

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Experimental and clinical rehabilitation of organic dysphonia

Andrea Tóth MD

Supervisor: István Sziklai PhD, DSc



UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2015

EXPERIMENTAL AND CLINICAL REHABILITATION OF ORGANIC DYSPHONIA

by Andrea Tóth, MD

Supervisor: Prof. István Sziklai, MD, PhD, DSc

Doctoral School of Clinical Medicine, University of Debrecen

Head of the **Examination Committee**: Prof. András Berta, MD, PhD, DSc

Members of the Examination Committee: Prof. Gábor Répássy, MD, PhD

Krisztina Mészáros, MD, PhD

The Examination takes place in the Library of Department of Ophthalmology, Faculty of Medicine, University of Debrecen, at 11.00 am, 25. 11. 2015.

Head of the **Defense Committee**: Prof. András Berta, MD, PhD, DSc

Reviewers: Prof. Zoltán Papp, MD, PhD, DSc

Prof. László Rovó, MD, PhD

Members of the Defense Committee: Prof. Gábor Répássy, MD, PhD

Krisztina Mészáros, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 13.00 pm, 25. 11. 2015.

1. Introduction

Voice is the most important human means of expression. During verbal communication, voice conveys ideas and emotions, and represents a very efficient way of interpersonal information exchange. In our modern society its importance is increasing in different areas of life such as work and spare time. The reduced voice quality has a negative influence on the social integration of the concerned person. In case of professional voice users (teachers, actors, singers, lawyers) the reduced voice quality can be detrimental to their careers. Total laryngectomy can compromise the quality of life, affect socialization and lead to a degree of isolation in activities with family members.

Voice therapy or voice rehabilitation is a way of treating voice disorders that involves vocal and physical exercises coupled with behavioral changes. The long-term goal of the voice therapy program may be to help the patient attain the best possible voice and compensate most effectively for the underlying disorder or disease that cannot be completely "cured."

1.1. Anatomy of sound production

Human voice is specifically a part of human sound production in which the vocal folds are the primary sound source. The mechanism for generating human voice can be subdivided into three parts; the lungs, the vocal folds within the larynx, and the articulators. The lung must produce adequate airflow and air pressure to vibrate vocal folds. The vocal folds are a vibrating valve that chops up the airflow from the lungs into audible pulses that form the laryngeal sound source. The muscles of the larynx adjust the length and tension of the vocal folds to 'fine-tune' pitch and tone. The glottal sound passes the cavities of pharynx, mouth, and nose which serve as resonators and thus shape the sound signal acoustically. The shaping depends on the actual vocal tract configuration. Speech is the vocalized form of human communication, when the voice production is continuous.

1.1.1. Laryngeal cartilages

The larynx serves three important functions in humans. In order of functional priority, they are protective, respiratory, and phonatory. The structure of the larynx is based on cartilagenous framework. The laryngeal skeleton consists of several cartilaginous structures the largest of which is the thyroid cartilage. The thyroid cartilage is composed of two rectangular laminae that are fused anteriorly in the midline. The incomplete fusion of the two laminae superiorly forms the thyroid notch. Attached to each lamina posteriorly are the superior and inferior cornua. The superior cornua articulate with the greater horns of the hyoid bone, while the inferior cornua form a synovial joint with the cricoid cartilage (cricothyroid joint). The cricoid cartilage is a signet ring-shaped cartilage which is the only laryngeal cartilage to encircle completely the airway. The cricoid cartilage articulates with the thyroid cartilage's inferior cornua on the cricothyroid joint facets. It joins the first tracheal ring inferiorly via membranous attachments. The face of the cricoid cartilage has a vertical height of only about 3–4 mm, while the lamina posteriorly stands about 20–30 mm high. The arytenoid cartilages are paired, pyramidal cartilages that articulate with the posterior lamina of the cricoid cartilage at the cricoarytenoid joint. Each arytenoid has both a vocal process medially and a muscular process laterally. These processes act as the attachment sites for the vocal ligament and the major intrinsic muscles of vocal fold movement respectively. The action of movement at the cricoarytenoid joints changes the distance between the vocal processes of the two arytenoids and between each vocal process and the anterior commissure. The cuneiform cartilages are crico-arytenoid joint paired elastic cartilages that sit on top of, and move with, the corresponding arytenoid. The soft tissue of the aryepiglottic folds covers these cartilages. The corniculates are small, paired, fibroelastic cartilages that sit laterally to each of the arytenoids, and are completely embedded within the aryepiglottic folds. These likely serve to provide additional structural support to the aryepiglottic fold. The epiglottis is

an oblong, feather-shaped fibroelastic cartilage that is attached, at its inferior end, to the inner surface of the thyroid cartilage laminae just above the anterior commissure. The major function of the epiglottis is to help in preventing aspiration during swallowing.

1.1.2. Laryngeal musculature

Muscles of the larynx are divided into *intrinsic* and *extrinsic* muscles. Intrinsic muscles of the larynx are responsible for altering the length, tension, shape, and spatial position of the vocal folds by changing the orientation of the muscular and vocal processes of the arytenoids with the fixed anterior commissure. Traditionally, the muscles are categorized into the following scheme: three major vocal fold adductors - *the lateral cricoarytenoid muscle (LCA)*, *thyroarytenoid muscle (TA)* – *internus and externus bellies* and *interarytenoid muscle (IA)* one abductor - *posterior cricoarytenoid muscle (PCA)*, and one tensor muscle - *cricothyroid muscle (CT)*. The extrinsic laryngeal muscles, such as the infrahyoid strap muscles (the sternothyroid, the sternohyoid and the thyrohyoid), the mylohyoid, digastric, geniohyoid, and stylopharyngeus muscles all act in concert to provide laryngeal stabilization, and indirectly may affect vocal fold position.

1.1.3. Cavities of the larynx

The laryngeal central cavity is tubular in shape and lined with mucosa. The superior aspect of the cavity (laryngeal inlet) opens into the pharynx. The inferior aspect of the cavity is continuous with the lumen of the trachea. The laryngeal cavity may be divided into 3 major regions: the vestibule, the middle, and the infraglottic space. The vestibule is the upper portion of the cavity, in between the laryngeal inlet and the vestibular folds. The middle portion of the cavity, or the voice box, is formed by the vestibular folds above and the vocal folds below. The infraglottic space is the lower portion of the cavity, in between the vocal folds and inferior opening of the larynx into the trachea. The quadrangular membrane is a

layer of submucosa. It contains the cuneiform cartilages. The membrane runs between the lateral aspects of the epiglottis and arytenoid cartilages on each side. The free inferior border of the quadrangular membrane is the vestibular ligament which is the vestibular fold when covered by mucosa. The superior border is the aryepiglottic fold. The conus elasticus (or elastic cone) is the lateral portion of the cricothyroid ligament. The lateral portions are thinner and lie close under the mucous membrane of the larynx; they extend from the upper border of the cricoid cartilage to the lower margin of the vocal ligaments, with which they are continuous. The vocal ligaments may therefore be regarded as the free borders of each conus elasticus, and extend from the vocal processes of the arytenoid cartilages to the angle of the thyroid cartilage about midway between its upper and lower borders.

