [39,40]. Other values obtained from spirometry such as Peak Expiratory Flow % (PEF%), Peak Inspiratory Flow (PIF), sole PEF, Total Peak Flow (TPF) and calculations of area under inspiratory and expiratory curves have also been investigated, but no single parameter has yet achieved consistent clinical use [41-43].

Despite its widespread availability, spirometry has not yet been established as a reliable diagnostic tool for SGS. Further studies are warranted to identify and validate spirometry-based parameters that could serve as a diagnostic standard for the evaluation of SGS.

1.3.3 Treatment options and prognosis

Treatment options for SGS in earlier years were limited and, in many cases, patients were often fitted with a tracheostomy. Surgical treatment options were developed in the 80:s and 90:s with open cricotracheal resection (CTR) being established as a successful alternative to tracheostomy [44]. Surgical management using both CTR and laryngoplasty (LP), with or without grafting, remains widely practiced[45]. These techniques are recognized for their comparatively low recurrence rates, with CTR generally considered the most effective and durable treatment option currently available [46]. Previous studies have evaluated the range of available treatment options, and the overall evidence indicates that CTR provides a markedly lower recurrence rate compared with endoscopic approaches. However, CTR is associated with longer hospitalization and an increased risk of postoperative swallowing and voice dysfunction [47,48].

Endoscopic surgical treatment is a well-established method of treating SGS. The exact protocol differs between articles but in general it can be divided in Balloon dilation and CO_2 laser treatment often combined with intralesional injection of corticosteroids[49-52]. Endoscopic surgical treatment regardless of balloon dilatation or CO_2 laser technology can be performed in an outpatient setting and is a low-risk procedure. The

significant downside of endoscopic treatment is the high recurrence rate with 80% relapsing within the first three years[53,54]. However, with a declining frequency of surgical interventions along the timeline, more specifically a reported decreased risk of reoperation by 7.7%/yr interpreted as decreasing disease activity over-time [55], we propose employing the least traumatic surgical procedure feasible.

Medical treatment options are limited to the application topically of Mitomycin and injection of corticosteroids[53,56]. Corticosteroid injections have in recent years also been made under local anaesthesia in the stenotic area with promising results both in reduced stenotic area and treatment intervals with improvement of patients' symptoms and quality of life [55,57]. One could argue that there is a place for both the more aggressive CTR in some complicated SGS cases and endoscopic options in others[58-61].

2. Purpose and aims

2.1 Purpose

To advance the understanding and management of subglottic stenosis through the integration of population-based characterization, evaluation of endoscopic strategies, patient-reported outcome measures, and exploratory whole-genome sequencing of stenotic tissue.

2.2 Aims

The specific aims for each of my four studies are as follows:

I. To determine the comparative clinical effectiveness of CO_2 laser surgery and balloon dilatation for the management of SGS, using surgical free intervals (SFI) as the primary outcome measure.
II. To comprehensively characterize health-related quality of life in individuals with SGS and to assess the clinical impact of balloon-based surgical intervention through validated PROMs.
III. To investigate the presence and composition of non-human genomic material in SGS tissue biopsies and to compare these findings with results from the mucosal film in the larynx to identify microbiota profiles in non-SGS cases.
IV. To enhance the phenotypic characterization of upper airway stenosis to reduce diagnostic inaccuracies and improve the precision of clinical assessment pathways.

3. Material and methods

3.1 Study I

Study I was conducted as a retrospective observational analytic case series. Designed to compare long-term outcomes of balloon dilatation and CO_2 laser surgery, two commonly used endoscopic surgical settings used in treatment of SGS. The extended study period from 1999 to 2021 enabled reliable evaluation of recurrence patterns over time, and a reflection of the long-term (> 2 yrs.) nature of SGS.

3.1.1 Participants and eligibility

Eligible participants were adults diagnosed with SGS who underwent endoscopic treatment during the study period. Potential cases were identified using predefined ICD-10 codes and subsequently validated through a comprehensive review of clinical records from the Department of Otorhinolaryngology, Umeå University Hospital, including findings from endoscopic examinations and radiological assessments. Inclusion required a verified soft tissue stenosis at the subglottic level, whereas multilevel stenosis, malignant obstruction, cartilaginous lesions, and insufficient documentation led to exclusion. A final cohort of 63 patients met all criteria and was included for analysis.

3.1.2 Surgical procedures

All interventions were carried out under general anaesthesia with rigid suspension laryngoscopy and jet ventilation. CO_2 laser surgery was the predominant method from 1999 until 2009 and consisted of radial laser incisions under microscopic guidance using a line-of-sight micromanipulator (Figure 3). From 2010 onward, balloon dilatation became the preferred technique, in accordance with research from that time. This procedure consisted of radial cold-knife incisions followed by balloon expansion using a high-pressure dilatation catheter, with intralesional methylprednisolone administered beforehand (Figure 4). Both methods aimed to restore airway patency while preserving the laryngotracheal framework.

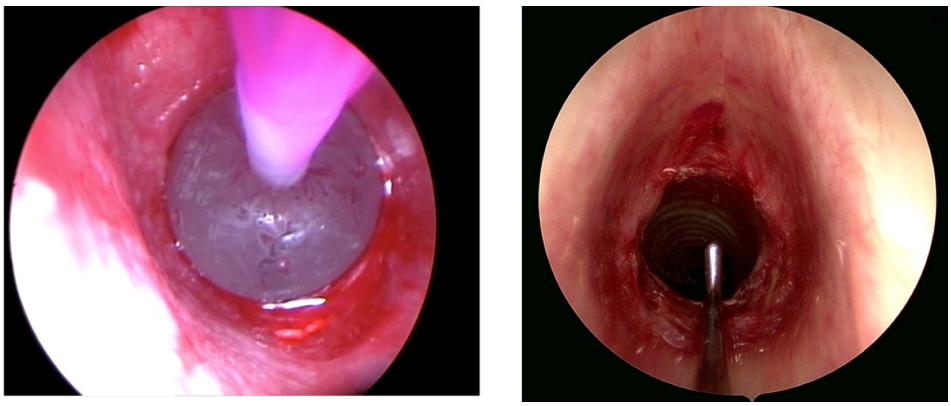

Fig 3: Ongoing balloon-dilatation (left) and postoperative view (right). The laryngeal hook measures 5 mm in length, the postoperative airway lumen measured 12 × 12 mm. Image reproduced with permission from Professor Katarina Olofsson.

