Aus der Klinik für Hals-Nasen-Ohrenheilkunde

der Medizinischen Fakultät Charité-Universitätsmedizin Berlin

DISSERTATION

CO_2-laserstapedotomy: Analysis of used energy and its influence on bone-conduction hearing levels

zur Erlangung des akademischen Grades

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CONTENTS

ABBREVIATIONS

AAO-HNS	American Academy of Otolaryngology - Head and Neck Surgery
ABG	Air Bone Gap
CO_2	Carbon dioxide
YAG	Yttrium aluminium garnet
KTP	Potassium titanyl phosphate (KTiOPO4)
dB	Decibels
sp	Super pulsed mode
cw	Continuous wave
STAMP	Stapedotomy minus prosthesis
SFS	Small fenestra stapedotomy
Hz	Hertz
kHz	Kilohertz
BC	Bone conduction
AC	Air conduction
mJ	Millijoule
mm	Millimeter
μm	Micrometer
PTA	Pure Tone Average
Rev	Revision Surgery
SD	Standard deviation

SUMMARY

CO_2-lasers are used by many otosurgeons to perforate the stapes footplate for the treatment of otosclerosis using the "multiple-shots-technique" or the "one-shot technique". The latter is successful in about 68% of all cases. However, in 78% of revision cases with a reossified footplate, multiple-shots are necessary.

The aim of study was to analyze the interdependence of the CO_2-laser used energy and post-operative bone conduction hearing thresholds to assess a possible laser-induced inner-ear damage.

A prospective analysis of the influence of cumulative laser energy on post-operative bone conduction hearing thresholds was performed with data from 391 primary cases that were divided into 261 single-shot and 130 multiple-shots CO_2-laserstapedotomies and 107 in revision cases which were divided into operations with simple change of prosthesis (n=35), single-shot perforation of a neomembrane (n=33) and multiple-shots for the perforation of reossified footplates (n=39).

Neither after primary nor in revision surgery significantly different changes in bone conduction hearing levels were found between the different groups that required an energy of 0, <1, 1-2 or >2 J after long-term follow up. In both tested time periods (short-term: 1~3 weeks; long-term: 1.5~6 months) the majority of patients, showed a lower bone conduction threshold which further improved over time.

A comparison of study results after stapedotomy available from the literature showed that our data were among the best results.

In conclusion, the application of the "one-shot technique" with energy ≤ 1 J impaired inner ear function less than the application of the "multiple-shots technique" during the short-term observation period. After long-term follow-up the influence of the total laser energy used during CO_2-laser stapedotomy performed as primary or revision surgery using the one-shot or multiple-shots technique had no energy-related damaging effect on bone conduction hearing levels. Therefore CO_2-laser stapedotomy performed according to the described technique can be considered as a safe procedure.

ZUSAMMENFASSUNG

CO_2-Laser werden von vielen Ohrchirurgen im Rahmen der Behandlung der Otosklerose zur Perforation der Stapesfußplatte mit der Mehrschuß- oder der Einschusstechnik verwendet. Letzteres gelingt in etwas 68% aller Fälle. Jedoch in 78% der Revisionen mit einer verknöcherten Fußplatte sind mehrere Schüsse notwendig.

Ziel der Studie war es, den Zusammenhang zwischen der verwendeten CO_2-Laserenergie und der postoperativen Knochenleitungshörschwelle zu analysieren, um einen möglichen laserinduzierten Innenohrschaden zu bestimmen.

Eine prospektive Analyse des Einflusses der kumulativen Laserenergie auf die postoperative Knochenleitungshörschwelle wurde mit Daten von 391 primären Fällen, die in Fälle mit 261 Einzelschuss- und 130 Fälle mit Mehrfachschuss-CO_2-Laserstapedotomien (n=130) geteilt werden konnten und in Revisionsfälle, die in Operationen mit einfachem Prothesenwechsel (n=35), Perforationen einer Neomembran durch einen Einzelschuss (n=33) und multiplen Schüssen für die Perforation von reossifizierten Fußplatten (n=39) aufgeteilt werden konnten, durchgeführt. In der Langzeitbeobachtung wurden weder nach Primär- noch nach Revisionsoperationen signifikante Änderungen der Knochenleitungsschwellen in den Gruppen, bei denen eine Energie von 0, <1, 1-2 oder >2 J aufgewandt wurde beobachtet.

In beiden untersuchten Zeiträumen (Kurzzeit: 1~3 Wochen, Langzeit: 1.5~6 Monate) wies die Mehrheit der untersuchten Patienten niedrigere postoperative Knochenleitungsschwellen auf, die sich im zeitlichen Verlauf noch verbesserten. Ein Vergleich von Studienergebnisse nach Stapedotomie, die aus der Literatur verfügbar waren zeigte, dass die von uns erhobenen Daten unter den besten waren.

Schlussfolgernd beeinträchtigte während der Kurzzeitnachbeobachtungsperiode die Anwendung der Einzelschusstechnik im Rahmen der Primäroperation mit einer Energie von ≤ 1 J die Innenohrfunktion weniger als die Anwendung Mehrschusstechnik.

In der Langzeitnachbeobachtungsperiode zeigte die totale verwendete CO_2-Laserenergie keinen energieabhängigen schädigenden Effekt auf die Knochenleitungsschwelle unabhängig davon, ob eine Primär- oder Revisionsoperation mit der Einzelschuss- oder Mehrfachschusstechnik durchgeführt wurde. Demzufolge kann die CO_2-Laserstapedotomie, mit der beschriebenen Technik, als eine für die Innenohrfunktion sichere Operation angesehen werden.

1. INTRODUCTION

1.1 Otosclerosis

Otosclerosis is a disease of the otic capsule in which new vascular spongy bone formation causes ankylosis or fixation of the footplate of the stapes and results in progressive conductive hearing loss. In 1704, Valsalva first described the concept of “stapes fixation” for the first time. Then in 1857, Toynbee was the first to link stapes fixation to hearing loss. In 1890, Katz first found microscopic evidence of otosclerosis, and in 1893 Politzer described the clinical entity of “otosclerosis” for the first time.

Conductive and/ or sensorineural hearing loss is caused by otosclerosis. This process usually starts around the oval window, causing fixation of the stapes, resulting in conductive hearing loss (**Figure 1**). In advanced cases, the bone surrounding the cochlea can also be affected causing sensorineural hearing loss (SNHL). Typically, otosclerosis will begin in one ear but will eventually affect both ears with a variable course.

Pathogeneses of otosclerosis are considered as multiple factors, including both genetic and environmental ones. It causes 5-9% of all the cases with sensorineural hearing loss and 18-22% of the cases with conductive hearing loss[1-3]. In 70-80% of the patients, this disease is bilateral and it is progressive over time[4]. The onset of hearing loss typically occurs between the ages of 15-45 years, with a higher prevalence in women than men: (2:1)[2,5,6]. Pregnancy seems to accelerate the process[1]. Clinical features are characterized by vertigo, tinnitus and hearing loss, or a combination of these symptoms.

Otosclerosis has some typical clinical signs, including normal tympanic membrane, progressive conductive hearing loss, no obvious middle ear inflammation and the Schwartz sign characterized by a faint pink tinge reflecting the vascularity of the cochlear promontory. According to these features, it’s not very hard to diagnose otosclerosis.

However, some cases with mixed or sensorineural hearing loss will be unclear if there is no imaging information. Therefore, imaging, especially high-resolution CT should be considered and performed to find the subtle bone when the hearing loss is sensorineural or mixed.

Otosclerosis visualized by CT can be graded using the grading system suggested by Symons and Fanning[7].

Grade 1: solely fenestral

Grade 2: patchy localized cochlear disease (with or without fenestral involvement) to either the basal cochlear turn (grade 2A), or the middle/ apical turns (grade 2B), or both the basal turn and the middle/ apical turns (grade 2C)

Grade 3: diffuse confluent cochlear involvement (with or without fenestral involvement)

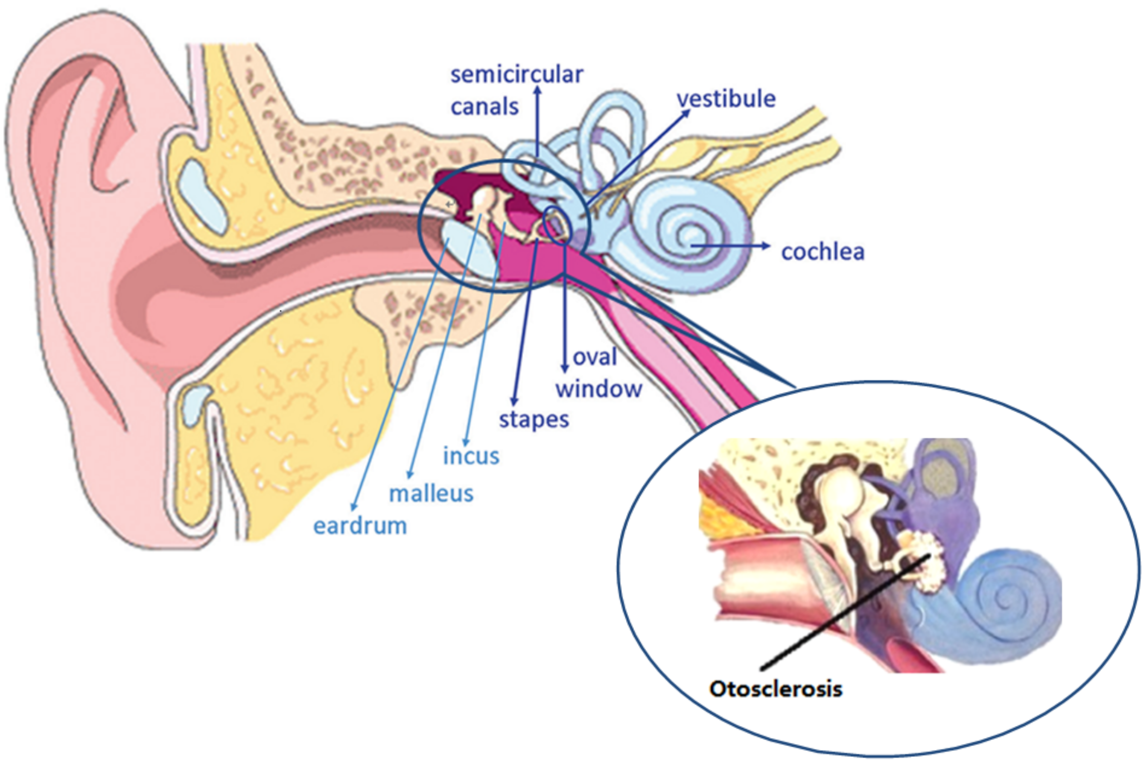

Figure 1: Anatomy of the ear and otosclerosis (modified from the picture found at http://www.hearingloss.ca/articles/otosclerosis/)

1.2 Stapes surgery

The two principal therapeutic approaches for the treatment of otosclerosis are stapedectomy and stapedotomy. The first description of stapedectomy was given by Shea, who performed it in May 1956 on a 54-year-old housewife who could no longer hear even with a hearing aid[8]. The main procedure of this surgical treatment is replacing the stapes bone with a micro-prosthesis after removing the stapes bone.

In the early 1960s, just a few years after the first successful stapedectomy, Marquet[9] and Shea et al.[10] separately introduced the concept of the “small-hole stapedotomy”. In this technique, a small hole, called fenestration, is made in the stapes footplate and the whole footplate is left in its oval niche. The prosthesis can be placed in this perforation, which is connected to the long process of the incus, regaining the mobility of the ossicular chain (**Figure 2**).