1.1.4. Vocal fold histology

Vocal folds are the voice generating element during phonation. Due to their histological structure self-sustained oscillations excited by the pulmonary airflow. The vocal folds have special vibrating qualities based on their unique multi-layered structure with different physical characteristics.

The different layers of the soft tissue are partitioned in three groups:

1. Squamous epithelial lining – The outermost thin layer (0.05-0.10 mm) is composed of stratified squamous cells.
2. Lamina propria – Lamina propria represents the transition layer between the epithelium and the vocalis muscle (musculus vocalis). It is divided into the *superficial*, *intermediate* and *deep layer* based upon histological composition. The superficial layer of lamina propria is referred to as the Reinke's space (0.5 mm) – it is composed of loosely organized elastin fibers and matrix. The intermediate and deep layer have a thickness of about 1-2 mm. The intermediate layer is composed of elastin

fibers mainly orientated in anterior-posterior direction, whereas the deep layer primarily comprises collagen fibers. The intermediate and the deep layer together form the vocal ligament.

3. Muscle – The thyroarytenoid muscle courses laterally to the lamina propria and represents the major portion of the vocal fold with a thickness of approximately 7 to 8 mm.

The stiffness, mass or mobility of these different layers has an important role during phonation.

1.1.5. Vasculature and innervation of the larynx

The arterial supply to the larynx comes from the superior and inferior laryngeal arteries, the venous supply mirrors the arterial supply. The superior laryngeal artery is a branch of the superior thyroid artery, which arises directly from the external carotid. The superior laryngeal artery branches from the superior thyroid artery at the level of the hyoid bone. This artery then courses medially with the internal branch of the superior laryngeal nerve and enters the thyrohyoid membrane 1 cm anterior and superior to the superior tubercle.

The second major arterial supply to the larynx comes from the inferior laryngeal artery, a branch of the inferior thyroid artery. This artery enters the larynx between fibers of the inferior constrictor muscle and anastomoses with branches of the superior laryngeal artery. Lymphatic vessels of the larynx are divided into two groups: superior and inferior groups. The superior vessels end in the superior deep cervical nodes. The inferior vessels reach the inferior deep cervical nodes. Some inferior vessels may end in the pretracheal and prelaryngeal nodes. The larynx is innervated by branches of the vagus nerve on each side. Sensory innervation to the glottis and laryngeal vestibule is supplied by the internal branch of the superior laryngeal nerve. The external branch of the superior laryngeal nerve innervates the cricothyroid muscle.

Motor innervation to all other muscles of the larynx and sensory innervation to the subglottis is originated from the recurrent laryngeal nerve. While the sensory input described above is (general) visceral sensation (diffuse, poorly localized), the vocal fold also receives general somatic sensory innervation (proprioceptive and touch) by the superior laryngeal nerve.

1.2. Speech sound and its physical properties

The most complex and highly specialized laryngeal function is sound production. Voice (or vocalization) is the sound produced by humans using the lungs and the vocal folds in the larynx, or voice box. It is generated by the airflow deriving from the lungs and while passing through the intralaryngeal space it cause vocal folds vibration. The sound emitted by the larynx is modified by the cavities and tissues located above the larynx (supralaryngeal vocal tract), which act as an acoustic filter relatively independent of the source characteristics. The vocal tract causes resonances - the formants - that reinforce energy at certain frequencies depending on the shape of the vocal tract. Different vowels correspond to different configurations of the articulators that yield different resonant properties of the vocal tract, and thus induce formants at different frequencies. Speech is produced by precisely coordinated muscle actions in the head, neck, chest, and abdomen. Muscles of the thorax and abdomen are also involved in the regulation of air pressure and flow.

Since sound is a wave, we can relate the properties of sound to the properties of a wave. The basic properties of sound are: *pitch, loudness and tone*.

One important physiologic parameter which must be noted during phonation is the mucosal wave. The vibration rate of the vocal folds determines the fundamental frequency of phonation (f_0) - this is the basic frequency at which a person phonates. The fundamental frequency in adult males is around 120 Hz while in adult females it is around 200 Hz. The frequency spectrum of this sound source contains energy not only at the f_0 , but also at all

integer multiples of the f_0 (harmonics). *Pitch* is the highness or lowness of a sound based on the frequency of the sound waves. The frequency of a sound wave is what our ear understands as pitch. A higher frequency sound has a higher pitch, and a lower frequency sound has a lower pitch. The *volume* of sound is principally a result of the pressure of the air that is blown past the vocal folds. A more forceful expulsion of air from the lungs raises this pressure and creates a louder sound. The amplitude of a sound wave determines its loudness or volume. A larger amplitude means a louder sound, and a smaller amplitude means a softer sound. Talking with a normal voice approximate to a sound pressure level of 70 dB , a raised voice to 76 dB , a very loud voice to 82 dB and a shouting voice to 88 dB .

Vocal range is the measure of the breadth of pitches that a human voice can phonate - the average untrained singer may have an octave or perhaps an octave and a half (strained), by male $70\text{-}170\text{ Hz}$, by women $120\text{-}260\text{ Hz}$. Professional singers (with extensive training) have a practice singing range of about 3 octaves (unstrained). It is extremely rare, but there are singers with 4 octave practice ranges.

Voice is similar to an instrument. Changing the shape of the vocal tract, its acoustic properties are altered leading to different harmonics being boosted or dampened. Vocal tract is a moveable, very agile resonator that can assume different shapes. This means that the formants can be quickly change, which gives us a huge range of sounds. Human voice has formant regions determined by the size and shape of the nasal, oral and pharyngeal cavities that permit the production of different vowels and voiced consonants. Three formants are generally required to create a vowel sound. These regions appear as dark horizontal bands on a spectrogram or amplitude peaks on a line spectrum diagram.

1.3. Voice analysis, voice parameters

There are several subjective and objective evaluation methods for voice analysis. The auditory-perceptual evaluation of voice is one of the most traditional approaches used to analyze voice quality. The evaluation is based on the auditory impression of the evaluator when listening to altered and nonaltered voices, and then it is further compared with physiological findings by a speech therapist. The GRBAS scale, which assesses overall dysphonia grade, roughness, breathiness, asthenia, and strain, is used worldwide in several fields as a means of vocal evaluation by clinicians and researchers. Further subjective parameters are breathing during speech, tone and starting of speaking. The computerized voice analysis tools allow clinicians to study the acoustic parameters of various types of voice disorders. The most important acoustic voice quality parameters used are fundamental frequency (F0), maximum phonation time (MPT), range of voice, perturbation (jitter and shimmer) and harmonic-to-noise ratio (HNR).

