Laryngeal response patterns during mechanically assisted cough in Amyotrophic Lateral Sclerosis

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Contents

1. Preface

Respiratory complications are life threating in Amyotrophic Lateral Sclerosis (ALS), and a proactive preventive approach is a key element in disease management. Non-invasive ventilatory support and mechanically assisted cough are mainstay therapeutic techniques. Mechanical insufflation–exsufflation (MI-E) is an efficient tool used to improve cough in most patients with neuromuscular disorders, but the method often fails in ALS, especially when bulbar involvement is present.

We do not fully understand why bulbar ALS patients do not seem to benefit from MI-E, and thus we do not have evidence based guidelines to advise how treatment can be modified to be clinically more acceptable and efficient. Although the upper airways have been a suggested cause of this treatment failure, comprehensive studies of the laryngeal response patterns to MI-E have not been previously reported.

Clinical respiratory physiotherapy and non-invasive ventilatory support is of vital importance to maintain respiratory health and quality of life in ALS, and it is therefore imperative that we gain more knowledge on how we can overcome the obstacles preventing MI-E from being effective in these patients.

Thus, the overarching aim of this thesis is to investigate if and in what way abnormal laryngeal responses can possibly explain the frequently experienced lack of clinical effect of MI-E when applied in patients with progressing ALS.

1.1 Scientific environment

The thesis originated from the PhD program of the Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway.

The Western Norway Regional Health Authority and Norwegian National Advisory Unit on Long-term Mechanical Ventilation, Thoracic Department, Haukeland University Hospital have funded the study.

The present study was carried out between 2011 and 2017 in collaboration with Norwegian National Advisory Unit on Long-term Mechanical Ventilation at Thoracic Department, Departments of Neurology, Otolaryngology, Pediatrics, Physiotherapy and Clinical Engineering at Haukeland University Hospital, Bergen, Norway and The Faculty of Health and Social Sciences, Western Norway University of Applied Sciences, Bergen, Norway.

The main research environment was the WestPaed Research Group at Haukeland University Hospital. Collaborative research environments were Norwegian National Advisory Unit on Long-term Mechanical Ventilation and The Bergen Respiratory Research Group.

The supervisors during this work have been:

Ola Drange Røksund, physiotherapist, professor at the Faculty of Health and Social Sciences, Western Norway University of Applied Sciences, Bergen, Norway.

Thomas Halvorsen, pediatrician, professor at Department of Clinical Science, Faculty of Medicine, University of Bergen, Bergen, Norway.

John-Helge Heimdal, otolaryngologist, professor at Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Bergen, Norway.

Ole-Bjørn Tysnes, neurologist, professor at Department of Clinical Medicine, Faculty of Medicine, University of Bergen, Bergen, Norway.

Maria Vollsæter, pediatrician, PhD, senior consultant at the Norwegian National Advisory Unit on Long-term Mechanical Ventilation and Department of Pediatrics, Haukeland University Hospital, Bergen, Norway.

Ove Fondenes, pulmonologist and the head of the Norwegian National Advisory Unit on Long-term Mechanical Ventilation, Haukeland University Hospital, Bergen, has served as a professional specialist and supervisor in relation to respiratory challenges and mechanical ventilation of patients with Amyotrophic Lateral Sclerosis.

Statistical longitudinal analyses were carried out under the supervision of:

Roy Miodini Nilsen, biostatistician, associate professor at the Faculty of Health and Social Sciences, Western Norway University of Applied Sciences, Bergen, Norway.

1.2 Acknowledgements

knowledge and all the enthusiastic discussions we have had trying to understand the pathophysiology of our findings.

Maria Vollsæter, thank you for being my co-supervisor during the last part of my PhD period and before that my research fellow and my colleague in the Norwegian Advisory Unit for Home Mechanical Ventilation (NKH); your brain is huge and your heart is even bigger. I am looking forward to our further working together, both clinically and in research.

Ove Fondenes, you have been my mentor and advisor since 2002 when you hired me as respiratory physiotherapist in NKH. I have so much to thank you for; for all the opportunities my work has given me, your endless belief in me and the freedom you have given me through the years which has pushed me to do my best work. We still have a lot to do together and I am looking forward to all the new adventures with you and rest of our team in NKH.

I am grateful to The Western Norway Regional Health Authority and NKH who have funded this study. I also want to thank the Thoracic department, and its head Kahtan Al-Azawy; and the Department of Physiotherapy, and head Else Sterndorff and my nearest leaders there Grete Ege and Theresa Johannessen, for allowing me to perform this study.

Ola, Thomas and John-Helge, in addition to developing this research field you have also created a unique research environment with positive, supportive and a highly inclusive atmosphere. You are good at linking researchers in positive collaborations, and have a genius for teambuilding. I am very grateful to be part of this group and I want to thank the whole group for your support. I am especially in great gratitude to Astrid Sandnes, Maria Vollsæter, Hege Clemm, Magnus Hilland and Lorenz Sandven for performing the laryngoscopies and to Thor-Andre (Tollis) Engelsen for editing the film clips and helping me with illustrations. Thank you so much! Also our funny Englishman Zoe Fretheim- Kelly, thank you for your friendly help with the proofing of my thesis.

Marit Renså and Gunvor Mo Norstein; our eminent nurses at the ALS outpatient clinic, thank you for coordinating the ALS clinic and making the project

1.3 Summary of thesis

Background: Patients with amyotrophic lateral sclerosis (ALS) are treated with mechanical cough assist devices using the technique of mechanical insufflation-exsufflation (MI-E) in order to improve cough and enhance clearance of airway secretions. The aim of treatment is to prevent lung infections and to provide a better quality of life. The technique often fails in ALS patients with bulbar involvement, allegedly due to upper airway malfunction. Laryngeal collapse during exsufflation has been proposed to explain the ineffectiveness of MI-E in bulbar ALS. However, there are a lack of studies utilizing comprehensive and verifiable methods to investigate the role played by the larynx of patients in whom MI-E appears to be non-successful.

Objectives: *Study #I:* To examine the feasibility of transnasal fiberoptic laryngoscopy (TFL) during ongoing MI-E in healthy volunteers, and to describe normal laryngeal response pattern(s) to MI-E. *Study #II:* To investigate laryngeal response patterns to MI-E in a cross-sectional study of ALS of different phenotypes. *Study #III:* To examine and describe changes in laryngeal response patterns to MI-E as ALS progresses, and to explore if treatment protocols can possibly be modified and improved in these patients.

Design: Population-based, explorative, descriptive, observational studies, with cross-sectional design in Study #I and #II, and prospective cohort design in the Study #III.

Subjects: *Study #I*: Twenty healthy medical students. *Study #II*: Twenty patients with ALS together with 20 healthy volunteers, matched for age and gender. *Study #III*: Thirteen eligible patients with ALS, recruited from Study II, prospectively followed during disease progression for up to 5 years.

Methods: ALS was phenotyped according to established international standards. Upper and lower motor neuron symptoms were characterized, and the respiratory function determined. Video recorded flexible TFL was applied during ongoing cough assisted by MI-E that was applied using various pressures according to a preset protocol. The video files were used to carefully assess and tabulate the laryngeal

movements. The examinations were performed at outpatient visits that were scheduled at set time-intervals.

Results: *Study #I*: The larynx could be studied with TFL during ongoing MI-E. The laryngeal responses to MI-E in healthy volunteers were compatible to that described in normal cough. *Study #II*: The laryngeal structures of patients with ALS and bulbar symptoms tended to adduct, especially during insufflation, which in some patients severely compromised the size of the laryngeal inlet, especially if high pressures were applied. *Study #III*: During ALS disease progression, the first signs of laryngeal adduction occurred with the highest insufflation pressures and *prior* to any clinically evident signs of bulbar involvement. Hypopharyngeal constriction during exsufflation was observed in all subjects regardless of bulbar symptoms, and later in the disease progression than the above described adverse events during insufflation. Cough gradually became less expulsive and also less synchronized at the laryngeal level. Triggering of swallowing reflexes by the positive air flow from the MI-E further complicated these matters. Attempts of careful individual tailoring of the MI-E therapy as the patients' condition deteriorated seemed to prolong its successful use.

Conclusions and interpretations: Laryngoscopy can safely be performed during ongoing MI-E, and appears a feasible tool to visualize the laryngeal responses to this therapy. In bulbar ALS, laryngeal structures are prone to adduct throughout the various pressure cycles of MI-E, especially if applying high insufflation pressures, thereby severely obstructing the airflow and thus hampering the effect of the treatment. Cough patterns alter as ALS progresses, and rapidly alternating MI-E pressure cycles may become challenging or even impossible to handle for patients. Individually tailored MI-E treatment can improve and may possible extend the use of non-invasive ventilatory support in ALS, and TFL can become a feasible and valuable tool in this respect.

1.4 List of publications

Paper I   Laryngeal Response Patterns to Mechanical Insufflation-Exsufflation in Healthy Subjects.

Andersen T, Sandnes A, Hilland M, Halvorsen T, Fondenes O, Heimdal J-H, Tysnes O-B and Røksund OD.

Am J Phys Med Rehabil 2013 Vol. 92: 920-9.

Paper II   Laryngeal response patterns influence the efficacy of mechanical assisted cough in amyotrophic lateral sclerosis.

Andersen T, Sandnes A, Brekka AK, Hilland M, Clemm H, Fondenes O, Tysnes O-B, Heimdal J-H, Halvorsen T, Vollsæter M and Røksund OD.

Thorax 2017 Mar; 72(3):221-229.

Paper III   Mechanically Assisted Cough in Progressing Amyotrophic Lateral Sclerosis.

Andersen T, Sandnes A, Fondenes O, Miodini Nilsen R, Tysnes O-B, Heimdal J-H, Clemm H, Halvorsen T, Vollsæter M and Røksund OD.

Respiratory Care, in press, accepted 28th November 2017.

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1.5 Abbrevations

AEF	Aryepiglottic folds
ALS	Amyotrophic Lateral Sclerosis
ALSFRS-r	ALS Functional Rating Scale – revised
BIS	Bulbar impairment scale
BS	Bulbar score
BMI	Body Mass Index
CI	Confidence interval
CLE	Continuous laryngoscopy during exercise test
CPAP	Continuous Positive Airway Pressure
CPF	Cough Peak Flow
CT	Computer Tomography
EILO	Exercise induced laryngeal obstruction
ENT	Ear-Nose-Throat
FEV_1	Forced expiratory volume in the first second
FVC	Forced vital capacity
EG	Epiglottis
MEP	Maximal expiratory mouth pressure
MI	Mechanical insufflation
MI-E	Mechanical Insufflation-Exsufflation
MIC	Maximal insufflation capacity
MIC>SCV	Calculated difference between MIC and SVC
MIP	Maximal inspiratory mouth pressure
MND	Motor neuron disease
NKH	Norwegian National Advisory Unit on Long-term Mechanical Ventilation
NIV	Non-invasive ventilation
PCA	Musculus cricoarytenoideus posterior
PCF	Peak cough flow
PEF	Peak expiratory flow
PI_{max}	Maximal inspiratory mouth pressure
PE_{max}	Maximal expiratory mouth pressure
RCT	Randomised controlled trial
RS	Respiratory score
SNIP	Sniff nasal inspiratory pressure
SVC	Slow vital capacity
TFL	Transnasal fiberoptic laryngoscopy
TVF	True vocal folds
USA	United States of America
WST	100-ml water swallow test

2. Background

2.1 Personal Introduction

Respiratory management of people with neurological disorders has improved significantly during the two last decades. This positive development has occurred for a range of reasons, but has primarily been due to an increased interest and volume of knowledge within the medical community, accompanied by technological advances. A better understanding of the pathogenesis of chronic respiratory failure, and the role played by weak cough have been important. In Norway, the *Norwegian National Advisory Unit on Long-term Mechanical Ventilation* (previously labeled *The Norwegian Centre of Excellence for Home Mechanical Ventilation*) was established in Bergen in 2002, with the aim to increase the quality of life of persons on long-term mechanical ventilation, and to facilitate equal access to evidence based treatment across Norway for patients with chronic hypoventilation. I have been employed by this institution as a respiratory physiotherapist with responsibility for airway secretion management since it was established.

The prevailing clinical impression is that MI-E in most neuromuscular patients contributes to more efficient clearance of airway secretions, improved management of chest colds and prevention of severe chest infections, and that requirement for hospitalizations and even tracheostomy can be reduced. However, patients with ALS and bulbar dysfunction have been particularly challenging to treat successfully with MI-E, allegedly due to disturbed laryngeal responses. This clinical observation is poorly understood, and has not been thoroughly investigated using comprehensive and verifiable methods. (Literature search has been done regularly since October 2010 and for the last time on 5th December 2017).

In our hospital (*Haukeland University Hospital*, Bergen, Norway), transnasal laryngoscopy is being extensively used as a diagnostic tool in a variety of functional tests; e.g., during ongoing maximal treadmill exercise to diagnose exercise induced

laryngeal obstruction (EILO). Inspired by these examinations, I decided to use laryngoscopy during ongoing mechanically assisted cough in patients with ALS in order to increase our understanding of why MI-E fails to help some of these patients, and - hopefully - to contribute to improving their airway clearance therapy. This idea constitutes the basis for my PhD thesis.

2.2 Amyotrophic Lateral Sclerosis

2.2.1 Definition

Amyotrophic Lateral Sclerosis (ALS) is a fatal, highly disabling and incurable neurodegenerative disease of upper and lower motor neurons.[1-3]

Charles Bell, a British anatomist and surgeon, reported the first ALS case in 1830, describing a condition that caused progressive paralysis of the limbs and tongue. The first to describe ALS as an entity was the French neurologist Jean-Martin Charcot; hence ALS is also known as Charcot disease. The condition gained more widespread attention after the very popular American baseball player Lou Gehrig was diagnosed with ALS in 1939.[4] In Europe and in USA the term *Amyotrophic Lateral Sclerosis (ALS)* is used, while in Australia and United Kingdom the term *Motor Neuron Disease (MND)* is preferred.[5]

The pathogenesis of ALS is poorly understood.[6] The disease is heterogeneous in its presentation and progression; e.g., with variabilities as regard the presence and the timing of onset of upper and lower motor neuron signs. Upper motor neurons have their origin in the motor cortex. Their axons travel through the great corticofugal tracts to the brain stem (corticobulbar neurons) and to the spinal cord (corticospinal neurons) to influence the patterned activity of the lower motor neurons.[7] Upper motor neuron abnormalities lead to spasticity and hyperreflexia, while abnormalities of lower motor neuron entail flaccidity and hyporeflexia in addition to muscle atrophy and fasciculation.[8,9] The motor deficits in ALS are often mixed and encompass both flaccid and spastic weakness.[8-11]

The initial clinical findings and symptoms in ALS are characterized either as *spinal onset* with clinical symptoms from the arms and/or legs, or as *bulbar onset*, with clinical symptoms primarily being difficulties with speech, swallowing and coughing. Regardless of the type of onset, muscle weakness and atrophy will, as the disease progresses, eventually involve all skeletal muscles.[3,12] *Spinal onset* ALS with gradually progressive weakness of arms or legs is the most common initial clinical presentation, occurring in about 70% of cases. About 25-30% of ALS patients have a *bulbar onset* disease, however, this is less common in younger patients and more common in older patients; i.e. occurring in 43% of those over 70 years.[13] The patients' history together with the clinical neurological examination commonly establishes the diagnosis and the subtype of ALS, in accordance with the El Escorial World Federation of Neurology revised criteria.[14,15] Some few ALS patients have documented signs of frontotemporal dementia, characterized by cognitive and behavioral dysfunction associated with changes in personal and social conduct.[4]

The causal factors leading to ALS are still unknown, and both environmental and genetic factors are assumed to be involved.[16,17] Authors have suggested that a high level of physical activity might modulate the risks of ALS, with increased risk among e.g. professional football players and in subjects with a body mass index (BMI) below average.[18,19] About 10% of ALS cases have a family history of the disease, and over 20 genes linked with familial ALS have been identified. Almost all familial cases have an autosomal dominant inheritance.[16]

2.2.2 Epidemiology

ALS is a rare disease; however, it is considered frequent among rare diseases.[16] A wide range of ALS incidence and prevalence rates have been reported in various populations.[17,20] In Europe, the reported annual incidence varies from 0.5/100 000 in Belgrade[21] to 3.6/100 000 in the Faroe Island,[22] and the prevalence varies from 1.1/100 000 (95% CI, 0.71–1.71) in "old" Yugoslavia[21] to 8.2/100 000 (95% CI 2.1–20) in the Faroe Islands.[22] The prevalence in Hordaland county in Norway, where our hospital is located, was 3.7/100 000 in 1989.[23]

ALS onset is typically around 60 years of age.[2] The incidence is highest in people aged 55–79 years, and onset below the age of 40 years is uncommon.[24] There is a modest male predominance. While bulbar onset has no gender prediction, the spinal onset is more common in males.[25] People older than 80 years have a standardized incidence of 10.2/100 000 in men and 6.1/100 000 in women.[26]

2.2.3 Prognosis

Involvement of respiratory muscles limits respiratory function and cough which leads to accumulation of secretions, increased risk of lung infections and eventually respiratory failure. In the end-stage of the disease, this strongly impacts quality of life and survival. Signs and symptoms of the disease increase as the disease progresses. Since there is no cure for ALS, the treatment is largely symptomatic and palliative.[1,12,13,27,28]

Most patients with ALS die within 2–3 years of developing the first symptoms, but 20% of patients survive for 5 to 10 years.[29] The median post-diagnosis survival time has increased over the last decade; 29 months for patients diagnosed before the year 2000 vs. 36 months for those diagnosed during 2000–2009.[30] This is most likely due to better access to multidisciplinary clinics and better treatment. A poor prognosis is associated with older age at disease onset, quick development of respiratory muscle weakness and bulbar onset.[5] Also rapid progression of bulbar or respiratory symptoms negatively influences outcome.[20] Contrary, limb onset, younger age at disease onset and longer diagnostic delay are independent predictors of prolonged survival.[29,31,32]

Care of the ALS patient focuses on maintaining function and quality of life.[24] Respiratory complications are life threating in ALS and a proactive preventive approach is a key element in disease management. Non-invasive ventilatory support and mechanically assisted cough are mainstay therapeutic techniques;[33,34] however, non-invasive methods are challenging to use and tend to fail in ALS when bulbar

dysfunction is present.[33,35-37] This is an unfortunate situation that hampers treatment success, particularly as the disease progresses.

2.2.4 Bulbar innervated muscel weakness

Muscles of the jaw, face, soft palate, pharynx, larynx and the tongue are innervated by neurons located in the so-called bulbar region of the brain stem.[11] The bulbar region of the brain encompasses the lower brainstem, pons and medulla.[38] Bulbar innervated muscle dysfunction leads to so-called *bulbar symptoms*, such as weakness of pharyngeal muscles, spasticity or lack of coordination of laryngeal or lingual muscles.[39,40]

Clinically, bulbar involvement in ALS can primarily be characterized by either spasticity or flaccid paresis and atrophy, depending on the extent of upper versus lower motor neuron involvement, the former usually labeled *pseudobulbar palsy* and the latter *progressive bulbar palsy,* or a combination. ALS mainly involving the upper motor neurons leads to *pseudobulbar palsy* which is clinically characterized by spasticity of the bulbar innervated muscles, emotional lability (e.g. pathological laughing and crying) and an enhanced masseter reflex (jaw jerk reflex). ALS mainly involving the lower motor neurons leads to *progressive bulbar palsy* which is clinically characterized by flaccid paresis and muscular atrophy, and fasciculation of the tongue (i.e. small, local, involuntary muscle contraction and relaxation) and/or tongue fibrillations (rapid, irregular, and unsynchronized contraction of muscle fibers).[11]

Dysarthria (difficulties to articulate and pronounce) and dysphagia (difficulties to swallow) are frequent features of bulbar symptoms in ALS, and can reduce quality of life and life expectancy. Dysarthria results from flaccid or spastic paresis of the bulbar innervated musculature. Dysphagia can result from weakness or spasticity of the muscles innervated by trigeminal, facial, hypoglossal, glossopharyngeal or vagal nerves.[11] Laryngospasm (uncontrollable muscular contraction of the vocal folds) is described to affect up to 19% of patients with ALS.[41]

Bulbar innervated muscle dysfunction is a huge challenge in the respiratory treatment of ALS, since upper airway stability is fundamental to the delivery of non-invasive respiratory aids to the lungs. Thus, bulbar muscle dysfunction is often associated with ineffective non-invasive respiratory management.[33,36,37,42] Bulbar insufficiency also corrupts effective cough, and thereby prevents removal of airway secretions.[35] ALS patients commonly report episodes of ineffective cough and episodes of choking when bulbar function deteriorates beyond a critical level.[43]

2.3 Larynx

The larynx functions as a valve to the airways with three major functions:

    1) To control airflow during respiration.

    2) To protect the lungs from aspiration.

    3) To play a key role in phonation.[44-47]

2.3.1 Anatomy

The larynx is located in the throat. The laryngeal inlet is situated at the inferior part of the pharynx (hypopharynx), and the larynx extends from the tip of the epiglottis, through the true vocal folds to beneath the border of the cricoid cartilage. See **Figure 1** for illustration.The larynx consists of rigid cartilage skeletons, ligaments and muscles for adduction and abduction.[44]
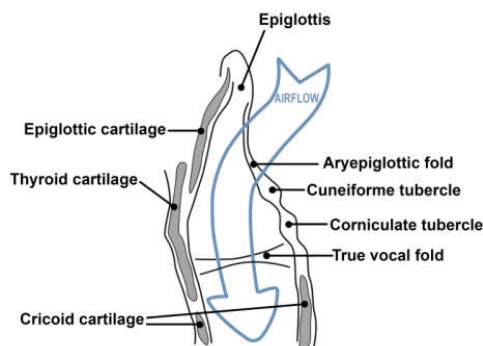
Figure 1. Side view of the larynx.

The intricate skeleton of hyaline and nine elastic cartilages are connected to each other by joints and elastic ligaments.[48,49] The aryepiglottic folds connect the epiglottis and arytenoid cartilage on either side of the midline.[50] Within the mucous membrane, there are two tubercles (corniculate tubercle and cuneiforme tubercle) formed by the two cartilages of the corresponding name. The laryngeal muscles can be described as either intrinsic or extrinsic, and they are under voluntary as well as involuntary (reflex) control. The larynx is innervated by the internal branch of the superior laryngeal nerve and the recurrent laryngeal nerves; both branches of the vagus nerve.[51]

2.3.2 Extrinsic and intrinsic muscles

The laryngeal *extrinsic* muscles can be referred to as strap muscles. They control the position of the larynx in the neck and are particularly important in swallowing, which involves elevating and depressing the larynx in the longitudinal axis.[52] Musculus cricothyroideus is the only extrinsic muscle that tightens and lengthens the true vocal folds.

The small *intrinsic* laryngeal muscles move the laryngeal structures in relation to each other. They are essential in breathing, speech, cough and swallowing where they interact in complex manners, but always act in concert.[48,49,53] The larynx has several small intrinsic adductor muscles, but only one abductor muscle; musculus cricoarytenoideus posterior (the PCA muscle). It operates in a coordinated and phasic relationship with the diaphragm, meaning that diaphragmatic vagal stimulation is coupled with increased activity of the PCA muscle, leading to laryngeal abduction and thereby opening of the laryngeal inlet immediately before diaphragmatic contraction.[54]

Abduction and adduction

Abduction of the glottis is fundamental for free airflow in and out of the lungs during respiration with the least possible resistance. During normal quiet breathing, the glottis widens during inspiration and narrows during expiration. This widening occurs ahead of the onset of inspiration, whereas the narrowing begins before the onset of

expiration.[55,56] Both forced inspiration and expiration are associated with increased activation of the intrinsic laryngeal muscles.[57] **Figure 2** demonstrates an open and closed glottis as viewed laryngoscopically.

OPEN (DEEP INSPIRATION)   CLOSED (GLOTTIC CLOSURE)
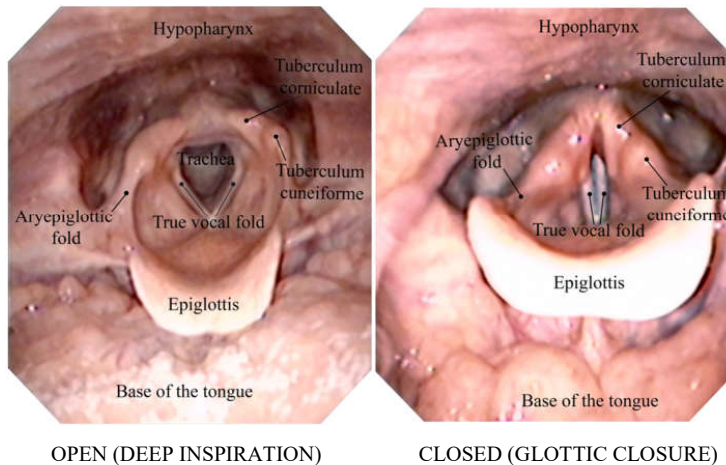
Figure 2. Top view of the larynx; open and closed glottis.