In recent decades, these two operations were used in different situations by different otorhinolaryngology surgeons. Compared to the stapedectomy, stapedotomy seems to be much safer and more effective in most situations. The advantages are as follows:

1. Same or better hearing improvement and reduction of the air-bone hearing gap (especially at higher sound frequencies).
2. Less prone to complications. In particular, the stapedotomy procedure greatly reduced the chance of a perilymph fistula (leakage of cochlear fluid).
3. Better prosthesis stability with significantly improved air conduction.

It still should be noted that stapedotomy is not applicable for relatively rare cases such as those involving sclerosis of the entire ossicular chain or congenital malformation of the stapes.

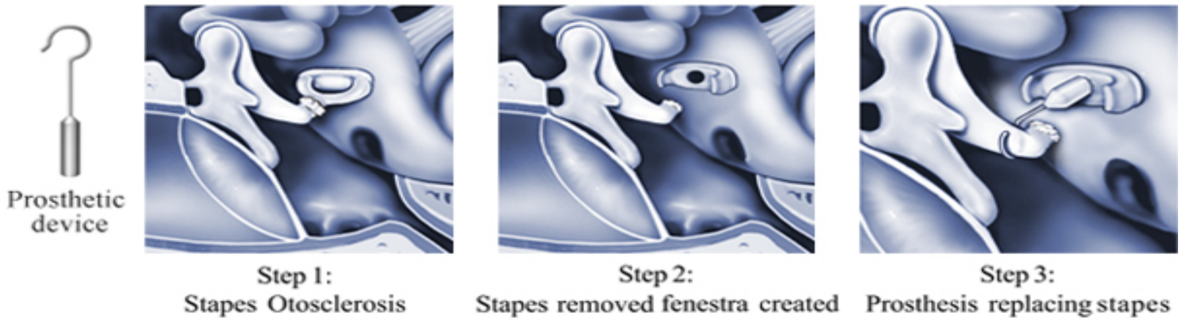

Figure 2: The stapes (left) otosclerosis and procedure of stapedotomy (modified from the picture found at http://osgonline.org/patient-information.html)

1.3 Laser in stapes surgery

The principle of stapedotomy has been applied over time as a classic surgical method, but the tools have been improved with the availability of lasers in the clinical setting.

Perkins performed the first argon laser stapedotomy in the 1978.[11] Since then, many different kinds of laser systems (CO_2 [carbon dioxide], KTP [potassium titanyl phosphate], Er:Yag laser [yttrium-aluminium oxide dotted with Erbium3^+ ions]) have been put to the test in otologic surgery. Compared to the conventional technique, laser stapedotomy was believed potentially to cause less ear damage and less complications, especially in the area of minimal invasive or keyhole surgery (**figure 3**).[12-15]

There are many different lasers available nowadays, sharing three unique features:

- Generation of monochromatic light, i.e. light of a specific wavelength. Thus, by selecting the specific wavelength, different effects can be achieved depending on the wavelength-specific absorption of tissue being treated. This allows for either high selectivity or homogeneous distribution in large volumes. The emitted wavelength is dependent of the medium of the laser, which can be in the following states: gas, liquid, solid or plasma.
- Generation of a highly collimated output beam, i.e. parallel without divergence. This beam can be transported with miniature mirrors and applied to a spot without direct contact. Further, it enables efficient coupling into small optical fibers providing transportation e.g. through flexible endoscopes anywhere in the body. However such fibers are not available for far infrared lasers (CO_2 or Er:YAG lasers).
- Generation of high energy capable of heating tissues to hundreds of degrees in a very short amount of time, generating explosions and high mechanical stresses.[16,17]

The goal of laser stapedotomy is to create a precise opening in the cochlea without damaging the inner ear or the remaining middle ear structures. Strict adherence to the recommended laser energy parameters will minimize the risk of thermal and/ or acoustic damage to middle and inner ear structures[18-20].

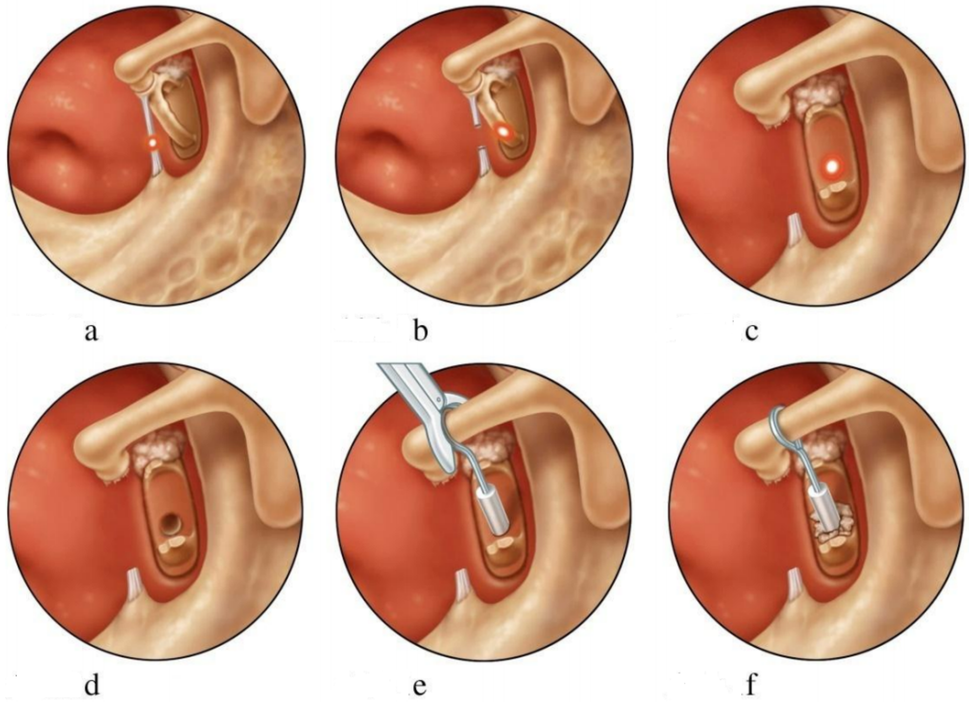

Figure 3a-f: Schematic representation of the laser stapedotomy and supply of a stapes piston (Jovanovic S. in Hildmann H., Sudhoff H. 2006)

a: Vaporization of tendon of stapedius muscle with 2-3 single pulses at low power of 2 W

b: Separation of the rear stirrup leg with 4-8 pulses with a power of 6 W and a pulse duration of 50 ms

c: Perforation of the stapes footplate with an "one-shot" CO_2 laser application using SurgiTouch® Scanners (20 W, pulse duration 40 ms, scanners diameter 0.6 mm)

d: Achievement of a round, smooth configured perforation of 0.4-0.7 mm in diameter

e: Insertion of a platinum-Teflon piston into the perforation and attachment of the prosthesis on the long incus leg

f: Sealing the oval niche with connective tissue or a blood clot

1.4 CO_2 Laser in stapes surgery

Otosclerosis is caused by abnormal growth of bone resulting in a reduced mobility or fixation of the stapes footplate in the oval window causing hearing loss. Stapes surgery was introduced as early as the end of the nineteenth century and many improvements to the technique were proposed[21]. Compared to the conventional surgical technique, the advantages of laser stapedotomy have already been confirmed by numerous authors. Lots of Clinical studies have demonstrated that laser stapedotomy, including the CO_2-laserstapedotomy, has a lower risk of causing inner ear damage and vertigo than conventional operations[5,13-15,20,22-24].

Meanwhile, the CO_2 laser is used in microsurgery more often than other lasers. This may be because of the greater absorption of the CO_2 laser beam at the footplate, resulting in higher effectiveness, better reproducibility and lower thermal side effects[25,26]. Additionally, it can be coupled to a surgical microscope and aimed with a micromanipulator. Moreover, the CO_2 laser is much more reliable and easier to operate when used in pulsed mode[27,28].

Consequently, CO_2-laser stapedotomy has become an established and widely used technique to perforate the stapes footplate during primary surgery of otosclerosis. Ideally the footplate is perforated with a single shot if the laser is combined with a laser-scanner[29]. However, with this technique, one or more laser applications are still required in 32% of primary operations to achieve a perforation in the footplate of adequate diameter for successful insertion of a stapes-prosthesis[29]. Similarly, according to a study conducted by our group, multiple applications were necessary in 78% of revision cases (**figure 4**)[30].

During each application, laser energy reaches the inner ear. Especially in cases of a partially perforated footplate, the perilymphatic fluid is directly irradiated and there is a potential risk of inner ear damage. Some surgeons therefore enlarge a small perforation with conventional instruments such as a drill, to avoid further irradiation of the perilymphatic fluid. However, this additional procedure exposes the patients to additional risks that potentially include a floating footplate or dislocation of bone fragments into the labyrinth.

Therefore, it is very important and necessary to determine the safe energy range for CO_2 laser applications, not only to achieve the goal of footplate perforation, but also to guarantee the safety of inner ear function and to reduce complications. The safe and effective parameters for CO_2 laser stapedotomy (type 40c with the Lumenis Acuspot 712 micromanipulator, **figure 5**) have been determined based on data obtained in the petrous bone, in the cochlear model, and in animals experiments[31,32]. Earlier experimental data indicated that the energy used for a single application should not exceed 1 J[32]. Additionally, the effect of CO_2 laser energy was experimentally tested on the cochlea of guinea pigs. Essentially, it was found that if the laser was set to continuous wave mode, even single applications at a pulse duration of 50 ms, an approximately four times higher power density (60,000 W/cm^2) than necessary for a stapedotomy (energies up to 1 J) and 20-fold applications of effective parameters for a footplate perforation (power density 16,000 W/cm2; energy 0.2 J) did not cause CAP changes[32].

Based on these data, further investigation in humans on the effects of laser energy on inner ear function and additional information on a safe and effective energy range for clinical application would be desirable. For this purpose, we collected prospectively data of different clinical settings to evaluate the interdependence between different CO_2 laser energies and post-operative bone conduction thresholds.

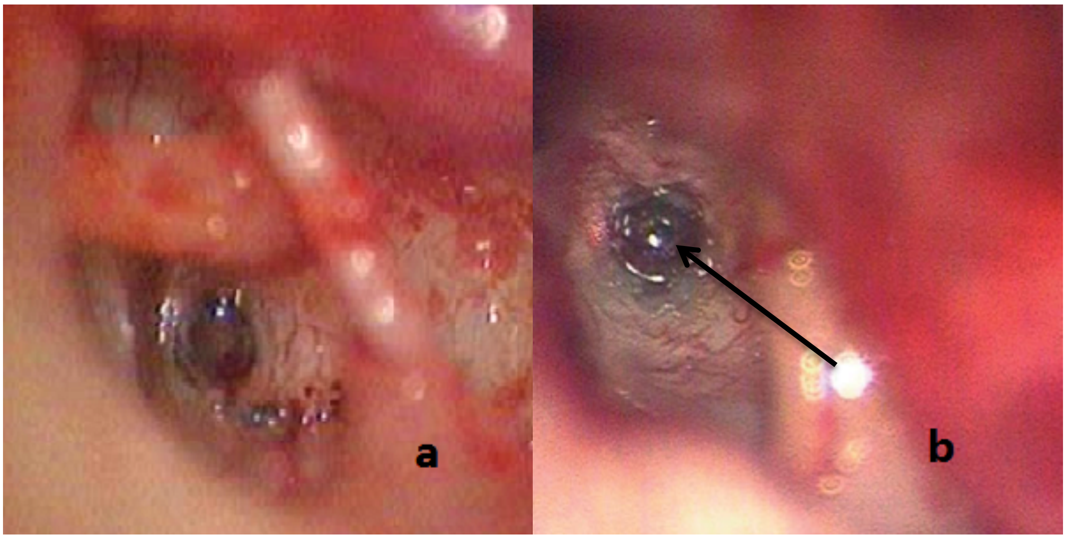

Figure 4a: Primary CO_2 laser stapedotomy, one-shot perforation of the stapes footplate approx. 0.6 mm in diameter (power: 20 W, pulse duration: 0.04 s)[33]

Figure 4b: Revision CO_2 laser stapedotomy of the connective tissue neomembrane with one laser application using the SurgiTouch scanner.[30] In the center of the perforation, perilymph is visible (black arrow).