1.4. Voice disorders (dysphonia)

Dysphonia is the medical term for disorders of voice: an impairment in the ability to produce sound using the vocal organs. Disturbances of the complex muscle interaction during phonation or variation of the organic constitution of the vocal folds induce a dysphonic voice. The dysphonic voice can be hoarse or excessively breathy, harsh, or rough. Dysphonia has either organic or functional causes.

1.4.1. Functional voice disorders

Functional dysphonia is the result of erroneously coordinated motions of the phonation apparatus. Functional dysphonia means poor voice quality without any obvious anatomical, neurological or other organic difficulties affecting the larynx or voice box. There are two types of functional dysphonia. Hypofunctional dysphonia - results from an incomplete

closure of the vocal cords or folds, hyperfunctional dysphonia - results from overuse of the laryngeal muscles and, occasionally, use of the false vocal folds.

1.4.2. Organic voice disorders

Organic dysphonia is characterized as a voice disorder due to morphological changes of the vocal folds. The causes for the anatomic or physiologic alteration are congenital anomalies, inflammations, edemas, papillomas, nodules, scars, nerve paralysees, endocrine diseases, side effects of drugs, different benign and malignant tumors, myopathy, diseases of the central nervous system and complications of operations.

2. Literary review

2.1. Dysphonia after vocal fold paralysis or total laryngectomy, voice rehabilitation methods

2.1.1. Causes and types of vocal cord paralysis

Recurrent laryngeal nerve (RLN) paralysis can occur due to different causes such as surgeries, accidents, viral disease and can be associated with tumors. Paralysis/immobility of the vocal cords can be unilateral or bilateral. In unilateral RLN paralysis the vocal fold mobility impairment can be partial or complete and the position of the paralyzed folds can vary from paramedian to lateral. The severity of voice and swallowing problems depends on where the nerve damage occurs. Typical symptoms include hoarseness, breathy voice, inability to speak loudly, limited pitch and loudness variations, voicing that lasts only for a very short time (around 1 second), choking or coughing while eating possible pneumonia due to food and liquid being aspirated into the lungs (the vocal cords cannot close adequately to protect the airway while swallowing). The unilateral type can be treated conservatively or surgically. Bilateral paralysis is less common but more serious as it leads to life-threatening breathing complications, causing suffocation.

2.1.2. Indications for total laryngectomy

Laryngectomy, the removal of the larynx is carried out in cases of subglottical laryngeal cancer, advanced laryngeal cancer (T3-T4), advanced hypopharyngeal tumors and sometimes in cases of severe laryngeal injury and chronic aspiration. In a total laryngectomy the entire larynx, hyoid bone and in a few cases some part of the pharynx are removed.

2.1.3. Conservative and surgical treatment in vocal cord paresis

Bilateral vocal fold paralysis is treated either by tracheostomy, arytenoidectomy or laterofixation. In case of unilateral vocal fold paralysis voice therapy with electrotherapy (corticosteroids) is normally the first treatment option. Many patients improve spontaneously enough during the first 6 months of follow-up to avoid surgical treatment. During these 6 first months after onset of paralysis, speech therapy is important to help restoring the voice. But speech therapy alone cannot rehabilitate a completely aphonic patient or reduce significantly a very breathy voice nor help in reducing level differences between the normal and the paralyzed VF. Usually, surgical treatment is postponed between 6 months and 1 year after onset of paralysis. At that time, denervation signs and poor response to speech therapy are obvious. The needs for early surgical treatment (before 6 months) are aspiration, important breathy hypophonia and ineffective cough. After voice therapy, the decision for surgery depends on the severity of the symptoms, vocal needs of the patient, position of paralyzed vocal folds, prognosis for recovery, and the cause of paresis/paralysis. Currently, static medialization techniques such as laryngeal framework surgery are performed to manage dysphonia and aspiration. Surgical procedures will try to improve the glottis closure and the glottis resistance during phonation. The procedures consist either in medialization techniques or in reinnervation procedures. In the case of bilateral damage, tracheostomy is often required to bypass the airway obstruction, but this severely impairs quality of life and may actually aggravate aspiration. Reinnervation can be performed only if the laryngeal muscles are

completely denervated. In case of partial denervation, neural sprouting is blocked by under expression of adhesion molecules by the innervated muscle fibers. The goal of these reinnervation procedures is to restore some muscle bulk, muscle tone and movement in the paralyzed vocal fold.

For the cases with no hope of recovery and with tight position of the VFs, several surgical procedures can be proposed to improve breathing : endoscopic VF lateralization, endoscopic laser cordotomy or CO2 laser arytenoidectomy. Patients have to be aware that, while in these procedures breathing improves as a consequence of the operations, voice quality will worsen. Recurrent laryngeal nerve paralysis due to different causes may be treated most effectively by reinnervation of the larynx. Successful reinnervation restores the normal phonation and secures airways. Several reinnervation techniques have been reported, including direct anastomosis, nerve grafting with nerves such as the phrenic nerve and ansa cervicalis, and the laryngeal nerve-muscle pedicle (NMP). So far the nerve anastomosis technique appears to be eligible for clinical application because it provides for regaining the bulk and tension of the vocal cord; however, the mobilisation is poor. Direct nerve anastomosis or nerve grafting technique results in nonselective reinnervation of abductor or adductor muscles and produces an uncoordinated, synkinetic movement of the vocal cords. The advantage of the NMP technique is a selective reinnervation and restitution of movement of different intrinsic laryngeal muscles.

The NMP was successfully used for reinnervation of the bilaterally paralyzed vocal cords. Transposition of NMPs from the sternohyoid muscle is capable of reinnervating the ipsilateral cricothyroid, thyroarytenoid and posterior cricoarytenoid muscles. Another NMP method is suturing a phrenic nerve-innervated omohyoid muscle flap to the paralyzed PCA muscle. The advantage of the of the NMP technique is a selective reinnervation and restitution of movement of different intrinsic laryngeal muscles. The NMP technique is recommended

especially for those patients who use their voice professionally (professional singers, actors, teachers) or have bilateral vocal cord paralysis. Tucker, Crumley, Broniatowski, Maniglis et al used different NMP for reinnervation of the thyroarytenoid muscle, cricoarytenoid muscle, and cricothyroid muscle.

Advantages of NMP reinnervation technique:

- the muscle island contains a large amount of undivided axons with functional motor endplates,
- the three-dimensional pedicle muscle in the NMP technique contains the transected axons,
- a piece of muscle in the NMP method would be a source of growth of new muscle tissue and a considerable regeneration will occur in a muscle graft (satellita-stem cells),
- pedicle muscle fibers can be a source of fibronectin and laminin, which create an excellent medium for the growth of regenerating nerve sprouts.

Previous studies show that 8 weeks after transection of RLN, the PCA muscle was fibrotized, suggesting that any attempt to reinnervate the muscle after 8 weeks of injury is unlikely to succeed. Contrary to this finding, other investigators showed that reinnervation is still possible 6-9 months after denervation.