Tight adduction of the true vocal folds, together with the activity of the aryepiglottic muscles lead to closure of the inlet of larynx by bringing the aryepiglottic folds tight together. This enables the larynx to withstand the high pressures that can be generated in the thorax, e.g. during cough.[58] Varying degrees of laryngeal occlusion are also involved in speech.[52] During swallowing laryngeal occlusion is combined with a lifting of the larynx and elevating of the epiglottis in order to prevent the food bolus (i.e. the mass of food formed in the mouth, mixed with saliva) from entering the airways.[58,59]

Opening and closure of the glottis require complex and concerted intrinsic laryngeal muscle contractions involving similarly complex neural interactions that are only partially understood.[60,61] Stimulation of extremely sensitive receptors in the supraglottic larynx induce complex adductor reflexes that, for example, prevent foreign bodies from entering the airways.[54]

The larynx is considered to mature throughout puberty and little is known regarding inter-individual variability throughout adult life in healthy individuals. A gradual development of laryngeal structure and function from infancy to adulthood has been described, characterized by maturation of the cough reflexes and improved coordination and strength of the muscles that are involved in cough.[62-64]

Cough at the laryngeal level

At the laryngeal level, a normal cough requires intrinsic laryngeal muscle contractions[60,61] and consists of three distinct phases:

> 1) An initial abduction to allow airflow into the lungs.
>
> 2) A closing phase (0.2 seconds) to allow build up of intrathoracic pressure.
>
> 3) A secondary abduction of the true vocal folds to allow high expiratory airflow. The expiratory phase of cough can also present with a varying number of sequential glottic closures and openings.[65-67]

During the closing phase, closure of the true vocal folds is followed by closure of the supraglottic structures. These movements are further aided by squeezing of the pharyngeal walls, collectively creating a supraglottic sphincter.[66] A reflex cough is mediated (afferent) through the vagal nerve, while volitional cough is mediated through corticobulbar pathways.[43]

2.3.3 Aerodynamics

The larynx can be described as the bottleneck of the airways[68] accounting for a significant fraction of total airway resistance. The type of the airflow, either laminar or turbulent, contributes to airflow resistance of the airways; turbulent airflow generating more resistance than laminar airflow. Most of the turbulent airflow is shown to be located in upper airways; i.e. in the nose during nasal breathing and in the mouth, pharynx, glottis, larynx and upper trachea during mouth breathing. Especially at higher airflow rates, the resistance in the larynx becomes proportionally larger than the resistance in the lower airways.[69]

Increased airflow through the larynx may result in an aerodynamically induced laryngeal narrowing, or even a collapse of laryngeal structures. This is explained by the Bernoulli principle witch states that as the velocity of gas increases, the pressure exerted by the gas decreases. Translated into the laryngeal environment, this means that increasing airflow velocity leads to an increasing negative pressure inside the laryngeal lumen and then to an increasing inward pressure on the laryngeal architecture. The consequence of this is that the high airflows through the larynx may lead to negative pressures that may overcome the stability of the organ and thus lead to the structures being sucked inwards.[70] To prevent this happening, the forces of muscular contraction and tissue elasticity/rigidity together try to keep the larynx open, allowing a passage for air in and out of the lungs.[44]

2.3.4 Laryngeal effects of mechanically applied pressures

In healthy awake subjects, passive progressive positive pressure ventilation results in progressive glottic narrowing, occurring especially in the absence of diaphragmatic activity. The glottic narrowing during positive pressure ventilation increases the inspiratory resistance and reduces progressively the fraction of delivered minute ventilation reaching the lungs.[71]

The Bernoulli principle at the laryngeal inlet have been suggested to be causally involved in the condition known as floppy epiglottis during Continuous Positive Airway Pressure (CPAP) therapy. In this condition, the epiglottis is sucked into the laryngeal inlet during inspiration assisted by an externally applied positive pressure.[72]

Negative pressures applied in the expiratory phase of the respiratory cycle in healthy subjects have been described to provoke partial or total narrowing of the upper airway at the pharyngeal and the oropharyngeal level, and thus decrease expiratory flow rates.[73-75]

2.3.5 Laryngeal evolvement in ALS

Several neurological conditions with impairment of sensory afferents, abnormal reflexes, poor coordination or motor weakness can severely disrupt laryngeal

function.[76] Thus, problems of upper airway function are frequently encountered in ALS[39,77] and laryngological presentations of ALS are described in the literature,[39,78-82] particularly in relation to distinguishing between bulbar versus spinal onset ALS.[39,78,83]

Bulbar innervated involvement in ALS leads to abnormalities of the control and strength of the laryngeal and pharyngeal muscles.[39,40] Hillel and Miller described the typical progressive pattern of bulbar ALS, affecting firstly tongue and lips, secondly the palatal-, jaw- and pharyngeal muscles, thirdly facial-, upper trunk-, and laryngeal muscles, and finally the extra-ocular muscles. As a result of the order in which the muscles are being affected, swallowing difficulties are one of the earliest complaints reported by patients with bulbar ALS.[84] The tongue muscle deficit has been suggested to be the major factor causing swallowing difficulties in ALS.[85] Signs of laryngeal involvement can also include failure of the larynx to move superiorly and anteriorly during the swallowing, leading to incomplete closure of the larynx and thus a risk of aspiration.[84] Additionally to the reduced muscle strength and control, abnormal sensory function at the laryngeal level has been described in ALS,[86,87] and sensory deficits are more frequent in bulbar than in spinal onset ALS.[87] Also an impaired laryngeal adduction reflex[43] (the sensorimotor response that protects the airways from aspiration during and after swallowing) has been demonstrated in ALS.[85]

Pharyngeal and laryngeal complications lead to increased risk of aspiration, and eventually a need for the patient being fed via a percutaneous endoscopic gastrostomy tube.[80] Inability to coordinate the true vocal folds as well as weak expiratory muscle strength, impairs the compressive and expulsive characteristic of the cough,[88] challenging non-invasive management of airway secretions.[33,36,37,42]

2.4 Respiratory management in ALS

Respiratory insufficiency and pneumonia are the primary causes of comorbidity and mortality in ALS. Home mechanical ventilation and various airway clearance

techniques are critical components of respiratory management in ALS, and are often used in conjunction.[33]

2.4.1 Respiratory muscle weakness

In patients with ALS, respiratory muscle function is a strong predictor of quality of life[28] and survival.[89] The respiratory muscle weakness is a major clinical feature of ALS[24,90] and may affect all the respiratory muscles; both inspiratory- and expiratory, and the muscles controlling the upper airways.[91] It decreases both voluntary and reflex coughing, and eventually leads to respiratory failure.[11]

The progressive picture of respiratory muscular weakness in ALS contributes to lower functional lung volumes, decreased gas exchange and alveolar ventilation. Respiratory muscle weakness leads to ineffective cough, leading to further accumulation of airway secretion, pulmonary infections, atelectases, pneumonias and disturbed gas exchange.[92]

Upper airway muscle weakness often leads to accumulation (pooling) of saliva or secretions in larynx, drooling (unintentional loss of saliva from the mouth) and aspiration (passage of material into the level of the vocal cords). Drooling is often regarded socially unpleasant, and may represent an additional burden to the more serious medical problems of aspiration. Aspiration of food, liquids or nasopharyngeal or oral secretions can lead to aspiration pneumonia.[10,92]

Figure 3 illustrates pathological consequences and interactions from respiratory muscle weakness observed in people with neuromuscular disorders. Since ALS is a very rapidly progressing disease, the lung function deteriorates similarly rapidly. In fact, the pathological processes may evolve so quickly that the respiratory management often becomes responsive to already established pulmonary complications.
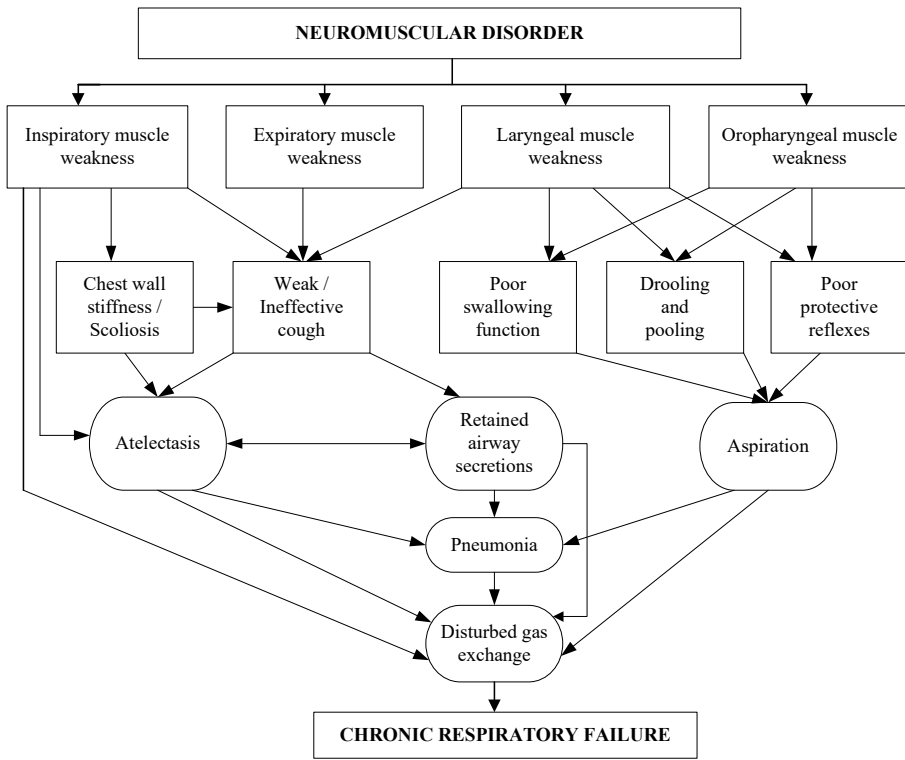
Figure 3. Pathologic consequences and interactions from respiratory muscle weakness in neuromuscular diseases.

2.4.2 Home mechanical ventilation

Mechanical ventilation is provided by positive pressure devices in the presence of daytime hypercapnia, symptomatic sleep-disordered breathing, and deteriorating pulmonary function. Technical development during last 100 years has provided ventilatory support that can be applied at home by means of small portable devices via a non-invasive interface, i.e. a nasal-mask, a face-mask or a mouthpiece.[36] Home mechanical ventilation is widely provided in neuromuscular disorders and predominantly delivered by *non-invasive mechanical ventilation* (NIV) without need for tracheostomy.[93] In contrast to other neuromuscular conditions, the use of ventilatory support in ALS has been evaluated using a randomized controlled study design.[94,95] NIV increases the average survival especially in patients with mild to moderate bulbar weakness and maintains quality of life. In patients with severe

bulbar impairment NIV had benefit in quality of life, but not in survival.[94] NIV may be most beneficial for quality of life when it is started before patients becomes symptomatic for hypoventilation.[95] Successful application of NIV requires adequate bulbar function to allow for sufficient airflow to the lungs,[42,96,97] and a preserved ability to cough and to remove airway secretions.[33,45,98]

Invasive mechanical ventilation and airway clearance via a tracheostoma cannula can compensate for these issues; however, it is less widely chosen in ALS since tracheostomy involves complex ethical issues and can have adverse effects on quality of life,[42] and also involves a greater burden of caregiving and financial costs.[33,99-104] Thus, to choose tracheostomy in ALS requires careful consideration in each individual patient.[105] There are no clinical guidelines that advice on the optimal timing to perform a tracheostomy in relation to increasing bulbar dysfunction and patients' deterioration.[106]

In summary, mechanical ventilation, both non-invasive and invasive, can extend life and improve respiratory symptoms in ALS.[37,91,94,107-111] However, non-invasive methods are often challenging in bulbar ALS patients.[37,94,107]

2.5 Airway secretions

2.5.1 Defense mechanism of the lungs

The respiratory tract from the nasal cavity to the bronchioles is covered by ciliated epithelium that contains mucus-secreting cells (goblet cells). Goblet cells together with submucosal secretory cells, produce a thick layer of mucus that lines all but the smallest conducting airways.[59] The airway mucus catches foreign particles, such as dust and micro-organisms that are inhaled into the lungs with the air during breathing. This mechanism prevents foreign particles from reaching the alveoli, where gas exchange takes place. Mucus is normally transported to the oropharynx by the airway clearance mechanism.[112-114] The airway secretions have an important role in the defense mechanisms of the lungs, and defective airway clearance can have serious

consequences as accumulation of airway secretions will lead to infections, atelectasis, increased airway resistance, increased work of breathing, hypoxemia, and eventually contribute to increased mortality.[88]

2.5.2 Normal airway clearance

Pheripheric airways

Airflow bias is the label used to describe an important phenomenon that occurs naturally in the airways during normal breathing. The mechanism implies that the diameter of the airways widens on inspiration and narrows on expiration, with unchanged inspiratory and expiratory volumes and times. Narrowing of the airways during exhalation will increase linear velocity and thus enhance the shearing forces in the airways during normal tidal breathing, as well as in deeper voluntary breaths and during sighs, thereby contributing to clearance of airway secretions. Airflow bias is also a contributing factor to the clearance of secretions from the larger airways, and it may be amplified during a cough.[114]

The ciliated epithelial cells contribute to moving the mucus layer towards the upper airways at the rate of 4 mm/min,[59] until the so-called isothermic saturation boundary is reached. This is the level of the airways where inspired gas is humidified to 100 % and heated to body temperature (37°), and is usually located at the 3rd to 5th generation of the bronchial tree during quit breathing.[115,116]

Typically, people with neuromuscular disorders have no abnormalities of these so-called mucociliary escalator mechanisms, unless a chronic aspiration scenario has been established or a chronic lung disease has evolved.

Proximal airways

Cough is a crucial element among the defense mechanisms of the airways,[117,118] particularly as regards removing airway secretions from the central airways.[119,120] The central airways contain irritant sensors that may become reflex triggered by mucus surplus or other physical or chemical irritants, subsequently inciting reflex cough.[43,67] An effective cough depends on coordinated and forceful movements from several muscle groups:

- Inspiratory muscles to increase the lung volume up to 85 – 90 % of the total lung capacity.

- Expiratory muscles to increase the thoraco-abdominal pressure to as high as 400 cmH$_2$O during glottic closure. Upon glottic opening, this enables extremely vigorous expiratory efforts and high expiratory flow rates, with peak cough flow in the range of 360–1200 liters/min.

- Laryngeal intrinsic muscles to facilitate finely tuned coordinated glottic openings and closures.[67,121,122]

During the expulsive phase of cough, the releasing pressures and the high expiratory flow rates shear the secretions from the airway walls and move the secretions upwards. This phase can be interrupted by short sequences of glottic closures and openings, each with compressive and expulsive phases, which can be seen as a series of *airflow spikes* on a maximal expiratory flow volume curve.[123] For details, please see **Figure 4**.

Peak cough flow is the highest measured airflow spike during cough, usually appearing immediately after the glottic opening phase of the cough cycle. When measuring peak cough flow, the person is simply encouraged to inhale deeply and then actively cough via a mask or a mouth piece into to a peak flow meter.[124] Peak cough flow is a measure of the person's ability and (indirectly) strength to cough, normal values in adults exceed 360 liters/min.[125]

Effectiveness of airway clearance in neuromuscular diseases has been shown to be related to peak cough flow.[120,126-128] Thus, techniques to augment coughing and to increase peak cough flow are recommended to prevent chest infections in neuromuscular disorders,[129-131] with peak cough flow of 160 liters/min considered a minimum for effective airway clearance.[120,126,132,133] Persons with neuromuscular disorders can be further weakened by acute episodes of viral illnesses. This has been linked to decreased inspiratory and expiratory muscle strength induced by the viral illness, leading to lower vital capacity and thus less effective cough.[134]
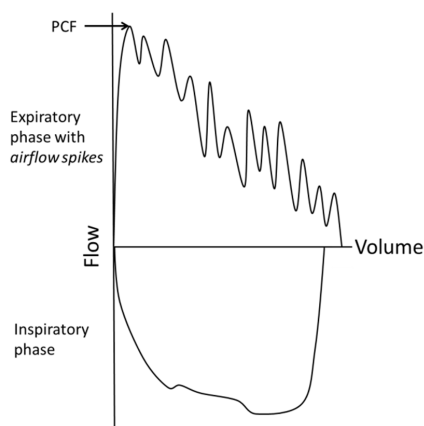
Figure 4. *Airflow spikes with the peak cough flow (PCF) during the expiratory phase of cough illustrated by the flow volume curve measured during a cough maneuver.*

2.5.3 Airway clearance in respiratory physiotherapy

Respiratory physiotherapy is a multifaceted discipline and consists of examination, assessment and evaluation of function, interventions, exercise and therapy to facilitate breathing, respiratory care and airway clearance, and - not least - education and advice on self-management.[130,135]

Airway clearance, also referred to as mucus clearance or secretion clearance, is one of the main elements in respiratory physiotherapy and a critical aspect of respiratory care.[88] Therapeutic airway clearance regimens should be effective, efficient, easy to use and teach, possible to carry out independently or with an assistant, and should improve lung function without being perceived as uncomfortable or causing hypoxemia. It should be flexible and adaptable to the changing needs of the individual patient.[136]

Physiotherapy techniques to clear airway secretions should support mucociliary clearing (peripheral techniques) and augment cough (proximal techniques).[104,124,136,137] The choice of technique depends on the patients' physiological and functional deficiencies rather than the medical diagnosis.[129] In primary pulmonary diseases, such as cystic fibrosis, primary ciliary dyskinesia, bronchiectasis and chronic obstructive pulmonary disease, the impaired airway

clearance is related to impaired mucociliary escalator mechanisms, and the physiotherapy techniques should therefore support peripheral airway clearance.[88] In neuromuscular disorders such as ALS, the impaired airway clearance is related to impaired cough due to respiratory muscle weakness, and the physiotherapy techniques should therefore focus on augmenting the cough. Some circumstances in neuromuscular disorders, for example during acute pulmonary infections, increased production of airway mucus and obstructions in the airways, the need for the institution of additional peripheral airway clearance techniques may be warranted.[124,137]

The aim of respiratory physiotherapy and airway clearance techniques applied to persons with neuromuscular disorders is to increase inspiratory volumes and expiratory flow.[129,130] Cough augmentation should target both of these aspects.[88,124,138]

The techniques physiotherapists use to assist cough often combine assisted insufflation of the lungs with expiratory support. Insufflation can be delivered by the technique of air stacking (repeated inspirations without breathing out) with manual resuscitator, mechanical device delivering positive pressure, or with glossopharyngeal breathing (self-air-stacking by using the mouth, tongue, pharynx and larynx to compensate for the weakness of the inspiratory muscles). Expiratory support can be provided either manually by thoraco-abdominal thrust or mechanically by exsufflation. Mechanical insufflation-exsufflation (MI-E) is a mechanical technique to assist cough combining both inspiratory and expiratory support.[88,124,139]

2.5.4 Airway clearance therapy in ALS

The aims of airway secretion therapy in ALS are to relieve the symptoms and the discomfort related to the accumulation of secretions, and to prevent and treat pneumonias. The need for assisted airway clearance techniques increases when the respiratory muscles become progressively weaker.[33,34]

In patients with ALS, peak cough flow below 270 liters/min during acute episodes suggests an ineffective cough, and therefore it is recommended that these patients

start using assisted cough techniques on a daily basis to prevent chest infections.[140] MI-E is regularly used in ALS.[132,141-145] Additional techniques that have been described are manually assisted cough, air stacking techniques, lung volume recruitment techniques, postural drainage and manual techniques (shaking, percussion and vibrations), active cycle of breathing technique, intrapulmonary percussive ventilation, humidification and mouth- and airway suction.[24,104,137,139,146-152]

2.6 Mechanical Insufflation-Exsufflation

MI-E is widely used in patients with neuromuscular disorders to assist and augment cough by applying positive and negative pressure changes to the airways, either non-invasively *via* a mask or invasively *via* a tracheostomy.[117,119,128,131,141,143-145,153-158]

When the patient inhales, the MI-E supports the inspiratory movement by delivering a positive pressure (insufflation), contributing to a better expansion of the lungs. This is followed by a rapid switch (10 milliseconds) to a negative pressure, supporting the expiration (exsufflation) of the patient's lungs. These positive and negative pressure swings are applied sequentially. The rapid switch from positive to negative pressure aims to simulate the airflow changes that occur during normal cough, potentially facilitating secretion clearance.[153] A voluntary cough maneuver is desired when using MI-E, and patients must learn to co-ordinate their own cough movements (i.e. glottic openings and closures) when the device switches from insufflation to exsufflation.[159] The treatment is tolerated by most patients with neuromuscular disorders, and it is considered a safe method for cough clearance.[119,155-157,160-162]

2.6.1 Clinical use

In Norway, MI-E devices became available in 2001, and currently the Norwegian public health care system provides MI-E for eligible patients in home setting.[163] The most commonly used MI-E device in Norway is *Cough Assist®* (Respironics, Murrysville, USA), and since 2013 *Cough Assist E70®* (Phillips Respironics, Murrysville, USA).

MI-E devices can in principle be operated in two modes: manual or automatic. In both modes settings entail pre adjustment of the positive and negative pressures (cmH$_2$O) and the inspiratory flow rate (liters/min). The automatic mode setting entails pre adjustment of the duration (timing) of the insufflation, exsufflation and the interval between the exsufflation and next insufflation (pause), when in manual mode this is operated during the therapy by the therapist. A new device from 2013 (Cough Assist E70®) also allows for patient triggered insufflation, as well as oscillations with interruptions of the insufflation and/or exsufflation pressures and flows.

Physiotherapists use MI-E to increase chest expansion (lung recruitment) during inspiration and to assist the expiratory cough movements as a part of the total respiratory physiotherapy treatment. The clinical use of MI-E is a dynamic process, involving to a large extent patient interactions. It is important to build up a good chest expansion by utilizing the assistance provided by the applied positive pressure during inspiration, and subsequently to adjust the negative pressure during exsufflation so that the audible quality of the cough reaches the desired and adequate levels. MI-E is often combined with manually assisted cough; i.e. the therapist manually providing abdominal thrusts coordinated with the patients cough movements during the exhalation phase.[159] The daily respiratory treatment of ALS patients usually takes place at the patients' home, and is usually performed by personal caregivers according to individually tailored procedures that are planned for home use. One treatment session with MI-E usually consists of several phases of coughing and rest, involving repeated cycles of insufflations and exsufflations, followed by approximately 30 seconds of rest. An additional thoraco-abdominal thrust applied by the therapist or by an assistant can be provided during exsufflation. These cycling periods are repeated several times or until secretions are substantially expelled.[153]

2.6.2 Settings

When using MI-E for individual patient treatment, the settings of positive and negative pressures, flow rates and time intervals should be adjusted to ensure reasonable patient comfort, and facilitate a deep inspiration that provides as good

chest expansion as possible, subsequently followed by timing and pressure settings during expiration that allow for optimal cough movements, capable of mobilizing secretions.[159]

Pressure

The literature varies regarding suggestions of what are optimal inspiratory and expiratory pressure settings, ranging from 20 to 60 cmH$_2$O in positive and negative pressures.[124,153] High pressures of ±60 cmH$_2$O have been described and used by Bach and co-workers.[128,133,157,164-167] However, lower pressures have been reported by other researches; ±30 cmH$_2$O[156] and +20 cmH$_2$O to -30 cmH$_2$O.[119,168] Pressure settings of ±40 cmH$_2$O appear to be a good compromise between efficacy and comfort.[117,151,153,169] Higher pressures have been proposed to be necessary when lung mechanics change during progression of the illness,[170] in scoliosis[117] or if used via a tracheostomy cannula.[171]

Insufflation flow

The insufflation flow rate used with the Cough Assist® device can be adjusted to either high flow (10 liters/min) or low flow (3 liters/min).[159] The optimal inspiratory flow settings when MI-E is applied in various disease conditions, have not been investigated in properly designed studies.