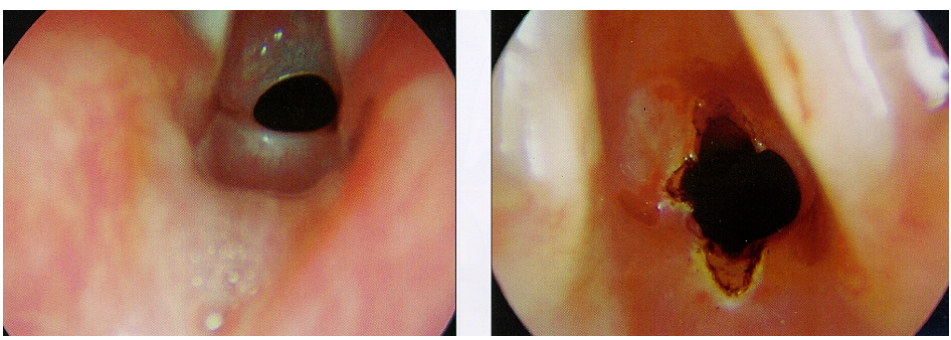

Fig 4: Showing a subglottic stenosis before and after CO_2 laser surgery. Image reproduced with permission from Professor Katarina Olofsson.

3.1.3 Outcome measure

The primary outcome was surgery free interval (SFI), defined as the time in days between consecutive endoscopic procedures for SGS. This measure reflects the durability of each technique over long-term follow-up.

3.1.4 Statistical analysis

Time-to-event modelling was performed using a Cox proportional hazards regression model with the Andersen–Gill extension, allowing

repeated events per patient. The proportional hazards assumption was evaluated using Schoenfeld residuals, and when violated, stratified Cox models were applied. Sensitivity analyses excluded one outlier with unusually frequent recurrences and explored the impact of removing cold steel dilatations and submucosal resections to preserve group purity. All statistical analyses were carried out using SPSS v28 and R (survival package). No formal power analysis was performed. The study included all available cases fulfilling the inclusion criteria's and performed treatment at our department during the study period and was therefore constrained by the number of eligible patients rather than by a predefined sample size target. Furthermore, the retrospective and exploratory nature of the study limited the usefulness of a priori power calculations.

3.2 Study II

Study II was a prospective descriptive study aimed at evaluating patient-reported outcomes (PROMs) following balloon dilatation for SGS. Participants were assessed pre-operatively and again three months after surgery, allowing quantification of symptom and quality-of-life changes. A follow-up period of three months was selected to minimize the influence of the recurrent natural course of SGS and to more specifically assess the effect of balloon dilatation. We also aimed to clearly delineate the quality of life experienced by untreated SGS patients, as captured with validated and robust PROMs.

3.2.1 Participants and eligibility

Adults with confirmed SGS scheduled for balloon dilatation at the Department of Otorhinolaryngology, Umeå University Hospital were consecutively recruited between September 2020 and August 2023. Eligibility required age ≥18 years, verified subglottic soft tissue stenosis, and the ability to complete Swedish-language PROMs. Patients with malignant stenosis, multilevel involvement, inadequate documentation, or inability to consent were excluded. Of the 40 eligible patients 33 completed both pre- and post-operative questionnaires and formed the analytic cohort.

3.2.2 Diagnostic confirmation

All diagnoses were established through flexible laryngoscopy and supplemented with high-resolution CT imaging. SGS was defined as a soft tissue narrowing at the cricoid level without significant extension into the glottic or tracheal regions.

3.2.3 Surgical procedure

All treatments consisted of endoscopic balloon dilatation performed under general anaesthesia using rigid suspension laryngoscopy under jet ventilation without catheter ventilation. Radial cold-knife incisions were made prior to balloon inflation. The balloon catheter (12–18 mm diameter) was inflated to predetermined pressures for approximately two minutes, during a monitored apnoea. Methylprednisolone (40 mg/ml) was administered as an intralesional injection before dilation.

3.2.4 Patient-reported outcome measures

Breathing-related disability was measured using the DI, a validated 10-item instrument specific to upper airway obstruction. Health-related quality of life was evaluated with the SF-36, covering eight physical and psychosocial domains. Both instruments were administered at the pre-operative visit and at three-month follow-up, either in person or through structured telephone interviews when needed. We chose to collect PROM data at 3 months postoperatively to evaluate treatment efficacy without the confounding effects of disease progression, as re-stenosis can be measured at 6 months in selected cases.

3.2.5 Statistical analysis

Changes in DI and SF-36 scores were analysed using the Wilcoxon signed-rank test due to non-normal score distributions. Results were summarised using median values and interquartile ranges. Statistical significance was defined as $p < 0.05$. Analyses were conducted using SPSS v28. No power analysis was performed due to the descriptive

design, the sample size was not adjustable, and the effect size and variance are unknown (pilot design).

3.3 Study III

Study III was a prospective cross-sectional study designed to characterise the microbiota present within stenotic subglottic tissue using high-depth WGS. The aim was to identify microbial signatures that may contribute to the inflammatory and fibrotic processes underlying SGS.

3.3.1 Participants and eligibility

Patients aged 18 years and older with confirmed SGS scheduled for endoscopic intervention were consecutively recruited between September 2020 and September 2023. Exclusion criteria included malignant or cartilaginous stenosis, multilevel airway involvement, incomplete documentation, or inability to consent. Of the 39 eligible individuals, 34 fulfilled all criteria and were included. Studies II and III were performed on largely the same participants, with one additional case included in study III. Diagnosis was verified by flexible laryngoscopy and high-resolution CT when required.

3.3.2 Biopsy procedure

Tissue samples were collected during endoscopic balloon dilatation performed under general anaesthesia. Using rigid suspension laryngoscopy with superimposed high-frequency jet ventilation, biopsies approximately 2 × 2 mm in size were taken from the stenotic segment under microscopic guidance. Samples were placed in sterile saline-moistened tubes and immediately stored at −80°C. All biopsies were collected prior to radial incisions, balloon inflation, or steroid injection to minimise procedural contamination. Sample handling occurred in a virology laboratory with strict contamination control, including sterile equipment and UV-sterilised workspaces.