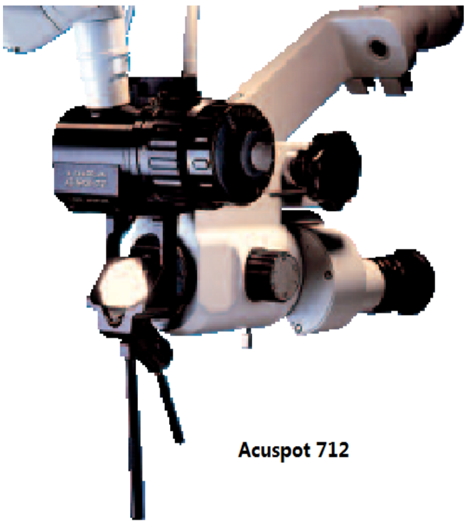

Figure 5: Lumenis Acuspot 712 micromanipulator (modified from the picture found at http://www.clarionmedical.com/wpcontent/uploads/2013/07/Luemnis_AcuSpot_Datasheet_Rev0812.pdf)

2. HYPOTHESIS AND AIMS

Hypothesis: The cumulative laser energy as required in CO_2-laser stapedotomy may have an energy-dependent effect on inner ear function. The effect may in part be temporary or permanent.

Aims

1. To compare the postoperative bone-conduction hearing thresholds with the preoperative bone-conduction hearing thresholds in clinical settings using different laser energies.
2. To compare the postoperative changes in bone-conduction hearing thresholds over time: short-term (1~3 weeks) and long-term (1.5~6 months).
3. To compare the postoperative changes in bone-conduction hearing thresholds between "one-shot" and "multiple-shots technique" in primary surgery.
4. To compare the postoperative changes of bone-conduction hearing thresholds between this and other studies available from the scientific literature.

3. MATERIALS AND METHODS

3.1 Patients

This study was approved by the ethics board of Charité -Universitätsmedizin Berlin, Berlin, Germany. The presented data are based on a total of 515 patients that received a CO_2 laser stapedotomy at our institution between the years 1999 and 2010. From this group, 446 patients received a primary stapedotomy while one or several revisions were necessary in 134 patients. About 80% of all operations were carried out by only one surgeon (S. J.) and all other interventions were supervised by him.

Detailed information about the distinct patient groups is listed in **table 1**.

Table 1: Overview of patient data and conducted operations

	All operations	Primary surgery	Revision surgery*
Number of surgeries	654	501	153
Right / Left ear	363 / 291	278 / 223	85 / 68
Number of patients	515	446	134
Age (mean and range)	45.6 (17 - 76) years	45.0 (20 - 76) years	47.4 (17 - 73) years
One / Both ears	458 / 57	391 / 55	130 / 4
Female / Male	321 / 194	277 / 169	86 / 48

(*Several revisions: 2 revisions n = 16, 3 revisions n = 2)

3.2 Surgical procedure

The following surgical procedures are based on experience and experimental data obtained in our department. The procedures have been described previously[29,30]. However, to provide the reader with a comprehensive picture, they are described again in the following.

3.2.1 Primary surgery

CO_2 laser stapedotomy was performed under general anaesthesia. Application of the CO_2 laser was preceded by some test shots on a wooden spatula to ensure aiming beam alignment with the CO_2 beam.

The stapedius tendon was first vaporized with 2 to 3 single pulses at a low power of 1 W (power density, 4000 W/cm^2) and a pulse duration of 0.05 seconds. With a completely fixed footplate, the incudostapedial joint was conventionally severed. Laser-assisted joint severance was performed in cases with only a partially fixed stapes. Vaporizing the head of the stapes with 8 to 14 single pulses of the laser beam at 6 W (power density, 24,000 W/cm^2) and a pulse duration of 0.05 seconds separated the incudostapedial joint. Because the CO_2 laser beam does not strike perpendicularly to the joint, the separation may sometimes not be complete. Any remaining tissue was severed with a 90° hook.

Vaporization of the posterior crus required 4 to 8 laser shots with a power of 6 W set at a pulse duration of 0.05 seconds. When severing the joint and posterior crus with this relatively high laser power, care must be taken that middle ear structures in the path of the beam and beyond the target (footplate, facial nerve canal, etc.) were not accidentally irradiated and damaged. Adequate protection was provided in such cases by filling the tympanic cavity with saline or covering of the non-target structures with a saline-soaked gelatin sponge (Gelita or Spongostan).

The anterior crus of the stapes was usually not directly accessible to the laser beam. It was therefore fractured with a hook. However, in some cases, the anterior crus was partially visible. Protecting the non-target areas, the crus was then vaporized with a CO_2 laser beam, using the same parameters as for the posterior crus. Should vaporization be incomplete, the residual bone was severed with a minimum of force, using a cold instrument. Footplate mobilization or even partial or total footplate extraction was avoided. The stapes supra-structure was removed with micro-forceps. The energy setting for footplate perforation with the one-shot application technique using the SurgiTouch™ scanner depended on the thickness of the footplate and the irradiation diameter of the scanning figure applied. Typically, a power setting of 20 W to 22 W (power density, 80,000-88,000 W/cm^2) with an exposure time of 0.03 to 0.05 seconds per pulse created a round perforation 0.4-0.7 mm in diameter.

In those cases in which the desired perforation diameter was not achieved with one-shot technique, the perforation was enlarged by additional laser applications either with or without the scanner system, depending on the perforation diameter already achieved. While a second shot with the scanner was applied at the same site for perforations up to 0.3 mm in diameter, enlargement to 0.4 mm or more was achieved with a few slightly overlapping multiple-shot applications of laser energy without the SurgiTouch™ scanner. With a beam diameter of 180 μ m, the power was set at 6 W (power density, 24,000 W/cm^2) and the pulse duration at 0.05 seconds.

Care was taken to ensure that the vestibulum was filled with perilymph for adequate protection of inner ear structures and prevention of damage by direct laser irradiation.

A 0.4-mm platinum Teflon piston was then inserted into the perforation and attached to the incus neck. In most cases, the prosthesis diameter was 0.1 to 0.2 mm smaller than the perforation diameter. Finally, the oval niche was sealed with connective tissue or a blood clot.

3.2.2 Revision surgery

Exposure of middle ear structures

The tympanomeatal flap was elevated, and the middle ear structures were inspected. In some instances, for proper exposure of the posterior half of the oval niche, the posterior wall of the outer ear canal was further reduced and the chorda tympani carefully mobilized by removing scar tissue. The malleus, incus, and the prosthesis were carefully examined and probed with a needle to assess their integrity and mobility. If soft-tissue adhesions covering the middle ear structures were present, they were vaporized with the CO_2 laser.

Exposure of the prosthesis and the oval niche

Exposure of the prosthesis was also achieved by laser vaporization of the adherent connective tissue. A power setting of 1 to 2 W and a pulse duration of 0.05 seconds was used to clean the oval window niche of connective and granulation tissue to visualize the margins of the footplate and to check if the piston was in the stapedotomy opening. To minimize mechanical trauma to the inner ear, only the laser was used, adhering to a non-contact technique. Next, the prosthesis was detached from the incus with a 2-mm-long 90-degree hook and was subsequently extracted. If, in the case of remaining parts of the stapes superstructure (e.g., posterior crus), bone needed to be vaporized, the laser was set to 6 W. In all patients, connective tissue surrounding the prosthesis and covering the oval niche was removed by using the laser. Direct irradiation of the prosthesis was minimized to reduce heat conduction to the inner ear. If the prosthesis contained Teflon components, direct laser irradiation of the prosthesis with 1 to 2 W was regarded as safe. However, higher laser powers larger than 6 W were avoided because it has been observed that Teflon prostheses can be damaged by heat.

Reperforation of the stapes footplate

For reperforation of the stapes footplate, three scenarios occurred:

1. A connective-tissue neomembrane covered the stapes footplate. A 4- to 8 W laser application with a pulse duration of 0.03 to 0.05 seconds, depending on the scanner diameter (0.4, 0.5, or 0.6mm), was applied using the one-shot technique. If no scanner was used, several shots with a power of 1 to 2 W (pulse duration, 0.05seconds) were applied.

2. In the case of a bony footplate or if the pre-existing perforation was too small (with or without membranous covering), a 20- to 22 W laser application with a pulse duration of 0.03 to 0.05 seconds was applied with the scanner system also using the one-shot technique.

3. If the existing perforation was only slightly too small, an enlargement of the perforation was achieved with multiple shots using a small focused laser beam (180 μm). Laser power of 1 to 2 W (membranous covering of the footplate) or 6 to 8 W (bony footplate) without using the SurgiTouch scanner was applied in a slightly overlapping pattern (pulse duration of 0.05 seconds) to achieve an appropriate perforation. Independent of the technique used, the tissue at the posterior part of the oval window was uniformly vaporized to create a perforation of 0.5 mm (piston diameter, 0.4 mm) or 0.7 mm (piston diameter, 0.6 mm) in diameter. After the laser procedure, vestibular perilymph was visible through the opening in the footplate (figure 4b). An irradiation of the empty vestibulum was avoided.

The length of the replaced prosthesis was determined by measuring the distance from the lower surface of the incus to the vestibule and by adding 0.2 mm. The most frequently used size was 4.5 to 4.75 mm. The length of the prosthesis was chosen to extend 0.1 to 0.2 mm into the perforation to prevent migration. A platinum-teflon or titanium prosthesis was inserted into the perforation and attached to the long process of the incus if the incus was intact. Finally, the oval window niche was sealed with connective tissue, and the tympanomeatal flap was replaced.

3.3 Laser settings

A CO_2 laser (type 40c, Lumenis Ltd., Yokneam, Israel) equipped with the SurgiTouch scanner and Acuspot 712 micromanipulator (Lumenis) was used. For all operations, the laser was set to continuous wave mode at a working distance of 250 mm with a focused beam diameter of 180 µm. The scanner system enables the irradiation and perforation of the area of the footplate adequately sized for subsequent placement of the prosthesis. Diameters of 0.5 mm to 0.7 mm are selectable.

Detailed descriptions of the laser parameters used for the surgery have been previously described[29,30]. **Table 2** shows a summary of the effective laser parameters typically used for a perforation of the footplate in primary and revision surgery.

Because the transmission of CO_2 laser irradiation via the hinged mirror arm and micromanipulator results in power losses varying from 20% to 30%, depending on the system used, the power indicated on the laser device is higher than the effective power that is actually applied to the tissue.