Oscillation

During the cycles of insufflation, exsufflation, or both, *the Cough Assist E70® device* can apply high-frequency oscillatory vibrations, generated by air pulses. Settings entail pre adjustment of the oscillation frequency within the range of 1-20 Hz, and the amplitude of oscillations within the range of 1-10 cmH$_2$O. Theoretically, these *high frequency oscillations* may facilitate the loosening of thick secretions, enabling them to be cleared.[172] This assumption is based on studies showing that the application of high frequency oscillations of airflow in the airway to change the viscoelastic properties of the secretions, making them more mobile.[173,174] Recently, a study by Sancho et al. demonstrated that addition of oscillations to MI-E does not have effect on the peak cough flow in medically stable patients with ALS.[172]

Time

The time settings applied with the various MI-E phases is mainly based on experience, and has not been investigated in properly designed studies. A study by Gomez-Merino et al. demonstrated that increasing the insufflation time is more important for optimal peak cough flow than increasing the exsufflation time.[175]

2.6.3 Effect

The effect of MI-E in neuromuscular disorders is mainly linked to its ability to increase peak cough flow to the level required to eliminate airway secretions, and thereby augment airway clearance.[117,119,128,131,141,143-145,153-158] A review from 2005[131] concluded that the use of MI-E is the most effective physiotherapeutic approach to increase peak cough flow and to maintain vital capacity in patients with neuromuscular disorder.[119,132,133,143,157]

A Cochrane systematic review of randomized controlled trials (RCT) using MI-E in neuromuscular disorders, failed to demonstrate any conclusive benefits as regards mortality, morbidity, or quality of life for patients or any serious adverse events from the treatment.[176] A recent review that also incorporated case series, cohorts and other pre-post trial designs other than RCTs, concluded that improvements of peak cough flow was consistently reported by all 12 studies; however, none of them reported survival advantages. Two of the studies (n=21 patients) found no change in hospital length of stay; however, two studies found a decrease in respiratory exacerbation rates, and improvements in quality of life were observed across a number of studies (n > 100 patients). None of the studies objectively measured sputum quantity. Further, the heterogeneity of the applied outcome measures complicated the summary statistics.[177]

Despite these limitations, most relevant international guidelines recommend the use of MI-E, supporting the view that *"absence of evidence of effect is not evidence of absence of effect"*.[137]

2.6.4 MI-E in ALS

Most literature suggests that MI-E is most efficient in ALS patients without bulbar dysfunction.[132,141-145] Particularly, in patients with bulbar involvement MI-E is less effective at increasing peak cough flow to the levels considered necessary for effective removal of airway secretions.[141,143] To increase understanding of this observation, Sancho et al. examined three patients with ALS at *baseline* conditions and during application of mechanical *exsufflation* with a computer tomography (CT) scanning of their upper airways (pharynx and oropharynx). They found that failure to increase peak cough flow to an adequate level was associated with a dynamic collapse of the upper airway during *exsufflation*. Although they did not examine directly the laryngeal level of the upper airways, they used their findings to suggest that coordinated movements of the glottis and intact bulbar function are necessary elements for effective use of non-invasive MI-E.[141]

It is a well-established clinical experience that the response to MI-E in patients with ALS is variable, but it is difficult to predict beforehand in whom the method will succeed and in whom it will not. Non-responders tend to express a sense of being *"unable to breath in or out"* with the device. In practice, a therapeutic trial is conducted in most neuromuscular patients with a manifested cough problem.

The knowledge obtained from the study of Sancho et al[141], and the assumptions made by other authors fit my own clinical experience as a respiratory physiotherapist, and it supports the hypothesis that preserved laryngeal function is crucial to the effectiveness of MI-E.

Nevertheless and surprisingly, laryngeal movements and patency during ongoing MI-E cycles applied in patients with neuromuscular disorders has yet to be studied.

2.7 Transnasal fiberoptic laryngoscopy

Transnasal fiberoptic laryngoscopy (TLF) was first performed in the late 1960s, and has since proved to be a well-tolerated examination that provides a direct view of the larynx. The findings can easily be video-recorded, and thus it is possible to scrutinize

and interpret outside the immediate examination context. This is particularly important in a study context, as the findings thereby can be made available for multiple assessors and also verifiable by others. TFL can relatively easily be performed by otolaryngologists or other trained medical doctors or speech therapists. It does not require sedation, and skilled hands can perform TFL with little discomfort and at no risk. TFL is *"the gold standard"* to visualize abnormal laryngeal movements.[68,178-184]

In addition to playing an important role in the otolaryngological examinations, TFL has allowed for development of several functional tests in which its use plays a primary role.[184] One of these is flexible endoscopic evaluation of swallowing; the laryngoscope is used to visualize the mechanics and efficiency of swallowing and to detect laryngeal malfunction and aspiration.[185,186] TFL is also used to diagnose exercise induced laryngeal obstruction (EILO), applied in the continuous laryngoscopy exercise test. This method was developed at our hospital, and is suitable for children as well as adults.[68,182,183] TFL has not routinely been used in ALS, but some studies have reported on vocal cord function during simple tasks such as vocalizing, spontaneous coughing, forced exhalation or swallowing.[39,76,78,80,87,90,187,188]

We hypothesized that visualizing the larynx by means of video recorded TFL applied during ongoing MI-E in patients with ALS could contribute to a better understanding of the functional role played by the larynx in this contextual setting. The ultimate aim of this project was to apply this knowledge to improve the clinical management of impaired airway secretion clearance in patients with ALS.

3. Aims of the thesis

The overall aim of this study was to explore the laryngeal response pattern(s) to mechanically assisted cough when applied in patients with ALS of different phenotypes and during the expected and rapidly progressing clinical course of the disease. Secondly, we aimed to use this knowledge to tentatively improve and possibly extend the use of MI-E in deteriorating patients with progressing ALS.

STUDY #I (Paper #I)

AIMS: To investigate the feasibility of TFL applied during ongoing MI-E, and to describe normal laryngeal response pattern(s) to MI-E, in healthy subjects.

> **Research question #1:**
> Can TFL successfully be performed during ongoing MI-E in healthy subjects?

> **Research question #2:**
> Are the laryngeal response patterns to MI-E in healthy subjects compatible to the laryngeal movements that are described for normal cough?

STUDY #II (Paper #II)

AIMS: To investigate the feasibility of TFL applied during ongoing MI-E, and to describe laryngeal response pattern(s) to MI-E in a cross-sectional study of patients with ALS of different phenotypes and to compare with healthy controls matched for age and gender.

> **Research question #3:**
> Can TFL be performed during ongoing MI-E in patients with ALS.

Research question #4:

Do the laryngeal response patterns to MI-E vary between patients with ALS with and without involvement of bulbar innervated muscle function and when compared to healthy controls?

STUDY #III (Paper #III)

AIMS: To investigate the feasibility of TFL applied during ongoing MI-E, and to describe changes of the laryngeal response pattern(s) to MI-E, in a longitudinal follow-up study of patients with progressing ALS, and to explore if treatment efficacy can be modulated and improved in these patients.

Research question #5:

Can TFL be performed during ongoing MI-E in deteriorating patients with progressing ALS?

Research question #6:

Do the laryngeal response patterns to MI-E evolve in ALS disease progression?

Research question #7:

Can the laryngeal response patterns to MI-E be modulated and improved by optimizing pressure settings, flow rates and time intervals?

4. Material and methods

4.1 Study design

This was a combined cross-sectional and longitudinal observational descriptive and explorative cohort design, applied in a field of medicine not previously studied with verifiable and objective methods.

Study #I (Paper #I)
A cross-sectional study of healthy volunteers.

Study #II (Paper #II)
A cross-sectional study of ALS patients and their age-and gender matched controls.

Study #III (Paper #III)
A prospective longitudinal cohort study of ALS patients.

4.2 Ethics

The study was based on voluntary participation and the subjects could withdraw from the study at any time. Written informed consent (Appendix 1 and 2) was obtained from all participants. The ethical considerations that were made when planning and conducting this study were based on the Declaration of Helsinki.[189] The study protocol was approved in 2011 by the Regional Committee for Medical Research Ethics of Western Norway (2011/784/REK vest).

Two years after the data collection had started, a new MI-E device was launched on the Norwegian marked with new options for pressure, flow rate, time and oscillation settings. We reasoned that it would be relevant to explore, within the frames of the present study, the opportunities that were provided by these new options, particularly

as this new MI-E device was made available by the Norwegian public health care system for home treatment of eligible ALS patients comparable to the enrolled patients. Therefore, based on the findings made in the second and cross-sectional phase of the project, a protocol amendment was made in 2013 to include additional and explorative use of MI-E settings thought to be beneficial to individual patients, applying to the third and longitudinal phase of the project. This protocol amendment was approved by the Regional Committee for Medical Research Ethics of Western Norway in 2013.

4.3 Sample size

Explorative studies have no *a priori* hypothesis (to be tested), but subsidiary hypothesis (to be explored), and calculation of sample size and final number of patients who should be included is difficult.[190,191] ALS is relatively rare and 100% fatal, and all diagnosed patients living in the area served by Haukeland University Hospital are routinely referred for followed-up at the hospital out-patient clinic. This setting enabled a population-based cohort design; cross-sectional in Study #II and longitudinal in Study #III. Same amount of healthy control subjects, individually matched for age and gender were recruited for Study #II.

4.4 Exclusion criteria

Prior to inclusion, all participants were screened for the following exclusion criteria: Age < 18 years, a history of laryngospasm, sensitisation to Xylocain® (anaesthetic used during laryngoscopy), pneumothorax, additional lung disease, cancer, acute infection of the chest one month before enrolment, frontotemporal paresis with cognitive changes and mental instability.

4.5 Participants

The thesis included three groups of participants who are described in **Table 1**.

Table 1. Overview of the study participants, design and publications.

	Population	*Study design*	*Publications*
Study #I	20 healthy medical students	Cross-sectional	**Paper #I**
Study #II/#III	20 ALS patients	Cross-sectional cohort	**Paper #II**
		Longitudinal cohort	**Paper #III**
Study #II	20 healthy volunteers (controls), matched for age and gender with the ALS patients	Cross-sectional	**Paper II**

4.5.1 Healthy medical students

Study #I included 20 healthy medical students, recruited to demonstrate the feasibility of TFL during MI-E, and to describe normal laryngeal response patterns to MI-E. Third year students at the Medical Faculty in University of Bergen, Norway received both verbal and written information about the study. Twenty students entered the study, after having signed the consent form.

4.5.2 ALS patients

Population

The participating patients were recruited from the multidisciplinary and multispecialized team at the ALS outpatient clinic at Haukeland University Hospital, which serves a population of approximately 500 000 inhabitants. We aimed to include as many ALS patients as possible during the 1.5-year recruitment period from December 2011 to June 2013, thus aiming to produce population-based data with low attrition rates. Approximately 20 ALS patients who have not undergone tracheostomy are usually enrolled at all times, and by experience approximately eight new patients are expected to be enrolled per year. All patients enrolled at the clinic are routinely rescheduled every three to six months. The patients who were enrolled at the clinic when the study was commenced had different disease phenotypes (i.e. both limb and bulbar onset) and they were all at variable stages of their disease progress.

Study #II

At the start of this study (December 2011) and during the 1.5 years inclusion period 37 patients without exclusion criteria received both verbal and written information about the study and were then invited to participate. Thirteen patients declined participation and four died soon after being invited, leaving 20 patients consenting to participate. Reasons for non-participation were severe disease and/or fatigue (n=7), or limb-onset ALS without bulbar symptoms and, therefore, no interest in participation (n=6) since treatment failure of MI-E in bulbar ALS was the focus of the study.

Study #III

All 20 patients from Study #II were asked to participate in the Study #III and 18 consented. One patient did not want to be examined further due to discomfort during the examination procedures, and one declined due to fatigue with disease progression. However, shortly after inclusion in Study #III, two patients became tracheotomised and three died. This resulted in a cohort of 13 ALS patients having repeated and scheduled examinations approximately every three to six months at the ALS outpatient clinic during the study period from 2011 to 2016. Patients were followed up until death, tracheostomy or they had become too weak to come to the out-patient clinic, or did no longer wish to participate.

4.5.3 Neurologically healthy age- and sex-matched controls

A separate control group, different from the one that was recruited for Study #I, and instead matched for age and gender to the participating ALS patients, had to be recruited for Study #II. The reason was that anatomical and physiological conditions change with age, and that data suggest that many factors affect the function of the larynx change after the age of 60.[192,193] Thus, 20 healthy individuals of comparable age and matched for gender to the participating ALS population were recruited through the network of our research group.

4.6 Study procedures

All the examinations (**Table 2**) were performed in conjunction to scheduled visits at the ALS outpatient clinic. In the Studies #I and #II, the data was obtained during one pre-planned visit. In Study #III, all data retrieval was performed longitudinally at planned visits to the out-patient clinic until death, tracheostomy, or withdrawal.

Table 2. Overview of the examinations used in Studies #I to #III.

Procedures	Study #I	Study #II	Study #III
Video recorded transnasal fiberoptic laryngoscopy during:	x	x	x
-Pre-set standardized protocol of MI-E settings used clinically	x	x	x
-Additional use of MI-E with new modalities using individualised patients settings for home treatment			x
Forced spirometry	x	x	
Slow vital capacity		x	x
Peak cough flow	x	x	x
Respiratory muscle strength	x	x	x
Maximal insufflation capacity		x	x
Clinical neurological examination		x	x
ALSFRS-r		x	x
Assessment of dysphagia		x	x

MI-E=mechanical insufflation-exsufflation, ALSFRS-r= ALS Functional Rating Scale –revised

4.6.1 Transnasal fiberoptic laryngoscopy examination

Transnasal fiberoptic laryngoscopy examinations were video-recorded in order to visualize the larynx during the use of MI-E and to provide opportunity to observe and describe laryngeal movements and patency retrospectively and in detail, both in real time and in slow motion.

Preparations and implementation

Participants were verbally informed about the examination both prior to the examination and during the complete MI-E protocol. Regular decongestant nasal spray (Otrivin®, GlaxoSmithKline Consumer Healthcare, London, England) was used

to facilitate the introduction of the laryngoscope. Examinations were performed by medical doctors specialized to perform TFL examinations; i.e. either ENT surgeons or trained pediatricians.

The flexible laryngoscope (Olympus ENF-P3, Tokyo, Japan), diameter 3.5 mm, was lubricated with local anesthetic gel (Xylocain®, Astra Zeneca, Södertälje, Sweden) and led through an oronasal mask, the nose and via the nasopharynx advanced until a good view of the larynx was obtained. A modified oronasal facemask (adult facemask for Cough Assist® ventilatory circuit, Respironics, Murrysville, USA) served to fasten the laryngoscope and to allow TFL video recordings to be obtained at the same time as the MI-E procedures were performed. In Study #I, the laryngoscope was attached to a head mount with a customized scope-holder during the examinations (**Figure 5**). In the Studies #II and #III, the laryngoscope was supported manually (**Figure 6**), instead of using the customized headgear.

The laryngoscope was connected to a video camera-system for continuous recording during the entire examination. The examinations were continuously filmed by two independent video cameras/recording systems:

- An endoscopic video camera system (Telecam, Karl Storz, Tuttlingen, Germany) via the transnasal fiberoptic laryngoscopy.

- An external video camera with an attached microphone, in order to document the MI-E control panel, the instructions given by the therapist, and the respiratory sounds produced by the patients.

The two video recordings and the sound track were stored as one file, which allowed for retrospective investigation of the laryngeal events during all MI-E pressure cycles. To ensure adequate technical quality of the recordings while they were performed, the complete set-up was shown real-time during the entire procedure on a television screen present in the same room as the procedure was performed (**Figure 6**; Samsung Electronics 55", Suwon, South Korea).

Figure 5. *The set up in Study #I with laryngoscope going through a modified interface and attached to a custom made headset.*

Figure 6. *The setup in Studies #II and #III with a laryngoscope passing through a modified interface with the laryngoscope supported and adjusted manually. Situation arranged.*

Standardized MI-E protocol during TFL examination

A standardized MI-E protocol was prepared, consistent with procedures commonly applied during therapeutic use in patients with neuromuscular disorders with a manifest cough problem. The MI-E device (Cough Assist®) was used both in an

automatic mode with applied mechanical insufflations and exsufflations (MI-E), and in a manual mode for applied mechanical insufflations (MI) only, followed by a manually assisted cough; i.e. thoracic thrusts applied by the therapist accompanied by instructions to cough. The automatic MI-E mode had the following settings: insufflation time of two seconds, exsufflation time of two seconds and pause time (between exsufflation and next insufflation) of one second. The protocol included 12 intervention arms, i.e. various combinations of pressures, patient instructions and manual thoracic thrusts (**Table 3**). Pressures of ± 20, ± 30, ± 40 and ± 50 cmH$_2$0 were used with instructions as follows.

1) With MI-E; Instruction to actively "inhale" (but not too deep) when insufflation was started and instruction to actively "exhale" when the device switched to exsufflation.

2) With MI-E; Instruction to actively "inhale" (but not too deep) when insufflation was started and instruction to actively "cough" when the device switched to exsufflation.

3) With MI: Instruction to actively "inhale" (but not too deep) when insufflation was started and instruction to actively "cough" while subjects received manually assisted thoracic thrust.

Each intervention arm was repeated two to five times to secure good quality video recordings. After each intervention arm, a quick break was held to prevent hyperventilation. In the case of patient discomfort, the procedure was stopped and higher examination pressures were not applied.

Table 3. Standardized protocol of conditions during the intervention with MI-E and MI.

Intervention arm	Pressure settings (cmH$_2$O): MI-E	MI	Instruction during exsufflation: active exhale	active cough	Manual thoracic thrust
1.	±20		x		
2.	±20			x	
3.		+20		x	x
4.	±30		x		
5.	±30			x	
6.		+30		x	x
7.	±40		x		
8.	±40			x	
9.		+40		x	x
10.	±50		x		
11.	±50			x	
12.		+50		x	x

MI-E=Mechanical insufflation-exsufflation, MI=Mechanical insufflation.
Intervention arms 1-12: respective pressures of MI-E or MI combined with instruction to either actively exhale or to cough during exsufflation. Additional manual thoracic thrust during cough was provided in combination with MI.

Additional MI-E use of individually titrated settings

New and individually customized MI-E settings were put into use for home treatment of ALS when a new MI-E device with new modalities became available on the market in 2013 (Cough Assist E70®; Respironics, Murrysville, PA, USA). Thus, we additionally tested these settings as an extended study arm of Study #III.

We tested patient triggered insufflation (on/off) and oscillation (frequency in the range of 5 to 10 Hz and amplitude in the range of 5 and 10 cmH$_2$O) along with asymmetric use of pressure settings (positive pressure range +15 to +40 cmH$_2$O combined with the negative pressure range of -30 to -40 cmH$_2$O) and reduced insufflation flow (high, medium and low). Each individual setting of interest was performed two to four times; whereof the cycle with best quality was studied retrospectively for laryngeal responses. **Table 4** illustrates the overview of individually tested home settings in each six cases.

Table 4. New and individually adjusted settings were put into use for ALS home treatment when a new MI-E device became available in 2013 (Cough Assist E70®; Respironics, Murrysville, PA, USA), and tested as an extended arm of Study #III

	Case no.					
	2	**3**	**5**	**6**	**7**	**13**
Patient triggered insufflation (On/Off)	On and off	On and off	On and off	On and off	On and off	On and off
Insufflation pressure (cmH$_2$O)	+15 to 40	+25 to 40	+20 to 40	+25 to 40	+25 to 40	0 to 40
Insufflation flow (L/M/H)	L/H	L/M/H	L/H	M/H	L/M/H	M
Insufflation time (sec)	2.0 to 3.5	1.8 to 3.0	2.0 to 3.0	2.0	2.0 to 3.0	2.0
Exsufflation pressure (cmH$_2$O)	-40	-30 to 40	-40	-40	-35 to 40	-40
Exsufflation time (sec)	1.0 to 2.0	1.8 to 2.0	1.7 to 2.0	2.0	2.0	2.0
Oscillation (On/Off)	Off	On/off	On/off	On/off	On/off	Off
Frequency (Hz)	-	10	5 and 10	10	10	-
Amplitude (cmH$_2$O)	-	10	5 and 10	10	5	-
Visits with CAE70 during TFL	1	6	2	4	4	1
(in the range of months)		(37)	(13)	(12)	(32)	

L=low, M=medium, H=high

4.6.2 Examination of pulmonary function and respiratory muscle strength

Pulmonary function and respiratory muscle strength were determined as baseline characteristics of all study participants, and were followed up during the disease progression of the ALS patients participating in Study #III.

Forced spirometry

Forced spirometry was performed in Study #I and #II. In Study #I, it was performed seated and in Study #II both seated and supine, starting at total lung capacity, and measured with Vmax 22® (SensorMedics, Yorba Linda, USA), applying standard quality criteria. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV$_1$) and peak expiratory flow (PEF) were recorded, and the values expressed as percentages of predicted.[140,194-197]

Slow vital capacity

Slow vital capacity (SVC) was measured in Study #II and #III, starting at total lung capacity, and in seated position using a Respirometer Wright 14® (nSpire Health,

Hertford, UK). The highest value of three or more attempts was chosen for analysis and standardized to percentages predicted.

Peak cough flow

Peak cough flow (PCF) was measured in all studies, starting at total lung capacity, and in seated position via a mask using a hand-held Peak Flow Meter (Vitalograph®, Ennis, Ireland). The highest value of three or more attempts was chosen for analysis.

Respiratory muscle strength

Plateau values (average of one second) of the maximal inspiratory (Pi_{max}) and expiratory (Pe_{max}) muscle strength were measured in all studies, and sniff nasal inspiratory pressure (SNIP) was measured in Study #II and #III, in seated position using a Respiratory Pressure Meter (Micro RPM®; Micro Medical, Rochester, UK). SNIP was measured at functional residual capacity, Pi_{max} at residual volume and Pe_{max} at total lung capacity. The highest value of three or more attempts was chosen for Pi_{max} and Pe_{max} measurements, while the highest value of ten attempts was chosen for SNIP; and finally all raw data was standardized to percentages predicted.

Maximal insufflation capacity

Maximal insufflation capacity (MIC) was measured via an oronasal mask and in seated position using a Respirometer Wright 14® (nSpire Health, Hertford, UK) in ALS patients in Study #II and Study #III. MIC was measured after air stacking; i.e. the patient taking a deep breath, holding it, and then consecutively delivering volumes of air from a manual resuscitator (Lærdal Medical, Lærdal, Norway) to the maximum volume that the patient could hold with a closed glottis. The patient then exhaled the maximally held volume of air into the respirometer for volume measurement. The difference between MIC and SVC was calculated (MIC>SCV). The highest value of three or more attempts was selected.

4.6.3 Neurological assessments

Neurological assessments were used to determine the baseline characteristics and the phenotype of the ALS patients when entering Study #I, and during the follow-up performed during Study #III.

Clinical neurological examination and categorization

ALS was diagnosed by neurologists in accordance with revised El Escorial criteria.[14,15] Details on diagnostics and description of symptoms and signs and their onset were obtained retrospectively from medical journals. The clinical neurological examination included identification of the relevant symptoms and signs when patients were enrolled and at each subsequent visit during the follow-up period. Particularly, the patients were phenotyped as regards presence or absence of upper and lower motor neuron degeneration, or if a combination of these features were present (**Table 5**).

In Study #II, patients were categorized by *the state of the disease* at enrollment (**Table 6**) classified as either *spinal ALS* or *bulbar ALS* that was further sub-classified as *progressive bulbar palsy* or *ALS with pseudobulbar palsy*.

In Study #III, patients were categorized in two groups *by the initial onset of the symptoms of ALS; i.e. spinal or bulbar onset* (**Table 7**). This data was collected either retrospectively from medical charts in those participants who had already been diagnosed and enrolled in the ALS outpatient clinic when the study was commenced, or at the time of being diagnosed for those who were included later. Bulbar symptoms were further classified as *progressive bulbar palsy* or *pseudobulbar palsy*, as described in **Table 6**.

Table 5. Patient characteristics used to determine bulbar upper or lower motor neuron involvement.

	YES	*NO*	*Notice*
Triggered jaw reflex?			
Tongue atrophy?			
Reduced force in the tongue?			
Reduced pace in the tongue?			
Considering the state of the disease essentially as:			
Spastic (pseudobulbar paresis)			
Peripheral (progressive bulbar paresis)			

Table 6. Categorization of the *state of the dis*ease at enrolment in the Study #II.

State of the disease	Symptoms and signs
Spinal ALS	Symptoms affects the limbs
ALS with Progressive bulbar palsy	Hypotonic bulbar symptoms with dysarthria Tongue atrophy Absence of the jaw reflex
ALS with Pseudobulbar palsy	Spastic bulbar symptoms with dysarthria No tongue atrophy Exaggerated jaw reflex

Table 7. Categorization of the *onset of the disease* retrospective from medical journals in the Study #III.