2.1.4. Voice rehabilitation possibilities after total laryngectomy

The first known successful laryngectomy on a human was undertaken by Billroth in 1873, so laryngologists have been faced with the challenge of voice restoration. Total laryngectomy result in an extreme form of voice disorder, namely an entire loss of voice. In this case the two requirements of normal phonation - active respiratory support and adequate glottic closure with normal mucosal covering of the vocal cord - are missing. Only the vocal tract

exists, anatomic structures for articulation and resonance are usually unaltered. In our days there are three main methods of vocalizing – the tracheoesophageal voice prosthesis, the esophageal speech and the electrolarynx – after total laryngectomy. Esophageal voice is one of the convenient methods of voice rehabilitation after total laryngectomy. It is cost effective, hands-free and it provides a natural sound. Seeman was the first, who in 1922 recognized the role of the cervical esophagus as the neoglottis, and also the air reservoir in the esophagus.

Several factors determine which type of vocalization technique can be chosen, including age and physical condition of the patient, social background, the type of surgery (e.g. laryngectomy combined with glossectomy, neck dissection) or radiotherapy. From among the three, voice prosthesis is the most widely used method worldwide, but many of the patients refuse another surgery. Selection of a method should be based on input from the surgeon, speech pathologist, and patient. The decision is best made keeping in mind the patient's communicative needs, physical and mental status, and personal preference. Esophageal speech, also mentioned in the literature as esophageal voice, is a method of speech production that involves oscillation of the esophagus. Advantage of this method of communication is that it does not require the purchase of any special equipment or any newer surgical procedure, but can be difficult to learn. The sound quality of esophageal voice is often described as husky, rough and low pitched. Because of the large vibrating mucosal surfaces and the altered resonance tract, the pitch of esophageal speech is low, usually between 50 and 100 Hz. In addition, initially the alteration of voice pitch and the mode of asking questions are very difficult. In our clinic acts once a week the so-called esophageal speech-club from 2002. The goal was to introduce the patients to the adequate breathing, swallowing and vocalization technique with psychological support.

2.2. Objective

Our aim was to examine the surgical reconstruction of the vocal cords and conservative voice rehabilitation with voice therapy in patients after unilateral vocal fold paresis or after total laryngectomy.

1. We examined the NMP technique for reinnervation of the PCA muscle. Our goal was to investigate the success of reinnervation and remobilization of paralysed vocal fold with laryngoscopic, electromyographic and histological methods.
2. We analyzed the applicability of ansa cervicalis – SH pedicle for reinnervation of the abductor muscle. We assessed the rate and the direction of vocal fold motion.
3. We assessed the histological changes of the denervated PCA muscle six months after the paralysis. It can largely influence the chance for reinnervation.
4. In our clinic acts the so-called esophageal speech-club from 2002 once a week. The goal of the club is to introduce the patients to the adequate breathing, swallowing and vocalization technique with psychological support. The sound quality of esophageal voice is often described as husky, rough and low pitched. Our aim was to reach a more natural speech quality, and help the patients in producing voice of the best possible pitch, loudness, and quality in relation to the gender.
5. One of the objectives of this study were to assess the success rate of the esophageal speech in patients after total laryngectomy.
6. The success of acquiring this technique was estimated by means of a voice analyzing program (pitch, sound-holding, loudness, spectrogram), and by the intelligibility via the telephone. We compared these results with data found in the literature.

7. The surgical reconstruction methods of the pharyngeal mucous membrane can have an influence on voice quality parameters. Thus the relationship of the different suture techniques and the voice quality parameters of different neoglottis forms were analyzed.

3. Material and methods

3.1. Laryngeal reinnervation with nerve-muscle pedicle

We investigated the reinnervation and remobilization of the vocal fold after one side denervation in canine model (all studies were performed under permission of the Animal Research Committee of the University of Debrecen - 2/2000/DEMÁB).

Surgical method: The experiments were performed on 8 mongrel dogs (4 male, 4 female; 12-23 kg). Each dog was anesthetized with atropine (0.04 mg/kg, subcutaneously) as premedication and then by ketamin (10 mg/kg, intramuscularly [IM] or intraveously [IV]) and xylasine (2%, 1 mg/kg, IM or IV), allowing spontaneous respiration. After the operation the dogs received allobarbital and aminophenason injection (0.5/10 kg, IM) for painkilling. Five dogs (dogs 1-5) underwent the operation with NMP technique. In 3 dogs, the recurrent laryngeal nerve was transected at a distance of 2.5 cm from the cricothyroid joint, and a portion of 2-3 cm was resected. NMP was prepared unilaterally from the ansa cervicalis branch to the sternohyoid muscle at the point of entry of the nerve into the muscle. The NMP had been completely mobilized. The larynx was rotated 90° around its longitudinal axis. The ansa cervicalis – SH muscle pedicle was prepared and sutured to the left PCA muscle with 3 interrupted microsutures using 5.0 Prolene (monofilament, polypropylene suture from Etichon, Inc., Somerville, New Jersey, USA). The skin was closed in 2 layers with Vicryl (monofilamnet, polyglactin from Johnson and Johnson Intl., Brussels, Belgium).

Three dogs (dogs 6-8) underwent a sham operation. The right unilateral vocal fold was denervated by severing the right RLN. Both ends were electrically cauterized to diminish the

possibility of reinnervation. One year later, the 5 dogs with NMP were returned for a 2nd surgery.

Videolaryngoscopy was performed, and electromyographic data were collected. The NMP was localized under anesthesia and stimulated electrically while the vocal cords were observed by a 0° endoscope (Karl-Storz GmbH, Tuttlingen, Germany), allowing monitoring of motions directly for video recording. Control dogs underwent the same examination 6 months after denervation surgery.

Electromyography (EMG): A Disposable Concentric Needle Electrode (26G, 37 mmx0.46 mm, Medtronic, Dantec) was placed directly into the PCA and other laryngeal muscles after rotation of the larynx by 90°. The reinnervating branch of the ansa cervicalis was electrically stimulated by a nerve stimulator (Ministim apparatus, home made) with a pulse duration of 0.1-1ms (individual quadrangle impulse). Electromyographic (EMG) activity was recorded by a digital oscilloscope (Agilent Technologies, CA) with a home made amplifier (150-180x, lower filter: 20Hz, upper filter: 10kHz) and a personal computer.

Histological methods: The cricoarytenoid posterior muscles were fixed in 4% phosphate buffered paraformaldehyde solution (pH 7.4). The specimens were either embedded into paraffin and the 6-7 µm sections were used for routine haematoxylin eosin staining, or they were used for immunohistochemical reaction. In this later case the tissue blocks were immersed into 10% and 20% sucrose and sectioned on a freezing microtome at 50 µm. Freefloating sections were treated with 5% bovine serum albumin and incubated with monoclonal anti-neurofilament 200 (Sigma, diluted 1:200) for 24 h at 4°. After incubation the sections were transferred to biotinylated anti-mouse IgG (dilution 1:500) for 2h followed by incubation in avidin-biotinylated peroxidase complex (Vector) for 1h. The immunoreaction was visualized by a diaminobenzidine chromogen reaction. Sections were collected on gelatine-coated slides, dehydrated and coverslipped.