Table 2: Laser parameters for a footplate perforation in primary and revision surgery

	With scanner system (Beam diameter 0.5–0,7 mm)	Without scanner system (Beam diameter 0.18 mm)
Primary surgery	20-22 W / 0.03 - 0.05 s	6 W / 0.05s
Revision surgery Bony reossification of the stapes footplate	20-22 W / 0.03 - 0.05 s	6 W / 0.05s
Revision surgery Connective tissue neo-membrane	4-8 W / 0.03 - 0.05 s	1 – 2 W / 0.05s

3.4 Audiometric examination

A pure-tone hearing test was performed in all patients one day before the operation. To assess the inner ear function after surgery and to differentiate between short and long-term postoperative effects, patients were examined 1 – 3 weeks and 1.5 – 6 months after the operation.

Air and bone conduction thresholds were determined for frequencies of 0.5, 1, 2, 3, and 4 kHz and four pure-tone averages (PTA) at frequencies of 0.5, 1, 2, and 3 kHz were calculated according to AAO-HNS guidelines.

For this study, only the bone conduction thresholds were used.

3.5 Data collection and analysis

Patient data together with specific information about the laser energy used were entered into a database together with pre- and post-operative hearing results. The alterations of bone conduction thresholds were calculated as $PTA_{post} - PTA_{pre}$.

To investigate a possible influence of laser energies, operation techniques or pathologies frequently encountered in revision stapedotomies on the inner ear function, the audiometric data were divided into several subgroups.

Subgroups according to applied total laser energies at the oval niche:

a) no laser application, energy 0 J

b) energy of less than 1 J

c) energy between 1 and 2 J

d) energy of more than 2 J.

Furthermore, primary surgery was broken down into operations with successful "one-shot" technique, which means that only one laser application was used to create a sufficiently large perforation, and all other operations: where several applications with or without scanner system were used ("multiple-shots technique"). Revision surgery was subdivided according to the structure covering the oval niche which consisted either of a bony reossification of the footplate or a neomembrane.

3.6 Statistical Testing

Statistical evaluation of the alteration of audiometric data included pre- versus post-operative results and data from the short- versus long-term postoperative period. Analyses were performed by using Wilcoxons' signed rank test for paired differences. For the comparison between two independent subgroups, the Mann-Whitney U test was used. The Kruskal-Wallis Test was used for the analysis with several independent groups. A significant difference was assumed for $P < 0.05$.

The distributions of the hearing results are presented as box-and-whiskers plots including minimum and maximum, 10-90% percentiles, 25-75% quartiles, median and mean marked with a star. In the tables, differences of preoperative minus postoperative pure-tone-average values were calculated (positive values indicate a better postoperative hearing threshold).

4. RESULTS

4.1 Energy applied for footplate perforation

The distribution of the number of applied laser applications and the resulting total energies required for an adequate perforation size of 0.5 to 0.6 mm are shown in **table 3** and **figure 6** for primary and revisions operations.

Table 3: Number of patients in relation to the number of applied laser applications required for a footplate perforation with a diameter of 0.5 to 0.6 mm in primary and revision surgery.

Laser applications	Primary surgeries	Revision surgeries
None	- - -	50 (33%)
One application with the scanner-equipped laser	330 (66%)	19 (12%)
Two applications with the scanner-equipped laser	54 (11%)	4 (3%)
Application with the scanner equipped laser and multiple applications without scanner	117 (23%)	53 (34%)
Multiple laser applications without scanner	- - -	27 (18%)

Primary surgeries: In 66% of the operations only one laser application was necessary for a sufficiently large perforation (**Table 3**). The median of the energy used was 0.8 J (min: 0.5 J, max: 1.4 J). Two applications were used in 11% of the cases with a median of total energy of 1.7 J (min: 1.2 J, max: 3.2 J). In the remaining cases (23%), the scanner application resulted in an insufficiently large perforation and further applications with a small focused laser beam (without the scanner system) were necessary to enlarge the perforation to an adequate size. This procedure resulted in higher cumulative laser energies and the median was 2.3 J (minimum 1.0 J, maximum: 9.6 J).

The distribution of the laser energies used in all primary operations is shown in **figure 6A**.

Revision surgeries: In revision surgeries different scenarios occurred:

a) No laser application at the footplate was necessary in 33% (**Table 3**). In 74% cases of this group, no replacement of the existing prosthesis was performed and in all other cases the prosthesis was changed without laser applications at the oval niche.

b) In the remaining cases (67%) the existing prosthesis was removed and the footplate was re-perforated with the CO_2 in laser combination with the SurgiTouch scanner. In 12% of all cases, a sufficient perforation was achieved with a single application, and in 3% with two shots. In most operations (52%), more laser applications with or without scanner were necessary (maximum of 44 in a case of obliterative otosclerosis).

Besides the number of laser applications, the single pulse energy used depending on the structure covering the oval niche is more important for the total energy (**figure 6B**). Therefore, the total energy used depended on two factors:

A) In cases of a bony reobliterated footplate, the total energy scattered extremely (Min: 0.7 J, Max: 22.4 J) depending on the individual intra-operative situation (**figure 7A**). The median was 3.3 J.

B) On the other hand, if a connective tissue neomembrane covered the oval niche, only a single pulse with low energies was used (**table 3**) resulting in lower total energies (**figure 7B**). The median was 1.1 J (Min: 0.3 J, Max: 3.4 J).

The distribution of the number of applications differed only marginally between these two groups.

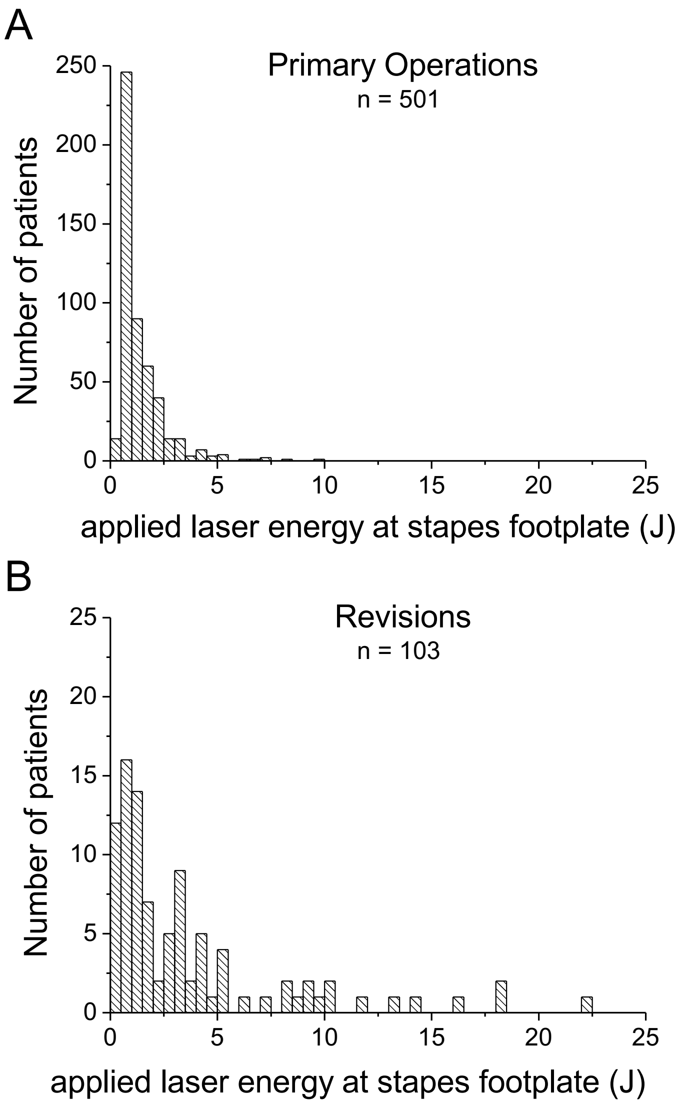

Figure 6: Distribution of cumulative energy used in 501 primary (A) and 103 revision surgeries (B) to achieve a perforation with a diameter of 0.5 to 0.6 mm. The cumulative laser energy is a result of one or several laser applications. The energy of a single application was always below 1.2 J.

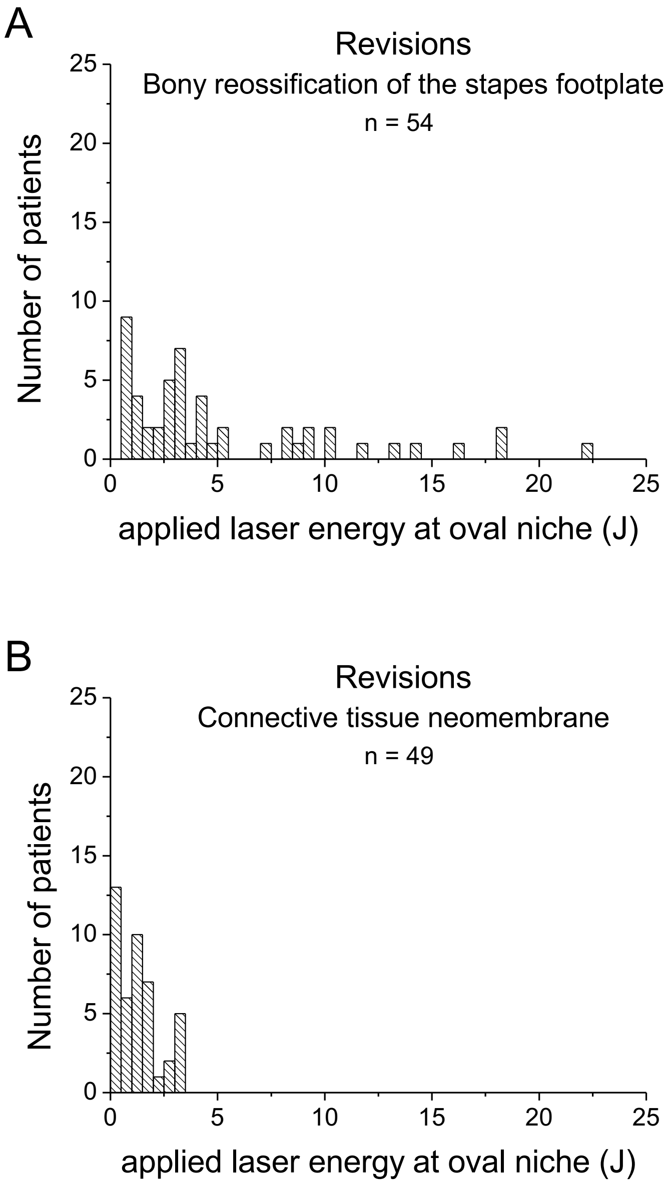

Figure 7: Distribution of cumulative energy used in revision surgeries (A) to achieve a perforation in a reossified footplate (n=54) and (B) to perforate a neomembrane (n=49) with a diameter of 0.5 to 0.6 mm.

4.2 Alteration of post-operative bone conduction hearing thresholds

4.2.1 Audiograms available for data analysis

For our analysis, from all 654 operations, 498 (76%) cases the audiograms of hearing results, were available in both postoperative periods examined (short-term: 1 – 3 weeks and long-term: 1.5 – 6 months).

For detailed analysis, the groups were subdivided according to the laser energy applied at the stapes footplate. **Table 4** and **table 5** show the number of available data presented in the next paragraphs.

Table 4: Available audiograms of primary surgeries: laser energies applied at the footplate in relation to the number of laser applications.

	Number of surgeries	0 J	≤ 1 J	1 – 2 J	≥ 2 J
Primary surgeries	391 (78%)	- - -	220	104	67
One-shot technique	261	- - -	220	41	0
Multiple-shots technique	130	- - -	0	63	67

Table 5: Available audiograms of revision surgeries: laser energies applied at the footplate in relation to the number of laser applications.