Onset of the disease	Symptom onset in	Signs
Spinal ALS	Limbs	Muscle weakness
Bulbar ALS	Bulbar innervated muscles	Voice harshness, defects of articulation, breathlessness, dysphonia, hyper nasality and swallowing difficulties

ALS Functional Rating Scale-r (ALSFRS-r)

Patients were assessed by the neurologists using the ALS Functional Rating Scale (ALSFRS-r). The ALSFRS-r is a validated measure of functional impairment in ALS,[198] nevertheless its sensitivity is discussed.[199] It is a questionnaire-based functional scale, containing 12 items rated from 0 (complete dependence for that

function) to 4 (normal function), divided into 3 sub scores (bulbar 12, spinal 24, and respiratory 12), with normal function defined by a maximum score of 48.[198]

Items from the ALSFRS-r involving functions of speech and swallowing (maximum score of 8) were used to rate the bulbar impairment scale (BIS) in Study #II. [200]

In Study #III the bulbar and respiratory scores were calculated from ALSFRS-r, where the items of speech, salivation and swallowing, dyspnoea, orthopnoea and ventilation were calculated (each with a maximum score of 12).[198]

Assessment of dysphagia

Dysphagia in Studies #II and #III was determined with 100-ml water swallow test (WST). Subjects were asked to drink 100-ml water and the timing (seconds) to complete the task was recorded. Swallowing speed < 10 ml/s was classified as dysphagia.[201,202]

4.7 Analysis of the videorecordings

4.7.1 Editing of the video clips

Video recordings from each subject were edited into a video file including film clips based on representative shots of each intervention arm. In the video evaluation, the MI-E cycles were cut into three phases of interest: the insufflation phase, the point when the MI-E device switches from positive to negative pressure (labelled the pressure drop), and the exsufflation phase or the voluntary cough with no negative pressure applied. The onset and offset of each phase was observed and defined from the parallel external video recording of the MI-E manometer (**Table 8**).

Table 8. Definitions for onsets and offsets of phases for insufflation, pressure drop, exsufflation and voluntary cough with no applied negative pressures

Phase of interest	Definition for onset	Definition for offset
Insufflation (Time settings for insufflation is 2 seconds)	The point when the positive pressure is started with the MI-E device. Observed from the manometer on the control panel of the MI-E device.	Offsets when the positive pressure on manometer turns off.
Pressure drop (Automatically very rapid phase)	The point when the MI-E device switches from positive to negative pressure. Observed from the manometer on the control panel of the MI-E device.	Offsets when the negative pressure commences and appears on the manometer on MI-E devices control panel.
Exsufflation (Time settings for exsufflation is 2 seconds)	The point when the negative pressure is applied with the MI-E device. Observed from the manometer on the control panel of the MI-E device.	Offsets when the negative pressure on manometer turns off.
Voluntary cough with no applied negative pressures	The point when the MI-E device has pressure of zero after an insufflation. Observed from the manometer on the control panel of the MI-E device.	Offsets when the last glottic closure in voluntary cough maneuver is observed.

4.7.2 Interpretation of the video clips

Since the laryngeal movements during MI-E have not been studied previously, there was no standardized assessment score available. The intention of the present study was to establish a scientific foundation and a tool for the assessment of the larynx during MI-E. Laryngeal movements were observed and assessed from the video files, and this process generated categorized data from these explorative examinations.

Laryngeal anatomy and movements at rest in Study #I and #II were evaluated by a senior otolaryngologist. Laryngeal responses during MI-E were evaluated by this PhD candidate in cooperation with a senior otolaryngologist. In Study #II recordings were initially evaluated together by two raters (Anne-Kristine Brekka, MSc student in cardiopulmonary physiotherapy, and this PhD candidate) and verified by a senior otolaryngologist.

The video clips were reviewed and evaluated both in real time and in slow motion as many times as needed, both in real time and in slow motion. The observed movements at glottic, supraglottic and hypopharyngeal levels (**Figure 7**) were

described both by words and with snapshots of the laryngeal positions during the movements. Thereby laryngeal responses were classified into explicitly defined categories, and an observation scheme was prepared (Appendix 3). Thereafter the video recordings were replayed as many times as needed and the responses observed in the individual participants were assessed and recorded by "yes or no" in the observation scheme. Laryngeal responses during MI-E were compared to "adequate laryngeal control", defined as described for normal cough in the literature,[65,67] and presented as initial inspiratory abduction of the true vocal folds and aryepiglottic folds, and thereafter glottic closure with subsequent rapid opening when coughing, abduction of the true vocal folds and aryepiglottic folds followed by sequential closures and/or narrowing in the exhalation phase of the cough.

In the Study #III, five laryngeal events were targeted and tested, and defined as adverse and typical "bulbar features" and depicted in **Table 9**. See **Figure 8** for the process chart.
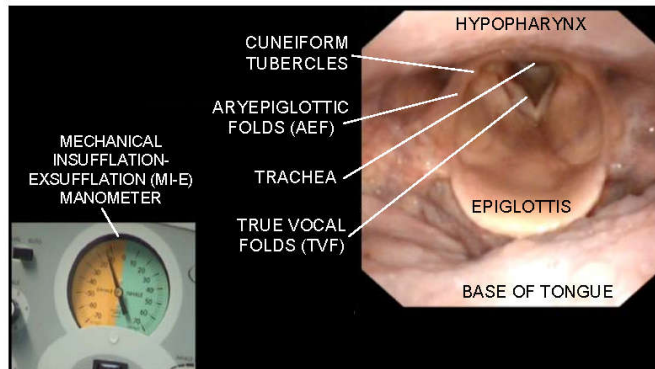
Figure 7. The examinations were recorded using two continuously running and synchronised video streams on one screen, depicting the laryngeal view in one and the various phases on the MI-E device in the other. Anatomic landmarks are illustrated on the laryngeal top view.

Table 9. Five laryngeal events during MI-E defined as adverse and typical bulbar features based on findings in Study #II, contrasting what has been described as normal cough.[65,67]

Laryngeal level		Adverse laryngeal response during MI-E	
Glottic	True vocal folds	Adduction of true vocal folds during insufflation; paradoxical movement of true vocal folds during inhalation creating either a slim glottic opening or a total closure of glottis.	
Supraglottic	Aryepiglottic folds	Medial rotation of the cuneiform tubercles accompanied by considerable adduction of aryepiglottic folds during insufflation, to the extent that it prevents observation of the glottic laryngeal level below.	
	Epiglottis	A retroflex movement of the epiglottis (a passive dorsal rotation) covering the glottis, either as a brief movement or lasting throughout the insufflation.	
Hypopharyngeal	Tongue base	Backward movement of the tongue base during insufflation constricting the laryngeal entrance.	
	Hypopharynx	A severe hypopharyngeal narrowing during exsufflation.	
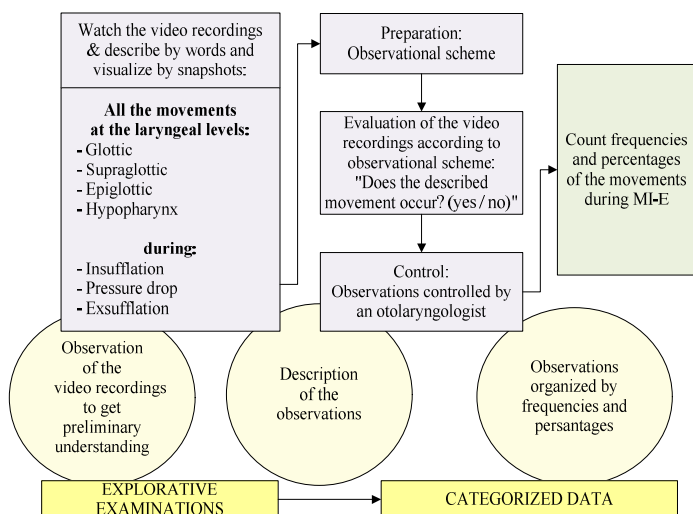
Figure 8. Process chart of analysing the laryngeal responses during MI-E from unstructured to structured data.

4.8 Statistical methods

The statistical analyses used in all studies are presented in **Table 10**. All p-values were two-sided and values below 0.05 were considered statistically significant.

Table 10. Statistical methods used in Studies #I to #III.

Statistical methods	Study #I	Study #II	Study #III
Group counts and frequencies	x	x	x
Mean and standard deviation	x	x	x
Median and range			x
The chi-square test or Fisher's exact test if expected cell counts < 5		x	
Depicted line plots of longitudinal continuous measures			x
Kaplan Meier survival function			x
Log rank test			x
Wilcoxon Signed Rank test			x

Study #I

Quantitative data of laryngeal movements during the MI-E were presented as percentages with the described movements. Baseline characteristics were presented as descriptive statistics in tables with means and standard deviations. Data from the assessment were processed in Excel for Windows Office version 2010 (Microsoft, Redmond, Washington, USA) and in SPSS v18.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, Illinois, USA).

Study #II

The number of subjects with the described patterns of laryngeal movements during MI-E was given as group counts and percentages. The chi-square test, or Fisher's exact test if expected cell counts were less than five, were applied to assess differences between groups with regard to categorical data. Background data were given as group means with standard deviations. Statistical analyses were conducted using SPSS v21.0 (Statistical Package for Social Sciences, SPSS Inc, Chicago, Illinois, USA).

Study #III

The number of subjects with the described patterns of laryngeal movements during MI-E was given as group counts. Individual longitudinal continuous measures were depicted using line plots. The Kaplan Meier survivor function was used to estimate the proportion of the various clinical and MI-E events as well as the corresponding median event times. To test for difference in survival functions across groups, the log-rank test was used. In the Kaplan Meier analyses, the time (in months) from ALS symptom start until the first occurrence of the relevant event was used as the measure of event time. A person's event time was "right censored" if he died or did not experience the event before study end, defined as December 31st 2016. To investigate if various laryngeal outcomes occurred before the onset of bulbar symptoms in time, we used Wilcoxon Signed Rank test. If an individual did not experience laryngeal events and/or bulbar symptoms, that individual was removed from the analysis. Patient group characteristics were quantified, and central tendencies given as means or medians with variability measures indicated by standard deviation, 95% confidence intervals, or range, as appropriate. All statistical analyses were performed using Stata version 14 (StataCorp LLC, Texas, USA) for Windows.

5. Summary of results

Background characteristics of the study participants (n=60) are described in **Table 11**, and the overview of the scheduled intervention arms and evaluated video clips of TFL examinations is presented in **Table 12**.

Table 11. Background characteristics of the study participants (n=60). Figures are group means with standard deviations.

	Healthy medical students (n=20)	*Healthy age- and gender matched controls (n=20)*	*ALS patients in the cross sectional study (n=20)*	*ALS patients in the cohort study (n=13)*
Male/female ratio	8/12	13/7	13/7	10/3
Age, years	24.4 (1.9)	66.9 (7.2)	68.7 (9.3)	67.1 (8.5)
BMI, kg/m^2	22.6 (2.0)	23.9 (2.4)	23.6 (4.3)	24.1 (3.4)
FVC, % pred	106.7 (8.5)	113.6 (16.0)	67.4 (22.1)	72.4 (18.7)
FEV1, % pred	99.1 (13.0)	107.4 (19.0)	70.6 (25.7)	74.5 (25.0)
SVC, l	-	4.15 (1.3)	2.92 (1.0)	3.4 (0.7)
SVC, % pred	-	110.6 (20.1)	76.1 (22.5)	82.5 (15.7)
PCF, l/min	501.0 (100.7)	484.5 (130.2)	266.8 (145.8)	318.5 (128.6)
Pi_{max}, cmH$_2$O	101.0 (23.9)	95.2 (23.6)	43.3 (20.9)	49.3 (20.5)
Pi_{max}, % pred	101.0 (24.3)	111.3 (24.9)	52.9 (23.7)	57.3 (19.5)
Pe_{max}, cmH$_2$O	132.6 (28.3)	140.8 (37.9)	50.4 (30.0)	61.2 (31.1)
Pe_{max}, % pred	105.1 (20.3)	140.1 (34.3)	49.4 (24.8)	56.3 (25.3)
SNIP, cmH$_2$O	-	91.2 (33.7)	38.6 (17.9)	42.0 (17.9)
SNIP, % pred	-	99.1 (34.6)	42.6 (19.0)	45.2 (20.0)
MIC>SVC, l	-	-	-	0.26 (0.44)
WST, ml/s	-	31.50 (7.7)	12.3 (11.4)	16.3 (11.0)
ALSFRS-r	-	-	36.7 (8.4)	38.5 (5.9)
BIS	-	-	6.0 (2.3)	6.38 (2.1)
BS	-	-	8.79 (3.9)	9.5 (3.1)
RS	-	-	9.95 (3.4)	10.2 (3.1)

ALS=amyotrophic lateral sclerosis, BMI=body mass index, FVC=forced vital capacity, FEV1=forced expiratory volume in 1 second, SVC=slow vital capacity, PCF=peak cough flow, Pi_{max}=maximal inspiratory mouth pressure, Pe_{max}=maximal expiratory mouth pressure, SNIP=sniff nasal inspiratory pressure, MIC=maximal insufflation capacity, WST=water swallow test, ALSFRS-r=ALS functional rating scale (revised), BIS=bulbar impairment scale, BS=bulbar score, RS=respiratory score

Table 12. Scheduled intervention arms and evaluated video clips of TFL examinations.

	Scheduled intervention arms	*Evaluated video clips from intervention arms*	*Technical failures*	*Technical failure percent (%)*
Study #I	240	239	1	0.4
Study #II	480	453	6	1.3
Study #III	763	751	12	1.6
Total in all studies	1483	1443	19	1.3

5.1 Paper #I (Study #I)

Laryngeal Response Patterns to Mechanical Insufflation-Exsufflation in Healthy Subjects

All participants (20 healthy volunteers, aged 21-29 years) completed all examinations according to plan. Visualization of the larynx with TFL did provide a good overview of the larynx, and it was possible to observe movements of the laryngeal structures throughout the complete MI-E intervention. In total 239 laryngoscopy recordings were analysed.

An initial abduction of the vocal folds was observed in all subjects, both during the insufflation and exsufflation phases. 19 of 20 subjects adequately coordinated glottic closure when instructed to cough. When instructed simply to exhale during exsufflation, the glottis stayed open in a majority. Subsequent to an initial abduction during exsufflation and cough, various obstructive laryngeal movements were observed in some subjects, such as narrowing of the true vocal folds, retroflexion of the epiglottis, hypopharyngeal constriction and backward movement of the base of the tongue.

In conclusion, the study showed us that video recorded TFL during MI-E was possible in healthy subjects, and also a feasible and well-tolerated method to characterize laryngeal movements. The laryngeal responses to MI-E were compatible with the laryngeal movements that are described for normal cough; however, there

were some heterogeneities. The findings from the study suggested that laryngoscopy may be of value in patients.

5.2 Paper #II (Study #II)

Laryngeal response patterns influence the efficacy of mechanical assisted cough in amyotrophic lateral sclerosis

All 20 patients with ALS (6 without bulbar symptoms and14 with bulbar symptoms; 7 with pseudobulbar palsy and 7 with progressive bulbar palsy) and 20 neurologically healthy controls, matched for gender and age, were tested. Four patients with bulbar symptoms completed only parts of the MI-E protocol due to discomfort from the applied pressures. In total 453 (94%) of 480 scheduled recordings were analyzed. The overall impression was that video-recorded TFL was a tolerable and feasible method to characterize laryngeal responses throughout MI-E in ALS patients.

In general, the larynx moved downwards during applied insufflation and upwards (cranially) during exsufflation. Healthy subjects and patients with ALS and no bulbar symptoms (n=6) coordinated their cough well during MI-E. The ALS patients with bulbar symptoms (n=14) adducted their aryepiglottic folds (supraglottic level) during insufflation. At the glottic level, initial abduction of true vocal folds followed by subsequent adduction was observed in all ALS patients (n=20) during insufflation and exsufflation. Hypopharyngeal constriction during exsufflation was observed in all subjects, most prominently in patients with ALS and bulbar symptoms. Differences in laryngeal movements between patients with pseudobulbar (n=7) and progressive bulbar ALS (n=7) were not statistically significant.

In conclusion, the study showed that laryngoscopy during ongoing MI-E was possible in patients with ALS. If bulbar symptoms were present, the larynx adducted, especially during insufflation but also during exsufflation, severely compromising the size of the laryngeal inlet in some. This effect seemed to be influenced by the MI-E settings in that higher pressures had a greater negative impact.

5.3 Paper #III (Study #III)

Mechanically Assisted Cough in Progressing Amyotrophic Lateral Sclerosis

The 13 ALS patients who were longitudinally followed up varied substantially according to ALS subtype and disease progression. Follow-up time was median 17 months (range 6-59). In total 751 TFL recordings from 67 individual examinations were analyzed (per patient median 4, range 2-11).

The following adverse laryngeal events developed with disease progression *during insufflation*: Adduction of aryepiglotic folds was observed in all patients, initially at the highest positive pressure (50 cmH$_2$O) and prior to onset of other bulbar symptoms in the majority. As cough became less expulsive with disease progression, aryepiglotic fold adduction occurred also at lower insufflation pressures (20 cmH$_2$O). In 5 patients with spinal onset and no bulbar symptoms at study entry, aryepiglottic fold adduction occurred at 50 cmH$_2$O at median (range) 38 (19-60) months after the ALS debut compared to 47 (30-57) months for other bulbar symptoms (p=0.046). Adduction of true vocal folds occurred in 8 of 9 spinal onset patients. Retroflex movement of the epiglottis was observed in 7 of 13 cases regardless of insufflation pressures and independent of bulbar involvements. Backward movement of the tongue base appeared regardless of insufflation pressures in all but one subject. *During exsufflation* constriction of the hypopharynx was observed in all patients regardless of the presence of bulbar symptoms, and appeared after the above listed adverse events during insufflation.

Adverse laryngeal response patterns developed before the onset of other bulbar symptoms in most patients. As the disease progressed, cough at the laryngeal level was observed to become gradually less synchronized during the MI-E, and the laryngeal cough movements were observed to become rigid and require longer time. MI-E treatment could also be complicated by swallowing, which seemed to be associated with retroflex movement of epiglottis.

Individually modified MI-E settings were explorative assessed with TFL in 6 patients (one bulbar and 5 spinal onset) as they met for their scheduled and consecutive

outpatient assessments, analyzing a total of 45 film clips from altogether 19 visits. Customized use of MI-E, applying lower insufflation pressures and flows, were observed to provide less adduction in the larynx, both at the supraglottic and glottic level, with more appropriate laryngeal responses to insufflation at least in some (possibly all) cough cycles. This prolonged the time for which the treatment was perceived efficient by patients. The use of one cough cycle at the time, allowing for a 'pause' prior to the next insufflation, and instructions to actively inhale prior to insufflation, was observed to induce better cough synchronization, particularly in cases of a retroflex epiglottis or spasticity at the laryngeal level.

In conclusion, the study showed us that laryngoscopy during ongoing MI-E was possible to perform repeatedly in deteriorating patients with progressing ALS. The study suggested that "blindly" applying high insufflation pressures during mechanically assisted cough in ALS can become counterproductive during disease progression; importantly also prior to the onset of bulbar symptoms.

6. Discussion

In this thesis we have demonstrated that it is possible to visualize the larynx by TFL during MI-E in healthy persons and in patients with ALS, and that the method was tolerated when applied repeatedly in deteriorating patients as their ALS progressed. Thus, laryngeal responses to MI-E can be observed, video-recorded and analysed retrospectively, allowing observation and description of dissimilarities between healthy subjects and ALS patients. The laryngeal responses to MI-E in patients with bulbar ALS differed from what we saw in healthy subjects in ways that were seemingly counterproductive in terms of treatment effect, and also unexpected compared to what has been suggested in studies performed by others.[141] When following 13 ALS patients throughout their disease progression we saw that adverse and seemingly counterproductive laryngeal response patterns developed in most of them, and that this occurred before development of the typical "bulbar ALS phenotype". The knowledge provided should be tested by others in larger studies. However, the findings suggest that clinical use of MI-E in ALS should be individualized, and if confirmed respiratory therapists will need to alter their clinical management of ALS patients. Our results indicate that individually tailored MI-E treatment can improve and possibly extend the use of non-invasive MI-E in ALS.

In the following, methodological strengths and limitations will be discussed and ethical issues considered. Thereafter there will be a general discussion of the main findings, clinical implications and suggestions for future research.

6.1 Methodological considerations

6.1.1 Strengths and limitations

The major strength of this study lies in the overall idea of filling an important knowledge gap that is of practical implication to a group of patients who suffer from a devastating, progressive and fatal disease. Thus, the study tested a novel method of utilizing TFL during ongoing MI-E in order to observe laryngeal response patterns in

health and disease. The method was used for comprehensive assessments during a wide range of MI-E settings combined with patient instructions that are regularly used in clinical practice. This is therefore the first study that can provide systematic visual information on how the larynx performs during ongoing MI-E in ALS patients, cross-sectionally as well as throughout their disease progression. This is an issue that potentially will be of great importance to these patients. The studies have engaged and created a multi-professional research group that has included specialists from otolaryngology, neurology and respiratory medical environments and respiratory physiotherapists.

The participating group of ALS patients was recruited from a complete geographical area, and the study was thus population based by design. The patients were followed longitudinally for as long as possible, limited only by disadvantageous manifestations of their progressing disease. Healthy volunteers, matched for age and gender with the participating ALS patients, served as control group.

The major weakness of the studies lies in their size; as with all small studies the findings must be cautiously interpreted. To some extent this uncertainty was compensated by the population based design and by recruitment of a highly typical group of ALS patients who did exhibit an expected clinical course during the study period. Nevertheless, the knowledge provided should be tested by others in larger studies. The explorative design also calls for repeated testing of the findings in other and unrelated but comparable patient populations. Moreover, neither intra- nor inter-observer repeatability of the findings was tested. These are all issues that prevent firm conclusions being made as regards internal and external validity, to be discussed in details below.

Another weakness, or rather a shortcoming, of the studies, was that we were unable to fully test the opportunities that lie within the options provided by the new technology becoming available in a rapidly developing market. Thus, this research field is still in its "embryonic" phase.

6.1.2 Study design

As current knowledge in this field is sparse, an explorative approach was chosen. A prospective observational descriptive design allowed observation - and thereby description - of hitherto unknown responses.

Cross-sectional study design (Study #I and #II)

This type of study design is used to document a status at a single time point for each participant within the study. Additionally, Study #II included a matched control group, suitable to compare the outcomes and the characteristics of the enrolled population based patient group.[203] The cross-sectional study design does have limitations, especially when examining patients with a progressive disease like ALS; the patient is examined at one time point, acknowledging that the results obtained might differ from results obtained at another time point.[203] This will influence the results, as it is practically not possible to compare patients at different stages of their disease progression at exactly the same phase. Nevertheless, the cross-sectional study design allowed for an overall assessment of the feasibility of the method in this patient group, and also for a description of laryngeal response patterns to MI-E in ALS patients of different phenotypes and how these patterns differed from those of the healthy control group.

Prospective cohort study design (Study #III)

A gradual development of bulbar innervated muscle dysfunction has been suggested to influence the extent of successful use of MI-E; thus, it was important to investigate the long term responses in a longitudinal design. A challenge facing all longitudinal studies is that data must be collected over long time periods. Considering a 5-year survival rate for ALS of approximately 20%, the five year follow-up period of the present study was considered adequate.[2,13] Cohort studies are suited to determine the natural history of a condition.[203] In the present Study #III, the data retrieval could not be performed continuously, but at pre-set outpatient appointments. Even so, adverse laryngeal events consistently preceded other clinical signs of bulbar involvement in

cases without bulbar symptoms at inclusion, indicating that the findings of the study were solid.

6.1.3 Subjects

External validity is the expression used to describe the extent to which the results of a study can be generalized to other populations or other patients with the same disease.[203] A population based sample implies that all participating patients with the disease under study are recruited from a specified population and that all patients with that disease and who belong to that particular population are invited to participate. In our studies, all patients with ALS were recruited from the area served by Haukeland University Hospital, and no patients with ALS who lived within that area remained uninvited.