Histomorphometry: On the hematoxylin eosin specimens the outlines of the cross sections of muscle fibers were drawn with the aid of the Camera lucida attached to a Reichert microscope at the magnification of 40x. The diameters of 200 fibers were measured from both the reinnervated, denervated and intact side.

Statistical comparisons were made using one way ANOVA test.

3.2. Voice rehabilitation after total laryngectomy

Patients and surgical methods

The 158 patients were trained for esophageal speech after total laryngectomy in our department between 2003 and 2009. Sessions were held every week and about 15 patients attended each session at a given time. While voice recordings were taken from many of the patients, voice recordings and fiberoptic examinations were carried out on 20 patients. All the 20 patients were able to acquire the esophageal speech technique successfully.

Every patient, who agreed to perform all the examinations which were necessary for voice quality analysis, was enrolled in this study. The age of the patients varied from 47 to 73 (61.8 ± 5.6 years, mean \pm SD). Four beginners were included (P15, P18, P19, P20) to show the development in the quality of esophageal speech by comparing their data to well-trained patients' results. The postoperative follow-up was longer than five years for eleven patients, four years for three patients and two for six patients. Two patients had voice prosthesis originally, but they could not use it because of recurrent fungal infection and tracheoesophageal fistula. After the removal of the prosthesis and the closure of the tracheoesophageal fistula they started to learn the esophageal speech.

During the operation pharyngeal mucosal defects were closed surgically in three different manners. Smaller defects could be closed as a straight line while larger defects were closed as

a T shape. To create a larger mucosal fold in some patients, the meeting point of the horizontal and vertical suture line of T fashion closure of the hypopharynx was performed in a way to create a ball like bulge toward the lumen.

Steps of learning esophageal speech

On average, patients started the voice rehabilitation program one month after the operation or the postoperative radiotherapy. The first step in speech learning was an explanation of the anatomical changes that occurred after surgery and the basic principles of voice rehabilitation. Subsequently a successfully trained patient introduced the esophageal speech technique to the newcomers. After several training sessions, the patients could practice expressions and sentences to perform fluent speech.

Since esophageal speech is not pulmonary in origin it cannot be initially as fluent as normal speech. The patients were, therefore, trained in the “air injection” technique. It makes continuous airflow possible, and thus the speech becomes more fluent. The essence of the technique is that during the pronunciation of plosive consonants ([p], [t], [k], [b], [d], [g]) the patient can press air into the esophagus. Using this method, the main disadvantage of the esophageal speech – the usable air volume for producing voice is only 40–80 cm³ –, can be eliminated. The “air injection” technique provides faster and less erratic speech.

Finally, after the patients had acquired easy phonation, more specific, consecutive, individual exercises have begun addressing intonation, loudness, fluency and pitch modulation, articulation in order to obtain a near normal and intelligible speech.

Voice analysis

Qualitative evaluation was performed by healthy volunteers (medical students, $n = 25$), who did not know that the speakers underwent total laryngectomy. They evaluated the tone and

continuity of the patients' voice and speech. A more detailed evaluation was carried out by two physicians (phoniatrian specialists). Intonation, loudness changes, continuity, natural pauses, speech rate, intelligibility were rated by the examiners. A telephone test by the two phoniatrian specialists – speech intelligibility judgment – was also carried out. The frequency, the acoustic energy, the breathiness, the noisiness of the voice, and singing were determined by spectrographic analysis. Phonation parameters were extracted from sustained sound (Hungarian [a]) in the analysis.

A microphone (Behringer XM1800S) with a pre-amplifier connected to a personal computer (Mac mini 1.66GHzDC/512MB/60GB/COMBO/AP/BT-IEA, with a built-in speaker) was used for recording sound and storing the data. Dysphonia index, introduced by Friedrich was used for quantitative measure of voice function (auditive, acoustic and subjective scale data). This index assesses hoarseness, vocal intensity range, voice pitch range, phonation time (how long the patient can hold a sound), and communicative impairment. Voice pitch range and vocal intensity range were derived using the Speech Analyzer software (SIL International, Dallas, USA). Voice pitch range was measured in half tones, vocal intensity range in decibels, and phonation time in seconds. All five parameters were rated on a scale from 0 to 3. Hoarseness was rated by the RBH system (Roughness, Breathiness, Hoarseness), as determined by a phoniatrian. Communicative impairment parameter was determined by the patients themselves by listening to one another. The dysphonia index was calculated as the mean of these five values.

In addition, since spectrograms have a significant use in the evaluation of treatments for voice disorders they were also analyzed by the Speech Analyzer software. Acoustic recordings were made under standardized conditions, in the same quiet room with less than 30 dB background noise. A microphone was placed 20 cm from the patient's mouth for recordings. All subjects were vocalizing a sustained Hungarian [a] vowel at a loudness and pitch comfortable to them

by a conversational intensity. The fundamental frequency (F0) and the values of the first three formants (F1, F2, F3) of the sustained sound were evaluated together with the number of simple syllables (as [ba], [ma]) the patient could utter with a single breath. As a control, the spectrogram of healthy volunteers, ten females and ten males, was also recorded and analyzed.

Nasal endoscopy

Imaging of the neoglottis during speech was performed with a nasal fiberoscope consisting of a camera combined with a flexible fiber optic light guide (Storz, 11001 UD), a cold light source (Storz Laryngostostole Modelle 8010B), and a video recorder. The image sequences were archived on a compact disc. Local anesthesia – 10% lidocaine hydrochloride spray into the patients' nose – was used before the insertion of the fiberoscope.

Assessing the quality of life

A detailed questionnaire was used to evaluate the patients' own feelings about their quality of life, their communication skill, and the quality of their voice.

Statistical analysis

Voice and fiberoptic recordings were analyzed as blind data, that is, the patients could not be identified by the person conducting the analysis. The results of the spectrographic analysis were compared among the three groups of patients with different types of neoglottis forms. Pooled data are presented as mean \pm standard deviation of mean.

4. Results

4.1. Results with NMP reinnervation technique of previously denervated posterior cricoarytenoid (PCA) muscle

All five animals (reinnervated group) survived more than 1 year (12-14 months) and the control dogs survived six months.

Videolaryngoscopy: Normal vocal fold mobility was observed preoperatively bilaterally in all animals. The video records demonstrated the return of motion of the denervated PCA muscle in all five dogs. As a selective reinnervation, it restored the abductor muscle function only. As we paralysed all the laryngeal intrinsic muscles and reinnervated only the PCA muscle, thus the vocal fold was slightly atrophic. Normal, symmetric vocal cord abduction was observed during spontaneous respirations. In each dog a visibly excellent vocal cord abduction was seen during electrical nerve stimulation. The vocal cord excursions were measured and the two sides were compared. The maximal abduction movement was compared to the normal control side. Due to technical reasons we have only done data evaluation of four dogs out of the five. The vocal cord abduction movements after reinnervation were 66-74% of the control side movements.