	Number of surgeries	0 J	≤ 1 J	1 – 2 J	≥ 2 J
Revision surgeries	107 (70%)	35	22	18	32
Neomembrane	33	- - -	15	13	5
Bony reossification	39	- - -	7	5	27

4.2.2 Primary Operations

■Short-term post-operative hearing results (1 to 3 weeks)

The mean postoperative bone conduction was determined between 1 and 3 weeks after surgery. Taking all patients together (n=391) and for the subgroups with cumulative laser energies of less than 2 J applied at the footplate, hearing results were slightly better than preoperatively, while the average of the group where more than 2 J were applied was equal to preoperative results (**figure 8**). The mean value for all patients was 0.8 ± 7.5 dB (mean $\pm$ SD) which was an improvement over the preoperative value indicating a statistically significant improvement ($P=0.021$). However, as shown in **figure 8**, some variation for individual results occurred.

Accordingly, a significant improvement in postoperative bone conduction was only found for the subgroup where laser energies lower than 1 J ($P=0.005$) were applied. For the subgroup “1 to 2 J” and “higher than 2 J”, no significant differences ($P=0.6$ and $P=0.9$) were found.

For the short-term postoperative hearing results (1 to 3 weeks), **table 6** shows the alteration of the postoperative bone conduction hearing threshold. Differences of preoperative minus postoperative Pure-Tone-Average values were calculated (positive values indicate a better postoperative hearing threshold).

■Long-term post-operative hearing results (1.5 to 6 months)

The mean postoperative bone conduction was determined between 1.5 and 6 month after surgery in all patients (n=391) and for the subgroups with different cumulative laser energies applied at the footplate (≤1 J, 1-2 J and >2 J), hearing results were about 5 dB better than pre-operatively (**figure 8**). However, as shown in **figure 8,** some variation for individual results occurred.

Accordingly, a significant improvement in postoperative bone conduction was found for all patients and the subgroups independent of the applied laser energy. This means the distribution of these laser energy-dependent subgroups did not differ significantly with regard to inner ear function in the long-term observation period.

The mean value for all patients was 4.7 ± 5.8 dB and improved over the pre-operative value, indicating a statistically highly significant improvement ($P<0.001$). However, the worst result for one patient was an increased hearing threshold of 11 dB.

Compared to the short-term hearing results (0.8 ± 7.5 dB), there was significant improvement for the long-term post-operative hearing results ($P<0.001$).

Furthermore, since no difference between the different subgroups according to the laser energies used became apparent, the conclusion can be drawn that there was no negative influence of the applied laser energy on the bone conduction thresholds following primary surgery.

For the long-term postoperative hearing results (1.5 to 6 months), **table 7** shows the alteration of postoperative bone conduction hearing thresholds.

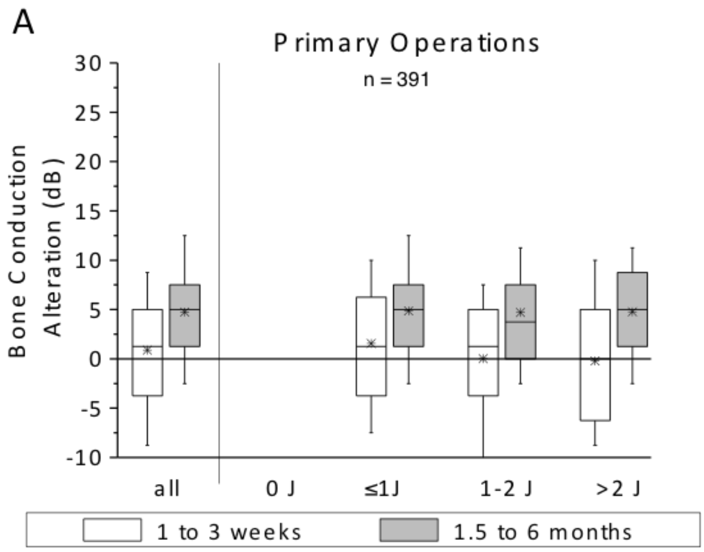

Figure 8: Alteration of short-term (1-3 weeks; empty bars) and long-term (1.5-6 month; filled bars) postoperative bone conduction hearing thresholds compared to preoperative results after primary stapedotomy for all patients (left) and in relation to the laser energies applied at the footplate (right).

To obtain the alteration in bone conduction, preoperative hearing thresholds were subtracted from postoperative Pure-Tone-Average values. Accordingly, positive values indicate a better postoperative hearing threshold. The boxes show the median and 25% to 75% quartile, the whiskers represent the 10% to 90% range and the stars represent the mean value.

Table 6: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing thresholds after a primary stapedotomy dependent on laser energies applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	-8.75	- - -	-7.5	-11.25	-8.75
25%	-3.75	- - -	-3.75	-3.75	-6.25
Median	1.25	- - -	1.25	1.25	0
75%	5	- - -	6.25	5	5
90%	8.75	- - -	10	7.5	10
Mean	0.78	- - -	1.57	0.03	-0.21
Std Dev	7.48	- - -	7.43	7.05	8.06
P value	0.021	- - -	0.005	0.6	0.9

Table 7: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing threshold after primary stapedotomy dependent on laser energies applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	-2.5	- - -	-2.5	-2.5	-2.5
25%	1.25	- - -	1.25	0.625	1.25
Median	5	- - -	5	3.75	5
75%	7.5	- - -	7.5	7.5	8.75
90%	12.5	- - -	12.5	11.25	11.25
Mean	4.70	- - -	4.86	4.69	4.77
Std Dev	5.75	- - -	5.95	5.64	5.47
P value	<0.001	- - -	<0.001	<0.001	<0.001

Bone conduction hearing results 1 to 3 weeks after the “one-shot” versus “multiple-shot technique”

Next, the patients were divided into two different groups according to the surgical technique used (“one-shot” or “multiple-shot technique” defined as either one or several application according to the one-shot technique with or without single applications without scanner as necessary to achieve an adequate perforation”).

A comparison of the alteration 1 to 3 weeks after surgery in bone conduction is shown in **figure 9**. The data are presented for all 391 subjects and the distinct subgroups (0 J, ≤1 J, 1-2 J and >2 J) according to the total applied laser energy. The one-shot technique was divided into two subgroups with applied energies of ≤1 J, 1-2 J and the multiple-shots technique was divided into subgroups with cumulative laser energies 1-2 J and >2 J. Only the subgroup with cumulative laser energies less than 1 J showed a significant improvement in postoperative bone conduction (mean: 1.7 dB, P=0.005). For the subgroups “1 to 2 J” and “higher than 2 J”, no significant improvements were found.

Table 8 and **table 9** show the detailed data and analysis of these two groups. A significant change was found only in the subgroup of ≤1 J, which was only achieved by the “one-shot” technique (P = 0.005). The other subgroups in either “one-shot” technique or multiple-shots technique showed no change compared to the preoperative hearing result.

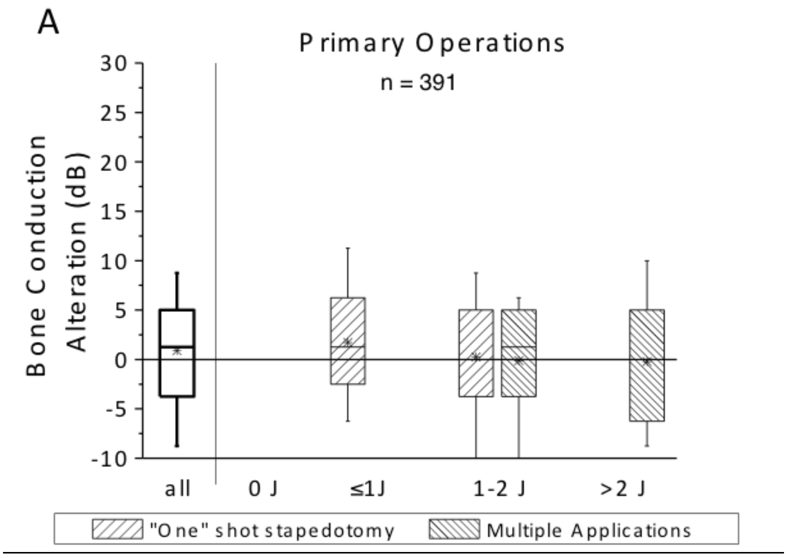

Figure 9: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing thresholds for both "one-shot" and multiple-shots technique groups in primary operations dependent on laser energies applied at the footplate and number of laser applications.

Table 8: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing thresholds for "one-shot" technique group in primary operations dependent on laser energies applied at the footplate.

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-8.75	-10	- - -
25%	- - -	- - -	-2.5	-3.75	- - -
Median	- - -	- - -	1.25	0	- - -
75%	- - -	- - -	6.25	5	- - -
90%	- - -	- - -	11.25	8.75	- - -
Mean	- - -	- - -	1.72	0.26	- - -
Std Dev	- - -	- - -	7.37	7.11	- - -
P value	- - -	- - -	0.005	>0.05	

Table 9: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing threshold for multiple-shots technique group in primary operations dependent on laser energies applied at the footplate.

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	- - -	-11.25	-8.75
25%	- - -	- - -	- - -	-3.75	-6.25
Median	- - -	- - -	- - -	1.25	0
75%	- - -	- - -	- - -	5	5
90%	- - -	- - -	- - -	6.25	10
Mean	- - -	- - -	- - -	-0.13	-0.21
Std Dev	- - -	- - -	- - -	7.07	8.06
P value	- - -	- - -	- - -	>0.05	>0.05

Bone conduction hearing results 1.5 to 6 month after "one-shot" versus "multiple-shots technique"

As described in the previous paragraph, the patients were also divided into two different groups according to the surgical technique used ("one-shot" and "multiple-shots technique").

A comparison of the alteration 1.5 to 6 month after surgery in bone conduction is shown in **figure 10**. The data are presented for all 391 subjects and the distinct subgroups (0 J: 0 subjects, ≤1 J: 221 subjects, 1-2 J: 104 subjects and >2 J: 72 subjects) according to the total amount of laser energy applied. The one-shot technique was used in two subgroups with applied energies of ≤1 J: 221 subjects and 1-2 J: 41 subjects, while the multiple-shots technique was used with cumulative laser energies 1-2 J: 63 subjects and >2 J: 72 subjects. The postoperative improvement of bone conduction was statistically highly significant in all subgroups (mean: 4.7 to 4.9 dB, all: $p < 0.001$). Furthermore, a statistical test of these three categories of laser energies used in relation the long-term period showed no significant difference (Kruskal-Wallis test: P= 0.7).

Table 10 and **table 11** show the detailed data and analysis of these two groups: the improvements of bone conduction in all subgroups were significantly better than the preoperational hearing result.