The data obtained from the ALS patients were compared with those of healthy matched volunteers who were recruited by a convenience sampling principle; i.e. drawn from a population that was easily accessible.[204] The control subjects participating in Study #I were young medical students, individuals whose health, social and socioeconomic status can be assumed to be above average. The control subjects participating in Study #II were recruited in order to match the age and gender of the participating patients with ALS. Both these control groups turned out to be fit and with low BMI, good lung function and high respiratory strength. As such, the control groups of Study #I and #II can be seen as "*too healthy or wealthy*", possibly increasing the differences versus the ALS group. However, we do not have information suggesting that factors such as fitness, BMI and social or socioeconomic status should influence laryngeal function and its responses to externally applied positive and negative airway pressures.

Concerning the ALS population, their clinical disease presentation is generally highly heterogeneous due to variability of upper and lower motor neuron involvement, the disease onset and its progression. This heterogeneity was found also in our ALS population. Their peak cough flow values were below 270 litres/min in most of the

examinations, indicating that they represent a fairly "typical" population with ALS in need of MI-E to improve their airway clearance.[140] There were 13 patients who declined and four who died soon after being invited to Study #II; a situation that can possibly have caused a selection bias.[203] Reasons for non-participation were either severe disease or absence of bulbar symptoms. The critical scenario in the longitudinal follow-up Study #III was to obtain data also from the most severely ill patients. We were able to follow these patients for long periods, also in relatively severe distress.

To conclude on these issues, we hold that our patient population was representative for ALS patients in general, and that the control groups adequately reflected the expected a normal responses to MI-E.

6.1.4 Sample size

A sufficient number of subjects and observations per subject are needed for adequate internal and external validity of a study.[203] A sample size of 20 ALS patients in the cross-sectional study and 13 ALS patients in the prospective cohort study reflects the relatively low prevalence of ALS and the small population base from which participants were recruited. As this was an explorative study set up to investigate hitherto unknown outcome data with unknown distributions, a priori power calculations or sample size estimation could not be undertaken.[190,191] The small size of the study complicated statistical handling of the data, and contributed to a situation were the generalizability of the results could be questioned. Particularly, these issues make the study vulnerable for type-II errors; i.e., failure to detect significant differences that may have been present.[190]

The heterogeneity of our findings suggests that it can be possible that even more nuances or variations or other types of responses imposed by MI-E in ALS could have been found, had the study population been larger. However, with numerous observations collected from our sample of 20 ALS patients, we found a clear pattern of laryngeal adduction occurring during insufflation in bulbar ALS patients when compared to patients without bulbar symptoms as well as when compared to the healthy controls. This pattern was observed also in the spinal onset patients during

their disease progression, as they gradually developed bulbar involvement, first at the highest MI-E treatment pressures, and later in their progression also at gradually lower pressures. Thus, as it seemed, a dose-response relationship between MI-E pressures and adverse laryngeal outcomes could be suspected as ALS proceeds.

Thus, we hold it to be relatively improbable that studies of larger ALS populations or other control groups would have reached other conclusions than ours. However, we acknowledge and certainly encourage, that our findings must be systematically tested in other and larger ALS populations, treated and followed at larger institutions than ours.

6.1.5 Visualization of the larynx

The results of this thesis are based on the visualization of the larynx with TFL during MI-E. Construct validity indicates to what extent the test or instrument measures what it is supposed to measure,[205] and refers to whether a study is able to truly answer the research questions.[203] TFL examination is the gold standard for visualization of larynx.[178-181,206] Several small studies have used TFL in ALS patients to describe laryngeal function during simple tasks.[39,76,78,80,87,90,187,188] Further, patients are referred to otolaryngologists and examined with TFL as a part of a diagnostic work-up for ALS due to laryngeal symptoms.[39] We used the TFL in a new setting, and experienced only a small amount of technical failures. Laryngeal responses and movements throughout the MI-E protocol were as previously described for normal cough in the healthy participants of Study #I,[65-67] and the findings were different in bulbar ALS patients. We are not aware of any other and better methods that could be used to examine the laryngeal responses during MI-E. Only one study has previously explored the role of the upper airway during MI-E; i.e., Sancho et al. who performed an observational study of three ALS cases using CT scans and observed dynamic upper airway collapse during *exsufflation*.[141] The limitations of their study are mainly linked to the limitations of using CT as a method to observe continuous cough cycles, and the fact that they did not examine the upper airways during *insufflation*, probably related to their hypothesis (i.e. upper airway collapse during the negative pressure).

Our results indicate that their study failed to reveal the complete picture of laryngeal responses to MI-E, as they did not focus on the inspiratory phase which is so important for the first phase of cough; the crucial 'filling up' of the lungs. Direct visualization of the larynx with TFL could provide a visual overview of the laryngeal structures throughout the complete MI-E interventions.

The video recordings were precious parts of the diagnostic set-up. Two continuous videos were running in parallel, showing respectively the internal laryngoscopy images and the external MI-E set-up with the pressure settings, both inputs synchronized and showed real time on one television screen and later stored as one file to be showed on a computer monitor. This allowed retrospective analyses, enabling fine nuances and rapid movements to be visualized during slow motion and the findings to be validated afterwards by other observers.[204,205]

Nevertheless, the examination with TFL during MI-E has limitations which can affect the validity of the findings. In all participants, the larynx tended to move upwards during the MI-E cycle, requiring adjustments of the position of the laryngoscope. In some subjects, anatomical characteristics could preclude visual access, such as a high standing epiglottis. In ALS patients we encountered additional challenges. As the larynx moved considerably more downwards and upwards during insufflation and exsufflation, dynamic adjustments of the laryngoscope position were required and the laryngoscopy was supported manually instead of using customized headgear as in Study #I. In some cases airway secretions led to poor-quality recordings and pre-treatment aiming to clear secretions could have been considered. This was more often observed with disease progression in Study #III. Further, the adduction of the aryepiglottic folds obscured the view of the true vocal folds, and therefore the glottic level was not always successfully visualized. To compensate for these challenges and to produce adequate recordings, we repeated the MI-E cycles several times, which led to good quality recordings in at least one MI-E cycle per intervention in almost all sessions. These issues need to be considered in future studies using TFL during MI-E.

We hold that direct clinical visualization of the larynx using TFL provides a good overview of the laryngeal responses to MI-E in patients who are difficult to treat.

This opinion recently gained support by a study by Sayas Catalàn et al.,[207] who postulated that titration of non-invasive ventilation (NIV) by means of visualization of the larynx with TFL leads to fewer obstructive events in subjects with upper-airway obstruction. Also Georges et al.,[208] inspected the larynx with TFL during NIV in 11 patients with ALS to increase the understanding of potentially counterproductive laryngeal response patterns.

6.1.6 Interpreation of the video recordings

There were no preexisting assessment scores that could be used directly in the evaluation of the video-recordings and the interpretations of the laryngeal responses were done by observing and describing the video-recordings which is usual in laryngeal inspection with TFL. This is solely an observational approach, which only requires a systematic knowledge of what to observe.[203] The knowledge on which the evaluations of the present study are based, mainly rest on clinical - and research based activities performed by professionals who are members of our research group, and on the knowledge obtained from close to 2000 tests performed for exercise induced laryngeal obstruction (EILO).[182] This work includes the EILO scoring tool, which has proved to be a reliable and valid tool in EILO.[183] Such a specific scoring system cannot automatically be applied in a different disease context; however, it did contribute to our interpretations of the laryngeal responses to MI-E. Development of a scoring tool is time consuming, and combined with a low number of participating patients it was considered beyond the scope of the present study to establish a graded scoring system specifically designed to cover laryngeal responses to MI-E in ALS. However, in our opinion the observation scheme prepared in Study #I and further developed in Studies #II and #III can be used in clinical examinations with TFL during MI-E. Important issues like validity and reliability of this tool should be tested in future studies.

Because the observer is 'the instrument', determining the reliability of observational measures is linked to determining intrarater and interrater reliability. Intrarater reliability could be assessed by using repeated MI-E cycles obtained from the same

study session, whereas interpretations of the video-recordings by different raters could be used to address interrater reliability. The measure is considered reliable if it repeatedly gives us the same result; assuming that what we are measuring is not changing. Repeatability is an integrated part of the validity of an assessment; a test can be reliable without being valid, but it must be reliable to be valid.[203]

We did have the data to perform analyses of intrarater and interrater reliability, but these features were not formally addressed in the present studies. We interpreted the video-recorded data with the most reliable existing method to date; by the trained and experienced human eye. To secure reliable evaluations, several professionals were involved, working together in a consensus based approach. We acknowledge that other investigators could have found nuances in the laryngeal observations not emphasized by us. In the future, specifically designed tracking tools (algorithms to detect certain responses) of video recordings could theoretically be developed to assess the observations more objectively. Formal tests of reliability and validity should be performed in future studies.

6.1.7 Functional value of the observed laryngeal closure

Both insufflating and exsufflating the lungs require an adequately sized laryngeal inlet,[44] and it is reasonable to assume that visually observed laryngeal adductions or even closures, are associated with corresponding obstructions to airflow and thus influence the efficiency of the MI-E treatment. However, we do not in fact know to what extent the laryngeal adductions observed during MI-E influences the airflow. This represents an important functional issue that was not addressed during the work with this thesis. Measurement of the airflow synchronized to the TFL examinations, could definitely shed light on these issues. Measurement of airflow near the facial mask has been used to measure upper airway obstruction during sleep, where reduced flow indicates upper airway obstruction.[209,210] Airflow curves have been constructed to monitor physiological effects in studies on MI-E.[119,155,211] Lacombe et al. presented an airflow profile curve obtained from a neuromuscular patient during coughing while being supported by MI-E cycles. By interpreting the flow changes on the curve, they marked laryngeal movements on this visual MI-E airflow profile curve.[211] Their

description of laryngeal movements is of course hypothetical since they did not directly observe the larynx, but relevant for our findings. Simultaneous monitoring of the airflow curve and visualization of the larynx during MI-E could reveal more information about the functional outcomes of TFL and potentially add validity to our results, and should be targeted in future research. We did collect data that can be used to construct airflow curves, but these issues were not addressed in the present studies but will be in the near future.

6.2 Ethical considerations

We need to perform clinical research in order to understand and to advance our management of diseases that affect quality of life, cause pain, suffering or death.[212] In the present thesis, both the included patients (with a fatal disease) and the examination method (of TFL used during MI-E) requires ethical considerations.[212]

6.2.1 Research projects including ALS patients

Relevance

ALS patients are vulnerable, facing a fatal disease which severely limits their life expectancy. Clinical research performed in such patients must be extraordinary well designed and should preferably also include elements that aim to improve the daily care of the participants.[212]

The main focus of the present study was to improve daily respiratory care of patients with ALS. Accumulation of mucus without the ability to cough is a huge psychological burden that massively and negatively impacts quality of life. Additionally, it may cause pneumonia - the main cause of death in ALS. Airway clearance therapy with MI-E has empirically proved effective in several other neuromuscular disorders, however is challenging in ALS patients; therefore it was important to investigate these issues.

Informed consent

Researchers whose study participants are terminally ill or very sick people must give extraordinary careful attention to the informed consent process in their research protocols as well as in their conduct and behaviour when interacting with eligible patients.[212] In the present study, all eligible patients received both verbal and written information about the study and a signed consent was required before inclusion. Although ALS is a neurological disease affecting also parts of the brain, the intellectual capacity of those affected is not influenced, unless they have frontotemporal dementia which is rare and also was one of the study exclusion criteria.[4] In the present study we carefully pointed out in a balanced way potential inconveniencies and disadvantages as well as potential advantages. We also emphasized that declining to participate would not in any way influence their treatment, and that knowledge gained from the study potentially could improve treatment, and would be made available also to them - to the extent possible. Patients had at least a week to think about participation, which prevented a rushed consent.

It is our opinion that eligible patients were carefully and conscientiously approached and informed about the 'pros and cons' of participation, and that patients for whom participation was perceived to involve some form of extra challenge were excluded.

Desperation and dependencies

The project manager worked as a respiratory physiotherapist at the ALS outpatient clinic at study initiation, and thus had a therapist-patient relationship with several of the eligible patients. This raised particular issues and concerns, as eligible patients may have been willing to join the research project because they perceived themselves as being in a dependent position, they may have been extraordinary primed to participate, or they may have been extraordinary anxious or even desperate to have the best available treatment, even for a highly distant prospect of personal benefit.[212] To prevent this, we were clear that declining participation would not affect the regular treatment at the out-patient clinic. Still, we experienced that patients familiar with MI-E treatment were particularly eager to participate, similar to patients with already developed bulbar symptoms. Consequently, warnings against unrealistic

expectations for own personal gains from participation were warranted.[212] Prior to the examinations, many of the included patients expressed that *if* they did not get any direct benefit for themselves, they wanted to improve the treatment of other patients with the same disease.

It turned out that patients participating in the study in fact did get individual benefits from the TFL examinations by more adequately and individually adjusted MI-E settings due to an understanding of their laryngeal responses that would not otherwise have been possible. Some of them also extended their use of MI-E, and potentially extended the use of NIV because of more successful non-invasive airway clearance.

In retrospect, it is our overall (humble) opinion that the study subjects participated because of a personal interest in the topic, and that their participation resulted in individual benefits for most of them. The use of their 'end-of-life time' to take part in the study examinations was considered meaningful and also had a clinical benefit.

6.2.2 Examination with TFL

TFL is well-tolerated, though it is an invasive procedure which may feel unpleasant, as the laryngoscope is introduced through the nose and upper airways. Our research group has extensive expertise in the use of TFL in a wide variety of circumstances, patient and age groups, and considered participation safe with minimal risks and relatively few nuisances. This view was supported by the present study, in that only two out of 20 patients enrolled in the cross sectional Study #II actively declined to enrol also in the longitudinal Study #III and only one of them gave as their reason as 'discomfort during the examinations'. This patient expressed that the discomfort was mainly related to the use of MI-E, which was unfamiliar for her, and not to the TFL per se.

To secure that TFL during MI-E with ALS patients was possible, we initially examined healthy individuals to increase our competence (Study #I). Skilled otolaryngologists or pediatricians who perform laryngoscopy routinely (every day/week), performed the procedure. We continuously explained the procedure to the

patient, and asked for feedback between procedures. The positive and negative pressures used during the test procedures with the MI-E were increased gradually, providing the subject with the necessary time to get familiar with the in- and exsufflations; similar to the MI-E pressure titration they are used to in their daily clinical use of this device. If the patient expressed any signs of discomfort, the procedure was stopped, and higher pressures were not applied. The intervention with new MI-E settings was used in the same way as we would have done without this study; i.e. the use of MI-E was reduced and eventually stopped when treatment tolerance decreased clinically. The TFL was performed as gently as possible, and under careful observation. Ethical aspects were a continual focus.

Before commencing on Studies #II and #III, we considered potential risks and discomfort to the participants to be minor when compared to the potential clinical gains for the individual participating patients, and the potential clinical gains for future ALS patients due to knowledge obtained from this study. In retrospect, it is our (humble) opinion that these reflections, made in advance, were in fact fulfilled.

6.3 Discussion of the main findings of the study

This study revealed i*nspiratory adduction of* supraglottic structures during insufflation in patients with bulbar ALS, the opposite of the *inspiratory abduction* observed in non-bulbar patients and in healthy controls. This might explain treatment failures in bulbar ALS. The compromised size of the laryngeal inlet obstructs the inspiratory airflow from the MI-E, resulting in poor filling of the lungs during the first phase of cough. With disease progression, supraglottic *inspiratory adduction* appeared in spinal onset ALS patients *before* the evolvement of other bulbar signs and symptoms. Generally, *the cough during MI-E altered* with disease progression, and became slower, less expulsive and desynchronized. *Individually customized settings* seemed to promote more optimal laryngeal response patterns to MI-E. Our findings may benefit vulnerable patients through better handling of their daily respiratory treatment.

6.3.1 Inspiratory closure

Contradicting previous suggestions that upper airway collapse during exsufflation should explain MI-E inefficacy in ALS patients,[141] our results suggest that the application of insufflation is the principle challenge, especially when bulbar dysfunction is present. Conceivably, the observed adduction prevents lung insufflation and leads to inadequate filling up of the lungs; a situation that will compromise attempts to assist the expiratory phase of cough through exsufflation, and thus lead to inefficient MI-E. However, the larynx is a highly complex organ that integrates a number of vital functions[44-47] and similarly, ALS is a complex disease involving several mechanisms.[6] Thus, inefficient MI-E in these patients is likely to be multifactorial and also vary between patients, and the relatively simple causal chain suggested above needs to be elaborated and substantiated by further and more comprehensive physiological study. Functional loss in ALS is often explained by the loss of motor control and strength,[11,213] and more recently by laryngeal sensory insufficiency.[87] Since ALS affects motor neurons in the brain and spinal cord, both afferent and efferent innervation may play a part in the adverse laryngeal response patterns during MI-E in these patients. In the following paragraphs, these aspects are discussed.

Aerodynamics

The aryepiglottic folds are relatively soft structures that are only provided with scattered muscle fibres. Therefore, adduction at the supraglottic level could, theoretically, be explained by the Bernoulli principle, i.e., increasing flows generating increasing negative pressures inside the laryngeal lumen, sucking the laryngeal structures inwards.[70] This mechanism may conceivably be particularly important in progressive bulbar ALS characterised predominantly by hypotonic paresis.

The Bernoulli principle may also play a role in epiglottic retroversion during insufflation. A retroflex floppy epiglottis has previously been described in ALS by Ito et al. in two patients during regular inspiration.[187] The epiglottis has also been shown to be pressed into the laryngeal inlet by the positive pressures applied when

continuous positive airway pressure (CPAP) is used to treat patients with obstructive sleep apnoea[214] and in patients with multiple system atrophy.[72] Considerably higher pressures are applied when using MI-E than in these CPAP studies; i.e. pressures of 20-50 cmH$_2$O compared to 4-7 cmH$_2$O, a factor that makes the Bernoulli principle even more relevant.

Motor control and strength

The size of the opening of the larynx is determined by a balance of forces between the abductor and adductor muscles. This balance can be influenced and disturbed by weakness of abductor muscles or increased activity of adductor muscles or vice versa. Essentially, the larynx has several small intrinsic adductors,[54] but only one abductor muscle. Several vital laryngeal functions involve occlusion,[58,59] and it has therefore been suggested that adductive movements by nature are dominant to abductive movements. This may be relevant for the findings of the present study; we found dominant adductive laryngeal movements in all patients with ALS compared to the healthy controls. Our results are supported by comparable findings from TFL examinations in other ALS patients. Polkey et al. reported two patients with bulbar dysfunction as having true vocal fold closure during both rapid spontaneous inspiration and expiration. Their interpretation was that this could be due to laryngeal abductor weakness, and suggested that when the weakness extends beyond a critical level, this may limit these patients ability to cough effectively.[90] Van der Graaf et al. described true vocal fold dysfunction with abduction weakness, paresis or laryngospasm in four patients with different disease courses (both limb and bulbar onset). After needle myography examination, the authors suggested that this was a result of both over activity of the adductors, and signs of re-innervation in the abductors.[188] Both these observations and our findings suggest that the motor deficit of intrinsic laryngeal muscles becomes weaker in ALS and that adductive movement becomes superior to the abduction.

Sensory insufficiency

Afferent activation in the larynx influences motor patterns.[52] In ALS, applied MI-E pressures may provoke reflex laryngeal adduction or swallowing. Stimulation of

extremely sensitive supraglottic receptors normally induces complex adductor reflexes that prevent foreign bodies from entering the airways.[54] This reflex circuit may be hypo- or hyper-responsive or dysregulated in ALS patients,[87] and therefore lead to inappropriate laryngeal closure, comparable to observations made in patients with Parkinson's disease or brainstem compression.[215,216] In fact, stimulation with positive air pressures has been used by otolaryngologists to provoke laryngeal reflex activities leading to both laryngeal closure and swallowing.[52,217,218] Positive pressure to the mucosa in the laryngeal vestibule activates afferents in the internal branch of the superior laryngeal nerve, releasing the laryngeal closure reflex.[217] Positive air pressure to the anterior facial area in the oral cavity activates glossopharyngeal afferents and elicit swallowing.[218] Amin et al. used positive pressure sensory testing (up to 14 cmH$_2$O) in combination with TFL to provoke swallowing in 22 ALS patients. More than half of these cases demonstrated abnormal laryngeal sensitivity.[86] The therapeutic use of positive pressures has previously been reported to provoke laryngeal narrowing. Jounieaux et al. revealed with laryngoscopic evaluation that positive pressure ventilation in healthy and awake subjects tended to result in progressive glottic narrowing, increasing the inspiratory resistance and thereby progressively reducing the fraction of air delivered to the lungs.[219] This was further aggravated during stable sleep stage, and even more during a deep sleep stage.[220] Delguste et al. reported complete upper airway obstruction in association with NIV-induced hypocapnia. The authors suggested that the use of positive pressure devices to extreme hyperventilation may increase the upper airway resistance and further proceed to a complete closure.[221]

These studies support the view that therapeutic positive pressures applied by MI-E may provoke afferent responses that may lead to adverse laryngeal adduction that prevents airflow from entering the lungs. The observation made in the present study that positive pressures triggered swallowing, support the notion that laryngeal adduction during MI-E may be reflex mediated and linked to afferent activation.

Type of muscular paresis

The type of muscle paresis may influence the laryngeal responses to MI-E. Motor innervation of the larynx is complex and only partially understood.[60,61] In ALS, both upper and lower motor neuron failure is possible, as are spastic or hypotonic muscular responses. Differences in the two subtypes of bulbar ALS may influence laryngeal response patterns to MI-E.[8-10] The observations made in Study #II indicated that in pseudobulbar ALS, laryngeal adduction occurs mainly at the glottic level. Due to small numbers of subjects, we were unable to separate predominant problems in hypotonic progressive bulbar palsy and hypertonic pseudobulbar patients. To our knowledge, these aspects are previously only examined in swallowing, but not during cough. Ertekin et al. examined swallowing in a heterogenic sample of 43 patients with ALS by electromyography. They found that the motor control of swallowing was disturbed, and indicated that hypo- or hyper reflexive states in the involved muscles could explain the findings. The analyses were unfortunately did not take into consideration bulbar symptoms or the type of bulbar symptoms, but focused on dysphagia.[213]

ALS affects both corticobulbar and corticospinal pathways, both crucial for voluntary efforts.[7] In patients with pseudobulbar paresis, patients are unable to perform forced manoeuvres (such as cough on command); however, reflex-induced cough may be preserved. Clinically, reduced coordination of laryngeal muscles will predispose the individual to the risk of choking and pulmonary aspiration.[43,222] It seems reasonable to suggest that MI-E more easily triggers laryngeal adductor reflexes in a disease that is predominantly spastic.

To conclude, MI-E can theoretically provoke a variety of responses at the laryngeal level in patients with ALS, which are capable of precluding non-invasive respiratory treatment efficacy. MI-E pressure application can lead to a disturbed balance between the forces that regulate the size of the laryngeal lumen, such as physical and aerodynamic forces relating to flow itself, muscle balances and reflex mechanisms. The present study was not set up to explain or disentangle these causal pathways. They are likely to be multifactorial, involving motor control and strength, sensory

insufficiency or abnormality, and aerodynamics in the larynx. These issues are important to investigate in order to understand how we can expand on the therapeutic alternatives that are available to these vulnerable patients.

6.3.2 Laryngeal responses in disease progression

Laryngeal responses evolves

As ALS progresses, also laryngeal responses evolve. Although longitudinal studies that include laryngeal examinations are lacking in ALS; laryngeal dysfunction has been suggested to occur both before and after the onset of other bulbar symptoms.[45,188] Van der Graaf et al. suggested that ALS patients with adequate vital capacity are at risk of glottic narrowing, and that vocal cord dysfunction in ALS is not always related to major bulbar involvement.[188]

The findings in the present longitudinal follow-up support this viewpoint. The larynx failed to respond adequately to externally applied positive pressures *before* this could be predicted by other signs generally used to signal bulbar involvement. Except from our Study #III, only one study has been published to date examining dysphagia with video fluoroscopy in ALS patients during disease progression. Higo et al. reported that parts of the swallowing movements slowed with disease progression. The authors suggested that this slowness of swallowing was mainly associated with tongue weakness.[223] Also inappropriate true vocal fold motion has been a feature of the disease, observed by laryngoscopic examination even in spinal onset ALS - without other clinical bulbar signs. In that a cross-sectional study Tomik et al. found that the function of true vocal folds was abnormal in both bulbar and limb onset ALS.[78] Both the studies of van der Graaf, Tomik et al. and Higo et al., in addition to the present Study #III, concur that laryngological examination may reveal early signs of vagus nerve involvement, and demonstrate "preclinical" bulbar failure in ALS. Thus, in respiratory management of ALS patients one should be aware that the larynx may exhibit unexpected and adverse performance before other clinical signs of bulbar involvement.