3.3.3 Nucleic acid extraction

Tissue was homogenised using a BeadBlaster 24 with steel beads. Nucleic acids, including bacterial DNA and viral RNA, were isolated using the NucleoMag Pathogen kit, optimised for microbial nucleic acid recovery. Extracts were eluted in RNase-free water and quantified using Qubit high-sensitivity fluorometry. All steps were performed in a controlled viral-laboratory environment to reduce the risk of bacterial contamination.

3.3.4 Whole-genome sequencing (WGS)

Library preparation was performed using the SMARTer ThruPLEX DNA-seq kit, suitable for low-input samples. Sequencing was carried out on an Illumina NovaSeq X Plus system, generating paired-end 150 bp reads with an average depth of approximately 290 million read pairs per sample. This deep sequencing enabled detection of both abundant and low-abundance microbial taxa.

3.3.5 Bioinformatic workflow

Taxonomic classification was performed using KrakenUniq within the Taxprofiler v1.2.4 framework, referencing the KrakenUniq MicrobialDB (August 2023). The definition of and rationale for the positivity cut-off of to distinguish pre-specified from exploratory was extensively discussed and settled to candidate taxa requiring both >1000 reads and high k-mer support to be considered biologically credible. All such taxa were further validated using BLAST against the NCBI nt database (October 2025). This multistep curation ensured robust discrimination between true intralesional microbes and environmental contaminants.

3.3.6 Comparative material

Ethical considerations precluded the collection of tissue biopsy specimens from anatomically unaffected regions of the larynx during SGS surgery. We also considered an intra-individual control design in which SGS patients would serve as their own controls, with mucosal

swabs obtained from anatomically distinct regions of the larynx. However, SGS procedures were performed under supraglottic jet ventilation, with ventilation frequencies approaching 2,000 breaths per minute. We anticipated that the high-velocity airflow generated by this technique would substantially alter the local mucosal environment and microbial composition, potentially resulting in artificially reduced microbial yields and misleading microbiological findings. Consequently, samples obtained under these conditions were considered unsuitable as valid comparison.

The use of post-mortem tissue was also considered; however, due to tissue processing procedures and their potential effects on nucleic acid integrity, particularly DNA degradation and fragmentation, post-mortem samples were deemed unsuitable for the purposes of this study.

Consequently, the comparative cohort finally consisted of mucosal swab samples obtained from macroscopically healthy laryngeal mucosa in patients undergoing general anaesthesia for indications unrelated to SGS, specifically respiratory papillomatosis (RRP). Ethical constraints against biopsies in RRP cases since there is a risk for implantation of Human Papillomavirus (HPV). In contrast to SGS procedures, RRP cases are ventilated using a jet ventilation technique in which a catheter was positioned below the vocal folds. This approach was considered less likely to alter the composition of the laryngeal mucosal microbiota through airflow-induced disturbance.

Although the comparative samples do not meet the criteria for true control specimens, owing to the methodological limitations outlined above, they nevertheless provide a valuable reference for the characterization of the laryngeal mucosal microbiota in individuals without SGS pathology and most importantly without posing any risk to harm the research persons.

The mucosal swab samples were processed using conventional culture at a clinical microbiology laboratory. Control findings provided a baseline for distinguishing common airway colonisers from species enriched specifically in SGS lesions.

3.3.7 Statistical approach

Given the exploratory aim, analyses were descriptive. Microbial presence, frequency, read counts, and k-mer metrics were summarised narratively and in tabular form. No inferential statistics were performed. Power calculation was not performed due to reasons clarified in study II based on the same research persons.

3.4 Study IV

Study IV was designed as a retrospective, descriptive, population-based observational study. The objective was to characterise the epidemiology, aetiology, anatomical distribution, and comorbidity burden of laryngotracheal stenosis (LTS). LTS cases admitted to the Department of Otorhinolaryngology, Umea University Hospital, a tertiary ENT centre were considered eligible. By systematically reviewing all cases labelled with diagnostic codes related to LTS diagnostics the study sought to describe the full spectrum of LTS encountered in routine clinical practice.

3.4.1 Study population and data sources

All patients who had visited our centre between January 1999 and December 2019 were screened for eligibility based on a predefined set of ICD-10 codes related to dyspnoea, stridor, laryngeal tumours, vocal fold immobility and subglottic stenosis. Each potential case underwent detailed review of the medical record to confirm the presence or absence of upper airway pathology at the LTS level. Diagnostic confirmation relied on documented endoscopic examinations, radiology reports, and operative findings. Cases in which the ICD-10 code suggested upper airway stenosis, but no anatomical narrowing was present were classified as mislabelled and excluded from further analysis. Likewise, patients with pharyngeal pathology or airway obstruction outside the larynx and upper trachea were excluded. The final dataset comprised 1071 confirmed cases of LTS. A formal power calculation was not performed. The study included all available cases fulfilling the inclusion criteria's

during the study period and was therefore constrained by the number of eligible patients rather than by a predefined sample size target.

3.4.2 Definitions and classifications

LTS classification is based on anatomical stenotic subsites in the larynx and/or trachea. These sites were assessed by operative notes, flexible laryngoscopy, or radiology. Aetiological categories were constructed from clinical diagnoses recorded in the medical chart, including benign and malignant causes. Vocal fold immobility was classified as benign unless a malignant lesion was confirmed histologically.

Comorbidity was assessed using the age-adjusted Charlson Comorbidity Index (ACCI), extracted from medical records. Smoking status was coded as current, former, non-smoker, or unknown, based on standard definitions used in Swedish healthcare documentation.

3.4.3 Data extraction

For all included cases, demographic variables, presenting symptoms, ICD-10 codes at presentation, diagnostic verification, anatomical site of stenosis, aetiology, comorbidity, and smoking status were extracted from the electronic medical record. Age was recorded at the time of first visit during the study period. When diagnoses pre-dated 1999, only data from the first visit within the study timeframe were included. Cases prior to 1999 were not included due to the transition from paper-based to digital records, which would have introduced a substantial risk of data loss.

The review process also captured misclassifications, particularly among patients labelled with dyspnoea (R06.0) or stridor (R06.1), where subjective symptoms often led to over-representation of non-stenotic cases. This systematic approach allowed quantification of diagnostic inaccuracy in routine coding.