In the denervated group the complete cross section of the recurrent laryngeal nerve resulted in a motionless fold in the paramedian position. Simultaneously, paralysis of the PCA muscle produced a subluxation of the arytenoid cartilage. Videolaryngoscopy at 6 months showed no movement of the paralysed right vocal cord during normal respiration and electrical nerve stimulation. The denervated PCA muscles looked pale and atrophic, the glottic structures were asymmetric as to mass, elasticity and resistance.

Electromyography: Postoperatively, the EMG activity in the reinnervated muscles was elicited electrically. In all five reinnervated animals a selective muscular activity was seen to electrical stimulation. The EMG data of these 5 animals showed evidence of reinnervation of

the PCA muscle with polyphasic potentials. We found physiologically appropriate firing of motor units in the PCA muscle to electrical stimulation, whereas one would observe voluntary motor unit action potentials, fibrillation potentials, positive sharp waves or isolated complex repetitive discharges in denervated muscles. Motor unit action potentials from the other laryngeal muscles in the reinnervated side couldn't be observed.

EMG records of neuromuscular activity under laryngoscopic control, indicated that reinnervation of PCA muscle is successfully obtained. This was further supported by the histochemical examination of the PCA muscle too, showing a reinnervation pattern.

Histologic examination: All dogs operated on with the NMP technique showed no histological differences between intact and reinnervated sides of the PCA muscles.

There were little differences in the appearance of the denervated and intact PCA muscles after 6-month denervation. The mass of the denervated muscles was less than those of innervated controls without fibrosis in each animal. The average values of the smallest diameters of muscle fibers were calculated. Muscle fiber size might normally be varied considerably in regions within these muscles. Because of this variability, the standard deviation for each group was also examined. Significant difference between the reinnervated and the intact muscle is not observed. Immunohistochemical reactions for neurofilament revealed nerve fibers and motor endplates in both sides. PCA muscle atrophy, on the basis of measurements of individual muscle fiber diameters, could not be demonstrated after six months denervation, in this study. Nerve fibers, neurofilaments or motor endplates couldn't be detected in the denervated PCA muscle by immunohistochemical examination against neurofilament antibody.

4.2. Results of voice rehabilitation with esophagus speech

Subjective evaluation of voice quality

Untrained listeners did not recognize that the patients were using esophageal speech. The examiners believed that the patients had flu, as the reason of the rough voice. Moreover, intonation, continuity, speech rate, and intelligibility were found good and in many cases close to normal by phoniatrian specialists. Only two patients (P15 and P18), who just started the esophageal speech training, performed poorly during these tests.

Objective, qualitative voice quality evaluation

The average dysphonia index for the 20 patients was 1.67 ± 0.38 . Patients who were judged as good speakers in the subjective voice quality evaluation obtained a score from 1.4 to 1.8. Two patients had a score of 2. Two others, a woman and a man, who just started the training in esophageal speech obtained scores above 2.

Next the voice of both male and female patients was analyzed and compared to those of healthy individuals. In our hands F0 for healthy volunteers was 220.5 ± 12.0 Hz ($n = 10$) in case of females and 115.2 ± 8.3 Hz ($n = 10$) in case of males.

All F0 values of both female and male patients were higher than those published previously (50 Hz) in the literature. F0 of female patients was around 140 Hz (139.5 ± 45.9 Hz, $n = 4$), while that of the male patients was around 90 Hz (89.7 ± 13.1 Hz, $n = 16$). Note, however, that the woman with high dysphonia index (2.4; P14) had a low F0 (72 Hz). The F1, F2, F3 values were also in the normal frequency range (data not shown).

Fmin (the lowest frequency) and Fmax (the highest frequency) frequencies were determined by having the patients ask questions and/or sing. Former observations generally gave a frequency range of a quint (diatonic fifth) that could be obtained by esophageal speech. For our patients it was extended to an octave by introducing singing exercises. The number of

continuously uttered syllables ([ba]-[ba]-[ba], [ma]-[ma]-[ma]) and the numbers (starting from one) uttered with one air intake were also counted. Since some patients could acquire the air injection technique, air intake was undetectable.

Regular, periodic vertical striations indicative of synchronous vocal cord movements with no irregularity of the vibratory pattern were observed in the spectrogram of healthy volunteers (both males and females). Pitch characteristics of the voice could be observed in the closeness of the vertical striations on the spectrogram. The top half of the spectrogram is relatively clear, indicating that no excessive breathiness exists. At the beginning of the esophageal speech training the patient produces inconsistent formants and noisy, breathy spectrograms. Technically adequate esophageal speech shows vertical striations on the spectrogram with little noise. Relative sound intensity is denoted by a continuous curve with small intensity changes. The fundamental frequency of the healthy male voice was 100 Hz in line with data published earlier, which did not differ significantly from that of the patient's F0 using esophageal speech (92 Hz). Similarly, negligible difference could be detected between the fundamental frequencies of a healthy females' voice and of the esophageal speech of a trained female patient. Furthermore, no significant difference between the spectrograms of normal and advanced speakers' could be recorded when singing, except for the slightly lower frequencies in esophageal speech.

Role of the neoglottis in esophageal speech

The shape of the neoglottis was examined by nasal fiberoptic. Three different neoglottis forms were distinguished. In some patients (patients P1–P12), a small 'button' evolved, which could vibrate and act as a valve instead of the vocal cords. In another group (patients P13, P14, P15, and P16) two mucosal 'battens' were visible in opposing positions, which could vibrate like the former vocal cords. In the third group (patients P17, P18, P19, and P20) one

pronounced mucosal 'lip' was visible. From the above three groups the dysphonia index was the smallest for those with mucosal 'button' (1.51 ± 0.16) while the largest for those with mucosal 'lip' (1.95 ± 0.41).

Assessing the quality of life by a questionnaire

Patients evaluated their own communication ability, quality of their voice and life. Most of them considered their communication as continuous or close to continuous which is tolerably or well understood by their listeners. These patients had little problem to reintegrate into society. Only a few, mostly the beginners, had problems with communication and were only speaking in front of family members or close acquaintances.

5. Discussion

Voice is one of the most important function of the human body, as it permits individuals to speak their thoughts and opinions and communicate with others. Dysphonia has either organic or functional causes due to impairment of any one of the vocal organs. Treatment of dysphonia can be conservative or surgical. The aim is to reach a normal or a near normal voice quality with adequate pitch and intensity.

We examined opportunity of surgical voice rehabilitation in case of vocal fold paresis in dogs, and voice rehabilitation of patients after total laryngectomy with esophageal speech..

Direct nerve anastomosis or nerve grafting technique results in nonselective reinnervation of abductor or adductor muscles and produces in an uncoordinated, synkinetic movement of the vocal cords. The advantage of the NMP technique is a selective reinnervation and restitution of movement of different intrinsic laryngeal muscles. Remobilisation of the paralysed vocal fold is also possible without having the distal stump of the RLN, since it is often difficult to identify and locate it due to heavy scarring. Reinnervation can restore a normal voice with adequate muscle tone, vocal fold compliance and arytenoid stabilization.