Age

Age of the patient may be a factor to consider when investigating the use of MI-E to assist cough. ALS patients are usually older than other neuromuscular patients who need to use a cough assist device. Age affects both strength and speed of the muscles that are involved in the cough process,[224] and the lungs, thorax and glottis are affected by age related structural alterations.[225] Expiratory strength is reduced, which affects the peak cough flow and relationship between two cough spikes.[226,227] Also swallowing is influenced by the normal aging processes; swallowing slows with advancing age, which is a consequence of the initiation of the movements in the larynx and pharynx become slower.[64,192] The sensitivity to trigger cough decreases and the risk of aspiration is increased in elderly people.[225,228]

The implication of age on laryngeal responses to cough assist devices should be investigated further; e.g. by comparing the findings from the two control groups of the present study.

Cough alters in ALS

Study #III shows us that cough alters with disease progression in ALS, becoming less expulsive and less synchronized, more rigid and slower. Presumably, this is related to a gradual loss of adequate cough flow spikes and lower peak cough flows. Previous studies that have measured airflow have described this development. Chaudri et al. demonstrated that bulbar ALS patients were unable to generate cough airflow spikes, contributing to their ineffective cough.[123] Polkey et al. also observed an inability to generate cough airflow spikes in patients with severe bulbar ALS and severe abdominal muscle weakness. The authors suggested that bulbar ALS patients are unable to achieve the necessary dynamic airway compression, and thus perform a huff instead of a cough. This may be related to muscular weakness and reduced glottic closure during cough maneuvers in ALS.[90] It has been suggested that diaphragmatic muscular force is involved in generating the expulsive force of cough, and ALS disease progression will eventually affect also diaphragmatic regulation.[43]

Inadequate dynamic airway compression, gradual loss of cough airflow spikes and lower peak cough flow combined with dysregulation of laryngeal muscles will

eventually all contribute to development of ineffective cough in all patients with ALS. Given the heterogeneous nature of ALS, it seems fairly evident that this development must be complex and multifactorial, and that the causal chain is clearly insufficiently understood and also probably differs between patients. Therefore, cough clearance techniques must be individualized and tailored to the development of each and every patient, preferably based on objective information. In our view, the suggestions put forward in the present study - that laryngoscopy is performed during cough assist devices testing - seems reasonable.

Swallowing during MI-E

Reflex triggering of swallowing may further complicate altered cough in ALS, as it might lead to aspiration. In ALS, swallowing is altered: patients swallow lower volumes with prolonged apnoeic pauses. Swallowing may also be followed by an inspiration, increasing the risk of aspiration. This contrasts normal swallowing, where the epiglottis covers the laryngeal inlet with an apnoeic pause of 0.6-2.0 seconds, and is followed by expiration. As a result of the order in which muscles are affected, swallowing difficulties are one of the earliest complaints reported by patients manifesting bulbar ALS[84] and swallowing is described to be most disturbed in pseudobulbar ALS.[45]

Why MI-E triggers swallowing can be related to laryngeal afferent activation as discussed previously. In addition, both backward movement of the tongue base and retroflex movement of the epiglottis were frequent findings during insufflation and could potentially induce swallowing. In ALS, tongue muscle insufficiency is suggested to cause swallowing problems.[85] In Study #III, retroflex movement of the epiglottis during insufflation could trigger swallowing and thus severely disturb timing and synchronizing of coughing. This can be related to the fact that the surface of the epiglottis is densely innervated by afferent fibres[229] which might contribute to activation of afferent loops of reflex arcs as ALS progresses. Still, the retroflex movement of epiglottis was clearly *not* triggering swallowing in healthy subjects even it was frequently observed in several healthy controls both in Study #I and

Study #II; thus, these mechanisms may have a more important role in progressing ALS.

To conclude, progressing ALS leads to disorganized laryngeal functions. Respiratory therapy in these patients therefore requires approaches that might differ from those applied in patients with other neuromuscular disorders without involvement of muscles with bulbar innervation. This study has indicated that when mechanical cough assist devices are used, great care must be taken to avoid applying pressures and cough cycles that the patients´ larynx is unable to handle, and that treatment needs to be individualized, preferably based on objective measures. Currently, in our opinion, direct laryngeal visualization during treatment is the best approach in challenging patients. This approach might be feasible also when titrating NIV for challenging ALS patients.[222,230]

6.3.3 Individually customized MI-E therapy

As dynamic collapse during exsufflation was previously thought to be the reason for ineffectiveness of MI-E in ALS, Kang and Bach suggested that ALS patients with bulbar dysfunction instead would benefit from a single deep insufflation combined with an abdominal thrust applied during the expulsive phase of cough.[138,231] We included this intervention in our protocol. However, it seemed to be useless. Instead, and based on observations made in the present study, we developed a protocol for MI-E titration that should prevent inspiratory closure during insufflation. **Figure 9** is a suggested algorithm for how to adjust MI-E settings based on observations in Study #II.
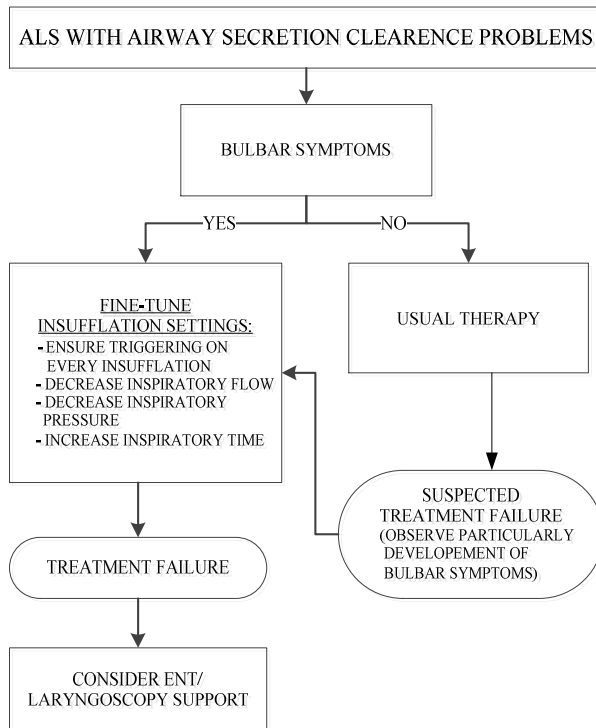
Figure 9. *A practical algorithm suggesting how to adjust the settings of MI-E when used to treat ALS patients for airway secretion clearance problems. MI-E=Mechanical insufflation-exsufflation, ENT=Ear-Nose-Throat. Reproduced with permission from Thorax. Andersen et al.[232]*

Asymmetrical pressure settings

We found that *asymmetrical settings* with lower insufflation pressures and flows appeared to be beneficial. To our knowledge, this is novel and has not been examined systematically in other studies including in ALS patients. Until recently, high *symmetrical* MI-E pressure settings have generally been considered to be most effective for neuromuscular patients,[170,175] and most studies and clinicians apply settings of 40cmH$_2$O or higher both for insufflation and for exsufflation.[131,176,233] We showed in Study #I that this symmetrical approach could lead to hypopharyngeal constriction during exsufflation in healthy medical students, indicating that this phenomenon alone could not explain treatment failure with bulbar ALS. Results of

Study #II revealed that ALS patients with bulbar affection tended to close supraglottic structures during insufflation, and indicated that high positive pressures were the most provocative. The results of Study #III supported the findings that high pressures were most provoking to the larynx in ALS, inciting laryngeal closure already prior to other clinical bulbar symptoms. These observations suggest that a "disorganized larynx" in bulbar ALS patients prevent adequate filling of the lungs with insufflation, due to laryngeal closure. Applying negative pressures after an unsuccessful insufflation is bound to create only discomfort and a vacuum in the upper airways, leading to laryngeal collapse.

Hence, we ended up exploring whether use of modified MI-E settings could succeed in keeping the laryngeal inlet open during insufflation for a prolonged period of time in patients with deteriorating ALS: we used lower insufflation than exsufflation pressures combined with more careful use of low insufflation flows. This approach is further supported by previous data published by Mustfa et al; they found that exsufflation alone could produce higher peak cough flow in bulbar ALS than insufflation alone; however, they did not examine other asymmetrical pressure settings.[143] Senent et al. used a ventilator in patients with ALS to show that use of a positive pressure of 30 cmH$_2$O prior to cough in fact produced *higher* peak cough flow than was achieved by using MI-E with ±40cmH$_2$O.[151] In Study #III we found further evidence suggesting that hypopharyngeal constriction occurred later in disease progression than laryngeal adduction during insufflation, thus supporting the theory that inability to fill the lungs with insufflation can create a vacuum-effect during subsequent exsufflation, and thereby aggravate hypopharyngeal narrowing. This emphasizes the importance of keeping the larynx open during insufflation to achieve a sufficient insufflation volume prior to cough. Increasing the insufflation time instead of the insufflation pressure is probably more feasible in achieving sufficient insufflation volumes; supported by a bench study by Gomez-Merino et al. who demonstrated that increasing the insufflation time is more important for optimal peak cough flow that increasing the exsufflation time.[175] These complex relationships may explain the diverging results that are reported from studies of bulbar ALS patients, where MI-E have been shown both to fail[141] and to succeed[151,234] in producing the

peak cough flow considered necessary for effective cough.[126] In our opinion, too high insufflation pressures seem to be counterproductive as they might lead to laryngeal closure and thereby lower instead of increased insufflation volumes.

MI-E cycle tailoring

Rapid MI-E cycles may be challenging or impossible to handle for patients with ALS. It seems reasonable that successful MI-E requires that the larynx is "reset" after exsufflation and that possible swallowing or closure reflexes have been brought to an end before the next insufflation. An increased time interval between exsufflation and insufflation, or the use of only one cough cycle at a time, might thus be more appropriate to prepare the larynx for the next insufflation. These findings are highly explorative - for obvious reasons. However, they represent an attempt to address these matters with verifiable methods and outcomes, and should be the subject of future research. Given the changing and progressive nature of ALS with development of laryngeal responses being particularly difficult to predict, direct inspection of the larynx during ongoing treatment appears to be a simple and reasonable approach in patients with signs or symptoms suggesting treatment failure.

6.4 Clinical implications

The results of the present thesis potentially have direct clinical implications on how airway clearance therapy should be performed in patients with ALS. As alluded to by Simonds; patients with bulbar ALS may not have failed their MI-E therapy, but instead the previous therapy may in fact have failed the patients, due to erroneously applied settings.[222] With the present studies we have achieved a better understanding of the laryngeal response patterns to MI-E in the various phenotypes of ALS, and we have been able to observe how these patterns evolve as the disease progresses. This study helped us - in our clinic - to establish better and individually tailored clinical respiratory treatment strategies for our participating patients, and we argue that our approach can successfully be applied also for patients with ALS in general.

Individually adjusted settings, carefully applied as patients deteriorate, may prevent adduction of laryngeal structures during insufflation, and thus prolong the period of successful non-invasive use of MI-E.

A similar approach may also be appropriate when performing "start-up titration" of NIV settings; high inspiratory pressures may generate airway closure and therefore pressure should be gently titrated upwards.[222] If problems are encountered, laryngoscopy can be a valuable tool in this process. This has already been preliminary reported by Farrero et al.; they adjusted ventilation parameters with the goal of achieving comfort for their patients in addition to adequate ventilation, and reported that over 50% of bulbar patients were successfully initiated on NIV. The authors attribute their success to a less "nihilistic attitude", in-patient initiation, and overnight adaptation of settings.[107] As alluded to before, therapeutic use of laryngoscopy may be a useful tool also during NIV titration, as suggested by the studies of Sayas Catalàn et al.[207] and Georges et al.[208]

In order to avoid bothersome, futile or even counterproductive handling of these vulnerable patients, we need proper and feasible methods that ensure optimal and extended use of non-invasive respiratory treatment. Various measures of lung function have also been suggested to this end.[134,140,235] Additionally, various neurological scoring systems and clinical signs that indicate bulbar involvement are used as signals to execute caution. By observing medial collapse of laryngeal structures during insufflation *before* the appearance of these bulbar signs and symptoms, this thesis certainly questions their usefulness, and instead suggests direct assessment of the organ in question; i.e. the larynx of patients who are difficult to treat. Hence, TFL can be a valuable tool for a variety of respiratory therapeutic interventions in selected patients who do not respond as expected.

There are no guidelines for the timing of tracheostomy, if that is a desired approach in a patient with ALS.[106] The present study has provided data that may benefit ALS patients in that non-invasive treatment can be prolonged, potentially postponing the need to consider tracheostomy. However, both the decision whether or not to perform

tracheostomy, and to optimize its timing, are exceedingly complex ethical and clinical issues that we did not set out to address in this thesis.

6.5 Future prospects

To examine functional outcomes of laryngeal adverse responses to MI-E by monitoring the airflow curve in parallel with TFL examination.

To study if asymmetrical treatment pressures are effective in ALS.

To develop and examine other outcome measures than peak cough flow for ALS patients that can be used to study the efficacy of MI-E or other cough augmentation techniques.

To examine if therapeutic video recorded laryngoscopy can be beneficial in NIV titration in patients with ALS.

To validate the findings of the present study in other and preferably larger patient populations.

To investigate further causal pathways of laryngeal responses to MI-E in larger patient populations.

7. Conclusions

In this thesis we have shown that video-recorded flexible laryngoscopy is a feasible method to characterize laryngeal responses throughout an MI-E protocol applied to assist cough in healthy individuals, in the various phenotypes of ALS, and throughout typical ALS disease progression. Laryngeal movements during MI-E in healthy individuals were found to be mainly as described for normal cough. We have concluded that treatment failure with MI-E in ALS patients with bulbar symptoms is likely to be caused primarily by laryngeal adduction during insufflation, predominantly at the supraglottic level. This response precludes air-filling of the lungs during insufflation, causing discomfort and subsequent inefficient exsufflation. In disease progression, this occurred prior to the development of clinically evident signs of bulbar involvement. Cough patterns at the laryngeal level altered in ALS as the disease progressed, and became less synchronized with the MI-E. Reflex triggering of swallowing by positive air pressures applied by the MI-E could further complicate these matters and rapid MI-E cycles can be challenging or impossible to handle for patients with ALS. Individually tailored MI-E treatment can improve - and may possible extend - the use of non-invasive MI-E in ALS. TFL may prove an efficient tool assessing patients whose laryngeal responses to treatment are suspected to complicate the MI-E treatment.

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9. APPENDIX

Forespørsel om deltakelse i forskningsprosjektet

Kartlegging og oppfølging av strupens funksjon
hos personer med Amyotrofisk lateral sclerose (ALS)

Bakgrunn og hensikt med denne studien

Dette er et spørsmål til deg om å delta som <u>frisk kontroll</u> i en forskningsstudie som skal kartlegge hva som skjer i strupen hos pasienter med Amyotrofisk Lateral Sclerose (ALS) når de puster og hoster - og spesielt når de bruker en såkalt "hostemaskin". Vi tror at strupen kan være et viktig hinder for god hostefunksjon og derved et hinder for å få opp slim fra lungene hos disse pasientene. Dette er uheldig for pasienter med ALS og derfor viktig å undersøke nærmere.

Tolkning av resultater fra undersøkelser av pasienter med ALS må baseres på sammenligning med tilsvarende undersøkelse av friske personer (kontroller). Derfor vil vi også kontakte friske voksne med spørsmål om å delta i et tilsvarende undersøkelsesprogram. Resultatene fra undersøkelsene av kontrollene vil bli sammenlignet med resultatene fra de som har diagnosen ALS.

Ca 200-300 personer har ALS i Norge. Det er en sykdom som rammer hjernen og ryggmargen og som etterhvert leder til problemer bl.a. med å hoste og puste på grunn av svekkelse av muskulatur i bryst, tunge og hals. Behandling av ALS tar sikte på å opprettholde eller forbedre livskvaliteten lengst mulig.

Hostemaskin er et hjelpemiddel til å få opp slim fra luftveiene. Den virker ved at luft først forsiktig blåses inn i lungene og deretter suges ut sammen med slim fra de sentrale luftveiene. Vi opplever at flere ALS pasienter får god hjelp av hostemaskinen, mens den fungerer dårligere hos andre. Vi tror at hindringen kan ligge i strupen, men dette har ikke blitt undersøkt tidligere.

Målet med denne studien er å kartlegge strupens funksjon hos ALS pasienter over tid gjennom undersøkelser ved de regelmessige polikliniske kontrollene ved Haukeland Universitetssykehus (HUS). Bedre kunnskap på dette området kan bidra til bedre og mer individuelt tilpasset behandling hos pasienter med ALS. Dette vil kunne forbedre kvaliteten på behandlingen, også hos pasientene som deltar i denne aktuelle studien. Vi ønsker å lære av våre erfaringer slik at de kan bli til nytte også for andre pasienter. Derfor utføres undersøkelsene systematisk og innenfor rammene av en forskningsstudie. Forespørsel om deltagelse i denne studien er sendt til alle pasienter som er tilknyttet ALS klinikken på HUS.

Deltagelsen av friske kontroller innebærer
- Kartlegging av lungefunksjon.
- Inspeksjon av strupen med laryngoskopi mens hostemaskin blir testet ut med forskjellige innstillinger. Laryngoskopi betyr inspeksjon av strupen ved hjelp av en myk slange med kamera som føres forsiktig inn gjennom nesen slik at man kan kikke ned på stemmebåndene. Undersøkelsen utføres rutinemessig mange ganger daglig ved sykehuset. Kamera gjør videoopptak av strupen som kan studeres etterpå.

Undersøkelsene utføres poliklinisk på en time avtalt sammen med deg.

Mulige fordeler og ulemper

Alle undersøkelser er trygge. Inspeksjon av strupen kan medføre ubehag ved neseskilleveggen og en følelse av kiling i halsen. For å redusere ubehaget vil neseslimhinnen behandles med vanlig nesespray som brukes ved forkjølelse, og deretter lokalbedøves ved hjelp av en gel (Xylocain®). Undersøkelsen utføres av en lege som utfører denne type undersøkelser av både barn og voksne med spørsmål om sykdommer i øvre luftveier.

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Frivillig deltakelse

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Mer informasjon om studiet kan du få ved henvendelse til:

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Overlege, senterleder [1, 2]

Ola Drange Røksund
Spesialfysioterapeut [6, 8]

Samtykke til deltakelse i studien

Jeg er villig til å delta som <u>frisk kontroll</u> i studien

Kartlegging og oppfølging av stemmebåndets bevegelse og stabilitet hos personer med Amyotrofisk lateral sclerose (ALS)

Navn _____
(Bruk blokkbokstaver)

Sted og dato_____

_____
Underskrift av prosjektdeltaker

Forespørsel om deltakelse i forskningsprosjektet

Kartlegging og oppfølging av strupens funksjon
hos personer med Amyotrofisk lateral sclerose (ALS)

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie som skal kartlegge hva som skjer i strupen hos pasienter med Amyotrofisk Lateral Sclerose (ALS) når de puster og hoster - og spesielt når de bruker en såkalt "hostemaskin". Vi tror at strupen kan være et viktig hinder for god hostefunksjon og derved et hinder for å få opp slim fra lungene. Dette er uheldig for pasienter med ALS og derfor viktig å undersøke nærmere.

Ca 200-300 personer har ALS i Norge. Det er en sykdom som rammer hjernen og ryggmargen og som etterhvert leder til bl.a. problemer med å hoste og puste på grunn av svekkelse av muskulatur i bryst, tunge og hals. Behandling av ALS tar sikte på å opprettholde eller forbedre livskvaliteten lengst mulig. Hostemaskin er et hjelpemiddel til å få opp slim fra luftveiene. Den virker ved at luft først forsiktig blåses inn i lungene og deretter suges ut sammen med slim fra de sentrale luftveiene. Vi opplever at flere ALS pasienter får god hjelp av hostemaskinen, mens den fungerer dårligere hos andre. Vi tror at hindringen kan ligge i strupen, men dette har ikke blitt undersøkt tidligere.

Målet med denne studien er å kartlegge strupens funksjon over tid gjennom undersøkelser ved de regelmessige polikliniske kontrollene ved ALS klinikken, Haukeland Universitetssykehus (HUS). Bedre kunnskap på dette området kan bidra til bedre og mer individuelt tilpasset behandling hos pasienter med ALS. Dette vil kunne forbedre kvaliteten på behandlingen, også hos pasientene som deltar i denne aktuelle studien. Vi ønsker å lære av våre erfaringer slik at de kan bli til nytte også for andre pasienter. Derfor utføres undersøkelsene systematisk og innenfor rammene av en forskningsstudie. Forespørsel om deltagelse i denne studien er sendt til alle pasienter som er tilknyttet ALS klinikken på HUS. Deltagelse innebærer stort sett bare undersøkelser som normalt uansett utføres ved ALS.

Tolkning av resultater fra undersøkelser av pasienter med ALS må baseres på sammenligning med tilsvarende undersøkelse av friske personer (kontroller). Derfor vil vi også kontakte friske voksne med spørsmål om å delta i et tilsvarende undersøkelsesprogram. Resultatene fra undersøkelsene av kontrollene vil bli sammenlignet med resultatene fra de som har diagnosen ALS.

Deltagelsen innebærer

Første kartlegging:
- Undersøkelse av en lege (spesialist i neurologi).
- Kartlegging av lungefunksjon.
- Inspeksjon av strupen med laryngoskopi mens man blir bedt om å gjøre enkle oppgaver som gir kunnskap om strupens funksjon. Hostemaskin blir testet ut med forskjellige innstillinger. Laryngoskopi betyr inspeksjon av strupen ved hjelp av en myk slange med kamera som føres forsiktig inn gjennom nesen slik at man kan kikke ned på stemmebåndene. Undersøkelsen utføres rutinemessig mange ganger daglig ved sykehuset. Kamera gjør videoopptak av strupen som kan studeres etterpå.
- Spørreskjema om hoste og pustefunksjon og forhold knyttet til ALS og livskvalitet

Oppfølging:
- Kort inspeksjon av strupen mens man blir bedt om å gjøre enkle oppgaver tilsvarende den første undersøkelsen.
- Oppfølging av funksjon i svelget, lungefunksjonen og sykdomsutviklingen.
- Oppfølgende spørsmål fra spørreskjema om hoste og pustefunksjon og forhold knyttet til ALS og livskvalitet

Inspeksjon av strupen første gang utføres poliklinisk på en time avtalt sammen med deg. De andre undersøkelser utføres når du har vanlige kontrolltimer ved ALS poliklinikken.

Mulige fordeler og ulemper
Alle undersøkelser er trygge. Du vil få en grundig vurdering av hvorvidt hostemaskinen er et aktuelt hjelpemiddel for deg. Inspeksjon av strupen kan medføre ubehag ved neseskilleveggen og en følelse av kiling i halsen. For å redusere ubehaget vil neseslimhinnen behandles med vanlig nesespray som brukes ved forkjølelse, og deretter lokalbedøves ved hjelp av en gel (Xylocain®). Undersøkelsen utføres av en erfaren øre-nese-hals lege som utfører denne type undersøkelser daglig av både barn og voksne med spørsmål om sykdommer i øvre luftveier.

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Samtykke til deltakelse i studien

Jeg tillater innsyn i min journal i den grad dette er viktig for å få fram opplysninger som er relevant for denne studien

JA ◯   **NEI** ◯

Jeg er villig til å delta i studien

Kartlegging og oppfølging av stemmebåndets bevegelse og stabilitet hos personer med Amyotrofisk lateral sclerose (ALS)

Navn _____
(Bruk blokkbokstaver)

Sted og dato_____

_____
Underskrift av prosjektdeltaker

Stedfortredende samtykke når berettiget, enten i tillegg til personen selv eller istedenfor

Sted og dato_____

_____
Signert av nærstående

Rotated title (left margin): MI-E AND LARYNGEAL RESPONSES – EVALUATION OF THE VIDEO RECORDINGS

APPENDIX 3

Intervention	Insufflation						Pressure fall						Exsufflation							Anatomic
	TVF abduction	TVF subsequent adduction	AEF abduction	AEF adduction	Epiglottis retroflex	Tongue-base backwards	TVF abduction	TVF adduction	AEF abduction	AEF adduction	Epiglottis retroflex	Hypo-pharyngeal constriction	TVF abduction	TVF subsequent adduction	Repetitive TVF closures (x times)	AEF abduction	AEF adduction	Epiglottis retroflex	Hypo-pharyngeal constriction	Define
Breathing																				
Forced expiration																				
Cough																				
1 20 I-E																				
2 20 I-C																				
3 20 I-sC																				
4 30 I-E																				
5 30 I-C																				
6 30 I-sC																				
7 40 I-E																				
8 40 I-C																				
9 40 I-sC																				
10 50 I-E																				
11 50 I-C																				
12 50 I-sC																				

Valsalva maneuvre: YES   NO

10. PAPERS I-III

II

OPEN ACCESS

ORIGINAL ARTICLE

Laryngeal response patterns influence the efficacy of mechanical assisted cough in amyotrophic lateral sclerosis

Tiina Andersen,[1,2,3] Astrid Sandnes,[3] Anne Kristine Brekka,[4] Magnus Hilland,[5] Hege Clemm,[3,6] Ove Fondenes,[1] Ole-Bjørn Tysnes,[7,8] John-Helge Heimdal,[5,8] Thomas Halvorsen,[3,6] Maria Vollsæter,[1,3,6] Ola Drange Røksund[4,6]

ABSTRACT

Background Most patients with amyotrophic lateral sclerosis (ALS) are treated with mechanical insufflation–exsufflation (MI-E) in order to improve cough. This method often fails in ALS with bulbar involvement, allegedly due to upper-airway malfunction. We have studied this phenomenon in detail with laryngoscopy to unravel information that could lead to better treatment.