3.4.4 Statistical analysis

Data were analysed descriptively using SPSS version 27. Frequencies, means, medians, and proportions were calculated for demographic and clinical variables. Cross-tabulations were used to evaluate distributions across anatomical subsites, aetiological groups, sex, smoking status, and Age-adjusted-Charlson Comorbidity index (ACCI) categories. Between-group differences were assessed using chi-square tests or two-sample z-tests where appropriate. Statistical significance was defined as $p < 0.05$. No imputation was performed for missing data.

No power analysis was performed. The study included all available cases fulfilling the inclusion criteria. Sample size was therefore constrained by the number of eligible research persons rather than by a pre-defined sample size target. The retrospective and descriptive nature of the study limited the usefulness of a power calculation.

4. Ethical considerations

All studies included in this thesis were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and followed all relevant national and institutional regulations governing research involving human participants. Ethical approval was obtained from the Swedish Ethical Review Authority prior to data collection for each study.

Study I was approved under reference number 2020-00253 (ratified 2020-04-14), permitting retrospective review of medical records. Study II-III, which involved the acquisition of subglottic tissue biopsies, whole-genome sequencing and accompanying PROMs, was approved under 2020-00253 and the additional reference (for an upcoming hormone study) 2024-03145-02 (ratified 2024-06-24). The use of the comparative materials was approved under 2021–04941 (ratified 2021-12-22, Lunds university). Updated Biobank Sweden latest 2023-01-09 document nr 472-23-001.

Study III. The transfer of human biological material from Clinical Genomics, Umeå University, to SciLifeLab, Uppsala University, for WGS analytical purposes is governed by a set of legal and regulatory documents ensuring compliance with applicable biobanking, data protection, ethical, and transportation requirements. These documents were prepared with guidance from and approved by legal counsel at Umea University. The transfer is regulated by a Material Transfer Agreement (EBF UMU-MTA 1-23-12-14), ratified on 31 January 2024. In addition, the transfer is covered by the Agreement on the Transfer of Human Biological Material for Research Purposes (approval no. 472-23-001), originally approved on 22 October 2019 and ratified on 8 March 2023. All packaging, transport, and logistical procedures have been conducted in accordance with the applicable guidelines and requirements established by SciLifeLab, Uppsala University and Umeå University.

Study IV was likewise conducted under approval 2020-00253, allowing retrospective epidemiological review of all relevant cases over the defined study period.

For all prospective components (Studies II and III), written informed consent was obtained from every participant prior to inclusion. Retrospective components (Studies I and IV) were granted waiver of consent in accordance with Swedish law governing medical record-based research. Data handling and storage across all studies adhered to the European Union General Data Protection Regulation (GDPR; EU 2016/679), ensuring secure processing of personal data, restricted access, and anonymisation or pseudonymisation where appropriate.

Collectively, these approvals and procedures ensured that all research activities were ethically sound, legally compliant, and implemented with full respect for participant autonomy, privacy, and safety.

A comprehensive Data Management Plan (DMP) is available at https://dmp.umu.se/plans/134727. The DMP outlines the procedures for the collection, management, storage, protection, documentation and sharing of research data throughout the project lifecycle. It ensures that data are handled in accordance with established principles of research integrity, legal and ethical requirements. Promoting transparency reproducibility and long-term accessibility.

5. Results

5.1 Study I

A total of 63 patients fulfilled all eligibility criteria and were included in the analysis. 49 (78%) were women and 14 (22%) men, with a mean age of 60.1 years. Across the study period (~1600 days or 4.4 yrs) there were 159 surgical events. In the primary time-to-event analysis including all cases and allowing for repeated procedures, there was no statistically significant difference in surgery-free intervals (SFI) between balloon dilatation and CO_2 laser surgery (hazard ratio [HR] for balloon vs laser 1.67, 95% CI 0.82–3.43; $p = 0.16$). Because the proportional hazards assumption was violated for the method variable, a stratified Cox approach was applied; in that analysis, the estimated survival curves overlapped with a transient early advantage for balloon dilatation up to ~700 days that did not persist thereafter (Figure 5).

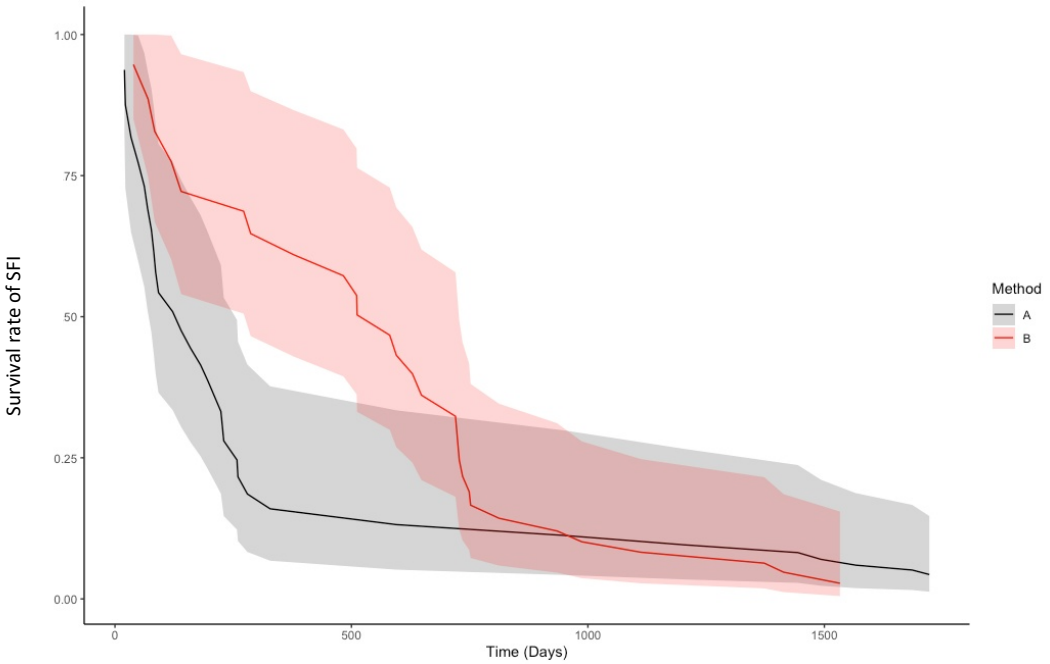

Fig 5: Estimated survival functions with corresponding approximate 95% confidence bands for the respective surgical method, obtained from stratifying the Cox PH analysis on method. Method B (balloon dilation) has a greater estimated survival of SFI up until approximately 700 days, after which method B (balloon dilatation) in fact has a lower estimated survival. However, the confidence bands for the respective methods are overlapping throughout. A = CO_2 *Laser treatment. B = Balloon dilatation. Published with written permission from Acta Oto-Laryngologica.*