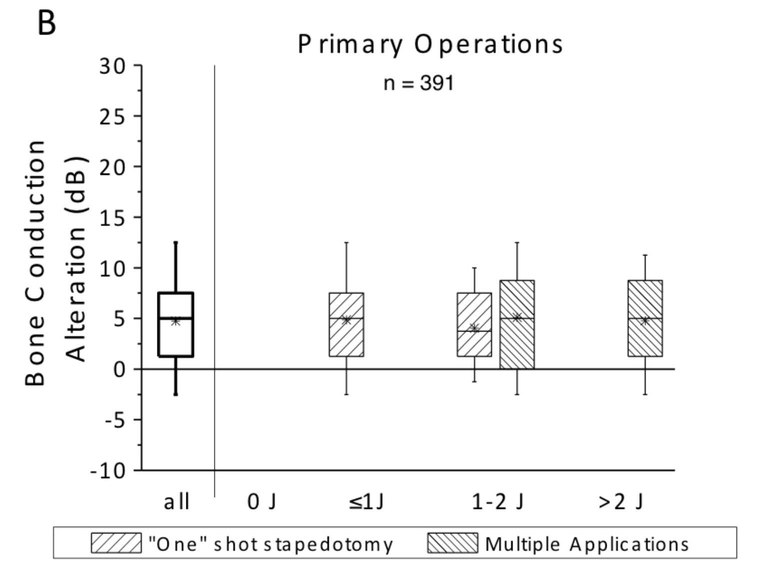

Figure 10: Alteration of long-term (1.5 to 6 months) postoperative bone conduction hearing threshold for both "one-shot" and multiple-shot technique groups in primary operations dependent on laser energies applied at the footplate and number of laser applications.

Table 10: Alteration of long-term (1.5 to 6 months) postoperative bone conduction hearing thresholds for the "one-shot" technique group for primary stapedotomy dependent on the amount of laser energy applied at the footplate.

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-2.5	-1.25	- - -
25%	- - -	- - -	1.25	1.25	- - -
Median	- - -	- - -	5	3.75	- - -
75%	- - -	- - -	7.5	7.5	- - -
90%	- - -	- - -	12.5	10	- - -
Mean	- - -	- - -	4.86	4.05	- - -
Std Dev	- - -	- - -	5.95	4.45	- - -
P value	- - -	- - -	<0.05	<0.05	- - -

Table 11: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing threshold for the multiple-shots technique group in primary operations dependent on the amount of laser energy applied at the footplate.

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	- - -	-2.5	-2.5
25%	- - -	- - -	- - -	0	1.25
Median	- - -	- - -	- - -	5	5
75%	- - -	- - -	- - -	8.75	8.75
90%	- - -	- - -	- - -	12.5	11.25
Mean	- - -	- - -	- - -	5.10	4.77
Std Dev	- - -	- - -	- - -	6.30	5.47
P value	- - -	- - -	- - -	<0.05	<0.05

4.2.3 Revision Operations

To investigate the influence on bone conduction of all surgical steps including the change of prosthesis during the procedure *except* the use of the laser, revision laser stapedotomies with only a change of prosthesis and no laser application were also included in the analysis.

a) Short-term post-operative hearing results (1 to 5 weeks)

The mean postoperative bone conduction was determined between 1 and 3 weeks after surgery for all patients (n=107; **figure 11**). All patients taken together showed an improvement in bone conduction of 3.44±8.02 dB (mean±SD). This indicated a better postoperative test result in the short-term period for the majority of revision patients compared to the preoperative result. No statistical significance was found between groups with different energy levels. However, the error bars indicate that cases with a loss in postoperative bone conduction thresholds tended to be in the subgroups with laser applications, which further increased with the total amount of laser energy applied. In turn, the groups of patients without laser application and just a change of prosthesis included only a few patients with a maximum loss of bone conduction less than 3 dB.

The analysis according to the applied laser energies led to different results, which were comparable to those found after primary surgery. In the short term period, the subgroups "< 1 J" and "1 – 2 J" showed no significant improvement as compared to preoperative hearing thresholds (mean: 2.0 dB and 3.2 dB; p = 0.3 and p = 0.1). However, surprisingly in the subgroup with the highest level of used laser energy (≥ 2 J), a significant alteration was measured (mean: 4.0 dB; p = 0.02).

For the short-term post-operative hearing results (1 to 3 weeks), **table 12** shows the alteration of postoperative bone conduction hearing thresholds after revision surgery. Differences of preoperative minus postoperative Pure-Tone-Average values were calculated (positive values indicate a better postoperative hearing threshold).

[redacted]Long-term post-operative hearing results (1.5 to 6 months)

The mean postoperative bone conduction was determined between 1.5 and 6 months after surgery for all patients (n=107; **figure 11**). All patients taken together showed a mean improvement in bone conduction of 4.8±7.2 dB (mean±SD), which was above the preoperative value and statistically significant. After revision stapedotomy, compared to the short-term hearing results

(3.4 ± 8.0 dB), there was no significant improvement in the long-term post-operative hearing results (P =0.20).

Interestingly, a similar result was observed for hearing results after an operation without laser application at the footplate. There was no difference between the short and long-term period ($p = 0.7$). However, a significant improvement compared to preoperative values was found (mean: 4.2 dB and 5.3 dB; $p = 0.02$ and $p = 0.003$).

There were significant improvements for all these three laser energy-dependent subgroups as compared to preoperative thresholds (mean: 3.2 dB, 3.2 dB and 5.9 dB; $p = 0.005$, $p= 0.03$ and $p < 0.001$).

The statistical comparison between these three laser energy-dependent subgroups and the group without laser application indicated that in the long term data, there was no significant difference (Kruskal-Wallis test: $p= 0.7$).

Table 13 shows the alteration of postoperative bone conduction hearing threshold after revision surgery for the long-term post-operative hearing results (1.5 to 6 months). Differences of preoperative minus postoperative Pure-Tone-Average values were calculated (positive values indicate a better postoperative hearing threshold).

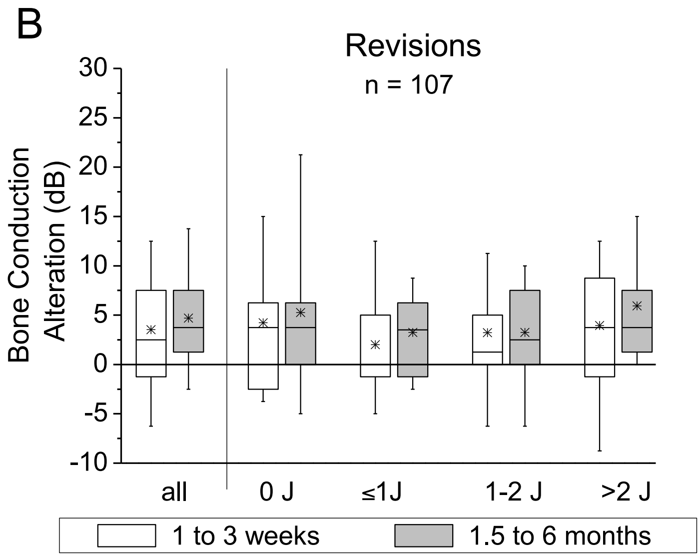

Figure 11: Alteration of short-term (1-3 weeks; empty bars) and long-term (1.5-6 month; filled bars) postoperative bone conduction hearing thresholds compared to preoperative results after revision stapedotomy for all patients (left) and in relation to the amount of laser energy applied at the footplate (right). Positive values indicate a lower hearing threshold as compared to preoperative values, as the differences of preoperative minus postoperative Pure-Tone-Average values were calculated to determine the bone conduction alteration. The boxes show the median and 25% to 75% quartile, the whiskers represent the 10% to 90% range and the stars represent the mean value.

Table 12: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing thresholds for all patients after revision operations in relation to the laser energy applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	-6.25	-3.75	-5	-6.25	-8.75
25%	-1.25	-1.875	-1.25	0	-1.25
Median	2.5	3.75	0.625	1.25	4.375
75%	7.5	6.875	5	5	8.75
90%	12.5	15	12.5	11.25	13.125
Mean	3.44	4.24	2.01	3.24	3.96
Std. Dev.	8.02	8.84	6.23	8.56	8.58
P value	< 0.001	0.02	0.3	0.1	0.02

Table 13: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing threshold for all patients in revision operations dependent on laser energies applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	-2.5	-5	-1.875	-6.25	0
25%	1.25	0	-0.625	0	1.25
Median	3.75	3.75	3.625	2.5	3.75
75%	7.5	6.25	6.875	7.5	8.75
90%	13.75	21.25	8.75	10	15
Mean	4.79	5.26	3.24	3.24	5.94
Std Dev	7.22	9.08	4.79	5.71	7.34
P value	< 0.001	0.003	0.005	0.03	< 0.001

■ Comparative analysis of the influence of distinct pathologies at the stapes footplate during the short-term post-surgical phase (1-3 weeks)

To comparatively investigate a possible influence of pathologies frequently encountered in revision stapedotomy, two groups were formed which were further subdivided according to the laser energy used: one with a neomembrane and one with a reossification covering the oval window (**figure 12**). With regard to inner ear function, no statistically significant difference was found among the different groups. Nevertheless, the mean value of bone conduction after perforation of neomembranes and comparatively the group of no laser application was consistently above the hearing level of bone conduction after perforation of reossifications.

Table 14 and **table 15** show the detailed data and analysis of these two groups: the improvements of bone conduction in all subgroups were significantly better than the preoperational hearing results.

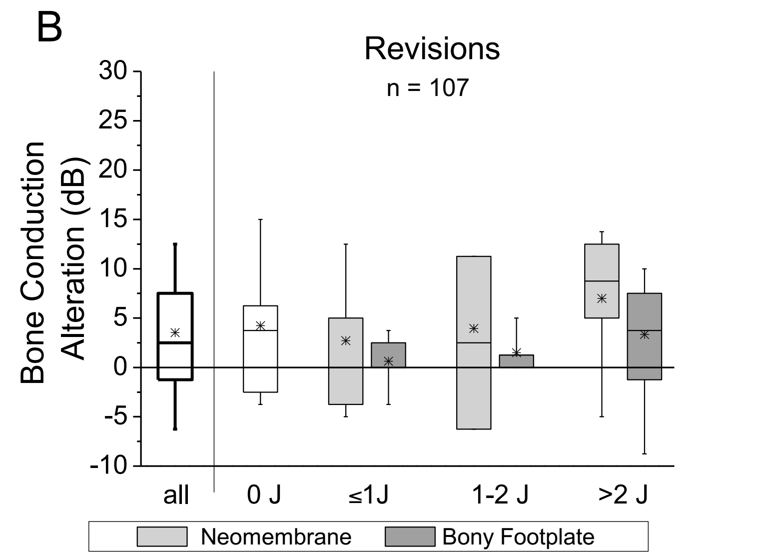

Figure 12: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing thresholds for the "neomembrane" and "bony reossification" groups in revision operations in relation to the laser energy applied at the footplate and the amount of laser energy (neomembrane: 0 J:0 subject, ≤1 J: 15 subjects, 1-2 J: 13 subjects and >2 J: 5 subjects; bony reossification: 0 J: 0 subject, ≤1 J: 7 subjects, 1-2 J: 5 subjects and >2 J: 27 subjects). The boxes show the median and 25% to 75% quartile, the whiskers represent the 10% to 90% range and the stars represent the mean value.

Table 14: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing threshold for the “neomembrane” group in revision operations dependent on amount of laser energy applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-5	-6.25	-5
25%	- - -	- - -	-2.5	-3.75	5
Median	- - -	- - -	0.625	3.125	8.75
75%	- - -	- - -	8.125	11.25	12.5
90%	- - -	- - -	12.5	11.25	13.75
Mean	- - -	- - -	2.71	3.96	7.0
Std Dev	- - -	- - -	7.44	10.15	7.53
P value	- - -	- - -	< 0.05	< 0.05	< 0.05

Table 15: Alteration of short-term (1-3 weeks) postoperative bone conduction hearing threshold for the “bony reossification” group in revision operations dependent on amount of laser energy applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-3.75	0	-8.75
25%	- - -	- - -	0	0	-1.25
Median	- - -	- - -	0.625	1.25	3.75
75%	- - -	- - -	2.5	1.25	7.5
90%	- - -	- - -	3.75	5	10
Mean	- - -	- - -	0.63	1.5	3.35
Std Dev	- - -	- - -	2.59	2.05	8.79
P value	- - -	- - -	> 0.05	> 0.05	< 0.05

Comparative analysis of the influence of distinct pathologies at the stapes footplate during the long-term post-surgical phase (1.5- 6 months)

The whole group of patients was divided into two subgroups: one with a neomembrane and one with a reossification covering the oval window (**figure 13**). No statistically significant difference was found among the different groups.