Methods We conducted a cross-sectional study of 20 patients with ALS and 20 healthy age-matched and sex-matched volunteers. We used video-recorded flexible transnasal fibre-optic laryngoscopy during MI-E undertaken according to a standardised protocol, applying pressures of ± 20 to ± 50 cm H_2O. Laryngeal movements were assessed from video files. ALS type and characteristics of upper and lower motor neuron symptoms were determined.

Results At the supraglottic level, all patients with ALS and bulbar symptoms (n=14) adducted their laryngeal structures during insufflation. At the glottic level, initial abduction followed by subsequent adduction was observed in all patients with ALS during insufflation and exsufflation. Hypopharyngeal constriction during exsufflation was observed in all subjects, most prominently in patients with ALS and bulbar symptoms. Healthy subjects and patients with ALS and no bulbar symptoms (n=6) coordinated their cough well during MI-E.

Conclusions Laryngoscopy during ongoing MI-E in patients with ALS and bulbar symptoms revealed laryngeal adduction especially during insufflation but also during exsufflation, thereby severely compromising the size of the laryngeal inlet in some patients. Individually customised settings can prevent this and thereby improve and extend the use of non-invasive MI-E.

Key messages

What is the key question?
► Mechanical insufflation–exsufflation (MI-E) is an efficient tool used to improve cough in most patients with neuromuscular disorders, but the method often fails when bulbar involvement is present.

What is the bottom line?
► We used laryngoscopy during ongoing MI-E and saw that patients with bulbar amyotrophic lateral sclerosis (ALS) were prone to adduct laryngeal structures throughout the various pressure cycles, thereby severely obstructing the airflow and the effect of the treatment.

Why read on?
► In patients with bulbar ALS, cough assistance with MI-E should be delivered carefully and according to the criteria suggested in the present study.

Linked

CrossMark

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an incurable and highly disabling neurodegenerative disease of upper and lower motor neurons. Treatment is largely symptomatic, and average life expectancy at the time of the diagnosis is 2–3 years unless ventilatory assistance is provided.[1]

ALS is classified as 'spinal' if symptom onset affects the limbs predominantly, and as 'bulbar' if the disease presents with difficulty in speaking, swallowing or coughing. Paresis to predominantly upper motor neurons leads primarily to spasticity, whereas paresis of lower motor neurons leads to flaccidity.[2] Regardless of the subtype, ALS progresses and eventually encompasses all skeletal muscles.[3] Involvement of respiratory muscles limits respiratory function and cough, thereby leading to secretion accumulation, lung infections and, eventually, respiratory failure.[3–6] Effective augmentation of cough is vital for clearance of airway secretions in these patients and fundamental for the prevention and treatment of pneumonias.[6 7]

In a voluntary cough, inspiratory muscles increase the lung volume, laryngeal muscles coordinate opening and closure of the glottis and expiratory muscles increase the thoracoabdominal pressure.[8] These interactions are disturbed in neuromuscular disorders.[7 9] Mechanical insufflation-exsufflation (MI-E) is used widely to assist cough mechanically by applying positive and negative pressure changes to the airways, either non-invasively via a mask or invasively via a tracheostomy.[10 11] It has been hypothesised that coordinated glottic movements are required for MI-E to be effective.[12] Non-invasive MI-E can be difficult to apply in patients with the

bulbar subtype of ALS. This problem may be due to dysfunction of bulbar-innervated muscles, but the basic mechanisms are not understood.

The laryngeal response to MI-E in patients with ALS has never been studied. Here, we investigated the laryngeal response patterns to MI-E in ALS to improve the treatment that we can offer to these severely ill patients.

METHODS
Neurological assessment and definitions
ALS was diagnosed by a senior neurologist (O-BT) in accordance with the revised criteria set by the El Escorial World Federation of Neurology.[13] [14] The disease was classified as 'spinal ALS', 'ALS with progressive bulbar palsy' (hypotonic bulbar onset with dysarthria, tongue atrophy and absence of jaw reflex) or 'ALS with pseudobulbar palsy' (spastic bulbar onset with dysarthria, exaggerated jaw reflex and no tongue atrophy). Patients were assessed using the ALS Functional Rating Scale-revised (ALSFRS-r).[15] Bulbar impairment score (BIS) was evaluated from the ALSFRS-r, from where the items of speech and swallowing were calculated.[16] Dysphagia was determined using the 100 mL water swallow test.[17] [18]

Subjects
This was a cross-sectional observational population-based study of 20 patients with ALS who had not undergone tracheostomy and 20 neurologically healthy age-matched and sex-matched controls. Exclusion criteria were age <18 years, history of laryngospasm, sensitisation to Xylocain (anaesthetic used during laryngoscopy), pneumothorax, additional lung disease, cancer, acute infection of the chest 1 month before study commencement and mental instability.

Approximately 20 patients with ALS who have not undergone tracheostomy are usually enrolled at all times at the ALS clinic at Haukeland University Hospital (Bergen, Norway), which serves a population of ≈500 000 inhabitants. At the start of this study, 17 patients were enrolled at the clinic and 20 new patients were diagnosed and enrolled during the 1.5-year recruitment period from December 2011 to June 2013. All 37 patients were informed about the study and invited to participate. Thirteen patients declined and four died soon after being invited, leaving 20 participants. Reasons for non-participation were severe disease/fatigue (n=7), or limb-onset ALS without bulbar symptoms and, therefore, no interest in participation (n=6). The study protocol was approved by the Regional Committee for Medical Research Ethics. Written informed consent was obtained from all participants.

Pulmonary function and respiratory strength
Spirometry was undertaken with a Vmax 22 Encore system (SensorMedics, Yorba Linda, California, USA). FVC, FEV1 and peak expiratory flow were measured seated, with a nose clip. Slow vital capacity was measured with a Respirometer (nSpire Health, Hertford, UK). Peak cough flow was measured using a hand-held Peak Flow Meter (Vitalograph, Ennis, Ireland). Plateau values (average of 1 s) of the maximal inspiratory (Pi_{max}) and expiratory (Pe_{max}) muscle strength and sniff nasal inspiratory pressure (SNIP) were measured seated using a Respiratory Pressure Meter (Micro RPM; Micro Medical, Rochester, UK). SNIP was measured at functional residual capacity, Pi_{max} at residual volume and Pe_{max} at total lung capacity. The highest value from three or more attempts was selected for analyses and standardised to predicted percentages.[19–22]

Video-recorded transnasal fibre-optic laryngoscopy during MI-E
Video-recorded transnasal fibre-optic laryngoscopy (ENF-P3; Olympus, Tokyo, Japan) was used to visualise laryngeal anatomy at baseline and response patterns during MI-E (Cough Assist; Respironics, Murrysville, Pennsylvania, USA). We used a set-up described in detail previously, except that the laryngoscope was supported manually (see online supplementary figure S1) instead of using a customised headgear.[23] A standardised MI-E protocol was used.[23] The protocol comprised 12 intervention arms with various combinations of pressures, instructions and manual thoracic thrust (see online supplementary table S2).

Pressures of ±20, ±30, ±40 and ±50 cm H_2O were used with specific instructions. For MI-E in automated mode with 2 s insufflation, 2 s exsufflation and 1 s pause, the instructions were to 'inhale' actively when insufflation was started and to (A) 'exhale' or (B) 'cough' actively when the device switched to exsufflation. For MI in manual mode with 2 s insufflation followed by manually assisted thoracic thrust, the instructions were to 'inhale' actively when insufflation was started and to 'cough' actively during the thoracic thrust.

In case of patient discomfort, the procedure was stopped and higher examination pressures were not applied.

Analyses of observations
Altogether, 480 recordings were scheduled for assessment, that is, one recording from 12 intervention arms in 20 patients and 20 control subjects. With respect to assessment of observations, MI-E cycles were edited into three phases of interest: (i) insufflation, (ii) pressure drop (from positive to negative) and (iii) active exsufflation or the voluntary cough with no negative pressure applied. The onset and offset of each phase were observed and defined from the parallel video recording of the MI-E manometer.[23] Video recordings were assessed systematically, as described previously,[23] by two trained raters (TA and AKB). Main features were described at glottic, supraglottic and hypopharyngeal levels (see online supplementary figure S3). Laryngeal anatomy and motion at rest were evaluated by a senior laryngologist (J-HH).

Statistical analyses
The χ^2 test, or Fisher's exact test if expected cell counts were less than five, were applied to assess differences between groups with regard to categorical data. Background data were given as group means with SDs. The number of subjects with the described patterns of laryngeal movements during MI-E was given as group counts and percentages. Statistical analyses were conducted using SPSS V.21.0 (IBM, Armonk, USA). The two-sided significance level was set at 0.05.

RESULTS
Patient characteristics
Of 20 participating patients with ALS, six had limb onset with no bulbar symptoms and 14 had bulbar symptoms (table 1); of these, seven had pseudobulbar (spastic) ALS and seven had progressive bulbar (hypotonic) ALS. Lung-function characteristics in ALS were lower than predicted (table 1). In patients with progressive bulbar ALS, 4/7 subjects had an abnormal epiglottis: three had a juvenile and high-standing epiglottis, and in one patient the epiglottis was considered 'floppy'. Retention of secretions/sputum was observed in 4/7 patients with progressive bulbar ALS, in 2/7 cases with pseudobulbar ALS, in 2/6 subjects with non-bulbar ALS and in 1/20 healthy controls.

Table 1 Background characteristics of the study participants (n=40)

	Healthy (n=20)	ALS (n=20)	ALS without bulbar symptoms (n=6)	ALS with bulbar symptoms (n=14)
Male/female ratio	13/7	13/7	6/0	7/7
Age, years	66.9 (7.2)	68.7 (9.3)	65.8 (9.2)	69.9 (9.4)
BMI, kg/m^2	23.9 (2.4)	23.6 (4.3)	23.5 (1.8)	23.6 (5.1)
FVC, % pred	113.6 (16.0)	67.4 (22.1)	73.5 (18.8)	64.5 (23.7)
FEV1, % pred	107.4 (19.0)	70.6 (25.7)	76.0 (22.0)	68.1 (27.7)
SVC, L	4.15 (1.3)	2.92 (1.0)	3.6 (0.7)	2.6 (1.0)
SVC, % pred	110.6 (20.1)	76.1 (22.5)	78.7 (12.9)	75.0 (26.2)
PCF, L/min	484.5 (130.2)	266.8 (145.8)	340.8 (198.6)	232.6 (108.4)
Pi$_{max}$, cm H$_2$O	95.2 (23.6)	43.3 (20.9)	54.2 (18.9)	38.6 (20.5)
Pi$_{max}$, % pred	111.3 (24.9)	52.9 (23.7)	58.3 (20.6)	50.6 (25.2)
Pe$_{max}$, cm H$_2$O	140.8 (37.9)	50.4 (30.0)	80.2 (32.1)	37.6 (18.3)
Pe$_{max}$, % pred	140.1 (34.3)	49.4 (24.8)	68.2 (30.3)	41.3 (17.6)
SNIP, cm H$_2$O	91.2 (33.7)	38.6 (17.9)	47.7 (22.2)	33.6 (13.8)
SNIP, % pred	99.1 (34.6)	42.6 (19.0)	48.1 (22.9)	39.7 (16.9)
WST, mL/s	31.50 (7.7)	12.3 (11.4)	25.8 (7.6)	5.5 (4.9)
ALSFRS-r	–	36.7 (8.4)	39.0 (7.5)	35.6 (8.9)
BIS	–	6.0 (2.3)	8.0 (0)	5.0 (2.3)

Figures are group means with SDs.
ALS, amyotrophic lateral sclerosis; ALS Functional Rating Scale-revised; BIS, bulbar impairment scale; BMI, body mass index; PCF, peak cough flow; Pe$_{max}$, maximal expiratory mouth pressure; Pi$_{max}$, maximal inspiratory mouth pressure; SNIP, sniff nasal inspiratory pressure; SVC, slow vital capacity; WST, water swallow test.

Laryngeal response to MI-E

In total, 453 (94%) of 480 scheduled recordings were analysed. Four patients with bulbar symptoms completed only parts of the MI-E protocol due to discomfort from the applied pressures, that is, one patient (progressive bulbar ALS) interrupted the protocol after pressures of ±20 cm H$_2$O (missing 9/12 intervention arms), one patient (pseudobulbar ALS) after ±30 cm H$_2$O (missing 6/12 intervention arms) and two patients (one pseudobulbar and one progressive bulbar ALS) after ±40 cm H$_2$O (both patients missing 3/12 intervention arms). Technical failures led to loss of video recordings in one healthy control at examining pressures of ±40 and ±50 cm H$_2$O (missing 6/12 intervention arms).

In general, the larynx moved downwards during applied insufflation and upwards (cranially) during exsufflation. (See table 2 for overall descriptions and online supplementary video 1 for the laryngeal response in a patient with non-bulbar ALS; online supplementary video 2 in a healthy control; online supplementary video 3 in a patient with progressive bulbar ALS; online supplementary video 4 in a patient with pseudobulbar ALS.) Adequate laryngeal control was defined as described for normal cough in the literature,[8] and presented as initial abduction of the true vocal folds (TVF) and aryepiglottic folds (AEF), and thereafter glottic closure with subsequent rapid opening when coughing, abduction of the TVF and AEF followed by sequential closures and/or narrowing in the exhalation phase of the cough.

Response at the glottic level

Observations at the glottic level were not possible in some patients with ALS and bulbar symptoms, because adduction of AEF and/or the hypopharyngeal area obscured the view of TVF, particularly in the high-pressure ranges of 40–50 cm H$_2$O. Observations at the glottic level were based on successful visualisation of MI-E cycles (TVF responses A, B, G, I, M, S, N$_1$, N$_2$ and N$_3$ in figure 1 and online supplementary tables S4, S5 and S6).

There were significant differences between patients with ALS and healthy controls with respect to TVF adduction subsequent to the initial abduction during insufflation (response B in figure 1 and in online supplementary table S4) and exsufflation. Varying the instructions (to cough or exhale during negative pressures or to cough without applied negative pressure) did not influence the groups differently (response N$_1$, N$_2$ and N$_3$ in figure 1 and online supplementary table S6).

Response at the supraglottic level

AEF responses are presented as C, D, H, J, O and P (figure 2 and online supplementary tables S4, S5 and S6). Medial rotation of the cuneiform tubercles accompanied by considerable adduction of the AEF was observed during insufflation (initially or subsequent to abduction) in all patients with bulbar ALS (online supplementary table S4 and response C and D in figure 2).

A retroflex movement of epiglottis (a passive dorsal rotation) was observed to partly occlude the laryngeal inlet in some cases, either as a rapid movement or lasting throughout the insufflation (responses E, K and Q (figure 2 and online supplementary tables S4, S5 and S6)).

Oesophageal opening was observed during insufflation in two patients with progressive bulbar ALS. Both subjects were observed to burp afterwards, suggesting that (part of) the insufflation volume ended up in the oesophagus and stomach instead of the lungs.

Response at the tongue base and at the hypopharyngeal level

There were significant differences between healthy controls and patients with ALS with regard to backward movement of the tongue base during insufflation and during the pressure drop (responses F and L in figure 3 and online supplementary tables S4 and S5).

Constriction of the hypopharynx during exsufflation was observed in healthy controls and in patients with ALS, regardless of the presence of bulbar symptoms. In patients with ALS and bulbar symptoms, hypopharyngeal constriction was more prominent in those with progressive bulbar paresis. The

Table 2 Description of laryngeal response patterns during the MI-E protocol (n=40)

| Subjects (N=20) | Glottic level | Supraglottic level | | Tongue base and hypopharyngeal level | |
	True vocal folds (TVF)	Aryepiglottic folds (AEF)	Epiglottis (EG)	Base of the tongue (BT)	Hypopharynx (HP)
Healthy (n=20)	Adequate control* in all	Adequate control† in all	Retroflex movement in 8/20	Backward in 4/20	Constriction in 12/20 of varying degrees
ALS without bulbar symptoms (n=6)	Adequate control* in all	Adequate control† in all	Retroflex movement in 1/6	Backward in all	Constriction in all of varying degrees
Progressive bulbar ALS (n=7)	Adequate control* in all	Adduction in insufflation in all	Retroflex movement +'floppy' in 1/7	Backward in 5/7	Constriction in all, and very narrow in 4/7
Pseudobulbar ALS (n=7)	Inadequate control§ in insufflation; in 3/7and in 1/7 in exsufflation	Adduction in insufflation in all (but in 4/7, only at higher pressures: $\geq$+40 cm H_2O)	Retroflex movement in 2/7	Backward in all	Constriction in all, and very narrow in 1/7

*Normal cough, that is, TVF abduction in insufflation, glottic closure when coughing and TVF abduction+sequential closures and/or narrowing in exsufflation.
†AEF follows the movements of the TVF.
§Very small TVF opening in insufflation or in exsufflation.
ALS, amyotrophic lateral sclerosis; MIE, mechanical insufflation–exsufflation.

hypopharynx was totally constricted in 4/7 patients with progressive bulbar paresis and in 1/7 patients with pseudobulbar paresis (responses R_1, R_2 and R_3 in figure 3 and online supplementary table S6).

Differences in laryngeal movements between patients with pseudobulbar and progressive bulbar ALS were not significant. A few significant values were observed between observations of healthy controls and patients with ALS and bulbar symptoms, and between patients with ALS with and without bulbar symptoms. Due to a multiple-testing problem, these results should be interpreted with caution. However, we saw a pattern in comparison between controls and patients with ALS and bulbar symptoms with regard to backward movement of the tongue base during the pressure drop and in subsequent adduction of TVF during exsufflation (see online supplementary tables S4–S6).

DISCUSSION

This is the first study to show that video-recorded flexible laryngoscopy is a feasible method to characterise laryngeal responses throughout MI-E in patients with ALS. Results clearly indicated that MI-E in patients with bulbar symptoms was associated with adduction of supraglottic laryngeal structures during insufflation, and that this seemed to compromise airflow. Backward movement of the tongue base during insufflation, potentially obstructing airflow at the hypopharynx, was more prominent in patients with ALS than in healthy controls. Moreover, patients with ALS, irrespective of subtype, were more likely to adduct the vocal folds during insufflation and exsufflation. Patients with ALS, without bulbar symptoms, could cough in a coordinated way, similar to that seen in healthy controls.

The main strength of this study was provision of important knowledge on a challenging clinical problem achieved using objective and verifiable methods in a population-based sample of patients whose data were compared with those of healthy matched volunteers. The small study cohort was a limitation, complicating statistical handling and rendering the study at risk of particularly type-II errors (ie, failure to detect significant differences that may have been present). A priori power calculation could not be undertaken, because the data distribution was not known when planning the study.[24]

Transnasal fibre-optic laryngoscopy during ongoing MI-E in patients with ALS has not been described previously, but has been used to describe the larynx during simple tasks (eg, vocalising, spontaneous cough and forced exhalation).[25][26] We

encountered some technical challenges. First, as the larynx moved downwards and upwards during insufflation and exsufflation, dynamic adjustments of the laryngoscope position were required. Sometimes, airway secretions led to poor-quality video recordings, and pretreatment aiming to clear secretions could have been considered. Adduction of supraglottic structures precluded visual access to the vocal folds in some patients.

The present study suggests that adduction of primarily supraglottic laryngeal structures during insufflation may be a critical issue when carrying out MI-E in patients with ALS and bulbar symptoms. Conceivably, the observed adduction prevents lung insufflation before exsufflation, thereby compromising the effect of MI-E. We cannot explain these response patterns, but can only speculate. There is only one abductor muscle in the larynx, the posterior cricoarytenoid muscle, but several small intrinsic adductors.[27] Intrinsic laryngeal muscles interact in a complex way during cough, speech and swallowing, but always act in concert. Stimulation of extremely sensitive receptors in the supraglottic larynx usually induces complex adductor reflexes that, for example, prevent foreign bodies from entering the airways.[27] This reflex circuit may be hyper-responsive or dysregulated in patients with ALS and, therefore, lead to inappropriate laryngeal closure, comparable with the observations made in patients with Parkinson's disease or brainstem compression.[28][29] Tomik et al[25] observed early dysfunction of the vagal nerve before any clinical signs of bulbar dysfunction in patients with spinal ALS. The observed vocal fold adduction in our study supports this finding.

Differences in the two subtypes of bulbar ALS may influence laryngeal response patterns to MI-E, that is, progressive (hypotonic) versus pseudobulbar (spastic) ALS. In pseudobulbar ALS, laryngeal adduction occurred mainly at the glottic level at relatively high insufflation pressures. It seems reasonable to suggest that positive pressures more easily trigger laryngeal adductor reflexes in a disease that is predominantly spastic. AEF are relatively soft structures provided with only scattered muscle fibres. Therefore, adduction at the supraglottic level could, theoretically, be explained by the Bernoulli principle: increasing airflow initiates negative intraluminal pressures that eventually cause medial collapse.[30] This mechanism may conceivably be particularly important in progressive bulbar ALS characterised predominantly by hypotonic paresis. An abnormal high-standing epiglottis may have a practical implication by compromising the laryngeal inlet during insufflation due to retroflex movements caused by the positive pressures, as demonstrated also during treatment with CPAP in patients with obstructive sleep apnoea.[31]
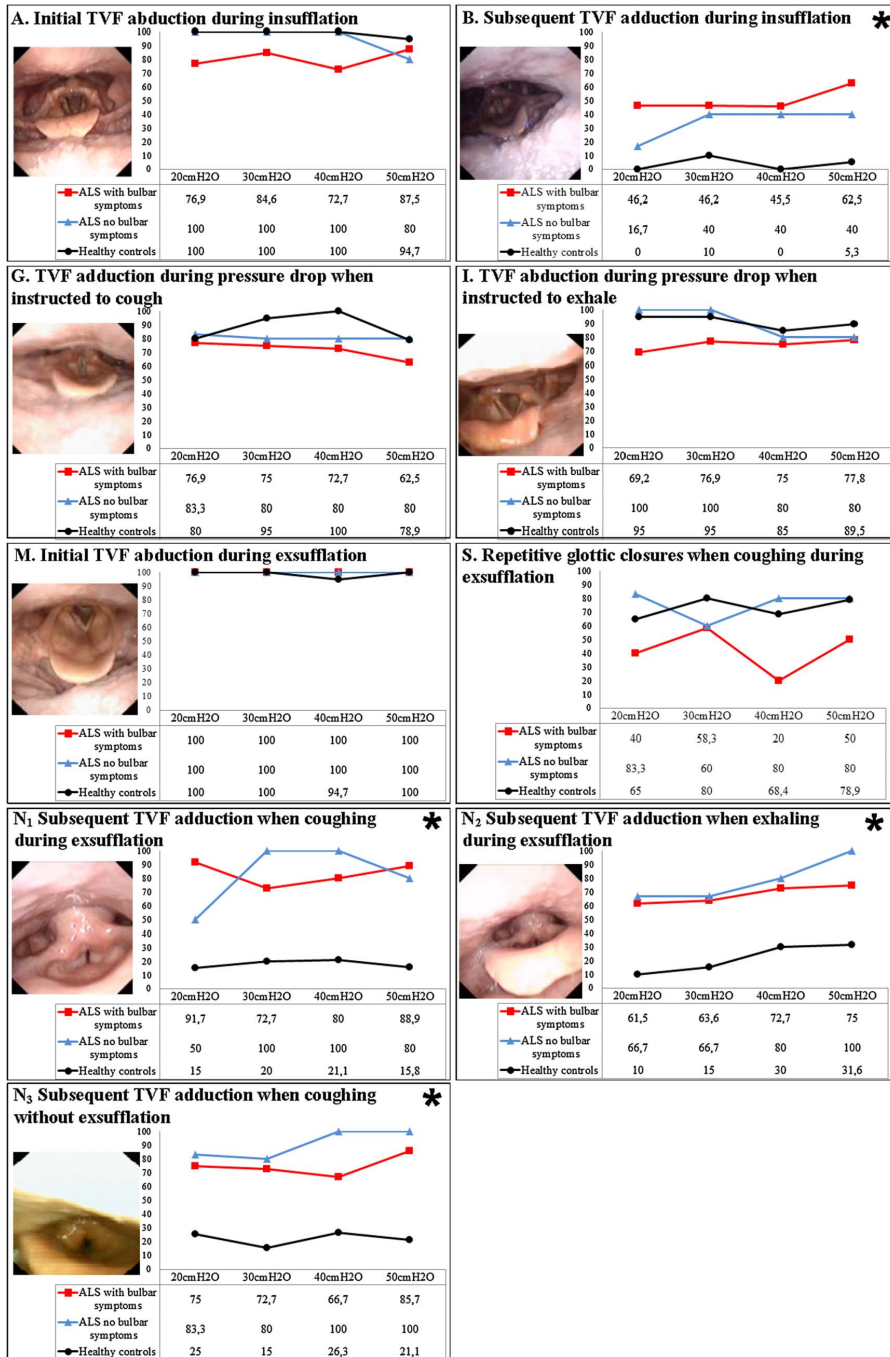
Figure 1 Laryngeal response at the glottic level. Figures are percentages of the sample with the described response. *Significant difference between healthy volunteers and patients with ALS. ALS, amyotrophic lateral sclerosis; TVF, true vocal folds.