Sensitivity analyses confirmed the main inference. Excluding one influential outlier with unusually frequent recurrences yielded (HR 0.91 95% CI 0.53–1.56; $p = 0.72$). When "cold" techniques (rigid dilatations and submucosal resections) were removed from the balloon dilatation group to preserve group purity, the association remained non-significant (HR 1.21, 95% CI 0.75–1.97; $p = 0.43$), with a similar early separation of curves up to ~700 days followed by overlap. In multivariable models from the purity analysis, female sex was associated with longer SFI (HR 0.19, 95% CI 0.07–0.54; $p = 0.002$), "unknown" smoking status with shorter SFI (HR 3.80, 95% CI 1.10–13.13; $p = 0.035$,

vs non-smokers), and mild comorbidity (ACCI 1–2) with longer SFI compared to ACCI 0 (HR 0.16, 95% CI 0.06–0.42; $p = 0.0002$). No procedure-related serious adverse events were recorded in the cohort.

5.2 Study II

Of 40 eligible patients, 33 completed both baseline and 3-month follow-up assessments and comprised the analytic cohort. 30 (91%) were women and 3 (9%) men. At baseline, Dyspnea Index (DI) scores were markedly elevated (median 31; mean 30.39), improving substantially to a median of 6 (mean 8.97) at three months. The pre- to post-operative change in DI was highly significant (Wilcoxon signed-rank, $p < 0.001$), with 32 of 33 patients showing improvement (Figure 6).

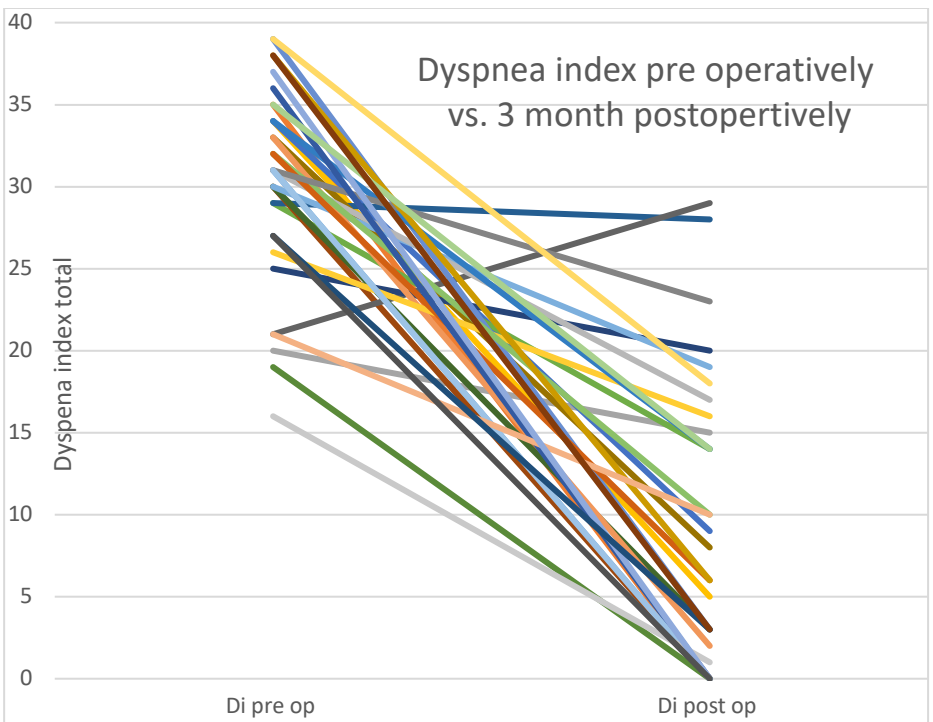

Fig 6: Graph showing the DI total score pre-operatively and post operatively at approximately 3 months. Each separate line represents one individual patient. Published with written permission from European Archives of Oto-Rhino-Laryngology.

Health-related quality of life as measured by Short Form Health Survey-36 (SF-36) improved across multiple domains at three months. After adjustment for multiple comparisons (Bonferroni for nine domains), statistically significant gains were observed in physical functioning, role-physical, role-emotional, energy/fatigue, emotional well-being, social functioning, general health, and perceived health change (all $p \leq 0.005$); the pain domain showed a smaller improvement that did not meet the corrected significance threshold ($p = 0.047$). No intra- or postoperative complications were recorded in the study cohort.

5.3 Study III

Thirty-four consecutive SGS patients, 30 (88%) females and 4 (12%) males, provided analysable biopsies. High-depth whole-genome sequencing (WGS) combined with curated taxonomic classification pipelines identified a consistent enrichment of *Cutibacterium acnes (C. acnes)* in 28 of 34 samples (82%). This finding was supported by high read abundance, robust k-mer-based classification evidence, and taxonomic assignment independently verified through BLAST-based sequence alignment. Additional verified taxa included *Schaalia odontolytica* (four cases), *Corynebacterium accolens* (one case), *Micrococcus luteus* (one case), and *Roseolovirus humanbeta6b* (one case).

Sampling for comparison from macroscopically healthy supraglottic mucosa in non- SGS cases, processed by conventional culture predominantly yielded *Staphylococcus aureus* (5/34, 15%), *Haemophilus influenzae* (3/34, 9%), and *group B streptococcus* (2/34, 6%). At least one of these species was detected in 29% (10/34) of controls. Analyses were descriptive and did not test causal associations.