It is an open surgical technique which requires a normal cricoarytenoid joint mobility. This technique may be considered either soon after loss of function or in selected cases after longstanding bilateral RLN or vagus nerve paralysis when spontaneous recovery is hopeless. Laryngeal NMP technique transfers intact functioning motor endplates from the SH muscle located at the end of the ansa cervicalis in our case. The reinnervated muscle takes the characteristics of its supplying nerve consequently, the selection of the donor nerve should account on the muscle fibre composition of the muscle to be reinnervated. . Branches of the ansa cervicalis to sternohyoid or sternothyroid muscle are suitable for PCA muscle reinnervation since these nerves are more active by inspiration and contain enough motor axons. Besides the selection of the appropriate NMP the accurate timing of the surgery is very important. In the clinical practice there is usually a considerable delay between injury to the recurrent laryngeal nerve (RLN) and presentation for treatment. A reduction in muscle fiber size as early as 2 weeks after denervation, followed by muscle atrophy after 8 weeks is reported. Duration of paralysis prior to surgery doesn't influence functional recovery as successful reinnervation was achieved 50 and 22 years after the paralysis.

Successful reinnervation seems to be dependent on the vacated neuromuscular junctions following motor endplates degeneration.

Our experimental results indicate that utilizing of an ansa cervicalis-sternohyoid muscle pedicle for selective reinnervation of the PCA muscle results in a remobilization in denervated vocal cord after one year. The reinnervated vocal cord movement was 66-74% of the control side movement. This difference between the two sides derived from the fact, that only abduction movement could be seen in the operated side due to selective PCA muscle reinnervation. On the control side not only abduction but adduction contributes to the vocal cord movement. Reinnervation is confirmed by immunohistochemical labelling of nerve fibers and endplates in reinnervated muscles, whereas a spontaneous reinnervation was

excluded in the control denervated muscles by the absence of immunoreaction. In a former study the behavior of laryngeal muscles following denervation was analysed, and demonstrated that after transection of the RLN the PCA muscle undergoes a rapid degeneration process over the first 2 to 3 weeks. Four weeks after RLN transection, however, the PCA muscle recovers its almost normal architecture. A significant difference in fiber diameter couldn't be noticed by us after six months. Our result indicate that application of the nerve-muscle pedicle in case of paralysis of the PCA muscle is clinically reasonable. This technique would eliminate the need of arytenoidectomy improving the quality of life and would reduce the development of synkinesis and paradoxical vocal cord movement.

Loss of voice after total laryngectomy leads to major changes in lifestyle. Esophageal speech is the most natural, unaided method to speak in the absence of the vocal cords. This method makes further surgical interventions avoidable and after learning how to improve tone and loudness, voice quality could become close to normal as compared to other solutions. From yearly operated 45 patients approximately 40 try to learn this speech technique and $\frac{3}{4}$ of them succeed. Furthermore, esophageal speech is a good alternative method for those patients, who had prosthesis insertion before, but the prosthesis had to be removed or they could not use the device. As a general finding, after the first 2–3 training sessions, one can predict who will be able to acquire this speech technique and who will not. It takes on average three months of learning and practicing to be able to speak fluently enough to be eligible for daily talk/conversations.

With the use of the 'air injection' technique, the initially rough and broken speech becomes more fluent and less exhausting. Besides teaching the correct speech technique, the aim was to improve the quality of the acquired voice. This means the widening of the frequency range, the improvement of the amplitude and intonation of voice. It was hard to reach the formal quality of speech, because of the thicker vibrating mucosal 'vocal cords' which develop and

cannot vibrate as synchronously and gently as the thin, specially architected vocal cords. Proper and careful surgical reconstruction of the pharyngeal mucous membrane has a beneficial influence on voice quality parameters. Best results were acquired from the group of patients with 'button' shape neoglottis form which was the result of the special suture technique in the wound closure. With individualized voice therapy could we reach more fluent, clear and understandable speech, which can the patient apply in everyday life.

New conclusions

1. The video records demonstrated the return of mobility of the PCA muscle reinnervated by the NMP. EMG data showed evoked polyphasic potentials which is also an evidence for the reinnervation of the PCA muscle. With immunohistochemical reaction (antineurofilament antibody+biotin) we could demonstrate neurofilaments and motor endplates in both sides in all five animals.
2. Our experimental results indicate that utilizing of an ansa cervicalis-sternohyoid muscle pedicle for selective reinnervation of the PCA muscle results in a remobilization in denervated vocal cord after one year. Ansa cervicalis-SH nerve-muscle pedicle is suitable for rehabilitation the movement of the larynx during inspiration and can secure the adequate position of the cricoarytenoid joint.
3. There wasn't significant difference in the appearance of the denervated and intact PCA muscles after 6-month denervation. PCA muscle atrophy, on the basis of measurements of individual muscle fiber diameters, could not be demonstrated after six months denervation, so successful reinnervation could be achieved months later after the paralysis.
4. It takes on average three months of learning and practicing to be able to speak fluently enough to be eligible for daily talk/conversations. With individualized voice therapy, with special excersises we can reach more fluent, clear and understandable speech.

5. From yearly operated 45 patients approximately 40 try to learn this speech technique and $\frac{3}{4}$ of them succeed. As a general finding, after the first 2–3 training sessions, one can predict who will be able to acquire this speech technique and who will not.

6. Qualitative and quantitative voice evaluation was performed. All F0 values of both female and male patients were higher than those published previously (50 Hz) in the literature. F0 of female patients was around 140 Hz (139.5 ± 45.9 Hz, $n = 4$), while that of the male patients was around 90 Hz (89.7 ± 13.1 Hz, $n = 16$). Former observations generally gave a frequency range of a quint (diatonic fifth) that could be obtained by esophageal speech. For our patients it was extended to an octave by introducing singing exercises.

7. Nasal fiberoptic examination revealed three different types of neoglottis forms – a small mucosal button, two mucosal battens, and a mucosal lip. Voice quality of the esophageal speech of the patients with the mucosal button was found to be the closest to normal by subjective and objective acoustical evaluation. These findings emphasize the importance of the proper wound closure technique which can facilitate the development of a special button shape neoglottis form and help to acquire esophageal speech with the best quality parameters shortly after total laryngectomy.