Interestingly, unlike the investigated interval of 1 to 3 weeks after surgery, the mean values of bone conduction after perforation of neomembranes and no laser application were closer and consistently above the hearing level of bone conduction after perforation of reossifications (with the exception of the subgroup 1-2 J).

Table 16 and **table 17** show the data and analysis of these two groups: the improvement of bone conduction in all subgroups was significantly better than the preoperational hearing results.

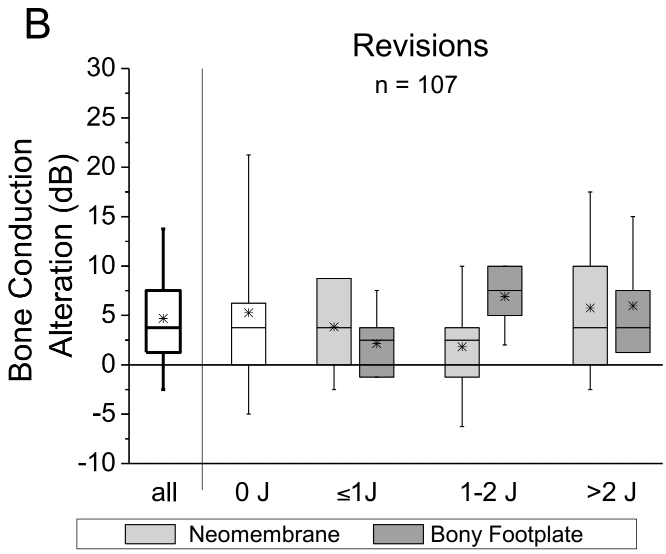

Figure 13: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing thresholds for "neomembrane" and "bony reossification" groups in revision operations dependent on laser energy applied at the footplate and the amount of laser energy applied (neomembrane: 0 J: 0 subject, ≤1 J: 15 subjects, 1-2 J: 13 subjects and >2 J: 5 subjects; bony reossification: 0 J: 0 subject, ≤1 J: 7 subjects, 1-2 J: 5 subjects and >2 J: 27 subjects). The boxes show the median and 25% to 75% quartile, the whiskers represent the 10% to 90% range and the stars represent the mean value.

Table 16: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing thresholds for "neomembrane" group after revision operations in relation to laser energy applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-2.5	-6.25	-2.5
25%	- - -	- - -	0	-1.25	0
Median	- - -	- - -	3.75	2.5	3.75
75%	- - -	- - -	8.75	3.75	10
90%	- - -	- - -	8.75	10	17.5
Mean	- - -	- - -	3.83	1.83	5.75
Std Dev	- - -	- - -	5.48	5.88	8.08
P value	- - -	- - -	< 0.05	< 0.05	< 0.05

Table 17: Alteration of long-term (1.5-6 months) postoperative bone conduction hearing threshold for "bony reossification" group after revision surgery in relation to the laser energy applied at the footplate

	all	0 J	≤ 1 J	1 – 2 J	≥ 2 J
10%	- - -	- - -	-1,25	2	1,25
25%	- - -	- - -	-1,25	5	1,25
Median	- - -	- - -	2,5	7,5	3,75
75%	- - -	- - -	3,75	10	7,5
90%	- - -	- - -	7,5	10	15
Mean	- - -	- - -	2,14	6,9	5,97
Std Dev	- - -	- - -	3,20	3,44	7,36
P value	- - -	- - -	< 0.05	< 0.05	< 0.05

4.2.4 Summary of study results after primary and revision stapedotomy

Table 18 summarizes the significant and insignificant differences in the bone conduction thresholds after primary and revision stapedotomy for the short- and long- term postoperative follow-ups compared to each other and to the preoperative bone conduction threshold. Subgroups were formed according to the energy and surgical technique used.

Table 18: Summary table of the study results

	Significant improvement in bone conduction threshold		
Primary stapedotomy	**Short term vs. preoperative**	**Long term vs. preoperative**	**Short term vs. long term**
One-shot (total)	Yes	Yes	Yes
Subgroup of one shot (≤1 J)	Yes	Yes	Yes
Subgroup of one shot (1-2 J)	No	Yes	Yes
Multiple-shots (total)	No	Yes	Yes
Subgroup of multiple-shots (1-2 J)	No	Yes	Yes
Subgroup of multiple-shots (> 2 J)	No	Yes	Yes
	Significant improvement in bone conduction threshold		
Revision stapedotomy	**Short term vs. preoperative**	**Long term vs. preoperative**	**Short term vs. long term**

No laser applied (0 J)	Yes	Yes	No
Neomembrane (total)	Yes	Yes	No
Subgroup of Neomembrane (≤1 J)	Yes	Yes	No
Subgroup of Neomembrane (≤1 J)	Yes	Yes	No
Subgroup of Neomembrane (1-2 J)	Yes	Yes	No
Subgroup of Neomembrane (> 2 J)	Yes	Yes	No
Bony reossification (total)	Yes	Yes	No
Subgroup of Bony reossification (≤1 J)	No	Yes	No
Subgroup of Bony reossification (1-2 J)	No	Yes	Yes
Subgroup of Bony reossification (> 2 J)	Yes	Yes	No

	Statistically Significant difference in bone conduction threshold	
	Short term	Long term
Multiple-shots technique vs. one-shot technique (total)	Yes (one-shot technique is safer)	No
Subgroup of 1-2 J	No	No
neomembrane vs. reossification covering the oval window (total)	No	No
Subgroup of ≤1 J	No	No
Subgroup of 1-2 J	No	No
Subgroup of > 2 J	No	No

	Significant difference in bone conduction threshold improvement among different laser groups (0 J, ≤1 J, 1-2 J, ＞2 J)	
	Short term	Long term
Primary surgery	Yes (energy≤1 J is better)	No
Revision surgery	No	No

5. DISCUSSION

In order to create a precise perforation by laser stapedotomy while at the same time preserving inner ear function and avoiding damage to the remaining middle ear structures, strict adherence to the recommended laser energy parameters is recommended to minimize the risk of thermal and/or acoustic damage to middle and inner ear structures[18-20].

These recommendations derive from previous experimental and clinical studies that produced supposedly safe and effective parameters for CO_2 laser stapedotomy (type 40c with the Lumenis Acuspot 712 micromanipulator)[29,31,32,34]. These data indicate that the energy used for a single application should not exceed 1 J[32].

Previous clinical investigations indicated that in about 32% of primary laser stapedotomies and in 78% of revision laser stapedotomies, one laser application according to the "one-shot" technique was not sufficient and more applications were necessary to achieve an adequate perforation to place a prosthesis[29,30]. In the present study, about 34% of primary and 55% of revision laser stapedotomies required multiple-shots to achieve an adequate perforation. The amount of applied laser energy increases with the increasing number of applications with and without scanner. 44% of primary cases required more than 1 J (26% 1~2 J, 18%> 2 J) total energy and 47% of revision cases required more than 1 J (17% 1~2 J, 30%> 2 J) total energy.

Until now, no study has systematically investigated clinically the validity of the introducing statement of the discussion. It was therefore investigated if there was a correlation between the total cumulative laser energy used for stapedotomy and postoperative bone conduction thresholds as an indicator for inner ear damage.

A) After primary CO_2 laser stapedotomy bone conduction thresholds improved during both observation periods.

The mean postoperative values for all patients treated with a primary stapedotomy were 0.8 ± 7.5 dB (short-term) and 4.7 ± 5.8 dB (long-term). Compared to the preoperative value, in both periods statistically significant improvements ($P = 0.021$ and $P < 0.001$) were observed. These results indicated that in a majority of patients in both tested periods bone conduction hearing levels improved postoperatively (short term: 62%; long term: 82% of the patients). Compared to the short-term hearing results, the results after long-term follow-up showed again a statistically significant improvement (P <0.001), demonstrating that the patients' bone hearing thresholds increased gradually with time and that this process may last several months.

According to the literature, some authors[35-37] indicated that the usage of a CO_2 laser causes temporary thermal damage to the inner ear. Brase et al[36] found that in patients one day after surgery, the bone conduction threshold was increased in almost all frequencies for both CO_2 laser and microdriller stapedotomy. The hearing threshold for some frequencies (2 kHz or 4 kHz) still remained higher as compared to the preoperative threshold after 4 days for both perforation techniques. However, thresholds in other frequencies had already recovered. In contrast, Sergi et al.[38] found bone conduction threshold to be stable even in data obtained 2 days after the operation.

Looking at the second observation period, the data indicated further improvement of bone conduction hearing levels over time, which led to the possible conclusion that a temporary negative effect due to the intervention possibly reversed over time. These observations are supported by findings of several other researches [35-37,39]. Especially Brase et al.[36], who used the same CO_2 laser instrument as that used in the present study (A type 40C CO_2 laser (Lumenis, Tel Aviv, Israel) with an AcuSpot 712 micromanipulator) and a similar surgical strategy (one-shot technique in most cases), found that immediately after surgery the bone conduction threshold was significantly increased but returned to the same or even better bone conduction levels 1 year post-operatively than those measured pre-operatively. Boonchoo et al.[35] also compared the early and late hearing outcomes after CO_2 laser stapedotomy and reached a similar conclusion: after CO_2 laser stapedotomy, the stability of cochlear function begins to improve in the early postoperative period and remains stable through the late postoperative period.

According to the literature, the cause of post-operatively increased bone conduction thresholds in the immediate phase after the operation maybe due to a transient labyrinthine hydrops, anoxia, vascular sludging, surgical trauma[40,41] or perilymph fistula (leakage of cochlear fluid) [42-44]. Most of the cases are described as self-limited and were observed to recover overtime. This can explain why the post-operative results improved during follow-up.

B) After revision CO_2 laser stapedotomy an improvement in bone conduction was observed, however no change over time was demonstrated.

The mean bone conduction hearing level for all patients that underwent revision surgery was 3.4 ±8.0 dB (short-term) and 4.8±7.2 dB (long-term). Both observed time periods were statistically significantly better than the preoperative value (P <0.001). Compared to the short-term hearing results, there was no significant improvement in the long-term post-operative hearing results (P =0.20). This allows to conclude that also revision stapedotomy resulted in an improvement in bone conduction hearing levels. However, for two subgroups of perforating on the bony-reossification with laser energy of ≤1 J and 1-2 J, there were no significant improvements compared to the preoperative bone conduction hearing result during short-term follow up. However, these two subgroups improved over time and in the long-term observation period to significantly better hearing thresholds than those observed preoperatively. This could also lead to the conclusion that temporary negative effect due to the intervention possibly reversed over time as discussed in the preceding paragraph.

C) No difference in bone conduction hearing level in relation to defined energy ranges were found in revision surgeries or in long-term follow-up.

With regard to the long-term observation period or for revision surgeries, the postoperative alterations in bone conduction hearing thresholds showed that there were no energy-dependent differences among the different groups (total energy: 0 J, ≤1J, 1-2 J and > 2 J (ANOVA, 5% level)). The improvement of bone conduction hearing level determined after the long-term period was statistically highly significant in all subgroups. This indicates that there was no significant energy-dependent influence of the total applied laser energy on the bone conduction thresholds if the surgery was conducted as described in the materials and methods section.