5.4 Study IV

Screening of 1,441 medical records identified 1,071 patients with confirmed laryngotracheal stenosis (LTS) over the 20-year study period; 58% (624/1071) were men and 42% (447/1071) women. Benign aetiologies predominated (73.9%), with malignant causes accounting

for 26.1%. The glottic level was the most frequent anatomical subsite of obstruction (~77%, 824/1,071). Unilateral vocal fold immobility was the single most common diagnosis (45.7%, 489/1,071), followed by glottic cancer (18.8%, 201/1,071). Notably, within the subgroup of subglottic stenosis, women were over-represented, consistent with published observations on subglottic stenosis (SGS). Misclassification was particularly common among cases initially labelled with ICD-10 codes for dyspnoea or stridor, underscoring the risk of diagnostic delay and miscoding when structural upper airway disease is not actively considered. Comorbidity burden (ACCI) was higher in malignant than in benign LTS.

6. Discussion

6.1 Principal findings of the thesis

The key advances in knowledge and the clinical relevance can be summarised as follows:

(i)No statistically significant difference in surgery-free intervals (SFI) was observed between balloon dilatation and CO_2 laser surgery. Balloon dilatation demonstrated a transient early advantage, but this benefit did not persist. *Relevance*: These findings support a surgical freedom of choice, allowing clinicians to select the intervention based on individual expertise and operative skills. Our data also indicate the possibility of spontaneous resolution of SGS, since the observed differences in SFI between surgical techniques was no longer evident after ~ 700 days (2 yrs) of follow-up. (ii) Using patient-reported outcome measures, we observed markedly reduced quality of life in the preoperative phase and pronounced postoperative improvement across multiple domains three months after balloon dilatation. By restricting outcome assessment to the three postoperative months, the influence of the disease's recurrent natural history is minimized, thereby increasing the likelihood that observed effects can be attributed to the intervention itself. *Relevance*: Because of its measurable impact on quality of life, healthcare utilisation, and societal consequences and relatively limited scientific literature this condition warrants further systematic investigation. (iii) Whole-genome sequencing is the most comprehensive genomic method currently in clinical use. WGS targeting non-human genome revealed the presence of *C. acnes* in 82% of analysed cases. *Relevance:* These curated and stable genomic data should be incorporated into future therapeutically oriented studies, potentially informing antimicrobial or adjunctive treatment strategies. (iv) We identified unilateral vocal fold immobility as the most prevalent underlying cause of laryngotracheal stenosis. *Relevance:* Increased clinical awareness of SGS aetiology may enhance diagnostic efficiency and reduce delays in appropriate management.

In Study I and II, we observed that balloon dilatation was associated with longer surgery-free intervals (SFI) for the first 700 days (2 yrs) and

significant improvements across multiple health-related quality-of-life domains. Beyond this time point, the estimated survival curves suggested a relative disadvantage for balloon dilatation compared with CO_2 laser treatment. Notable, the confidence intervals for both groups overlapped throughout the entire follow-up period. A short-term superiority of balloon dilatation over CO_2 laser treatment has been reported by Ntouniadakis et al. (2023)[62] which is in accordance with our findings however our study had a significantly longer follow up period which eliminated this difference. Differences in surgery-free intervals may not be attributable to the surgical technique or setting alone but could instead reflect a general decrease in disease activity over time. Chorell et al. (2026) [55] reported a 7.7% annual reduction in the risk of requiring a new surgical intervention along the timeline with SGS. Comparisons across different studies regarding surgical technique have yielded divergent findings [51,58,62], primarily attributable to methodological differences and limited sample size. Another plausible explanation for this inconsistency is the lack of clearly defined diagnostic criteria.

These strands cohere around a simple conclusion: managing SGS effectively requires integration of early recognition, clear consensual diagnostic criteria, pragmatic treatment decision-making, patient-centred outcome assessment, and attention to underlying biology. A purely mechanical paradigm focused on lumen such as the Cotton-Myer classification [63] and device choice does not fully explain, nor sustainably mitigate, the clinical course experienced by patients.

6.2 Diagnostic challenges and disease definition

As discussed in Section 6.1 for Study I-II consensual diagnostic algorithms are essential in clinical science since these criteria promote standardization and consistency, ensure reliability and validity, and ultimately secure comparability across studies and clinical settings. These diagnostic algorithms are grounded in several fundamental principles (i) temporal precedence, causes must occur before their effects; (ii) counterfactual inference, the disease state should be expected to differ if a putative causal factor were altered (iii) mechanistic

explanation, establishing the pathological processes. Together, these fundamental diagnostic principles underscore the importance of causal research within SGS pathology to improve treatment options.

Upper airway stenosis is an umbrella term for narrowing in the breathing passage from the nose to the trachea. Laryngotracheal stenosis (LTS) encompasses scarring and narrowing of both the larynx and trachea. Subglottic stenosis (SGS) is a specific type of soft tissue LTS affecting only the region directly below the vocal fold. An algorithmic classification based on indisputable anatomy rather than conceivable etiological factors and accurate subsite identification is not a matter of taxonomy alone—it influences prioritisation, technique selection, expected durability, and the balance between airway and voice outcomes.

The term *upper airway stenosis* is reflected in the title of our thesis because it is an inclusive terminology but most importantly aligns with the patient-reported outcome measures (PROMs) available for addressing the research question, which are designed for upper airway stenosis rather than for specific stenosis subtypes. As upper airway stenosis anatomically encompasses both LTS and soft tissue SGS, we propose that classification of SGS should primarily be based on anatomical location rather than on presumed pathophysiological mechanisms, which remain incompletely understood and may overlap between disease entities. Idiopathic SGS is a subgroup of SGS based on unequivocally presumed pathophysiology. iSGS remains a diagnosis of exclusion with variable operational definitions across studies, this variability propagates into incidence estimates and hinders comparisons.

The epidemiological mapping in Study IV highlights how broad, systematically applied inclusion criteria reduce selection bias and expose the clinically relevant mix of pathologies that reach an ENT service. In sum, earlier structural evaluation of refractory dyspnoea and explicit, level-specific classification of stenosis are likely the most underused diagnostic tool in the LTS area. With these fundamental principles in mind, we present diagnostic data identifying unilateral vocal fold immobility as the most common specific cause of LTS. Previous studies have either excluded malignant causes[64] or unilateral vocal fold

immobility [65] as causes to LTS therefore no direct comparable study does exist to our knowledge.

Diagnostic criteria play a critical role in clinical science (i) they ensure standardization and consistency by enabling researchers to identify the same condition in a uniform manner (ii) they enhance reliability and validity by providing evidence-based frameworks that reduce misclassification. Diagnostic criteria are also essential for generating well-defined, homogeneous cohorts in clinical research. This, in turn, is fundamental for evaluating new therapeutic approaches and for ensuring comparability of results across studies and institutions.