List of publications related to the dissertation



UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY



Registry number: DEENK/132/2015.PL
Subject: Ph.D. List of Publications

Candidate: Andrea Tóth
Neptun ID: W42KZD
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Tóth, A.**, Csernoch, L., Sziklai, I., Szűcs, A.: The role of the different neoglottis forms in the development of esophageal voice.
Acta Physiol. Hung. 101 (3), 291-300, 2014.
DOI: <http://dx.doi.org/10.1556/APhysiol.101.2014.004>
IF:0.747 (2013)
2. **Tóth, A.**, Németh, T., Szűcs, A., Szöllösi, Z., Sziklai, I.: Retropharyngeal superficial angiomyxoma.
J Laryngol. Otol. 124 (9), 1017-1020, 2010.
DOI: <http://dx.doi.org/10.1017/S002221510999274X>
IF:0.697
3. **Tóth, A.**, Szűcs, A., Harasztosi, C., Matesz, K., Pucsok, K., Mikó, I., Sziklai, I.: Intrinsic laryngeal muscle reinnervation with nerve-muscle pedicle.
Otolaryngol. Head Neck Surg. 132 (5), 701-706, 2005.
DOI: <http://dx.doi.org/10.1016/j.otohns.2005.01.045>
IF:1.218

List of other publications

4. Szűcs A., Batta J.T., Fekete A., Makó L., **Tóth A.**, Sziklai I., Jókay I.: Homloküreg gyulladás talaján kialakult szubperioszteális tályog kezelése két eset kapcsán.
Otorhinol. Hung. 58 (4), 169-171, 2012.



Address: 1 Egyetem tér, Debrecen 4032, Hungary Postal address: Pf. 39. Debrecen 4010, Hungary
Tel.: +36 52 410 443 Fax: +36 52 512 900/63847 E-mail: publikaciok@lib.unideb.hu, □ Web: www.lib.unideb.hu

List of other publications



UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY



5. Szűcs A., Fekete A., **Tóth A.**, Sziklai I., Jókay I.: Orrszárny-rekonstrukció porcátültetés nyeles lebény felhasználásával.
Magyar Traumatol. Ortop. Kézseb. Plaszt. Seb. 55 (4), 279-284, 2012.
6. Szűcs A., Batta T., Szappanos H., **Tóth A.**, Szigeti G., Panyi G., Csernoch L., Sziklai I.: Zajterhelés hatása a külső szőrsejt kalcium homeosztázisára és sejtfalmerevségére.
Fül-Orr-Gégegyógy. 56 (2), 81-88, 2010.
7. Szűcs A., Batta T., Somodi S., **Tóth A.**, Szigeti G., Csernoch L., Panyi G., Sziklai I.: A Deiters sejtek kifelé irányuló K⁺ áramának karakterizálása.
Fül-Orr-Gégegyógy. 55 (1), 28-33, 2009.
8. Szűcs A., Batta T., Somodi S., **Tóth A.**, Szigeti G., Csernoch L., Panyi G., Sziklai I.: Az eltérő morfológiai típusú Deiters sejtek elektrofiziológiai különbségei és szerepük a belső fülben.
Fül-Orr-Gégegyógy. 55 (2), 63-67, 2009.
9. **Tóth A.**, Szűcs A., Harasztosi C., Pucskó K., Mikó I., Csernoch L., Sziklai I.: Gége izom reinnerváció ideg-izom lebénnyel állatkísérletes modellen.
Fül-Orr-Gégegyógy. 55 (2), 68-72, 2009.
10. Szűcs A., Batta T., Szappanos H., **Tóth A.**, Szigeti G., Panyi G., Csernoch L., Sziklai I.: Az ATP hatása a külső szőrsejtek kalcium homeosztázisára.
Fül-Orr-Gégegyógy. 55 (3), 130-135, 2009.
11. Szűcs A., Somodi S., Batta J.T., **Tóth A.**, Szigeti G., Csernoch L., Panyi G., Sziklai I.: Differential expression of potassium currents in Deiters cells of the guinea pig cochlea.
Pflugers Arch. 452 (3), 332-341, 2006.
DOI: <http://dx.doi.org/10.1007/s00424-005-0038-1>
IF:4.807
12. Szűcs A., Szappanos H., Batta J.T., **Tóth A.**, Szigeti G., Panyi G., Csernoch L., Sziklai I.: Changes in purinoceptor distribution and intracellular calcium levels following noise exposure in the outer hair cells of the guinea pig.
J. Membr. Biol. 213 (3), 135-141, 2006.
DOI: <http://dx.doi.org/10.1007/s00232-006-0045-y>
IF:2.112
13. Bráth E., Németh N., Papp L., Békési L., Varga S., **Tóth A.**, Mikó I., Furka I.: Mikrosebészeti oktatás a kutatás és a klinikai gyakorlat szolgálatában.
Magyar Seb. 58 (2), 111-115, 2005.

Address: 1 Egyetem tér, Debrecen 4032, Hungary Postal address: Pf. 39. Debrecen 4010, Hungary
Tel.: +36 52 410 443 Fax: +36 52 512 900/63847 E-mail: publikaciok@lib.unideb.hu, Web: www.lib.unideb.hu



14. Szűcs, A., Szappanos, H., **Tóth, A.**, Farkas, Z., Panyi, G., Csernoch, L., Sziklai, I.: Differential expression of purinergic receptor subtypes in the outer hair cells of the guinea pig. *Hear. Res.* 196 (1-2), 2-7, 2004.
DOI: <http://dx.doi.org/10.1016/j.heares.2004.04.008>
IF:1.578
15. Szűcs A., **Tóth A.**, Kollár J., Szöllösi L., Sziklai I.: Izolált inveterált papilloma a sinus sphenoidalisban. *Fül-Orr-Gégegyógy.* 48, 43-45, 2002.
16. **Tóth A.**, Galuska L., Kovács J., Kollár J., Sziklai I.: A különböző képzőanyagok szerepe a fej-nyak tájéki vasculáris malformációk preoperatív diagnosztikájában. *Fül-Orr-Gégegyógy.* 48, 64-67, 2002.

Total IF of journals (all publications): 11,159

Total IF of journals (publications related to the dissertation): 2,662

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

25 June, 2015



Posters and lectures related to the dissertation

Tóth A., Szűcs A., Harasztosi Cs., Matesz K., Pucsok K., Mikó I., Sziklai I. Laryngeal reinnervation in animal experiments. 37th Congress of the European Society for Surgical Research, 2002, Szeged

Tóth A., Szűcs A., Harasztosi Cs., Matesz K., Furka I., Mikó I., Sziklai I. Gége reinnerváció ideg-izom lebennyel. Fül-, Orr-, Gégeklinika Éves Ülés, 2001, Debrecen

Tóth A., Szűcs A., Harasztosi Cs., Matesz K., Furka I., Mikó I., Sziklai I. Gége reinnerváció állatkísérletes modellen. Magyar Fül-, Orr-, Gégeorvosok Egyesületének 37. Nemzeti Kongresszusa, 2002, Siófok

Tóth A., Tóth Á., Szűcs A., Sziklai I. Gastrooesophagealis reflux a dysphonia hátterében. Foniátria Kongresszus 2003, Nyíregyháza

Tóth A., Dr. Szűcs A., Dr. Jókai I. A nyelvőcsőbeszéd oktatással kapcsolatos tapasztalatok. Magyar Fül-, Orr-, Gégeorvosok Egyesületének 42. Nemzeti Kongresszusa 2012, Pécs