Hypopharyngeal constriction during exsufflation was observed to varying extents in all study subjects, as well as healthy controls. This finding confirms reports of upper-airway narrowing at pharyngeal and oropharyngeal levels upon application of negative pressures during exhalation in healthy subjects.[32–34] This phenomenon has been used to explain the
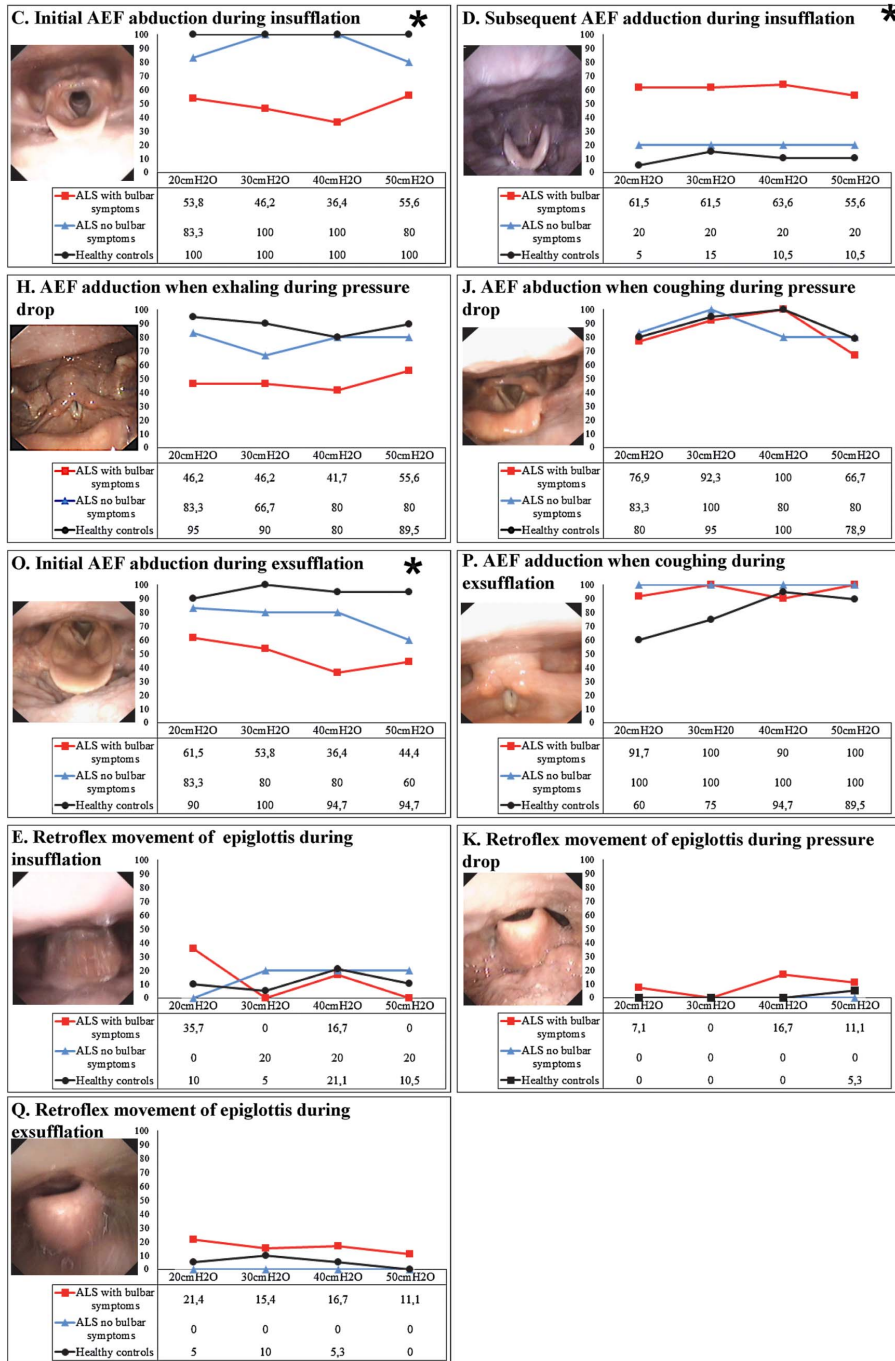
Figure 2 Laryngeal response at the supraglottic level. Figures are percentages of the sample with the described response.*Significant difference between healthy volunteers and patients with ALS. AEF, aryepiglottic folds; ALS, amyotrophic lateral sclerosis.

ineffectiveness of MI-E in patients with ALS.[35] Sancho et al undertook CT during MI-E at baseline and during exsufflation in three patients with ALS. They reported varying reductions

of the lateral diameter at the level of nasopharynx, uvula and pharynx during the exsufflation phase at −40 cm H_2O.[12] The response during insufflation was not examined. They suggested
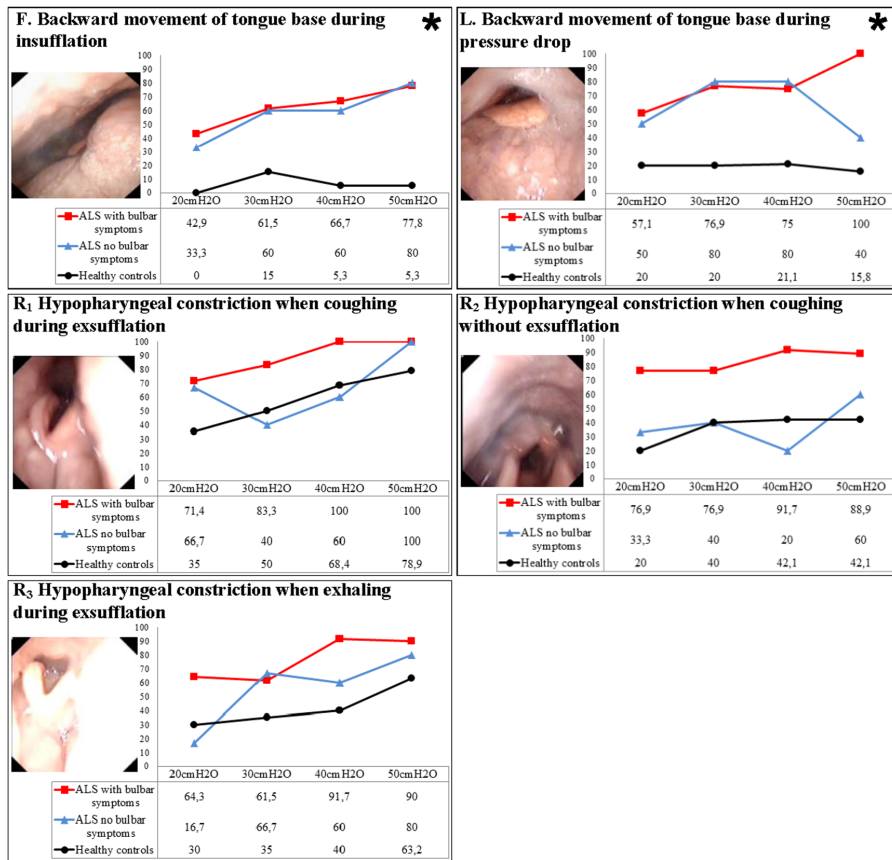
Figure 3 Laryngeal response at the tongue base and hypopharyngeal level. Figures are percentages of the sample with the described response. *Significant difference between healthy volunteers and patients with amyotrophic lateral sclerosis (ALS).

that MI-E should be carried out by applying a single insufflation followed by a manually assisted cough instead of active exsufflation with negative pressures.[35][36] Hypopharyngeal constriction during exsufflation was observed in healthy controls and patients with ALS in the present study; so, this phenomenon alone cannot explain treatment failure in bulbar ALS. Moreover, inability to fill the lungs during insufflation because of the observed supraglottic adduction would create a vacuum during the subsequent active exsufflation, and thereby aggravate hypopharyngeal constriction. If this hypothesis is correct, a single insufflation followed by a manually assisted cough cannot help patients with bulbar ALS to cough more effectively, but will be both uncomfortable and unproductive.

The present study suggests that an individual approach to MI-E used in respiratory airway therapy is highly important. Lower positive pressures and airflow combined with longer inspiratory times may contribute to better laryngeal stability during insufflation, perhaps by preventing or reducing the impact of protective laryngeal reflex circuits and the intraluminal suction forces induced by the Bernoulli effect (figure 4). Patients with bulbar insufficiency may, therefore, be more likely to obtain sufficient inspiratory volumes, a situation that would improve the conditions for exsufflation of the lungs. The phasic relationship

that exists between the posterior cricoarytenoid muscle and diaphragm is a feature that could, theoretically, be exploited clinically. That is, when the diaphragm contracts, the activity of the posterior cricoarytenoid muscle increases in a coordinated manner due to vagal stimulation, thereby abducting the larynx.[27] If the patient is instructed to inhale actively before active insufflation with MI-E, this act would, theoretically, lead to better laryngeal abduction and facilitate airflow. Recently, MI-E devices with a 'trigger' function linked to insufflation have become available, and these mechanisms should be studied closely.

A better understanding of laryngeal dysfunction as ALS progresses in its various phenotypes can help establish better (and hopefully individually tailored) clinical respiratory treatment strategies for these patients, and perhaps also for other patients with bulbar-innervated muscle dysfunction.

CONCLUSION

Video-recorded flexible laryngoscopy is a feasible method to characterise laryngeal responses throughout an MI-E protocol in patients with ALS. Treatment failure with MI-E in patients with bulbar symptoms is likely to be caused primarily by laryngeal adduction during insufflation, predominantly at the supraglottic level. This response precludes air-filling of the lungs during

Figure 4 A practical algorithm suggesting how to adjust the settings of mechanical insufflation–exsufflation (MI-E) when used to treat patients with amyotrophic lateral sclerosis (ALS) for airway secretion clearance problems, based on observations in the present study.
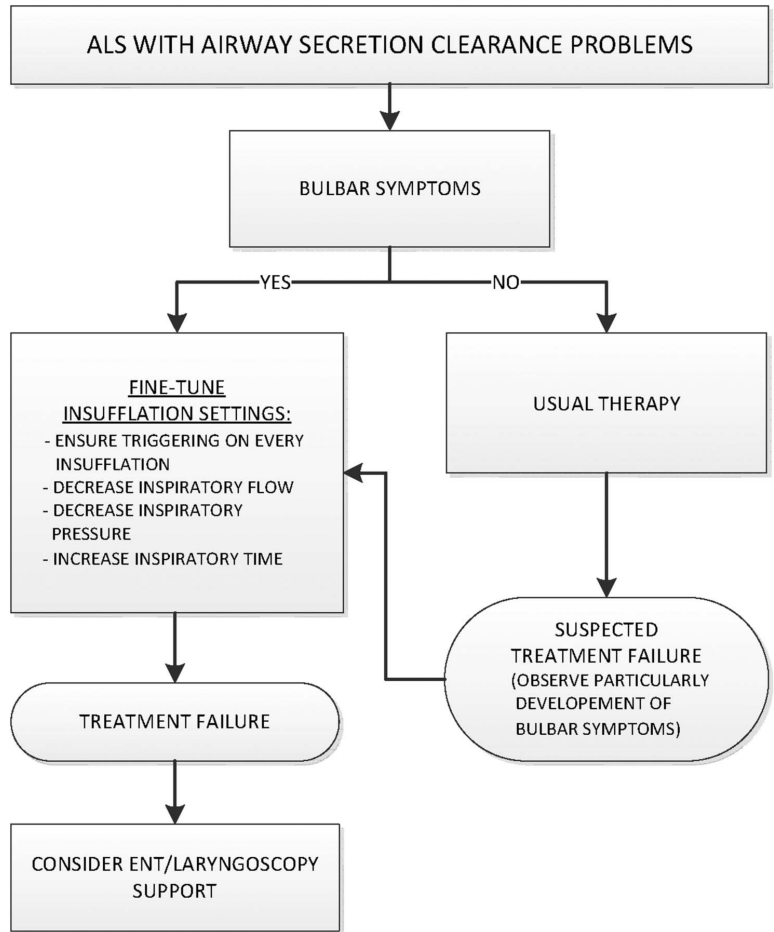
insufflation, causing discomfort and subsequent inefficient exsufflation. We propose that individually customised settings for pressure and flow can improve and extend the use of non-invasive MI-E in ALS, and that flexible laryngoscopy can be an efficient tool in this respect in selected patients who do not respond as expected.

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THORAX

Laryngeal response patterns influence the efficacy of mechanical assisted cough in amyotrophic lateral sclerosis

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Notes

SUPPLEMENTARY FIGURES

Figure S1. The setup with a laryngoscope passing through a modified interface with the laryngoscope supported and adjusted manually. Situation arranged.

Figure S2. Examination was recorded with two continuous videos on the same screen, and showed the laryngeal view and phases on the MI-E device synchronously. Anatomic landmarks are illustrated on the laryngeal top view.
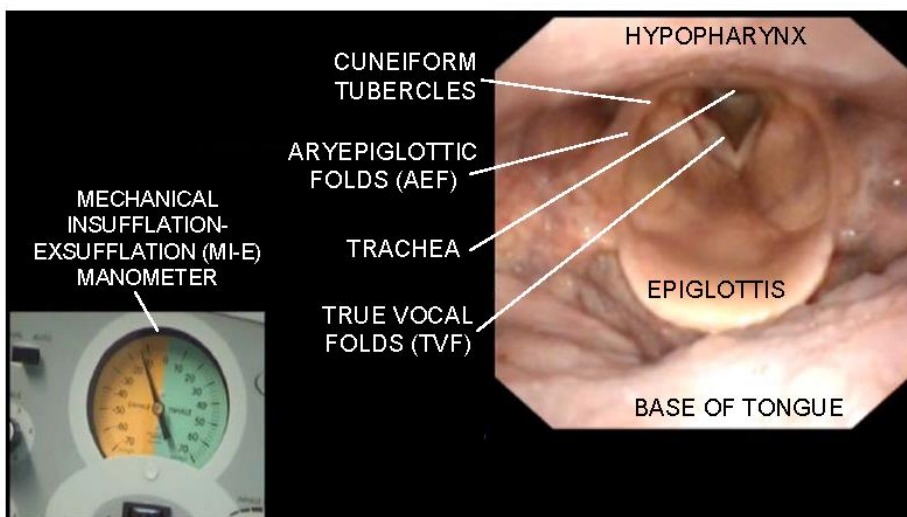
LEGENDS FOR SUPPLEMENTARY VIDEO FILES

Supplementary Video 1. Laryngeal response to mechanical insufflation–exsufflation (MI-E) in a patient with non-bulbar ALS visualised in real-time and slow motion by video-recorded transnasal fibreoptic laryngoscopy.

Supplementary Video 2. Laryngeal response to mechanical insufflation–exsufflation (MI-E) in a healthy control visualised in real-time and slow motion by video-recorded transnasal fibreoptic laryngoscopy.

Supplementary Video 3. Laryngeal response to mechanical insufflation-exsufflation (MI-E) in a patient with progressive bulbar ALS visualised in real-time and slow motion by video-recorded transnasal fibreoptic laryngoscopy.

Supplementary Video 4. Laryngeal response to mechanical insufflation–exsufflation (MI-E) in a patient with pseudobulbar ALS visualised in real-time and in slow motion by video-recorded transnasal fibreoptic laryngoscopy.

Supplementary Tables

Supplementary Table S1. Standardised protocol of conditions during intervention with MI-E and MI

Intervention arm	Pressure settings (cmH$_2$O):		Instruction during exsufflation:		Manual thoracic thrust
	MI-E	MI	active exhale	active cough	
1.	±20		×		
2.	±20			×	
3.		+20		×	×
4.	±30		×		
5.	±30			×	
6.		+30		×	×
7.	±40		×		
8.	±40			×	
9.		+40		×	×
10.	±50		×		
11.	±50			×	
12.		+50		×	×

MI-E=mechanical insufflation–exsufflation, MI=mechanical insufflation. Intervention arms 1–12: respective pressures of MI-E or MI combined with instruction to either exhale actively or to cough during exsufflation. Additional manual thoracic thrust during cough was provided in combination with MI.

Supplementary Table S4. Laryngeal response patterns during mechanical insufflation according to applied pressures. P-values are from comparisons between the ALS group and the control group, between the control group and the ALS group with bulbar symptoms, and between ALS without and with bulbar symptoms.

DURING INSUFFLATION				
MI-E pressures (cmH₂O)	±20	±30	±40	±50
Comparisons between ALS patient group (n=20) and healthy control group (n=20)				
A. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.
B. Subsequent adduction of TVF	.003	.03	.002	.003
C. Initial abduction of AEF	.003	.003	.002	.008
D. Adduction of AEF	.003	.04	.02	.002
E. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.
F. Backward movement of BT	.003	.006	<.001	<.001
Comparisons between healthy (n=20) *vs* ALS with bulbar symptoms (n=14)				
A. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.
B. Subsequent adduction of TVF	n.s.	n.s.	n.s.	n.s.
C. Initial abduction of AEF	n.s.	n.s.	n.s.	n.s.
D. Adduction of AEF	n.s.	n.s.	n.s.	.02
E. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.
F. Backward movement of BT	n.s.	n.s.	n.s.	.02
Comparisons between ALS without bulbar symptoms (n=6) *vs* ALS with bulbar symptoms (n=14)				
A. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.
B. Subsequent adduction of TVF	n.s.	n.s.	n.s.	n.s.
C. Initial abduction of AEF	n.s.	n.s.	.03	n.s.
D. Adduction of AEF	n.s.	n.s.	n.s.	.02
E. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.
F. Backward movement of BT	n.s.	n.s.	n.s.	n.s.
Comparisons between pseudobulbar ALS (n=7) *vs* progressive bulbar ALS (n=7)				
A. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.
B. Subsequent adduction of TVF	n.s.	n.s.	n.s.	n.s.
C. Initial abduction of AEF	n.s.	n.s.	n.s.	n.s.
D. Adduction of AEF	n.s.	n.s.	n.s.	n.s.
E. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.
F. Backward movement of BT	n.s.	n.s.	n.s.	n.s.

P-values were calculated using the chi-square test or Fisher's exact test if expected cell counts were less than five. MI-E=mechanical insufflation–exsufflation, TVF=true vocal folds, AEF= aryepiglottic folds, EG=epiglottis, BT=base of the tongue, n.s.=non-significant

Supplementary Table S5. Laryngeal response patterns during the pressure drop according to applied pressures and instructions. P-values are from comparisons between the ALS group and the control group, between the control group and the ALS group with bulbar symptoms, and between ALS without and with bulbar symptoms.

DURING THE PRESSURE DROP

Instruction during exsufflation:	Cough				Exhale			
MI-E pressures (cmH$_2$O)	±20	±30	±40	±50	±20	±30	±40	±50
Comparisons between ALS patient group (n=20) and healthy control group (n=20)								
G. Adduction of TVF	n.s.	n.s.	.04	n.s.	n.s.	n.s.	n.s.	n.s.
H. Adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	.01	n.s.	n.s.
I. Abduction of TVF	n.s.	n.s.	n.s.	n.s.	.008	n.s.	n.s.	n.s.
J. Abduction of AEF	n.s.	n.s.	n.s.	n.s.	.008	n.s.	n.s.	n.s.
K. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L. Backward movement of BT	.048	.001	.002	<.001	n.s.	.002	.008	<.001
Comparisons between healthy (n=20) and ALS with bulbar symptoms (n=14)								
G. Adduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
H. Adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
I. Abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
J. Abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
K. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L. Backward movement of BT	n.s.	.02	.02	.02	n.s.	n.s.	n.s.	<.001
Comparisons between ALS without bulbar symptoms (n=6) *vs* ALS with bulbar symptoms (n=14)								
G. Adduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
H. Adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
I. Abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
J. Abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
K. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L. Backward movement of BT	n.s.	n.s.	n.s.	.03	n.s.	n.s.	n.s.	n.s.
Comparisons between pseudobulbar ALS (n=7) *vs* progressive bulbar ALS (n=7)								
G. Adduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
H. Adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
I. Abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
J. Abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
K. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L. Backward movement of BT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

P-values were calculated using the chi-square test or Fisher's exact test if expected cell counts were less than five. MI-E=mechanical insufflation–exsufflation, TVF=true vocal folds, AEF=aryepiglottic folds, EG=epiglottis, BT=base of the tongue, n.s.=non-significant

Supplementary Table S6. Laryngeal response patterns during mechanical exsufflation according to applied pressures and instructions. P-values are from comparisons between the ALS group and the control group, between the control group and the ALS group with bulbar symptoms, and between ALS without and with bulbar symptoms.

DURING EXSUFFLATION

Instruction during exsufflation:	Cough				Exhale				Cough with no exsufflation			
MI-E pressures (cmH$_2$O)	±20	±30	±40	±50	±20	±30	±40	±50	+20	+30	+40	+50
Comparisons between ALS patient group (n=20) and healthy control group (n=20)												
M. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N. Subsequent adduction of TVF	<.001	.001	<.001	<.001	.001	.003	.02	.005	.003	.001	.007	<.001
O. Initial abduction of AEF	n.s.	.003	.005	.005	.008	.003	.002	.001	.008	.02	.001	.002
P. Subsequent adduction of AEF	n.s.	.048	n.s.	n.s.	.016	.002	.02	.005	n.s.	n.s.	n.s.	n.s.
Q. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
R. Hypopharyngeal constriction	.03	n.s.	n.s.	n.s.	n.s.	n.s.	.02	n.s.	.010	n.s.	n.s.	n.s.
S. Repetitive glottic closures	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons between healthy (n=20) and ALS with bulbar symptoms (n=14)												
M. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N. Subsequent adduction of TVF	.03	.004	.002	.002	.03	n.s.	n.s.	n.s.	.03	.009	.05	<.001
O. Initial abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P. Subsequent adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Q. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
R. Hypopharyngeal constriction	n.s.	n.s.	n.s.	n.s.	n.s.	.008	.007	n.s.	n.s.	n.s.	n.s.	n.s.
S. Repetitive glottic closures	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons between ALS without bulbar symptoms (n=6) *vs* ALS with bulbar symptoms (n=14)												
M. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N. Subsequent adduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
O. Initial abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	.02
P. Subsequent adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Q. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
R. Hypopharyngeal constriction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	.01	n.s.
S. Repetitive glottic closures	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Comparisons between pseudobulbar ALS (n=7) *vs* progressive bulbar ALS (n=7)												
M. Initial abduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N. Subsequent adduction of TVF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
O. Initial abduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
P. Subsequent adduction of AEF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Q. Retroflex movement of EG	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
R. Hypopharyngeal constriction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
S. Repetitive glottic closures	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

P-values were calculated using the chi-square test or Fisher's exact test if expected cell counts were less than five. MI-E=mechanical insufflation–exsufflation, TVF=true vocal folds, AEF=aryepiglottic folds, EG=epiglottis, BT=base of the tongue, n.s.=non-significant

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