Symptom profiles in glottic-level disease often overlap with lower airway disorders such as asthma and COPD and the static cross-sectional reduction may underestimate functional compromise during exertion, contributing to misclassification as asthma and to therapeutic delay. The thesis therefore takes a pragmatic stance and rigorous set of diagnostic principles. In adults with refractory dyspnoea, structural upper airway pathology should be considered early, flexible laryngoscopy and CT scan deployed sooner rather than later.

6.3 Surgical management and long-term outcomes

Balloon dilatation appeared favourable restoring airway patency in earlier phases (< 700 days or 2 yrs) of follow-up, but this advantage did not persist over long-term observation (cut-off at ~1600 days or 4.4 yrs), with confidence bands overlapping between methods. The observed convergence by number of surgeries over time for both balloon and CO_2 laser technology after approximately 700 days, together with the progressive prolongation of treatment intervals in both groups, may indicate a reduction in underlying disease activity over time rather than a sustained effect attributable to either surgical technique. We conclude our data on surgical management along the timeline as follows:

(i)Our outcome supports a pragmatic surgical freedom: centres may prioritise the modality that is safest and most reproducible within the team, (ii) short follow-up can overstate differences driven by learning curves and scheduling practices, (iii) the increased time between surgical

treatment along the timeline may reflect diminished disease activity, suggesting surgical caution and the use of the least hazardous surgical technique.

6.4 Patient reported outcomes and the lived experience

In this thesis, validated PROMs captured substantial improvements in dyspnoea and health-related quality of life (HRQoL) after endoscopic balloon dilatation (Study II), which is in accordance with previous studies [66]. These findings are consistent with the observation that the perceptual and emotional salience of breathlessness—including fear of suffocation, anticipatory anxiety, and activity avoidance—can amplify disability beyond what is explained by lumen diameter alone. Accordingly, anatomical or interval metrics are necessary but insufficient as sole indicators of success in SGS care. PROMs should be considered integral to both evaluation and follow-up. A practical corollary is that the timing of re-intervention should be based on patient's symptoms rather than objective measurements and classification. We implemented a 3-month follow-up period to evaluate surgical performance and quality of life without the confounding influence of an SGS recurrence.

6.5 Biological considerations and disease persistence

The exploratory tissue sequencing reported in this thesis (Study III) identified a reproducible enrichment of *Cutibacterium acnes* within SGS lesions. While these results do not establish causality, they are biologically plausible and align with a wider literature in which *C. acnes* acts as a low-virulence, immunomodulatory organism implicated in chronic inflammation and fibrosis [67]. *C. acnes* have been identified as a contributing factor in extra-laryngeal settings such as orthopaedic surgery and capsular contracture after breast augmentation [68-70]. Several mechanistic bridges could link such a microbial signal to SGS persistence: (i) amplification of local cytokine circuits (e.g., IL-17A/IL-23) and profibrotic mediators (e.g., TGF-β), (ii) promotion of dysregulated wound healing with excess extracellular matrix deposition, and (iii) episodic re-activation of local immune responses following micro-trauma or instrumentation[18,19,21]. At the same time,

alternative explanations must be acknowledged such as surface contamination or bystander colonisation, a problem also described in reports regarding orthopaedic surgery and *C. Acnes* [68,71]. To minimize contamination biopsy sampling was done under clean but not sterile conditions, followed by immediate freezing at −80 °C. DNA extraction was performed in a safety hood that was decontaminated with UV light before and after the procedure and all steps were carried out using customary protective equipment in a virology laboratory in order to reduce bacterial contamination by experienced laboratory staff.

6.6 Integrating the findings – a conceptual model

Bringing together the epidemiological, surgical, patient-reported, and biological strands this thesis supports a conceptual reframing of SGS. Rather than a purely structural defect, it is best understood as a chronic inflammatory condition with recurrent mechanical manifestations. We present a conceptual model to outline the theoretical core components and interactions in relation to our research outcome on SGS in comparison to published data, deliberately ignoring details. (i) Cellular injury is caused by invasion of *C. acne* with loss of membrane integrity and release of intracellular molecules (Damage-Associated Molecular Patterns, DAMPs) serving as endogenous signals of tissue damage [72]. (ii) The release of extracellular DAMPs is recognized by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), expressed on macrophages and other innate immune cells[73] . (iii) Engagement of these receptors initiates intracellular signalling pathways that activate the inflammatory response [74]. (iv) This cascade induces the production and release of pro-inflammatory cytokines and chemokines, including Tumour Necrosis Factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6)[75] . All mediators able to amplify local inflammation and promote the recruitment of additional immune cells to the site of injury in this case SGS. The resulting inflammatory response facilitates the clearance of damaged tissue and cellular debris. However, if inadequately regulated or prolonged, DAMP-mediated inflammation may contribute to ongoing tissue injury and pathological remodelling, all pathophysiological hallmarks for SGS.

The mechanical consequence is a progressive reduction in airway calibre, resulting in exertional airflow limitation that is commonly experienced by patients as distressing dyspnoea. Endoscopic intervention can restore airway patency and provide symptomatic relief; however, it does not eliminate the underlying pathophysiological driver(s), thereby predisposing patients to recurrent stenosis.
This integrated view explains why long-term durability is not strongly technique-dependent (Study I), why patient-perceived benefit may diverge from anatomical appearances (Study II), and why biologically targeted strategies could be required to improve durability in selected phenotypes (Study III), all against the backdrop of a broad and diagnostically challenging LTS spectrum (Study IV).