Several reasons are possible:

1) The far-infrared emission of the CO_2 laser has the main advantage of a strong absorption by aqueous solutions, resulting in a shallow penetration depth of only 0.01 mm from the irradiated surface. This property of CO_2 laser light is particularly useful in stapes surgery. During a stapedotomy, the perilymph absorbs the CO_2 laser energy and thus protects the inner ear structures from direct injury.

2) Generally, an overall temperature rise over 4°C is considered harmful. In some animal experiments it was demonstrated that if the temperature rose over 3°C, the inner ear function still stayed stable and the thermal inner ear damage was reversible [45,46]. Meanwhile, Gardner G, Lesinski SG and Kodali S et al.[5,47,48] separately measured the local heat during the CO_2

laser stapedotomy. The temperatures measured in the vestibule or below the footplate in these studies showed much lower results (1.0°C, 0.3°C and 1.8°C) than the temperature presumed to be harmful.

3) In experimental laboratory studies, Kamalski et al.[49] addressed the question of dynamic thermal damage by different lasers (KTP, Thulium, and CO_2 laser) in an inner ear model using a special technique combining high-speed and thermal imaging. The CO_2 group showed that in continuous wave mode, the CO_2 laser energy was mostly absorbed in water, showing only minimal penetration of heat into the vestibule. Because the distance from the footplate to the wall of the vestibule is above 3 mm, even local temperature rises along the footplate after using 10.6 μm continuous wave CO_2 laser (energy output: 2 W, pulse time 100 ms; our setting in primary operations without scanner system (6 W, 50 ms)), no thermal effects on the cochlear are likely.

4) Usage of the CO_2 laser in the continuous wave (cw) mode. Because the application of the CO_2 laser in super pulse mode with peak pulse powers of approximately 300 W in stapedotomy appears to be more unreliable and dangerous for the inner ear, continuous wave (cw) mode has been believed to be much safer than the super pulsed mode[32]. Almost all perforations in primary surgery and most of the perforations in revision surgeries are achieved by laser irradiation with microprocessor-controlled rotating mirrors, the so-called scanner systems (SurgiTouchTM, ESC Sharplan Co), a spiral figure is traced within the defined pulse duration. This device enables the CO_2 laser to achieve a much higher power density with minimal side effects. Typically, the laser energy used with the scanner system is 20-22 W. For the multiple-shot technique, laser energies with a maximum 6 W and a pulse duration of 0.05 s were used. The energy of a single pulse never exceeded 1 J.

D) After primary surgery, in the short-term observation period, better results were found if the "one-shot technique" with energies ≤ 1 J was used than in other subgroups

Although the CO_2 laser stapedotomy has been proven to be a safe and reliable operation as shown in the long term follow up. Data from the short-term period after primary surgery showed an improvement only in the subgroup of patients, where the one-shot technique with ≤ 1 J was applied. All other subgroups with higher energies (either one-shot technique or multiple-shots technique) resulted in no significant improvement during the shot-term follow up indicating that there is a temporary negative effect most likely caused by the laser energy.

E) No difference was observed in bone conduction hearing thresholds relative to distinct pathologies at the stapes footplate -as encountered in revision surgery-

Next, we compared the influence on bone conduction hearing thresholds as a possible result of distinct pathologies encountered at the stapes footplate. These pathologies required different amounts of total laser energy during surgery (0, ≤1 J, 1-2 J and > 2 J). A change of prosthesis required 0 J, while some re-ossifications of the stapes footplate required more that 2 J. Despite these large differences, the statistical analysis showed no significant difference between groups in either tested period.

This analysis also allowed for an investigation and comparison of the influence of the revision surgery without laser application to those with laser application.

There are several possible explanations for these findings:

A) In primary surgery, as a result of multiple laser applications (34% of the primary surgery), the cumulative laser energy may be much higher than that required for one or two applications, however the energy used for each application was always below 1 J. In revision surgery, the amount of energy used for each single application depended on the structure covering the oval niche. Although in cases of a bony re-obliterated footplate, the total used energy was extremely high (max 22.4 J), the energy of a single application was always below 1.2 J (the amount of single laser used to reopen a neomembrane was even lower). Confirming our clinical observations, investigations of the effect of CO_2 laser irradiation in a guinea-pig cochlear model demonstrated safety with laser energies of up to 2 J applied in the cw mode[32].

B) Furthermore, because the energy of the CO_2 laser is not only well absorbed by aqueous fluids but also by connective tissue (like the neo-membrane or the small bony structures), it can be used to vaporize these tissues, minimizing mechanical trauma. When perforating the footplate either bony or covered with a neomembrane, the fluid underneath absorbs most of the laser energy with these bony or neomembrane tissues thus preventing an irradiation of the cochlear.

C) Interestingly, the greatest variability in hearing results was found in the group of patients in which only a replacement of the prosthesis using the pre-existing perforation was performed (revision, 0 J). The postoperative bone conduction hearing threshold was 22 dB better. This observation indicates the existence of factors influencing hearing independent of the perforation procedure. Until these have been elucidated much care should be taken during this step of the operation.

To confirm the above findings and to put them into the wider perspective, a review of recent studies that included data about bone conduction hearing level change before and after stapedotomy with different techniques was performed and compared to our study results (**table 19**).

Table 19: Comparison of bone conduction hearing levels before and after stapedotomy extracted from the literature to our study results.

No	Study	Method	Patients (N)	Follow-up time	BC change[5] (PTApre-PTApost)	Primary or Revision surgery	Difference compared to study results
1	Merán Gil JL et al.[50]	Perforator or microdriller	46	Mean 2.65 months	2.1 dB	Primary surgery	0.002*
2	Boonchoo R[35]	CO_2 laser	26 CO_2 laser	1 year	0.7±9.5 dB	Primary surgery	≤0.001**
3	Freni F et al.[37]	CO_2 laser	49 classic steps 35 reversal steps	1 year	4.01±6.88 dB 4.29±6.01 dB	Primary surgery	0.439 0.687
4	Buchman CA et al.[23]	Argon laser VS CO_2 laser	59 Argon laser 65 CO_2 laser	Mean 4.7 months	5.6±8.0 dB 1.0±8.2 dB	Primary surgery	0.290 ≤0.001**
5	Sperling NM et al.[51]	Argon laser	45 Argon laser	6 months	-3.15 dB	Primary surgery	≤0.001**
6	Vincent R et al.[52]	Argon laser	583 Argon laser	Mean 25.2 months	-3.5 dB	Revision surgery	≤0.001**
7	Acar GO et al.[53]	KTP laser or Argon laser	16 STAMP* 67 SFS*	1 year	4.3±3.7 dB 1.9±7.0 dB	Primary surgery	0.783 ≤0.001**

8	Mangham CA Jr et al.[54]	KTP laser or microdriller	144 platinum piston 44 nitinol piston	1 year	3.8±7.2 dB 2.9±7.4 dB	Primary surgery	0.134 0.057
9	Timoshenko AP et al.[39]	KTP laser VS Er: YAG laser	137 KTP laser 54 Er: YAG laser	3 months	-0.1±8.6 dB -1.5±7.8 dB	Primary surgery	≤0.001** ≤0.001**
10	Galli J et al.[55]	Er:YAG laser VS microdriller	29 Er:YAG laser 41 microdriller	1 year	1.7 dB 0.22 dB	Primary surgery	0.005* ≤0.001**
11	Current study	CO_2 laser	391 Primary 107 Revision	1.5~6 months	4.70±5.75 dB 4.79±7.22 dB		

1 STAMP means stapedotomy minus prosthesis; SFS means small fenestra stapedotomy.

2 No 1, 8, 10 were perforator or microdriller;

3 NO 2~4 were CO_2 laser; No 4~9 were Argon or KTP laser (have the same wavelength);

4 No 9 and 10 were Er:YAG laser.

5 positive values indicate a postoperative improvement in bone conduction levels

* P≤0.05 study results were significantly or ** P≤0.001 highly significant better as compared to the cited literature.

The data presented in **table 19** show the change of the postoperative bone conduction after laser stapedotomy compared to classic footplate opening techniques using manual perforators or microdriller or other lasers. Positive values indicate an improvement in postoperative bone conduction thresholds. Some authors also compared the bone conduction result between the classic and reverse steps stapedotomy or different surgical procedures such as stapedotomy minus prosthesis and small fenestra stapedotomy. Independent of the methods that were used, compared to these studies, the results of our study were similar or better after long-term (1.5 to 6 months) post operation. In particular, we achieved better results compared to the studies of 1, 2, 5, 6, 9 and 10. In addition, when compared to the studies of 3, 4, 7, and 8, we achieved a similar or slightly improved result.

Taking these data together allows the conclusion that based on our and other groups' data, using the CO_2 laser within a defined laser energy range for each application, even repeated applications with high total laser energy are not harmful to the inner ear.

6. CONCLUSION

According to our analysis of the used energy for CO_2 laser stapedotomy, relative to the surgical procedure and time after surgery, we found on average an improved bone conduction hearing threshold in the short-term follow-up period (1~3 weeks) and the long-term follow up period (1.5~6 months).

After dividing patients into groups according to the total used laser energy, our results indicated that 1 J for each laser application should not be exceeded for best bone conduction results.

However, as observed during the long-term follow up period, a perforation of the footplate with repeated laser applications in primary or revision operations within the tested energy range had no negative influence on postoperative inner ear function.

The analysis of the two observed time periods showed no significant difference between distinct pathologies at stapes footplate for revision surgery. While on average not being significantly different compared to the other groups, a change of prosthesis showed a high variation of postoperative bone conduction hearing thresholds, indicating that factors other than the laser application influence bone conduction as well and that the change of the prosthesis is a critical step of the surgery.

The comparison of the data obtained during this study to data extracted from studies of other groups confirmed that other groups achieved similar results, confirming the results of this study.

In conclusion, the “one-shot technique” with energies ≤ 1J was superior to the “multiple-shots technique” for primary surgery in the short-term observation period.

However, the influence of total laser energy during CO_2 laser stapedotomy performed as primary or revision surgery using the one-shot or multiple-shots technique had no energy-related damaging long-term effect on bone conduction hearing levels and can therefore be considered as safe.

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8. AFFIDAVIT

"I, [Weiming, Hu] certify under penalty of perjury by my own signature that I have submitted the thesis on the topic [CO_2-laserstapedotomy: Analysis of used energy and its influence on bone-conduction hearing levels] I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My interests in any publications to this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author correspond to the URM (see above) and I am solely responsible.
The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date Signature

Declaration of any eventual publications

[Weiming Hu] had the following share in the following publications:

1. Sun Z, **Hu W**, Xu J, Kaufmann AM, Albers AE. MicroRNA-34a regulates epithelial-mesenchymal transition and cancer stem cell phenotype of head and neck squamous cell carcinoma in vitro. Int J Oncol. 2015 Aug 31. doi: 10.3892/ijo.2015.3142. Responsible: Co-author for drafting the manuscript and data collection.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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9. CURRICULUM VITA AND PUBLICATIONS

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publications:

1. Sun Z, **Hu W**, Xu J, Kaufmann AM, Albers AE. MicroRNA-34a regulates epithelial-mesenchymal transition and cancer stem cell phenotype of head and neck squamous cell carcinoma in vitro. Int J Oncol. 2015 Aug 31. doi: 10.3892/ijo.2015.3142. Responsible: Co-author for drafting the manuscript and data collection.

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