6.7 Clinical implications

Several practice-oriented implications follow:

- Earlier structural evaluation in refractory dyspnoea. In adults with persistent "asthma-like" symptoms, consider upper airway stenosis early and lower the threshold for flexible laryngoscopy and CT scan, thereby reducing diagnostic delay (Study IV).
- Apply pragmatic endoscopy. Given the absence of sustained surgical free intervals (SFI) superiority between balloon dilatation and CO_2 laser over long horizons (Study I), centres may prioritise team experience, safety, and reproducibility.
- Apply PROMs. Incorporate Dyspnoea Index and a generic HRQoL measure (e.g., SF-36 or equivalent) into baseline and follow-up to guide timing of re-intervention and to capture treatment value beyond anatomy (Study II).
- Apply whole-genome sequencing (WGS) in the diagnostic workup of subglottic stenosis (SGS) to identify potentially actionable microbial signatures and disease-associated genomic variants, with the aim of enabling personalized therapeutic strategies (Study III).
- Re-run WGS data (Study III). In the long term, continued bio-sampling of patients with SGS and reanalysis of the WGS dataset may further strengthen the validity of our findings. The

robustness of a scientific conclusion depends not only on reproducibility, defined as obtaining the same results using the same data and analytical methods, but also on replicability, defined as the ability to achieve consistent findings in an independent study using newly collected data.

- Apply risk-adapted follow-up. Tailor surveillance intervals to phenotype and comorbidity rather than fixed calendars alone. Consider triggers for earlier review based on PROM change or functional testing.

Collectively, these steps represent low-risk, high-yield changes that in part can be implemented without waiting for definitive disease-modifying therapies.

6.8 Strength and limitations

Strengths

Across all four research objectives (RO:s) we articulate clear and well-defined research questions, explicit aims, and clinically as well as societally relevant rationales. Each RO targets a specific and relevant research question, thereby avoiding the pitfalls of vague or overly broad research questions. All four RO:s demonstrates theoretical and evidence-based grounding, with comprehensive familiarity to prior research. In our presentations we have applied relevant theories and conceptual frameworks appropriately and articulated on how each study contributes to reducing existing knowledge gaps through replication/reproducibility, extension, or refinement of previous work. We have ensured methodological rigor by employing well-justified study designs, valid and reliable measurement instruments, and adequate sample sizes. The quantitative studies include transparent descriptions of methodological procedures, sampling strategies, and systematic approaches to data analysis. Together, these elements strengthen the credibility, robustness, and replicability of our findings. All studies adhere to high ethical standards as clarified under “Ethical considerations”, section 4. Finally, we ensure transparent and appropriate sampling across all studies. We clearly describe how

participants were identified and recruited and evaluate the representativeness and relevance of each sample. Since weak or inadequately described sampling strategies can compromise an entire study, we provide sufficient methodological detail to support the rigor and validity of the research.

Limitations

First, the observational design of the surgical comparisons cannot fully eliminate selection and calendar-time effects; although addressed through multivariable and stratified analyses, unmeasured confounding remains possible.

Second, although patient-reported outcome measures (PROMs) provide a direct assessment of the impact on SGS and health-related quality of life by capturing the patient's own perspective without interpretation of the researcher, PROMs remain susceptible to response bias. Therefore, PROM findings should be interpreted in conjunction with objective measures of airway function and disease severity whenever available.

Third, the biological findings are exploratory, whole-genome sequencing provides taxonomic signals rather than causal proof. Issues of contamination, spatial localisation, and activity status (active vs residual DNA) require dedicated validation. The comparison material used alongside the WGS biopsy samples in study III cannot be considered a true control material, as it does not fulfil the methodological and scientific criteria required of an appropriate control group. We acknowledge this limitation and discuss the associated scientific dilemma, including the rationale for our methodological choice, in section 3.3.6.

Fourth, the epidemiological mapping, though broad, reflects a tertiary-centre cohort, which may limit generalisability to other settings. Recognising these boundaries does not diminish the contribution, instead it clarifies where the next methodological investments should be made.

Fifth, the obvious limitations with a retrospective design. Data quality and completeness, we have relied on records not created for research often means missing-, inconsistent-, or inaccurate information. It is difficult to interpret uncontrolled variables (confounders) that may influence results. Misclassification it is an obvious risk of information bias if outcomes are misclassified or incorrectly documented.

6.9 Future directions

Future research should prioritise prospective phenotyping that combines precise subsite classification with objective functional testing. Validated patient-reported outcome measures (PROMs) and whole-genome sequencing (WGS) should be considered for incorporation into the routine diagnostic and clinical evaluation of SGS, given their potential to provide complementary patient-centred and molecular insights.
Mechanism-informed interventional studies in which endoscopic airway restoration is paired with targeted adjuncts e.g. anti-inflammatory or antimicrobial therapy. The specific objective is to discriminate pathogenic drivers from biologically incidental bystanders, thereby establishing when, and in which patient subgroups, biologically targeted therapy is scientifically and clinically justified.
Finally, outcome frameworks should move beyond single metrics; composite, patient-centred endpoints that integrate surgery-free intervals, change in PROMs and functional measures, together with cost-utility analyses, will better reflect therapeutic value and support service planning in a disease with high cumulative resource use. Methodologically, remote PROM capture and simple home-based flow metrics may facilitate earlier detection of relapse and more individualised timing of re-intervention.

7 Conclusions and relevance

Surgery-free intervals (SFI) did not differ significantly between balloon dilatation and CO_2 laser surgery. Balloon dilatation showed a short-term advantage, but this effect was not sustained with time. These findings support procedural equivalence in long-term follow up, allowing clinicians to choose the technique based on operative expertise. Robust patient-reported outcomes demonstrated substantial improvement across multiple domains three months after balloon dilatation. Whole-genome sequencing identified *C. acnes* in majority of cases. The genomic signatures could inform future treatment-oriented studies. Unilateral vocal fold immobility emerged as the most common underlying cause of laryngotracheal stenosis in the cohort. Greater awareness of this aetiology may reduce diagnostic delays and improve management pathways.

This thesis supports a conceptual shift in how subglottic stenosis is understood and managed. Rather than viewing the condition solely as a structural narrowing treated through mechanical dilatation or excision, advancing the field will depend on prospective phenotyping, interdisciplinary collaboration, and targeted approaches that address both the structural and biological dimensions of the disease.

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I never considered myself a researcher (I don't know if I do it now) and when I finished medical school research seemed very distant.
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9 AI-Declaration

Microsoft Copilot, based on OpenAI's large-scale language models (cf. https://chatgpt.com/), was employed exclusively for linguistic refinement, including improvements to grammar, syntax, and stylistic clarity at the level of individual sentences. All substantive content was authored solely by Anders Erlandsson. The tool was not used for generating new text, conducting analyses, or formulating interpretations or conclusions. Following the use of the language model, all text was thoroughly reviewed, verified, and approved by the author.

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